Antimicrobial therapy in prosthetic joint infection: an approach to the most relevant and current clinical problems

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ANTIMICROBIAL THERAPY IN PROSTHETIC JOINT INFECTION: AN APPROACH TO THE MOST RELEVANT AND CURRENT CLINICAL PROBLEMS

Memoria presentada por

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El Dr. Javier Ariza Cardenal, Profesor de la Facultad de Medicina de la Universidad de Barcelona y Jefe Clínico del Servicio de Enfermedades Infecciosas del Hospital Universitario de Bellvitge, y el Dr. Oscar Murillo Rubio, médico adjunto del Servicio de Enfermedades Infecciosas del Hospital Universitario de Bellvitge, hacen constar que la tesis titulada

"Antimicrobial therapy in prosthetic Joint infection: an approach to the most relevant and current clinical problems"

que presenta el licenciado Jaime Lora-Tamayo, ha sido realizada bajo su dirección en el campus de Bellvitge de la Facultad de Medicina, la consideran finalizada y autorizan su presentación para que sea defendida ante el tribunal que corresponda.

En Barcelona, febrero de 2014

Dr. Javier Ariza Cardenal

Dr. Oscar Murillo Rubio

A mis padres

A mis hermanos

A Celina

Sin su amor no es posible un
clima para la ciencia

Existen, sin duda, profesionales de distintos campos que cultivan asiduamente, por encima de la profesión misma, el estudio de los principios científicos que van influenciando su fisonomía, y esto ennoblece y dignifica su ejercicio profesional, que adquiere con ello rango y categoría superiores.

Manuel Lora-Tamayo, *Un clima para la Ciencia*

If you can force your heart and nerve and senew
To serve your turn long after they are gone,
And so hold on when there is nothing in you
Except the Will which says to them 'Hold on'

Rudyard Kipling, *If…*

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La esencia fundamental de esta tesis no comenzó con la elaboración de los trabajos científicos que aquí se recogen. Se gestó en el estudiante de tercero y en sus primeras aproximaciones a la clínica, de la mano del Maestro. La mayúscula no es casual, y se erige en modesto reconocimiento del acompañamiento de estos años, de las enseñanzas, de la medicina, de la vida. La puerta siempre abierta, saber mirar para encontrar, buscar la magia, esculpir la realidad. Javier Ariza, sabio jesuítico en la penumbra progresiva, ha sido un incombustible director de tesis, jefe y amigo, y es el artífice fundamental de estos trabajos. Mi profundo agradecimiento a Oscar Murillo, también director de esta tesis, ejemplo de transversalidad, oficio clínico y rigor científico, que me descubrió el universo del tan de moda biofilm bacteriano.

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- **RESUMEN**

1. Introducción

La infección es la principal complicación tras el implante de una prótesis articular. Supone una catástrofe para el paciente y para el sistema de salud, por cuanto su tratamiento exige numerosas cirugías y prolongados tratamientos. Se prevé un incremento del número absoluto de infecciones protésicas en el futuro. Son infecciones difíciles de tratar, fundamentalmente por la presencia de biofilm bacteriano en el que las bacterias se tornan menos susceptibles a los antibióticos. Esto obliga a un tratamiento agresivo, médico y quirúrgico, que con frecuencia requiere la retirada de la prótesis. Por su forma de presentación clínica, las infecciones protésicas se clasifican en

- Post-quirúrgicas precoces: aquéllas que acontecen durante el primer mes tras la colocación de la prótesis (algunos autores amplían este margen a los primeros 90 días)
- Hematógenas: infección de la prótesis en el curso de una bacteriemia en cualquier momento tras la colocación del implante
- Post-quirúrgicas tardías: aquéllas cuyos síntomas comienzan pasados los primeros 30 días tras la colocación de la prótesis articular
- y Cultivos Intraoperatorios Positivos: infecciones documentadas a partir de cultivos positivos durante la revisión en un tiempo de una prótesis articular, sin sospecha previa de infección

Los dos primeros tipos, agrupados como “infecciones agudas”, son debidos a microorganismos virulentos como *Staphylococcus aureus*, estreptococos o bacilos Gram-negativos (BGN). Se presentan con frecuencia con floridos signos inflamatorios que facilitan el diagnóstico. En estos casos puede optarse por un tratamiento conservador, consistente en desbridamiento, retención del implante y antibióticos (DAIR). Las dificultades para curar al paciente con este planteamiento son mayores, por cuanto se conserva el cuerpo extraño, pero ofrece importantes ventajas: supone una cirugía menos agresiva que el explante protésico, así como un número potencial de cirugías menor; evita el gasto de reserva ósea del paciente, y mantiene las

posibilidades de recambio protésico ulterior; finalmente, es una aproximación menos costosa.

Las infecciones crónicas se presentan con pocos signos inflamatorios. Frecuentemente el dolor articular es la única manifestación, haciendo que el cuadro sea difícil de distinguir del aflojamiento aséptico. Esto se debe a la naturaleza menos virulenta de los microorganismos responsables [estafilococos coagulasa-negativos (CNS) o *Propionibacterium acnes*]. El diagnóstico se basa finalmente en un compendio de datos clínicos, analíticos, radiológicos, histopatológicos y microbiológicos. El tratamiento exige con frecuencia la retirada del dispositivo articular e, idealmente, la sustitución por una nueva prótesis.

El tratamiento antibiótico que acompaña la estrategia quirúrgica debe ser activo frente a bacterias estacionarias e intracelulares, y debe difundir bien a través del biofilm y del tejido óseo. Además, no debe ser tóxico y debe permitir su administración durante largos períodos de tiempo.

El conocimiento clínico que tenemos de estas infecciones, y en particular del tratamiento antibiótico, procede en su mayor parte de estudios observacionales retrospectivos, con frecuencia realizados con muestras escasas y heterogéneas. Muchos de estos estudios no han sido diseñados para evaluar la actividad antibiótica. La escasez de estudios prospectivos y controlados se debe a las dificultades que existen para conseguir muestras algo más abultadas sin perder homogeneidad en la patología estudiada, y también por necesidad de largos períodos de seguimiento.

2. Objetivos.

A. Tratamiento antibiótico en la infección de prótesis articular manejada con retención del implante

A.1. Infección por *Staphylococcus* spp.

Objetivo 1 – Medir el impacto de rifampicina en el pronóstico de una gran cohorte de pacientes con infección protésica por *S. aureus*, incluyendo MRSA.

Objetivo 2 – Evaluar la eficacia de un tratamiento corto con levofloxacino y rifampicina en la infección estafilocócica de prótesis articular.

Objetivo 3 – Determinar el papel de la combinación de daptomicina más rifampicina para la infección de prótesis articular por estafilococos resistentes a fluoroquinolonas.

A.2. Infección por bacilos Gram-negativos

Objetivo 4 – Medir el impacto de las fluoroquinolonas en el pronóstico de una gran cohorte de pacientes con infección protésica por bacilos Gram-negativos.

A.3. Infección de prótesis articular en ancianos

Objetivo 5 – Análisis comparativo de la eficacia antibiótica en pacientes portadores de hemiartroplastia de cadera frente a pacientes con prótesis total de cadera

B. Tratamiento antibiótico en la infección de prótesis articular manejada con retirada del implante

Objetivo 6 – Evaluar el papel de linezolid en la infección de prótesis articular por microorganismos Gram-positivos manejada con un recambio en 2 tiempos.

C. **Actividad antimicrobiana en biopelículas de microorganismos Gram-negativos multirresistentes.**

Objetivo 7 – Evaluación de la actividad de colistina frente a biofilm de *P. aeruginosa* multirresistente en un modelo experimental *in vitro*.

3. Métodos

3.1. Investigación clínica.

Los estudios clínicos realizados en esta tesis se han desarrollado en la Unidad de Infección Osteoarticular del Hospital Universitario de Bellvitge, donde el doctorando ha podido colaborar en calidad de consultor de enfermedades infecciosas. Dirigida por el Dr. Xavier Cabo, se trata ésta de una unidad interdisciplinar, de referencia nacional para casos de difícil tratamiento, constituida por médicos traumatólogos, reumatólogos, microbiólogos, radiólogos e internistas especialistas en enfermedades infecciosas. Cuenta además con una dotación de enfermeras especializadas en las curas de estos pacientes. Desde el año 2003, la información de todos los casos de infección asociada a prótesis articular se almacena en una base de datos prospectiva.

Los estudios clínicos de carácter multicéntrico se han realizado en el marco de la Red Española de Investigación en Patología Infecciosa (REIPI), que cuenta con un Grupo para el Estudio de la Patogénesis y Tratamiento Antibiótico de la Infección de Prótesis Articular, coordinado por uno de los directores de la presente tesis doctoral, el Dr. Javier Ariza. Los hospitales integrados en este grupo comparten un protocolo clínico que ha permitido homogeneizar el manejo de pacientes con infección protésica. Durante el período 2003-06 compartieron asimismo una base de datos prospectiva de casos con infección protésica.

Los estudios clínicos de esta tesis incluyen 4 estudios observacionales y 2 ensayos clínicos. Aquéllos se han realizado mediante el análisis retrospectivo de información prospectivamente recogida. Los ensayos clínicos fueron debidamente registrados y aprobados por los comités éticos de los hospitales participantes.

3.2 Investigación experimental.

En el Laboratorio de Infección Experimental de la Universidad de Barcelona (Campus Bellvitge), vinculado al Servicio de Enf. Infecciosas del H. U. Bellvitge, se ha venido desarrollando en los últimos años un modelo de infección de cuerpo extraño en rata, en el que el doctorando ha podido participar. Este modelo permite una aproximación a las dificultades del tratamiento de la infección asociada a biofilm.

Además, el doctorando se trasladó durante 10 meses al Monash Institute of Pharmaceutical Sciences (Monash University, Melbourne, Australia), donde puso en marcha un modelo dinámico *in vitro* para estudiar la actividad de colistina y doripenem frente al biofilm generado por 3 cepas distintas de *P. aeruginosa* (una cepa de referencia, susceptible a carbapenemes, y 2 cepas clínicas, resistentes a carbapenemes; las tres heterorresistentes a colistina). El modelo está basado en el CDC Biofilm Reactor.

Para los experimentos se emplearon dos concentraciones de colistina clínicamente relevantes (1.25 mg/L y 3.50 mg/L), doripenem y sus combinaciones. La actividad de los distintos regímenes fue evaluada mediante recuento de bacterias viables en cultivo convencional. También se evaluó la emergencia de resistencia a colistina durante el tratamiento.

4. Resultados por objetivos

Objetivo 1 - Medir el impacto de rifampicina en el pronóstico de una gran cohorte de pacientes con infección protésica por *S. aureus*, incluyendo MRSA.

Artículo 1 – *A Large Multicenter Study of Methicillin-Susceptible and Methicillin-Resistant* Staphylococcus aureus *Prosthetic Joint Infections Managed with Implant Retention*. **J. Lora-Tamayo**, O. Murillo, J. A. Iribarren, A. Soriano, M. Sánchez-Somolinos, J. M. Baraia-Etxaburu, A. Rico, J. Palomino, D. Rodríguez-Pardo, J. P.

Horcajada, N. Benito, A. Bahamonde, A. Granados, M. D. del Toro, J. Cobo, M. Riera, A. ramos, A. Jover-Sáenz, J. Ariza. Clinical Infectious Diseases 2013; 56: 182-194.

Capítulo de libro – *Systemic treatment options for medical device-associated* infection. O. Murillo, **J. Lora-Tamayo**, J. Ariza. In: *Biomaterials associated infection*. T. F. Moriarty, S. A. J. Zaat, H. J. Busscher (eds), Ed. Springer. New York, 2013.

En este estudio observacional, retrospectivo, multicéntrico (17 hospitales en España), se incluyeron todos los casos de infección de prótesis articular por *S. aureus* manejados con desbridamiento, antibióticos y retención del implante (DAIR) entre 2003 y 2010. Durante este período, se dieron 345 casos (41% hombres, edad mediana 73 años), de los que 81 (23%) fueron MRSA.

Hubo una mayor proporción de infecciones hematógenas entre los casos con infección por *S. aureus* sensible a meticilina (MSSA), así como también infecciones de rodilla. La infección por MRSA ocurrió en pacientes más mayores y con más frecuente comorbilidad. Ambos tipos de infección recibieron combinaciones de rifampicina por igual, aunque la combinación específica de rifampicina fue distinta en cada caso: beta-lactámicos y fluoroquinolonas en el caso de MMSA; glucopéptidos, clindamicina, cotrimoxazol o linezolid en el de MRSA.

Se documentó fracaso del tratamiento en un 45% de los casos, tras una mediana de 1257 días. Los parámetros independientemente asociados con el fracaso fueron el tratamiento inmunosupresor, la presencia de bacteriemia, la infección polimicrobiana, la cifra de proteína C-reactiva, la necesidad de 2 ó más desbridamientos y el recambio de los componentes móviles (papel protector).

El tratamiento con rifampicina se asoció de forma independiente con un mejor pronóstico (HR 0.49), tanto para MSSA como para MRSA. Globalmente, los casos de infección por MSSA y MRSA tuvieron una tasa similar de fallo (44% vs 46%, p=0.778). Sin embargo, la dinámica de fracaso fue muy distinta: mientras que los casos de infección por MSSA fracasaron fundamentalmente una vez concluido el tratamiento

antibiótico, en el caso de la infección por MRSA el fracaso se produjo durante el tratamiento.

El pronóstico de los pacientes con infección post-quirúrgica y edad protésica entre los 30 y 90 días fue similar a aquéllos con edad protésica <30 días. Finalmente, los pacientes tratados durante >90 días presentaron un pronóstico similar a aquéllos tratados entre 60 y 90 días, y a aquéllos tratados <60 días.

Objetivo 2 - Evaluar la eficacia de un tratamiento corto con levofloxacino y rifampicina en la infección estafilocócica de prótesis articular.

Comunicación oral - *Short vs long duration of levofloxacin plus rifampin for acute staphylococcal prosthetic joint infection managed with implant retention: preliminary results of a clinical trial.* **J. Lora-Tamayo,** G. Euba, J. Cobo, J. Horcajada, A. Soriano, E. Sandoval, N. Benito, D. Rodríguez-Pardo, L. Falguera, M. del Toro, J. Palomino, J. Iribarren, A. Jover-Sáenz, M. Sánchez-Somolinos, A. Ramos, J. Baraia-Etxaburu, M. Fernández, M. Riera, C. Pigrau, J. Ariza. 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy. Denver, USA, 10th-13th September 2013. Comunicación oral (H-1005) de los resultados prelimiares del ensayo clínico (reclutamiento de paciente completado, seguimiento en curso, manuscrito en preparación).

Con la hipótesis de que un tratamiento de 8 semanas podría ser no inferior a un tratamiento de 3 ó 6 meses (para caderas o rodillas, respectivamente) en la infección aguda estafilocócica manejada con DAIR, realizamos un ensayo clínico abierto, comparativo, aleatorizado y multicéntrico (17 centros españoles). A los pacientes, tras ser sometidos a desbridamiento, se les asignaba al azar un tratamiento con levofloxacino y rifampicina durante 8 semanas (rama corta) o 3 ó 6 meses (cadera y rodilla, respectivamente; rama larga). El reclutamiento de pacientes comenzó en 2009 y finalizó en 2013; actualmente el estudio está en fase de seguimiento.

Durante el período de reclutamiento hubo 172 pacientes con infección aguda estafilocócica, de los que 63 (37%) fueron aleatorizados [33 en la rama larga, 30 en la corta (intención de tratar)].

De los 63 pacientes aleatorizados, 19 (30%) no pudieron completar el tratamiento planeado por diferentes motivos, en casos 10 por toxicidad de los antibióticos. En el análisis por intención de tratar de estos 63 pacientes, en 41 (65%) se observó curación, sin diferencias entre las dos ramas: 19 (58%) fracasos en la rama larga, y 22 (73%) en la rama corta (p=0.190); el tiempo medio al fracaso fue de 30 meses (95CI 22-38 meses) en la pauta larga y 33 meses (95CI 25-39 meses) en la pauta corta (p=0.156).

En el análisis por protocolo se incluyeron 44 pacientes (20 en la rama larga y 24 en la rama corta). Se observó curación en 41 pacientes (93%) tras un seguimiento mediano de 355 días (IQR 193-697): 1 (5%) paciente fracasó en la rama larga, y 2 (8%) lo hicieron en la rama corta (p=1.0); el tiempo medio libre de fracaso en la rama larga fue de 45 meses (95CI 41-50 meses), y en la corta fue de 39 meses (95CI 34-43 meses) (p=0.848). Similares resultados se observaron en el subanálisis por tipo de prótesis (rodilla y cadera) tanto en el análisis por protocolo como en el análisis por intención de tratar.

Objetivo 3 - Determinar el papel de la combinación de daptomicina más rifampicina para la infección de prótesis articular por estafilococos resistentes a fluoroquinolonas.

Artículo 3 – *Efficacy and Safety of High Doses of Daptomycin (10 mg/kg/d) plus Rifampin for the Treatment of Staphylococcal Prosthetic Joint Infection Managed with Implant Retention*. **J. Lora-Tamayo**, J. Parra-Ruiz, D. Rodríguez-Pardo, J. Barberán, A. Ribera, E. Tornero, C. Pigrau, J. Mensa, J. Ariza, A. Soriano. En evaluación para su publicación.

Se realizó un estudio observacional en 5 centros españoles entre 2010 y 2012, incluyendo todos los casos de infección protésica por estafilococos resistentes a

quinolonas, manejados con DAIR y que recibieron como tratamiento de primera elección la combinación de daptomicina (10 mg/kg/d) más rifampicina durante 6 semanas. Veinte casos fueron inicialmente incluidos, pero 2 pacientes (10%) no pudieron completar el tratamiento por toxicidad. Así, dieciocho (90%) pacientes recibieron daptomicina + rifampicina durante 6 semanas sin efectos adversos relevantes.

Nueve casos (50%) se consideraron curados tras un seguimiento mediano de 749 días [rango intercuartílico (IQR), 387-970 días]. Por tanto, 9 casos (50%) fracasaron. En 5 de éstos se recuperó el mismo estafilococo responsable de la infección inicial (28% fracaso microbiológico). No se observó aumento de la MIC de daptomicina o rifampicina.

Esta serie se comparó con 44 controles históricos de infección protésica estafilocócica, también tratados con retención del implante y otra combinación de rifampicina. Ambas series fueron comparables en sus características basales y presentación clínica, así como en las tasas globales de fracaso clínico y microbiológico. Sin embargo, el 73% de los fracasos en la cohorte histórica ocurrieron cuando los pacientes aún estaban bajo tratamiento, mientras que esto sólo ocurrió en un 22% de los fracasos de la cohorte daptomicina-rifampicina (p=0.033).

Objetivo 4 - Medir el impacto de las fluoroquinolonas en el pronóstico de una gran cohorte de pacientes con infección protésica por bacilos Gram-negativos.

Artículo 3 – *Gram-negative prosthetic joint infections: outcome of debridement, antibiotics and implant retention approach. A large multicenter study.* D. Rodríguez-Pardo, C. Pigrau, **J. Lora-Tamayo**, A. Soriano, M. D. del Toro, J. Cobo, J. Palomino, G. Euba, M. Riera, M. Sánchez-Somolinos, N. Benito, M. Fernández-Sampedro, L. Sorli, L. Guio, J. A. Iribarren, J. M. Baraia-Etxaburu, A. Ramos, A. Bahamonde, X. Flores-Sánchez, P. S. Corona, J. Ariza. En evaluación para publicación.

Realizamos un estudio observacional multicéntrico (16 hospitales españoles) incluyendo todos los episodios de infección de prótesis articular causados por BGN

entre 2003 y 2010, y se realizó un subanálisis de episodios manejados con DAIR. Se identificaron 242 episodios. Se realizó DAIR en 174 (72%) pacientes. El microorganismo más frecuente fue *E. coli*, seguido de *P. aeruginosa*. La infección fue polimicrobiana en un 20% de casos, más frecuentemente cuando existió infección por *P. aeruginosa*.

Tras un seguimiento mediano de 25 meses (IQR 15-39 meses), un 68% de pacientes seguía libre de fracaso. Los parámetros independientemente asociados con la probabilidad de fracaso fueron la insuficiencia renal crónica y el tratamiento con ciprofloxacino (HR 0.23). El uso de quinolonas también se asoció con un mejor pronóstico en el subgrupo de infección por *P. aeruginosa* y, de forma no significativa, en el grupo de bacterias productoras de beta-lactamasas de expectro extendido. La edad de la prótesis en los casos post-quirúrgicos no se asoció a un peor pronóstico.

Objetivo 5 - Análisis comparativo de la eficacia antibiótica en pacientes portadores de hemiartroplastia de cadera frente a pacientes con prótesis total de cadera

Artículo 4 – *Infected Hip Hemiarthroplasties and Total Hip Arthroplasties: Differential Findings and Prognosis*. **J. Lora-Tamayo**, G. Euba, A. Ribera, O. Murillo, S. Pedrero, D. García-Somoza, M. Pujol, X. Cabo, J. Ariza. Journal of Infection 2013; 67: 536-544.

Realizamos un estudio observacional de todos los casos de infección de prótesis de cadera asistidos en el H. U. Bellvitge (2003-2011), con un subanálisis de los casos manejados con DAIR. Como dispositivos de cadera, se consideraron las prótesis totales (THA) y las prótesis totales de cadera (HHA), y dentro de éstas los dispositivos cementados (C-HHA) y los no cementados (NC-HHA).

En total hubo 210 episodios (63% mujeres, edad mediana 74 años): 148 (61%) THA y 62 (39%) HHA. Los pacientes con HHA fueron más mayores y presentaron más comorbilidades. El tipo de infección en las HHA fue fundamentalmente post-quirúrgica precoz, siendo más variado entre las THA.

Ciento veintitrés (59%) pacientes fueron tratados con DAIR: 72 THA y 51 HHA. Las infecciones por *S. aureus* fueron más frecuentes en THA, mientras que los BGN fueron

más frecuentes entre los pacientes con HHA. La presencia de BGN resistentes a quinolonas y de MRSA más frecuente en portadores de HHA, especialmente en las C-HHA. El tratamiento quirúrgico fue similar para los dos tipos de prótesis, excepto por un menor recambio de componentes móviles en los casos de THA.

El fracaso global de la estrategia DAIR fue del 37%. En este estudio, los parámetros independientemente asociados con la probabilidad de fracaso fueron la infección hematógena, la cifra de leucocitos, la necesidad de 2 ó más desbridamientos, la infección por MRSA y la infección por enterococo. No hubo diferencias significativas en cuanto a fracaso clínico entre los tipos de prótesis (THA 41% vs HHA 31%, p=0.261). Realizamos un subanálisis comparando casos post-quirúrgicos de THA y C-HHA en los que se realizó recambio de componentes móviles, observando una tendencia no significativa a un mayor tiempo libre de fallo para las THA.

Finalmente, se observó una mayor tasa de mortalidad cruda y relacionada con la infección entre los pacientes portadores de HHA (21% vs 4%, p=0.01), especialmente aquéllos con C-HHA en comparación con NC-HHA (32% vs 9%, p<0.01).

Objetivo 6 - Evaluar el papel de linezolid en la infección de prótesis articular por microorganismos Gram-positivos manejada con un recambio en 2 tiempos.

Artículo 5 – *Linezolid in Late-Chronic Prosthetic Joint Infection Caused by Gram-Positive Bacteria*. J. Cobo, **J. Lora-Tamayo**, G. Euba, A. Jover-Sáenz, J. Palomino, M. D. del Toro, D. Rodríguez-Pardo, M. Riera, J. Ariza. Diagnostic Microbiology and Infection 2013; 76: 93-98.

Realizamos un ensayo clínico prospectivo, multicéntrico (7 hospitales españoles), abierto, no comparativo, en el que pacientes con infección crónica de prótesis articular por microorganismos Gram-positivos, sometidos a un recambio en dos tiempos (con espaciador sin vancomicina) fueron tratados durante 6 semanas con linezolid 600 mg/12h vo. Se estudio la tasa de curación clínica y la esterilidad del lecho quirúrgico en

el momento de reimplante. Para estudiar la toxicidad hematológica, se compararon los casos incluidos con controles históricos.

Se reclutaron 25 pacientes, la mayoría de ellos con infección protésica estafilocócica. La presencia de efectos adversos fue frecuente (76%), aunque la mayoría fueron de carácter leve o moderado, y típicamente se presentaron tras 2-3 semanas de tratamiento. Aunque sólo 1 paciente desarrolló plaquetopenia grave, el conjunto de pacientes, en comparación con los controles históricos, desarrolló un descenso gradual y significativo de la cifra de plaquetas. En 3 casos (12%) linezolid dio lugar a toxicidad grave, aunque reversible tras su retirada.

Los 22 pacientes restantes pudieron completar las 6 semanas de tratamiento. Dos casos (9%) fracasaron. De los 20 (91%) que fueron considerados como curados, en 1 (5%) las muestras quirúrgicas del segundo tiempo fueron positivas para microorganismos no presentes originalmente. Globalmente, la tasa de curación (clínica y microbiológica) fue de 19 pacientes (86%).

Objetivo 7 - Evaluación de la actividad de colistina frente a biofilm de *P. aeruginosa* multirresistente en un modelo experimental *in vitro*.

Artículo 6 – *Activity of Colistin Combined with Doripenem at Clinically Relevant Concentrations Against Multidrug-Resistant* Pseudomonas aeruginosa *in an* In Vitro *Dynamic Biofilm Model*. **J. Lora-Tamayo**, O. Murillo, P. J. Bergen, R. L. Nation, A. Poudyal, X. Luo, H. Y. Yu, J. Ariza, J. li. Sometido a revisión menor en el Journal of Antimicrobial Chemotherapy

Artículo 7 – *PK/PD Models in Antibacterial Development*. T. Velkov, P. J. Bergen, **J. Lora-Tamayo**, C. B. Landersdorfer, J. Li. Current Opinion in Microbiology, 2013 Jul 18 [Publicación aceptada].

Para estudiar la actividad de colistina, sola y en combinación con doripenem, frente a biofilm de *P. aeruginosa*, se puso en marcha un modelo dinámico *in vitro* basado en el

CDC Biofilm Reactor. Se utilizaron 3 cepas distintas de *P. aeruginosa* (una cepa de referencia susceptible a carbapenemes y dos cepas clínicas resistentes a carbapenemes), heterorresistentes para colistina.

Las monoterapias de colistina lograron una disminución transitoria del recuento bacteriano, con recrecimiento posterior y emergencia de resistencias, y sólo las concentraciones más altas de colistina (3.50 mg/L) lograron actividad bactericida inicial. Doripenem en solitario fue activo solamente contra la cepa de referencia (susceptible a carbapenemes), sin alcanzar actividad bactericida.

La combinación de doripenem y colistina mejoró la actividad de las monoterapias, especialmente cuando colistina se administró a mayores concentraciones, logrando sinergia en varios puntos horarios frente a las tres cepas, inclusive en las dos cepas clínicas resistentes a carbapenemes. Se observó menor recrecimiento y ausencia de emergencia de resistencias entre las células estacionarias del biofilm.

La actividad de los distintos regímenes sobre las bacterias obtenidas del caldo del reactor (planctónicas) fue menor.

5. Discusión

5.1. Tratamiento antibiótico en la infección de prótesis articular manejada con retención del implante.

5.1.1 La influencia del tratamiento antibiótico en el pronóstico.

La experiencia recogida en los dos trabajos con grandes cohortes de pacientes con infección por *S. aureus* y por BGN nos ha permitido comprobar la influencia del tratamiento antibiótico. Así, el tratamiento con rifampicina redujo significativamente la probabilidad de fracaso en la infección estafilocócica, y en la infección por BGN el tratamiento con ciprofloxacino se demostró también beneficioso.

Estos dos trabajos abundan en la idea de que el manejo con retención del implante puede intentarse mientras se disponga de antibióticos con actividad frente a las

bacterias estacionarias del biofilm. Es este sentido, fue interesante comprobar que el efecto protector de ciprofloxacino se extendió a patógenos específicos, como *P. aeruginosa*.

En el caso de *S. aureus,* observamos que los casos producidos por MRSA también se beneficiaron significativamente del tratamiento con una combinación de rifampicina. Aunque podría esperarse un peor pronóstico para estos casos, el resultado global fue similar para MRSA y MSSA. Sin embargo, mientras que los casos por MSSA (tratados fundamentalmente con quinolonas más rifampicina) fracasaron con mayor frecuencia tras retirar los antibióticos, los casos por MRSA (tratados con combinaciones alternativas de rifampicina) fracasaron fundamentalmente cuando aún estaban recibiendo tratamiento. Esto podría indicar que no todas las combinaciones de rifampicina tienen la misma actividad, y que levofloxacino más rifampicina fue más activa por cuanto consiguió diferir el fracaso, a diferencia de las otras combinaciones, que no lograron hacerlo.

5.1.2 Combinaciones de rifampicina alternativas para la infección de prótesis estafilocócica

Así, nuestra serie abunda en la indicación de quinolonas más rifampicina en la infección estafilocócica de prótesis articular manejada con DAIR. Cuando este último antibiótico no se puede administrar, como ocurre con frecuencia en la infección por MRSA, diversos modelos animales sugieren que la combinación de rifampicina con dosis elevadas de daptomicina es el tratamiento más activo.

Apenas existe experiencia clínica con esta combinación, cuya actividad queda avalada por el trabajo presentado en esta tesis. Aunque la tasa de fracaso fue similar a la cohorte histórica con la que se comparó, fue interesante observar que daptomicina más rifampicina apenas dio lugar a fracaso durante el tratamiento, a diferencia de combinaciones alternativas. Esto sugiere, como en el caso de levofloxacino-rifampicina comentado arriba, que daptomicina-rifampicina es efectivamente más activa que otras combinaciones de rifampicina. La combinación daptomicina-rifampicina podría ser un

tratamiento inicial en este contexto clínico, idealmente secuenciado por otra combinación por vía oral que permitiera ofrecer un tratamiento algo más prolongado.

5.1.3 Tasa de éxito con retención del implante y la importancia del algoritmo de Zimmerli

Las tasas de curación descritas en nuestros artículos están en un punto medio respecto a publicaciones previas, aunque ninguna de éstas tiene tamaños muestrales tan abultados. Dos aspectos importantes deben considerarse: en primer lugar, la definición de fracaso utilizada en nuestros trabajos es muy amplia y esto ha podido sobredimensionar la tasa de fracaso en comparación con otros estudios.

En segundo lugar, los dos trabajos recogieron todos los casos tratados con DAIR, tanto aquéllos que cumplían los criterios de Zimmerli para ser manejados con retención del implante, como los que no. Esto refleja de forma más realista la práctica clínica cotidiana. Los criterios de Zimmerli se basan en parámetros pronósticos, pero los puntos de corte establecidos podrían ser discutibles. Efectivamente, el algoritmo de Zimmerli de 2004 permite identificar a los pacientes que con toda probabilidad se beneficiarán de DAIR. Sin embargo, es menos evidente cómo debe procederse con los casos que no cumplen estos criterios. En este sentido, es significativo que un 48% de pacientes con infección estafilcócica que no cumplían los criterios de Zimmerli se curaron, como también ocurrió en un 53% de los casos con infección por BGN.

Por último, nuestros estudios inciden en la definición de caso post-quirúrgico ‘agudo’: estos dos trabajos avalan el plazo de 90 días de antigüedad de prótesis para indicar DAIR en la infección post-quirúrgica, más generoso que el de 30 días sugerido por algunos autores.

5.1.4 La eficacia de pautas más cortas de tratamiento

Esta tesis también aborda el problema de la duración de tratamiento. Las recomendaciones actuales de tratamiento (por ejemplo, 3 ó 6 meses de levofloxacino y rifampicina en la infección estafilocócica de cadera o rodilla, respectivamente) están basadas exclusivamente en datos empíricos y no en estudios controlados. Varias publicaciones reflejan tasas de éxito similares con tratamientos más cortos, y algunos estudios observacionales han sugerido que alargar el tiempo de tratamiento antibiótico sencillamente pospone el fracaso. Por otro lado, reducir la duración del tratamiento disminuye la posibilidad de toxicidad, el impacto ecológico y la selección de microorganismos resistentes en el entorno, y evidentemente reduce también los costes derivados del tratamiento.

Nuestro estudio es el primer ensayo clínico aleatorizado dirigido a demostrar que un tratamiento de 8 semanas de levofloxacino y rifampicina es tan eficaz como un tratamiento estándar de 3 ó 6 meses (cadera o rodilla, respectivamente). Nuestros resultados avalan esta hipótesis.

Sin duda, la mayor limitación de este estudio es la falta de potencia estadística. Con todo, la calidad de la evidencia es alta, y en cualquier caso incluye una cohorte homogénea de 24 pacientes tratados sólo durante 8 semanas y con una tasa de éxito superior al 90%.

5.1.5 La influencia del tratamiento antibiótico en pacientes especiales.

El tratamiento antibiótico debe ser individualizado de acuerdo con etiología de la infección, que a su vez se ve condicionada por las particularidades del huésped y el tipo de dispositivo infectado. En este sentido, nuestro estudio caracteriza las particularidades de la infección asociada a hemiartroplastia de cadera en el paciente anciano.

La microbiología responsable de la infección de HHA presentó una mayor frecuencia de BGN y microorganismos resistentes, algo que se explica por el mayor contacto con el

sistema sanitario de estos pacientes, su comorbilidad previa y su edad, y la colocación del implante como procedimiento de emergencia.

El resultado del manejo DAIR en HHA y THA, aparentemente similar, se puede explicar por la presencia de distintos factores de mal pronóstico entre los pacientes con uno y otro dispositivo. Aunque los casos de HHA se dieron en pacientes más mayores, con más comorbilidad y por microorganismos más resistentes, entre los casos de THA hubo una mayor frecuencia de infección por *S. aureus*, infecciones hematógenas, y menor tasa de recambio de piezas móviles. Así, el subanálisis realizado con una cohorte más homogénea, mostró una tendencia a un mejor pronóstico de las THA.

Resultó también llamativa la elevada tasa de mortalidad cruda y relacionada entre los pacientes con C-HHA. La fractura de cadera es un marcador de fragilidad y de mortalidad a medio plazo, y la infección supone en muchos casos el agravante necesario para este desenlace fatal. El hecho de que los pacientes con NC-HHA no tuvieran tan mal pronóstico puede explicarse porque, a pesar de ser más mayores, tenían menos comorbilidad; además, estos pacientes fueron sometidos a un recambio de la prótesis en 1 tiempo, lo que sin duda ofreció un mejor desbridamiento y control de la infección.

5.2. Tratamiento antibiótico en la infección protésica manejada con retirada del implante protésico.

5.2.1 Menores tasas de cultivos positivos en la reimplantación con un tratamiento oral

Nuestro estudio avala el uso de linezolid como tratamiento antibiótico en las infecciones de prótesis articular manejadas con recambio en 2 tiempos. Entre los pacientes clínicamente curados, sólo 1 (5%) presentó cultivos positivos del lecho quirúrgico en el momento de reimplante, una tasa inferior a la publicada por otros grupos.

La interpretación de los cultivos del segundo tiempo es controvertida. El hallazgo de microorganismos en el momento de reimplante, frecuentemente CNS resistentes a la antibioterapia previamente recibida, sugiere que la infección original podría ser en realidad policlonal, o bien el resultado de una superinfección por cepas de CNS resistentes a los antibióticos que el paciente estaba recibiendo. Ambas hipótesis sugieren la necesidad de un tratamiento antibiótico de amplio espectro, activo contra todos los posibles clones de CNS, como pueda ser vancomicina, daptomicina o linezolid.

La ventaja de linezolid radica en sus características farmacocinéticas, fundamentalmente su excelente biodisponibilidad que permite un tratamiento oral, y por tanto potencialmente ambulatorio. Nuestra experiencia abunda en esta posibilidad, y cuestiona la necesidad de un tratamiento endovenoso en estas infecciones.

Sin embargo, linezolid ha demostrado su potencial tóxico, de carácter acumulativo. En nuestra experiencia, linezolid fue razonablemente tolerado, salvo en 3 (12%) pacientes que precisaron su retirada. En este sentido, nuestro estudio apoya el uso de pautas que incluyan linezolid hasta 6 semanas, si bien exige un cierto grado de vigilancia clínica y analítica.

5.3. Actividad antibiótica contra biopelículas de bacilos Gram-negativos multirresistentes.

5.3.1. Colistina mejora la actividad de doripenem frente a biofilm de P. aeruginosa resistente a carbapenemes.

Colistina es, con una frecuencia creciente, el último antibiótico disponible frente a infecciones por BGN multirresistentes, incluyendo aquéllas que asocian biofilm. Colistina presenta importantes problemas PK/PD: las concentraciones plasmáticas que se logran con la dosificación optimizada de su prodroga (CMS) son a menudo

subóptimas, y además se ha descrito en diferentes especies el fenómeno de heterorresistencia. Ello ha conducido a la recomendación de administrar CMS en combinación con un segundo antimicrobiano.

Nuestro estudio supone la primera experiencia de la combinación de carbapenemes y colistina frente a biofilm de *P. aeruginosa*. De forma similar a la observada frente a bacterias planctónicas, el tratamiento en combinación logró sinergia frente a las tres cepas estudiadas (incluyendo las 2 cepas resistentes a carbapenemes). Esto es relevante, porque las dificultades descritas de colistina se ven notablemente incrementadas en el contexto de biofilm. En un modelo de infección pulmonar asociada a biofilm en rata, se requirieron concentraciones de 128 mg/L de colistina para lograr el descenso de 1 logaritmo en el recuento de unidades formadoras de colonias. Estas concentraciones de colistina quedan muy por encima de lo que puede esperarse con las dosis optimizadas de CMS endovenoso (aprox. 10 mg/L). Nuestros estudios abundan en esta pobre actividad de colistina en solitario frente a bacterias del biofilm, cuando se administra a concentraciones clínicamente realistas. Sin embargo, la combinación con doripenem logró una actividad más intensa y sostenida. Fue especialmente interesante la supresión de emergencia de resistencias entre las bacterias del biofilm con terapia combinada.

Esta mejor actividad de la combinación puede obedecer a una sinergia de subpoblaciones, en que cada antibiótico erradica las bacterias que son resistentes al otro, y viceversa. Además, en el caso de las combinaciones de colistina, podría añadirse el efecto permeabilizador de este antibiótico o sinergia 'mecanicista'. Efectivamente, las polimixinas alteran la permeabilidad de la membrana externa bacteriana, y pueden facilitar la entrada de otras moléculas, en este caso la del segundo antibiótico. Esto podría recuperar la actividad de algunos antibióticos frente a los que la bacteria se ha vuelto resistente, especialmente si el mecanismo de resistencia implicado está relacionado con una menor permeabilidad al antibiótico.

La realización de nuestros experimentos con tres cepas distintas, incluyendo dos resistentes a carbapenemes con mecanismos de resistencia diferentes, incrementa el valor de nuestras observaciones. La distinta respuesta apreciada entre las cepas puede obedecer a esto último, además de a la diferente habilidad de cada cepa para generar biofilm. Además, las tres cepas mostraron perfiles distintos de heterorresistencia frente a colistina.

6. Conclusiones (por objetivos)

A. Tratamiento antibiótico en la infección de prótesis articular con retención del implante.

A.1. Infección por *Staphylococcus* spp.

Objetivo 1 – Medir el impacto de rifampicina en el pronóstico de una gran cohorte de pacientes con infección protésica por *S. aureus*, incluyendo MRSA.

1.1. El uso de rifampicina mejoró de forma independiente el pronóstico de los pacientes con infección estafilocócica tratada con retención del implante.

1.2. Los casos debidos a MRSA también se beneficiaron del tratamiento con una combinación de rifampicina, siendo su pronóstico similar al de los casos producidos por MSSA.

1.3. La dinámica de fracaso fue distinta dependiendo de la combinación de rifampicina específica, sugiriendo una mejor actividad de la combinación fluroquinolonas más rifampicina.

Objetivo 2 – Evaluar la eficacia de un tratamiento corto con levofloxacino y rifampicina en la infección estafilocócica de prótesis articular.

2.1 Un tratamiento de 8 semanas con la combinación levofloxacino más rifampicina para la infección de prótesis articular estafilocócica aguda

manejada con retención del implante no es inferior al tratamiento estándar de 3 ó 6 meses.

Objetivo 3 – Determinar el papel de la combinación de daptomicina más rifampicina para la infección de prótesis articular por estafilococos resistentes a fluoroquinolonas.

3.1 El uso de dosis altas de daptomicina más rifampicina en el contexto de la infección de prótesis articular aguda por estafilococos resistentes a fluoroquinolonas manejada con retención del implante puede ser un tratamiento alternativo a la terapia estándar.

3.2. El porcentaje de fracasos con esta combinación durante el tratamiento fue bajo en comparación con la experiencia previamente publicada, sugiriendo una mejor actividad que otras combinaciones de rifampicina sin fluoroquinolonas.

A.2. Infección por bacilos Gram-negativos

Objetivo 4 – Medir el impacto de las fluoroquinolonas en el pronóstico de una gran cohorte de pacientes con infección protésica por bacilos Gram-negativos.

4.1. El uso de ciprofloxacino mejoró de forma independiente el pronóstico de los pacientes con infección de prótesis articular producida por bacilos Gram-negativos manejados con retención del implante.

4.2. Este beneficio se hizo extensivo a especies específicas de bacilos Gram-negativos, incluyendo *P. aeruginosa*

A.3. Infección de prótesis articular en ancianos

Objetivo 5 – Análisis comparativo de la eficacia antibiótica en pacientes portadores de hemiartroplastia de cadera frente a pacientes con prótesis total de cadera

5.1. Los pacientes con infección de hemiartroplastia de cadera e infección de prótesis total de cadera, además de distinguirse en el tipo

de implante ortopédico, son diferentes en términos de enfermedades de base, presentación clínica y etiología de la infección.

5.2. El resultado final del manejo con retención de la prótesis en ambos tipos de implante fue similar, probablemente debido a un reparto de distintos factores del mal pronóstico.

5.3. La infección de una hemiartroplastia de cadera supone una complicación de mal pronóstico y una mayor mortalidad, especialmente en los portadores de hemiartroplastias cementadas.

B. Tratamiento antibiótico en la infección de prótesis articular manejada con retirada del implante.

Objetivo 6 – Evaluar el papel de linezolid en la infección de prótesis articular por microorganismos Gram-positivos manejada con un recambio en 2 tiempos.

6.1. La administración oral de linezolid durante 6 semanas es un tratamiento eficaz en el manejo de estas infecciones.

6.2. La tasa de cultivos positivos del lecho quirúrgico en el momento del reimplante fue del 5% entre los pacientes con curación clínica. Esta tasa es menor que la publicada en estudios previos.

6.3. La seguridad y tolerancia de linezolid fue aceptable.

6.4. Linezolid podría considerarse como tratamiento de elección es este tipo de infecciones, aunque nuestros datos deberían confirmarse en estudios comparativos

C. Actividad antimicrobiana en biopelículas de microorganismos Gram-negativos multirresistentes.

Objetivo 7 – Evaluación de la actividad de colistina frente a biofilm de *P. aeruginosa* multirresistente en un modelo experimental *in vitro*.

7.1. Colistina en monoterapia, administrada en concentraciones clínicamente relevantes, presenta distintos grados de actividad sobre el biofilm de *P. aeruginosa*, en función de cada cepa, siendo el recrecimiento bacteriano un fenómeno frecuente.

7.2. La actividad de colistina se ve mejorada en combinación con doripenem, logrando un efecto sinérgico con las concentraciones más elevadas de colistina. Este efecto también se observó frente a cepas de *P. aeruginosa* resistentes a carbapenemes.

7.3. Con la combinación de colistina y doripenem se observó un menor recrecimiento en el biofilm de *P. aeruginosa* y se evitó la emergencia de cepas resistentes a colistina.

7.4. Nuestros datos apoyan el uso de la combinación de colistina más doripenem para las infecciones por *P. aeruginosa* con participación de biofilm, aunque estos resultados deberían ser validados en modelos experimentales animales.

- **SCIENTIFIC PRODUCTION**

Scientific production

Most of the studies included in this thesis hve been published in scientific journals and/or presented in national and international scientific conferences.

Publications in scientific journals

1. *A Large Multicenter Study of Methicillin-Susceptible and Methicillin-Resistant Staphylococcus aureus Prosthetic Joint Infections Managed with Implant Retention***. J. Lora-Tamayo**, O. Murillo, J. A. Iribarren, A. Soriano, M. Sánchez-Somolinos, J. M. Baraia-Etxaburu, A. Rico, J. Palomino, D. Rodríguez-Pardo, J. P. Horcajada, N. Benito, A. Bahamonde, A. Granados, M. D. del Toro, J. Cobo, M. Riera, A. Ramos, A. Jover-Sáenz, J. Ariza. Clinical Infectious Diseases 2013; 56(2): 182-194.
2. *Efficacy and Safety of High Doses of Daptomycin (10 mg/kg/d) plus Rifampin for the Treatment of Staphylococcal Prosthetic Joint Infection Managed with Implant Retention*. **J. Lora-Tamayo**, J. Parra-Ruiz, D. Rodríguez-Pardo, J. Barberán, A. Ribera, E. Tornero, C. Pigrau, J. Mensa, J. Ariza, A. Soriano. Submitted for publication.
3. *Gram-negative Prosthetic Joint Infections: Outcome of Debridement, Antibiotics and Implant Retention Approach. ALlarge Multicenter Study.* D. Rodríguez-Pardo, C. Pigrau, **J. Lora-Tamayo**, A. Soriano, M. D. del Toro, J. Cobo, J. Palomino, G. Euba, M. Riera, M. Sánchez-Somolinos, N. Benito, M. Fernández-Sampedro, L. Sorli, L. Guio, J. A. Iribarren, J. M. Baraia-Etxaburu, A. Ramos, A. Bahamonde, X. Flores-Sánchez, P. S. Corona, J. Ariza. Submitted for publication.
4. *Infected Hip Hemiarthroplasties and Total Hip Arthroplasties: Differential Findings and Prognosis*. **J. Lora-Tamayo**, G. Euba, A. Ribera, O. Murillo, S. Pedrero, D. García-Somoza, M. Pujol, X. Cabo, J. Ariza. Journal of Infection, 2013; 67: 536-544.
5. *Linezolid in Late-Chronic Prosthetic Joint Infection Caused by Gram-Positive Bacteria*. J. Cobo, **J. Lora-Tamayo**, G. Euba, A. Jover-Sáenz, J. Palomino, M. D. del Toro, D. Rodríguez-Pardo, M. Riera, J. Ariza. Diagnostic Microbiology and Infection Diseases 2013; 76(1): 93-98.
6. *Activity of Colistin Combined with Doripenem at Clinically Relevant Concentrations Against Multidrug-Resistant* Pseudomonas aeruginosa *in an* In

Vitro *Dynamic Biofilm Model*. **J. Lora-Tamayo**, O. Murillo, P. J. Bergen, R. L. Nation, A. Poudyal, X. Luo, H. Y. Yu, J. Ariza, J. li. Submitted for publication.

7. *PK/PD Models in Antibacterial Development*. T. Velkov, P. J. Bergen, **J. Lora-Tamayo**, C. B. Landersdorfer, J. Li. Current Opinion in Microbiology, 2013 Jul 18 [Epub ahead of print].

Book chapters

1. Book chapter – *Systemic treatment options for medical device-associated* infection. O. Murillo, **J. Lora-Tamayo**, J. Ariza. In: *Biomaterials associated infection*. T. F. Moriarty, S. A. J. Zaat, H. J. Busscher (eds). New York: Ed. Springer; 2013.

Papers at scientific conferences

1. *Linezolid (LNZ) in late-chronic prosthesis joint infection (PJI) by Gram-positive bacteria (GPB)*. **J. Lora-Tamayo**, J. Cobo, G. Euba, A. Jover, J. Palomino, D. del Toro, D. Rodríguez, M. Riera, J. Ariza. 50th ICAAC. Boston, USA, 12^{th}-15^{th} September 2010. (L1-346).
2. *Prosthesis joint infection (PJI) by* S. aureus *(SA): prognosis after debridement, antibiotics and implant retention (DAIR).* **J. Lora-Tamayo**, O. Murillo, A. Soriano, M. Sánchez, D. Rodríguez, A. rico, A. Granados, M.D. del Toro, M. Riera, J. Cobo, J. Horcajada, A. Ramos, A. Bahamonde, J. Palomino, N. Benito, A. Jover, J. Ariza. 51^{st} ICAAC. Chicago, USA. 17^{th}- 20^{th} September 2011. (K-241).
3. *Infected hip hemiarthroplasties and total hip arthroplasties: differences in clinical characteristics and prognosis*. **J. Lora-Tamayo,** G. Euba, O . Murillo, A. Ribera, S. Pedrero, F. Tubau, M. Pujol, X. Cabo, J. Ariza. 52^{nd} ICAAC. San Francisco, USA 9^{th}-12^{th} September 2012. (K-254).
4. *Activity of colistin alone and in combination with doripenem at clinicall-achievable concentrations against* Pseudomonas aeruginosa *in an* in vitro *dynamic biofilm model.* **J. Lora-Tamayo,** O. Murillo, J. Jacob, J. Ariza, R. L. Nation, J. Li. 23^{rd} ECCMID. Berlin, Germany, 27^{th}-30^{th} April 2013. (P1113).
5. *A large multicentre study of Gram-negative prosthetic joint infections: outcome of debridement, antibiotics and implant retention*. D. Rodríguez-Pardo, C.

Pigrau, **J. Lora-Tamayo**, A. Soriano, M. D. del Toro, J. Cobo, J. Palomino, G. Euba, M. Riera, M. Sánchez-Somolinos, N. Benito, M. Fernández-Sampedro, L. Sorli, L. Guio, A. Bahamonde, X. Flores-Sánchez, J. Ariza. 23rd ECCMID. Berlin, Germany, 27th-30th April 2013. (eP815).

6. *Eficacia y seguridad de daptomicina a dosis elevadas más rifampicina en la infección aguda estafilocócica de prótesis articular manejada con retención del implante.* **J. Lora-Tamayo**, J. Parra-Ruiz, D. Rodríguez-Pardo, A. Ribera, J. Ariza, A. Soriano. XVII Congress SEIMC. Zaragoza, Spain, 29th-31st May 2013. (Comuncation 140).
7. *Short vs long duration of levofloxacin plus rifampin for acute staphylococcal prosthetic joint infection managed with implant retention: preliminary results of a clinical trial.* **J. Lora-Tamayo,** G. Euba, J. Cobo, J. Horcajada, A. Soriano, E. Sandoval, N. Benito, D. Rodríguez-Pardo, L. Falguera, M. del Toro, J. Palomino, J. Iribarren, A. Jover-Sáenz, M. Sánchez-Somolinos, A. Ramos, J. Baraia-Etxaburu, M. Fernández, M. Riera, C. Pigrau, J. Ariza. 53rd ICAAC. Denver, USA, 10th-13th September 2013. (H-1005).

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ABBREVIATIONS

95CI: 95% confidence interval

ASA: American Society of Anesthesiologists

AUC: area under the curve

C_{max}: peak concentration

CBR: CDC biofilm reactor

CMS: sodium colistin methanesulphonate

CNS: coagulase-negative staphylococci

CRP: C-reactive protein

DAIR: debridement, antibiotics and implant retention

ESBL: extended-spectrum beta-lactamase

ESR: erythrocyte sedimentation rate

GNB: Gram-negative bacilli

HHA: hip hemiarthroplasty

IQR: inter-quartile range

MBC: minimal bactericidal concentration

MBEC: minimal biofilm erradication concentration

MBIC: minimal biofilm inhibitory concentration

MIC: minimal inhibitory concentration

MRSA: methicillin-resistant *Staphylococcus aureus*

MSSA: methicillin-susceptible *Staphylococcus aureus*

NNIS: National Nosocomial Infection Surveillance

PK/PD: pharmacokinetic/pharmacodynamic

PIOC: positive intra-operative cultures

PJI: prosthetic joint infection

REIPI: Red Española de Investigación en Patología Infecciosa (Spanish Network for Research into Infectious Diseases

THA: total hip arthroplasty

- **INTRODUCTION**

1. Prosthetic joints: infection and epidemiology

1.1 Arthroplasties

The implant of joint prostheses or arthroplasties has significantly improved patients' quality of life. The most frequent indications are joint degenerative diseases, such as arthrosis, rheumatoid arthritis or aseptic necrosis of the femoral head. Prosthetic joints are also the treatment of choice for certain types of femoral head fracture, especially among the elderly, allowing a rapid recovery and avoiding long periods of bed rest (1). The most frequently used prostheses are the ones placed in the knee or hip joints, but virtually all extra-axial joints may be substituted by a prosthetic device: shoulder, elbow (2), ankle (3), inter-phalanx joints (4), metatarsal-phalanx joints (5), wrist (6) …

The placement of a prosthetic device requires complex surgery in which the bone components of the joint are substituted by a medical device. Prosthetic joints may substitute the proximal and distal bone components of the joint (total prosthesis) or only a part of it, as in the case of hip hemiarthroplasties or unicompartmental prosthetic knee joints. Prostheses may be made from different materials or alloys (titanium, stainless steel, etc), and these metallic components may be stabilized by cementing them to the bone or using other methods so the new formation of bone around the prosthesis provides a solid anchorage. According to the number of arthroplasties performed in a given joint, surgeries are referred to as either primary or revision arthroplasty; a primary arthroplasty is the first substitution of the native joint, and a revision arthroplasty used when the primary device has become loosened some years after its placement.

1.2 Epidemiology of infection and risk factors

The placement of these orthopedic devices may be followed by serious complications, such as luxation of the device, bleeding, or loosening. However, the most feared complication is prosthesis infection. The overall likelihood of infection is 0.5-4% (7-12).

Infections may be acquired during surgical implanation or via hematogenous seeding from a distant foci; more rarely, they may also be caused by spread from contiguous foci. These different routes of infection reflect the fact that the risk of infection persists throughout the life span of the prosthesis. The risk has been quantified as 5.9 cases per 1,000 prosthesis and year during the first two years, and then 2.3 cases/1,000 prosthesis-year during the next 10 years (13).

Several risk factors for PJI have been described, including surgical site infection, wound discharge, systemic immunosuppressive therapy, high ASA or NNIS scores, previous native septic arthritis or the placement of a revision prosthesis (11, 12, 14). The risk of hematogenous seeding in the course of a bloodstream infection is variable when all microorganisms are considered (1-15%), but it may be particularly high in the setting of *S. aureus* bacteremia (30-40%) (15, 16).

The aging of the population, the increasing experience of the orthopedic surgeons, and the diversity and the improvement in the technology has led to a significant increase in the number of prostheses placed. In parallel, the absolute number of prosthetic devices infected has also increased (9, 10). The aging of the population has also led toan increase in the number of revision prostheses, which, as noted above, are exposed to a higher risk of infection (14).

1.3 Prosthetic joint infections: a health-care problem

The infection of the prosthesis is a catastrophe for the patient. Although the mortality rates may not be high, the related morbidity is always serious due to the difficulties for eradicating the infection. The management of PJI usually requires supplementary surgeries and long antimicrobial therapies, readmission to hospital and in many cases removal of the device. Although the prosthesis may be either kept or replaced by another device with good functional results (17, 18), this is not always guaranteed. This complex process also carries a significant psychological burden (19). Infections occuring in old, fragile patients with significant baseline comorbidity may easily worsen their condition and may, sometimes, lead them to death. Therefore, optimal treatment

of PJI must be provided by multidisciplinary units, comprising especialized orthopedic surgeons, infectious diseases specialists and microbiologists.

Most of our knowledge of PJI and its treatment comes from observational retrospective studies. Very few controlled trials very have been performed. Furthermore, many of these observational studies have used very heterogeneous samples of patients: in many instances, etiologies are mixed together, as well as acute and chronic episodes. What is more, the analysis of cases treated with implant retention and cases managed with prosthesis removal makes it difficult to draw firm conclusions. Notably, there are very few studies with large samples, and the statistical analysis of the majority of studies is not very robust. All in all, the scientific evidence of PJI treatment is not entirely reliable, and there are still many areas of uncertainty.

In particular, the role of antimicrobial treatment is not well defined among the huge number of prognostic factors. In many instances, the specific combination of antibiotics and length of therapy for a specific clinical scenario remains uncertain, as well as its actual influence on the patients' prognosis. Clinical research able to standardize and homogenize large samples of patients is urgently needed in order to address these questions.

2. Microbiology

2.1. The biofilm paradigm

Prosthetic joint infections are described as difficult-to-treat, mainly due to the presence of bacterial biofilm (7, 8). Biofilm consists in a bacterial population adhering to a surface and embedded in a self-produced glycoprotein matrix. The development of biofilm is a universal bacterial adaptive response that allows microorganisms to survive in hostile environments (20).

Formation of biofilm starts with bacteria attaching to inert surfaces and then excreting an extracellular matrix of glycoproteins that surrounds them in a protected, nutrient- and oxygen-restricted environment. As a result, bacteria undergo a phenotypical change and significantly reduce their metabolism: they consume less energy, and stop

being replicative (20, 21). However, far from being a passive adaptive form, the biofilm structure is a complex dynamic 3-D matrix, with inner channels that allow the flow of water and substances to deeper and more distant sites (20, 22, 23). Biofilm-embedded bacteria compose a complex community capable of communicating via molecular signaling comprising the so-called *quorum sensing* (20, 22, 24). Thanks to this chemical communication, bacteria are able to specialize and accomplish a specific mission within the biofilm community. While bacteria in the deeper layers are metabolically less active, the ones in the more superficial layers of the biofilm may be released and may recover their planktonic properties; they may finally colonize and attach to new inert surfaces, thus extending the biofilm structure (20, 22).

Biofilm-embedded bacteria become particularly less susceptible to antimicrobials for three main reasons (21, 22, 24):

1. Biofilm-embedded bacteria experience a dramatic phenotype change and become intrinsically less susceptible to antimicrobials, mainly due to their lower rate of replication (25).
2. The antibiotics may not reach their bacterial targets. This may be due to difficulties in spreading through the glycoproteic matrix and/or to inactivation of the antibiotic through this process of diffusion. This is the case of beta-lactams, which are inactivated by extracellular beta-lactamase excreted into the biofilm by bacteria (24, 26), and aminoglycosides, which are less active in acidic pH (27).
3. The complementary activity of the immune system is impaired in the biofilm. Adaptive resistant bacterial forms, such as persisters, are usually cleared by macrophages once antibiotics have substantially reduced the bacterial inoculum. However, phagocytic activity of white cells is inhibited by the biofilm.

Moreover, horizontal gene transmission is elevated in biofilms, thus increasing the likelihood of resistance development (24). In addition, although the rate of cell replication is significantly decreased for biofilm-embedded cells, some bacteria may increase tehir mutation frequency, especially when it is not very high in the planktonic state (28, 29).

2.2. The intracellular problem

In addition to the existence of biofilm, some bacteria may be present as intracellular microorganisms. Indeed, these intracellular bacteria have also been described in the setting of prosthetic joint infection (30) and in experimental models of foreign body infection (31). Intracellular microorganisms increase the difficulties in treating these infections and become infection reservoirs (32). Notably, phagocytosis-surviving bacteria are not exposed to the activity of the cellular and humoral immune system. Furthermore, many antibiotics are not able to penetrate the cell, or may not reach the specific cell compartment harboring the bacteria, or may be inactivated once inside the cell (32). As in the biofilm, intracellular microorganisms may be in a quiescent state or in other adaptive forms such as small colony variants (30, 33), and may therefore be more resistant to antibiotics.

The practical consequence of all this that antimicrobials are unable to cure infections when foreign bodies are present, as is the case of prosthetic joint infections or peace-maker infections, ventriculo-peritoneal shunt infections, vascular and urinary catheter infections, breast prosthesis infections, and so on. Many of these infections need a surgical approach to mechanically remove the biofilm from the foreign body or, frequently, to directly remove the foreign body itself.

2.3. The usual suspects in PJI

The specific microbiology responsible for the infection episode will determine the type of PJI (see below). Overall, Gram-positive cocci are the most frequent etiology of PJI (7, 8, 34-36). In a large series of PJI in Spain over 10 years, Benito *et al* reported coagulase-negative staphylococci (CNS) to be responsible for 34% of cases and *Staphylococcus aureus* for 25% of cases (36). Staphylococci, namely CNS, are part of the common skin flora of human beings, and possess a formidable ability to adhere to biomaterials and form a biofilm (37); this means that they are frequently responsible for device-

associated infections. Streptococci are less frequent (8-10%) (35, 36) and are usually acquired hematogenously in the context of bacteremia in old and fragile patients (38). Enterococci have been reported in 7% of PJI (36), and are usually polymicrobial (Soriano et al, data not published). Frequency of PJI by Gram-negative bacilli (GNB) ranges from 10% to 28% (35, 36, 39). The number of cases has increased in recent years (36), they being more frequent among the elderly (39). Anaerobic microorganisms account for fewer than 10% of infections, although their frequency may be underestimated (40, 41). Among them, *Propionibacterium acnes* is a relatively common etiology of chronic PJI. Like CNS, *P. acnes* belongs to the normal skin flora and typically colonizes sebaceous follicles; it is a characteristic etiology of shoulder arthroplasty infections (41-43).

3. Clinical aspects

3.1. Clinical presentation and classification

As mentioned above, the clinical features of an episode of prosthetic joint infection depend on the virulence of the microorganism responsible and on the route of acquisition.

Acute infections are normally due to pyogenic, virulent microorganisms, such as *S. aureus*, *Streptococcus* spp, *Enterobacteriaceae* spp and *Pseudomonas aeruginosa*. In these cases, a high inflammatory pattern of clinical presentation is the rule: there is pain and redness in the joint area, sometimes fever and bacteremia, and purulent discharge is frequently observed through the surgical wound. Purulent material surrounding the prosthesis may also be found. Diagnosis of these infections is normally not problematic, although a theoretical differential diagnosis may be made with superficial wound infection of the prosthesis.

By contrast, chronic infections are usually caused by less virulent microorganisms, usually pertaining to the normal skin flora, such as CNS and *P. acnes* (7, 8, 34, 35). This is why the symptoms and signs begin much later and the clinical pattern is less

inflammatory than in the case of acute infections. Pain is frequently the only symptom referred by the patient, and a difficult differential diagnosis needs to be made with aseptic loosening of the prosthesis, which also presents with joint pain. While the presence of sinus tract is very characteristic of late-chronic PJI, there is no gold-standard diagnosis for chronic infection, as it comprises a composited evaluation of each case based upon clinical, analytical, radiological, histological and microbiological data (7).

Tsukayama et al (35) proposed the following classification of episodes of infection:

- **Early post-surgical**: microorganisms colonize the prosthesis at some point during the surgery or soon afterwards, and symptoms of infection begin within the first 30 days after the placement of the prosthesis.
- **Hematogenous**: microorganisms reach the prosthesis and produce the infection via the bloodstream from a distant infectious focus. This may happen at any moment during the life-span of the prosthesis.
- **Late-chronic**: as in early post-surgical infection, microorganisms colonize the device during surgery, but symptoms of the infection begin after the first 30 days of the prosthesis placement – typically months after, and sometimes years.
- **Positive intra-operative cultures (PIOC)**: this is reserved for patients submitted to a one-step prosthetic revision procedure due to prosthetic loosening. The infection is unexpectedly diagnosed on the basis of positive cultures taken from the surgical site. These cases share the same pathogenesis and etiology as late-chronic infection, but the symptoms and signs of infection are so subtle that the diagnosis is not made until the revision procedure.

Alternative classifications to Tsukayama's are also based on clinical presentation. The Swiss school of PJI has proposed another widely used classification (8):

- **Early**: post-surgical infections with symptoms beginning within the first three months after surgery for placement – similar to Tsukayama's early post-surgical infection.

- **Late**: post-surgical infections with symptoms beginning from 3 to 24 months after the placement of the device – similar to Tsukayama's late-chronic infections and PIOC infections.
- **Delayed**: symptoms beginning more than 24 months after the placement of the prosthesis; the infection is usually hematogenous.

These classifications are clinically very relevant, since they allow the physician to choose one or other surgical and medical treatment (see below). Although the two classifications are quite similar, they differ in some aspects, such as the limit of time regarding early post-surgical infections. Indeed, it is controversial to define infections beginning beyond the first 30 days after the implant as early: while some groups decide to remove the implant, others may feel that the infection can be managed with implant retention.

Finally, a marginal aspect of these clinical classifications is that neither of them refer to PJI by contiguous septic foci – probably because this scenario is rare. However, in some instances such as infection of skin and soft tissues adjacent to the device, the prosthesis may eventually be involved in the infection and will need specific treatment. The pathogenesis of this particular type of PJI is probably similar to the acute forms (hematogenous and early post-surgical), and so they will probably benefit from a similar surgical and medical approach.

3.2. Diagnosis of PJI

The diagnosis of acute forms of PJI is not usually problematic. Inflammatory local signs are frequently evident: joint redness, pain and swelling bring the attention of the clinician. In the case of early post-surgical infections there may be dehiscence of the surgical wound and purulent discharge. A theoretical differential diagnosis with superficial wound infection may be made sometimes, but infllamatory signs normally reflect a deeper infection, and frequently the only way to discriminate is a surgical exploration of the joint. Both early-post surgical and hematogenous infection may

present with systemic signs of infection, fever and bacteremia, and sometimes even shock. Blood cultures must be taken in these patients (7, 8).

Chronic forms of PJI imply a more difficult and challenging diagnosis. Although the presence of a sinus tract is highly suggestive of infection, this is not always frequent, chronic insidious pain being the most frequent complain and making it difficult to distinguish from an aseptic prosthetic loosening. As mentioned, there is not a gold standard test, the diagnosis being based upon the composited results of the clinical presentation, blood tests, radiological aspect of the prosthesis, microbiological cultures and histopathological samples.

PJI leads to prosthetic loosening, and as mentioned, joint pain may not only be infammatory but also mechanic, and so not easy to distinguish from aseptic loosening. Joint pain starting early after the prosthesis placement is a valuable clue, suggestive of infection (7, 8). The same is valid for radiographic signs of loosening, such as periprosthetic radiolucency >2mm or periprosthetic osteolysis: the earlier they are observed, the more likely they are due to infection. Some other radiographic signs are more characteristic of infection, such as periostic reaction (7). Referral gammagraphy for the study of PJI is made with ^{111}In-marked leukocytes. Sensitivity is 80%, although in non-cemented prosthesis it may lead to a significant percentage of false-positive results, its specifity being ameliorated by adding ^{99m}Tc with sulphur colloid BMS. Newer techniques such as gammagraphy with anti-granulocyte antibodies or ^{18}F-fluodeoxyglucose positron-emission tomography may increase the sensitivity (7, 8).

Among blood tests, acute-phase reactants, such as the erythrosedimentation rate (ESR) or the C-reactive protein (CRP), may be of utility. They are sensitive tools but quite unspecific, especially in the presence of chronic inflammataroy joint diseases. When negative, though, they make the diagnosis of PJI very unlikely (44, 45). The leukocyte count is not reliable for diagnosing infection.

Among pre-operative cultures, swabs from sinus tract have a low predictive value, since they may be reflecting the microbiological colonization of the fistula from the patient's skin flora. When the swab is taken early after the development of the sinus

tract, or when it yields *S. aureus*, the predictive value is higher (7). Joint aspiration is more reliable – microbiology may be interpreted as when isolated from surgical cultures (see below). Synovial fluid cell count may be of utility, indicating infection when there are >1,700 leukocyes/mm3 or if >65% neutrophils (sensitivity of 94 and 97%, specificity 88 and 98%, respectively) (46).

Positive intraoperative cultures during the revision procedure are of high value, but specificity may be a problem due to the potential contaminant nature of common etiologic agents in this setting, such as CNS or *P. acnes*. Therefore, it is recommended to take at least 5 or 6 periprosthetic samples. The isolement of the same microorganism (as identified by species and antibiotic susceptibility profile) in ≥3 samples has a 99.6% specificity, and in ≥2 samples it has a 97% specificty (47). The rate of positive cultures may be ameliorated by sonication of the prosthetic components (48). Both aerobic and anaerobic media must be employed for surgical cultures. They must be incubated for at least 7 days (7). Extending this time to 14 days may ameliorate the rate of positive cultures, especially for slow-growing and fastidious bacteria (49). Incubating samples in media for mycobacteria and fungi is also recommended.

The presence of 1 to 10 polymorphonuclear leukocytes per high-power field at a magnification of 400 in samples of periprosthetic tissues defines acute inlammation. In the absence of other inflammatory joint diseases, it is suggestive of infection (50). A cutoff of ≥5-10 polymorphonuclear leukocytes has a sensitivity of 67-80% (7).

4. Surgical management

The relevance of the biofilm formed on the surface of the prosthesis has already been discussed. Antibiotics alone have a very low chance of successfully treating the infection. Therefore, a combined medical and surgical management is required. In this regard, it is not easy to decide whether surgery should be limited to a debridement and excision of necrotic tissues, or whether all foreign materials, including the joint prosthesis, should be removed.

4.1 Debridement, antibiotics and implant retention (DAIR)

DAIR consists in debridement surgery retaining the prosthetic device, followed by a long course of antibiotics. Debridement should include a thorough removal of necrotic tissues and purulent material, hematoma and debris, as well as the exchange of the removable components of the prosthesis (e.g. the polyethylene liner) (51, 52).

While removing the prosthesis is likely to heal the patient, the alternative management based on DAIR involves a more demanding treatment scenario, and the infection is less likely to be cured. However, DAIR may be desirable in some instances for several reasons: it is a less aggressive operation than the removal of a soundly cemented, fixed prosthesis; it also avoids further operations for the patient and loss of bone stock; and finally, it reduces the economic burden of the infection (34, 53-55).

Zimmerli's algorithm has been particularly useful for identifying the patients who are most likely to benefit from a DAIR approach (8). DAIR is considered in soundly fixed prostheses, and Zimmerli's criteria are based on the chronicity of infection, the duration of symptoms, the microorganism responsible for the infection and the condition of the periprosthetic soft tissues (8, 56).

The chronicity of the infection is indeed a key factor in deciding its surgical management. Acute infections present with young immature biofilms attached to the prosthesis, which are easier to remove with surgical debridement and are more susceptible to antimicrobials; by contrast, in chronic infections bacteria have had time to develop mature and complex biofilms which debridement surgery will not be able to remove – in these cases, the removal of the whole prosthesis is recommended. As mentioned above, the definition of 'acute' is not universal: while Tsukayama's classification states a strict limit of 30 days, Zimmerli's definition of an early post-surgical infection extends to the first 90 days after the prosthesis placement (8, 35).

The duration of symptoms is based on the same rationale as the age of the prosthesis: the longer it takes to perform the debridement surgery, the more mature and difficult

to treat the biofilm will be. Again, there is some controversy on the limit of symptom duration a PJI must have to be a candidate for DAIR. For instance, Brandt et al (57) found that a delay of >2 days in staphylococcal PJI significantly worsened the prognosis, while Zimmerli's algorithm allows a delay of 21 days (8, 58).

The odds of curing a patient with DAIR also depend on the microorganism. Zimmerli's algorithm discourages attempting implant retention when biofilm-active antimicrobials cannot be used (i.e. MRSA, *Acinetobacter* sp or multi-resistant *P. aeruginosa*).

As mentioned above, all these criteria are useful for selecting patients who will clearly benefit from DAIR, and they are widely accepted (56). However, many of these criteria are not evidence-based but are only estated empirically, and so it is controversial to deny DAIR to patients who do not meet these criteria. For instance, the time interval between the prosthesis placement and symptom onset, or between symptom onset and debridement surgery, is rather arbitrary. Also, the contraindication for performing DAIR in front of resistant microorganisms such as MRSA or *P. aeruginosa* may be seen as relative. Although useful, the algorithm simplifies a very complex reality, and the final decision of whether to submit a patient to DAIR is taken by the attending medical team (56).

In this regard, two other aspects must also be taken in consideration: a) the removal of a soundly-fixed recently-cemented prosthesis may require aggressive surgery with significant bleeding, sometimes including transfemoral osteotomy. For some patients this situation may be excessive, especially if they are old and fragile, and are in a septic condition, whereas debridement surgery may help to stabilize the sepsis and control the infection. And b) the failure of a DAIR management does not necessarily preclude salvage therapy where the prosthesis is finally removed.

4.2 Prosthesis removal

4.2.1 Prosthesis exchange

Patients who are not candidates for DAIR usually undergo a prosthesis exchange. The gold standard for this is a two-step exchange procedure (7, 8): in the first procedure, the prosthesis is removed and a thorough debridement is performed. In order to avoid joint collapse and to provide the patient with some mobility while awaiting the second procedure, a cement spacer is placed in the surgical bed. This spacer may be loaded with antibiotics with a double mission: first, to avoid the attachment of bacteria, since the spacer is a foreign body in which microorganisms may potentially restart the growing of biofilm; and second, to provide high local concentrations of antibiotic that spread over several days to the contiguous tissues and complement the role of the systemic antibiotics with which the patient is treated for a period of six weeks. Finally, some time after the end of therapy, the patient will undergo a second surgical procedure to implant the new prosthesis.

The rationale for such a complex exchange procedure is to provide a sterile surgical site for the new prosthesis, thus minimizing the likelihood of re-infection. While no ideal way of measuring the sterility of the surgical site has been standardized, some authors take samples for culture at the time of reimplantation. The results of these cultures have proved to be positive in 6-20% of cases (59-63), with CNS being the most frequent microorganism isolated. The demonstration of microorganisms in the surgical site has clinical consequences, since it may increase the likelihood of relapse (62) or the need for a new long course of antibiotics (59). Retrospective observational studies suggest that the use of local (in the spacers) or systemic antibiotics with wide anti-Gram-positive bacterial activity, including all *Staphylococcus* spp, would significantly decrease the rate of positive cultures at this stage (60, 63). However, prospective comparative data are lacking.

Overall, the odds of curing the patient with a two-step exchange procedure are between 80-90% (7, 8). Alternatively, a one-step exchange procedure may sometimes be attempted. While there are potential risks of infecting the new device by implanting

it in a non-sterile surgical bead, this strategy carries certaim advantages for the patient – namely one less operation, and a faster recovery. This approach is usually reserved for patients without badly damaged periprosthetic soft tissue, and with a less complex PJI by previously identified non-virulent microorganisms (7, 8).

4.2.2. Resection arthroplasty and limb amputation.

Sometimes the placement of a new prosthesis after the removal of the infected one is not feasible. This may be due to a lack of bone stock, poor condition of the soft tissues, the persistence of the infection in spite of foreign body removal, the fragility of the patient or a combination of all these factors. Orthopedic alternatives for these patients may include a two-step arthrodesis (i.e. in knee joints), Girdlestone arthrodesis (i.e. in hip joints) or even limb amputation.

5. Antimicrobial treatment

5.1. General principles

As discussed above, antibiotics are less active against adherent biofilm-embedded cells than against planktonic bacteria: therefore the need for surgery. The minimal bactericidal concentration (MBC) increases significantly when biofilm-embedded bacteria are tested (64). As a result, the pharmacodynamic parameters on the efficacy of antibiotics usually employed for planktonic bacteria are not always useful for predicting the antibiotic efficacy in the setting of PJI. In this regard, some authors have performed *in vitro* studies with stationary and/or biofilm-embedded bacteria. They propose the terms minimal biofilm inhibitory concentration (MBIC) and minimal biofilm eradicative concentration (MBEC) (65) or, alternatively, minimal bactericidal activity in the stationary phase (MBCstat). Given the difficulty of treating these infections, the use of high doses of antibiotics, sometimes in combination and usually for long periods, has been recommended (8, 56).

The activity of each antibiotic against biofilm-embedded bacteria is not impaired to the same degree. The increase of MBIC and MBEC depends on the specific antimicrobial-microorganism interplay. Taking into account the particularities of foreign body infection (presence of biofilm and intracellular infection) and the characteristics of prosthetic joint infection (extra-vascular infection, involvement of periprosthetic joint and bone tissue), the ideal antibiotic should possess the following properties:

- good diffusion in bone tissue.
- good diffusion in biofilm.
- activity in front of biofilm-embedded bacteria.
- activity in front of intracellular bacteria.
- good safety profile allowing its use for long periods.

Good diffusion in bone is important for an antibiotic to be effective in the treatment of PJI. Bone has a particular composition, different from other tissues in the body, and it is also less vascularized. Numerous techniques have been used for bone sampling and drug analysis in bone tissue, but no specific guidance has been published, and many of the techniques have not been validated (66). Many of these PK studies have been conducted in uninfected bone samples obtained during aseptic surgery, and no information is available on the intracellular/extracellular bone ratio or on the free fraction of antibiotic (67). Thus, there is some uncertainty regarding the actual concentrations of a particular drug in bone and its activity. Considering these limitations, drugs achieving higher concentrations in bone (in relation to their counterpart concentration in serum) are fluoroquinolones, macrolides and linezolid (serum:bone ratio 0.30-1.2), followed by cephalosporins and glycopeptides (0.15-0.30) and finally penicillins (0.10-0.30) (66)

Clinical comparison of different antibiotics for PJI is scarce. As mentioned, the majority of clinical experience with antimicrobial treatment comes from observational retrospective studies which usually include different types of infection and in which patients are treated sequentially with different antibiotics. Most of these studies were

not designed to assess the impact of the antimicrobial therapy, and so the conclusions drawn from them are not very reliable. Comparison of different antibiotic regimes has been possible in animal experimental models such as the tissue-cage model (31), osteomyelitis models (68), and prosthetic joint infection models (69).

5.2 Specific antibiotics for specific microorganisms

5.2.1 Staphylococcal PJI

Most of our knowledge of the activity of antibiotics in staphylococcal PJI is based on experience with *S. aureus*. Specific results with CNS infection are more limited.

Rifampin has shown to be key in the treatment of staphylococcal orthopedic device-associated infections (58). However, rapid development of resistance has been observed when rifampin is administered alone, and therefore it should be given combined with a second antimicrobial (70). In a cornerstone study published by Zimmerli *et al* (58), the combination of rifampin plus ciprofloxacin was significantly more effective than ciprofloxacin alone for treating patients with orthopedic device-associated infections managed with implant retention. Since then, this combination has been the treatment of choice for staphylococcal prosthetic joint infections, although ciprofloxacin has been frequently replaced by newer generation fluoroquinolones with better activity against staphylococci (i.e. high doses of levofloxacin or moxifloxacin), based on data from experimental models and limited clinical information (31, 71).

Current guidelines recommend the use of rifampin 450 mg twice daily (8, 56, 58), although the use of lower doses (600 mg/d or 300 mg/12h) has been accepted (56, 58). Actually, these doses are based on empirical experience and not in pharmacokinetic/pharmacodynamic studies. In fact, the most appropriate regime of rifampin in difficult-to-treat *S. aureus* infection is not well established and the issue remains unresolved. The area under the curve (AUC)/MIC ratio seems to correlate better with the activity of rifampin (72), and so two aspects must be taken into consideration. The first is the most appropriate fraction of the daily dose: a fasting

single morning dose seems to offer the best PK profile. It has been reported that the liver drug transport of rifampin is saturable, thus giving rise to non-linear increases in the C_{max} and the AUC for single doses beyond 300-450 mg (70).The second is the total daily dose: the values of $AUC_{0\text{-}12h}$ after a dose of 450 mg and 600 mg are 30.7 $\mu g \cdot h \cdot mL^{-1}$ and 40.2 to 57.3 $\mu g \cdot h \cdot mL^{-1}$ respectively, suggesting a similar PK profile for 450 mg twice daily and 600 mg once daily (73, 74). Furthermore, doses of 900 mg/d could be followed by a higher rate of adverse events (75).

Most of the clinical experience with rifampin for PJI has been described for methicillin-susceptible staphylococci. The experimental models suggest that it is also useful in the setting of MRSA infection (69, 76, 77), but clinical experience is scarce. Notably, MRSA are usually resistant to quinolones, and the best rifampin-based combination in this setting is yet to be defined. In this regard, animal models have suggested that the combination of rifampin with high doses of the new lipopeptide daptomycin could be a powerful alternative (69, 76). Daptomycin possesses intense bactericidal activity against Gram-positive bacteria, including biofilm-embedded staphylococci (78, 79). Although there is concern regarding daptomycin's penetration in bone tissue (80), its diffusion in biofilm is good (79), and its intracellular activity is comparable to that of other anti-Gram-positve microorganism antibiotics (81). However, clinical experience is scarce and refers mainly to daptomycin in solitary or at low regular doses (82, 83).

Also recently released, linezolid possesses a wide anti-Gram-positive bacteria spectrum, including all CNS species. It also has good diffusion in bone tissue. One of its most attractive advantages is its 100% bioavailability which allows oral administration, and so patients do not need to be hospitalized (84, 85). These properties make it a suitable alternative for the treatment of PJI, although more experience is needed. A major drawback is its toxicity, which is cumulative, especially in the field of bone and joint infection where long therapies are commonly needed (86-88).

5.2.2. Other Gram-positive microorganisms

It is uncertain whether other Gram-positive microorganisms, such as *Streptococcus* spp, *Enterococcus* spp or *P. acnes* may also benefit from the use of rifampin. In spite of the general principles described for the treatment of biofilm-associated infections (see above), antimicrobial therapy is based in beta-lactams (56), and the overall results are highly dependent on the specific microorganism involved. PJI by streptococci is usually treated with penicillins or cephalosporins, with an overall good prognosis even when the more demanding DAIR management is attempted (38, 89). Clinical series including cases treated with rifampin plus fluoroquinolones have also reported good results (90).

Ampicillin plus aminoglycosides are recommended for serious enterococcal infections (91). However, in the case of orthopedic device-associated infections this benefit has not been proven (92). Alternatively, it has been suggested that the use of ampicillin plus ceftriaxone may be better than ampicillin alone (93), as happens in endocarditis when aminoglycosides cannot be administered (54). The combination of fluoroquinolones plus rifampin has also been reported for the treatment of enterococcal PJI (95).

5.2.3 Gram-negative bacilli

Some retrospective studies suggest that quinolones significantly improve the prognosis of patients with PJI by GNB undergoing DAIR. This is probably due to the good diffusion of ciprofloxacin plus its good activity in response to biofilms (96). Aboltins *et al* reported a 2-year survival rate free of treatment failure of 94% [95% confidence interval (95CI) of 63-99%] in a small cohort of 17 patients managed with DAIR and ciprofloxacin (97). This contrasts with Hshieh's cohort, in which only 15 of 27 patients managed with DAIR received ciprofloxacin, and the 2-year survival rate free of treatment failure was 27% (95CI 16-34%) (39). Furthermore, in an observational study involving 47 patients with PJI by GNB managed with DAIR, Martínez-Pastor *et al* showed that treatment with fluoroquinolones was independently associated with good

outcome [OR 9.09 (95CI 1.96-50)] (98). The long-term analysis of these patients still showed benefits for cases due to fluoroquinolone-susceptible GNB (99).

Therefore, in the absence of prospective trials comparing fluoroquinolones regimes with alternative treatments, the available evidence favors the use of fluoroquinolones (7, 8). However, ciprofloxacin resistance is not rare, and the best treatment when quinolones cannot be used is uncertain: co-trimoxazole, tigecycline, beta-lactams, or colistin. Nor is it clear the better activity observed for quinolones also applies in the case of other specific pathogens, such as extended-spectrum beta-lactamase (ESBL) producing GNB, or *P. aeruginosa*.

5.2.4 Colistin for biofilm infections

Identifying the real options for treating multi-resistant Gram-negative microorganisms is a question of particular concern. As already mentioned, DAIR management is discouraged in the case of infections caused by microorganisms that cannot be treated with biofilm-active antibiotics (8, 56). So, in other words, the question is: what is the activity against biofilm-embedded bacteria of the antibiotics used for multi-resistant microorganisms (e.g., colistin for multi-resistant *P. aeruginosa* PJI)?

Indeed, the progressive emergence of multi-resistant microorganisms has overtaken the discovery of new antibiotic drugs which can be used against them (100). This has led physicians to use old forgotten antibiotics such as the polymyxins and, specifically colistin (101). Discovered in the late 1940s and used during the 1960s, colistin was withdrawn because of its toxicity and the introduction of safer and more active drugs. It has re-emerged in the last 10-15 years as the only possible antimicrobial therapy in many clinical scenarios (101, 102).

Colistin has a wide anti-Gram-negative microorganism spectrum, including *P. aeruginosa* (101, 102). It produces a bactericidal effect that is concentration-dependent, with the ratio AUC/MIC being the best PK/PD index to predict its activity (103). In addition to its potential toxicity (mainly nephrotoxicity) colistin has two other

major problems. First, heteroresistance is not infrequent, and has been demonstrated for *Klebsiella* sp, *Acinetobacter* sp and *P. aeruginosa* (103-105). Microorganisms exposed to suboptimal concentrations of colistin may amplify their resistant subpopulations, and eventually lead to clinical failure.

Second, colistin is not directly given to patients because of its toxicity, but is administered in the form of its inactive pro-drug, sodium colistin methanesulphonate (CMS). CMS is highly inefficient: 70% of it is excreted unchanged in the urine (by both glomerular filtration and tubular secretion), and a small part is hydrolized to colistin (106, 107). Final concentrations of colistin in plasma at current recommended usages are rather low (107-109). Significantly, inter-patient variation is high, and depends on the patient's renal function (107). The optimized dosage of CMS achieves suboptimal colistin serum concentrations (108), and increasing CMS doses is precluded by toxicity (110). These problems are aggravated in the biofilm scenario, where the minimal inhibitory and bactericidal concentrations are higher (111, 112).

In view of these problems, current opinion supports the use of colistin in combination with a second drug, seeking a synergistic effect (102, 113l). The rationale is based on two types of potential synergism. On the one hand there is subpopulation synergy, in which each drug would target the subpopulation that the other drug is not able to kill, and vice versa. On the other, there is a mechanical synergy based upon colistin's mechanism of action (113). As a cationic peptide, colistin targets the bacterial external membrane, and enhances its own uptake and that of other molecules. This change in the permeability of the membrane may enhance the penetration of other antibiotics in the cell (114-117).

The benefits of synergism have been demonstrated *in vitro* for both planktonic (113, 118, 119) and biofilm-embedded bacteria (120, 121). Recently, Covec et al have shown the benefits of combining colistin with tigecycline, fosfomycin or gentamycin in an animal tissue-cage experimental model (122). However, there is no experience with the combination of colistin plus carbapenems against *P. aeruginosa* biofilm.

5.3. Goals, route and length of antimicrobial therapy

The intravenous route of administration has classically been preferred in order to achieve high antibiotic concentrations at the surgical site. However, oral administration may obtain similar concentrations as long as the antibiotic used is highly bioavailable, as is the case with rifampin, quinolones, clindamycin, co-trimoxazole or linezolid, among others.

The use of antimicrobials may pursue either the eradication of the infection, as a complementary therapy to surgery, or the suppression of the symptoms in non-curable infections.

For the first goal, it is uncertain how long antibiotics should be administered. Since these are difficult-to-treat infections, and biofilm-embedded bacteria are less susceptible to antimicrobials, it is reasonable to think that a long course of antibiotics will be needed. The same rationale is applied to other biofilm related infections, such as endocarditis, or other bone and joint infections, such as chronic osteomyelitis. Thus, PJI are treated for long and variable periods of time. Originally, intravenous antibiotics were usually given for at least six weeks (57, 123). In recent years, the use of drugs with high bioavailability and reasonable tolerance, such as rifampin or fluoroquinolones, has led to the empirical recommendation of long therapies of more than three months (8, 51, 56, 58).

However, the recommendations for the duration of these treatments are empirical and have not been based on randomized clinical trials. In addition, the potential drawbacks discourage the use of long therapies: adverse events may occur (75, 124), cost may increase and there may be an impact in bacterial ecology (125, 126). Furthermore, some evidence suggests that such long treatments may be not necessary in order to achieve cure. Certain groups have reported their experience with shorter courses of antibiotics achieving similar rates of success (127-129), and some retrospective studies have shown that long therapies are not associated with better outcomes (51, 130). It is possible that, beyond a certain threshold, extending the antibiotics does not increase the likelihood of success, but simply delays the moment

of failure. Comparative studies are warranted in order to reduce the length of therapy in the setting of PJI.

A different scenario is that of patients in whom complete cure of the infection is assumed to be impossible, but long-term antimicrobial suppressive therapy is administered in order to improve the functional outcome (34). This treatment, considered for an indefinite period of time, aims to reduce the inflammatory signs such as pain or purulent discharge, so the patient is able to use the prosthesis in spite of the persisting infection. Ideally, the antibiotics used should be bioavailable, biofilm-active, and well tolerated.

- **HYPOTHESIS AND RATIONALE OF THE DOCTORAL PROJECT**

As noted above, prosthetic joint infections are a first-order problem for patients, assisting physicians and the health-care system as a whole. The prominence of the bacterial biofilm in these infections makes their treatment a difficult challenge for the infectious diseases specialist.

The lack of prospective studies, including clinical trials, cannot be stressed enough. It is due to the difficulties in collecting large samples, the insidious nature of the infection which leads to long therapies, and the need for long follow-up periods. In addition, retrospective observational studies usually include heterogeneous samples which commonly mix acute and chronic infections, etiologies and treatments, with the result that their conclusions lack strength. In particular, few previous studies have addressed the impact of antimicrobial treatment in these infections, as their design is not optimal for this. Therefore, many relevant clinical questions remain unanswered. A fuller understanting of these controvesial or poorly explored issues may lead to changes in the management of these infections, and also in the prognosis of patients.

The aims of the present thesis address some of these areas from the perspective of an infectious diseases specialist. The role of the antimicrobials which have become available in recent years, such as daptomycin, is yet to be defined. The long-term efficacy and safety of linezolid in PJI managed with a two-step exchange procedure has not been addressed, nor its ability to provide a sterile surgical site for reimplantation. Also, rifampin has been shown to be key in the treatment of staphylococcal infections, but the best rifampin-based combination and its impact on methicillin-resistant staphylococci are still to be determined. In addition, some data suggest that shorter courses of antibiotics could be as effective as standard long-term treatments, but conclusive evidence is lacking. In the setting of PJI by GNB, fluoroquinolones seem to be crucial, but again more evidence is needed; little is known regarding specific types of GNB. In addition, the role of colistin for GNB foreign body infection as an alternative in the case of resistant microorganisms is unknown. Finally, the specific antibiotics required for special populations or devices, such as elderly patients carrying hip hemiarthroplasties, has not been addressed to date.

Providing answers to these clinical problems requires the participation of an experienced clinical team, expert in the management of prosthetic joint infections. The Bone and Joint Infection Unit at the Hospital Universitario de Bellvitge, directed by Dr. Xavier Cabo and comprising a multidisciplinary team, has many years of experience in the management of PJI. The doctoral candidate has had the chance to be a part of this medical team and has been involved in the everyday clinical work of the Unit, attending to patients and creating and updating the clinical databases on which the clinical studies have been based.

In addition, to conduct studies with large, homogeneous samples, a multicenter design is essential. Five of the clinical studies presented here are multicenter and have been carried out in the setting of the Spanish Network for Research into Infectious Diseases (REIPI). Researchers belonging to this network share a common protocol for PJI management, thus enhancing the quality of the clinical studies without losing sample homogeneity. The coordinator of the Group for the Study of Prosthetic Joint Infection inside the REIPI is Prof. Javier Ariza, one of the directors of this thesis. Therefore, the doctoral candidate has had the opportunity to participate in the direction, coordination and design of these clinical multicenter studies.

Last but not least, experimental models provide essential information on the role of the antimicrobial therapy when clinical studies are not feasible. In this regard, the experimental foreign body infection in rats developed in the Laboratory of Experimental Infection (linked to the Bone and Joint Infection Unit, Hospital Universitario de Bellvitge, Universidad de Barcelona) provides an excellent platform for approaching the clinical problem. Furthermore, the collaboration with Prof. Roger Nation and Prof Jian Li's group at the Monash Institute of Pharmaceutical Sciences (Monash University, Melbourne, Australia) has given the doctoral candidate the chance to build an *in vitro* model for the study of *P. aeruginosa* biofilm.

The funding needed to conduct these studies has been obtained from various public competitive grants and private sources, thanks to the existence of a previously consolidated clinical group of researchers. This support has enabled our group to perform these studies addressing important trending topics in the field of PJI. All the

studies presented in this doctoral thesis provide new information on different clinical aspects and therapeutical approaches.

- **AIMS**

A. Antimicrobial therapy in prosthetic joint infection managed with implant retention

A.1. Infection by *Staphylococcus* spp.

Aim 1 – To measure the impact of rifampin in the outcome of a large cohort of PJI by *S. aureus*, including MRSA.

Aim 2 – To assess the efficacy of a short schedule of levofloxacin plus rifampin in staphylococcal PJI.

Aim 3 – To evaluate daptomycin plus rifampin for fluoroquinolone-resistant staphylococcal PJI.

A.2. Infection by Gram-negative bacilli.

Aim 4 – To assess the impact of fluoroquinolones in the outcome of a large cohort of PJI by Gram-negative bacilli.

A.3. Infection in the elderly.

Aim 5 – Comparative evaluation of the antibiotic efficacy in patients carrying total hip prosthesis or hip hemiarthroplasties.

B. Antimicrobial therapy in prosthetic joint infection managed with implant removal

Aim 6 – To evaluate linezolid in PJI by Gram-positive microorganisms managed with a two-step exchange procedure.

C. Antimicrobial activity on biofilms of multi-resistant Gram-negative bacilli.

Aim 7 – To study the activity of colistin against multi-resistant *P. aeruginosa* biofilm in an *in vitro* experimental model.

- **MATERIAL & METHODS**

1. Clinical Research

1.1. Setting

1.1.1. The Bone and Joint Infection Unit of the Hospital Universitario de Bellvitge

The Hospital Universitario de Bellvitge is an 800-bed teaching hospital in the urban area of Barcelona, Spain, with a referral population of one million. Cases with bone and joint infection are attended by a multidisciplinary team of orthopedic surgeons, infectious disease specialists, rheumatologists, microbiologists and radiologists at the Bone and Joint Infection Unit (Fig 1 and 2).

Fig 1. Medical doctors and nurses in the Unit of Bone & Joint Infection

This unit, which is a Spanish nation-wide reference for difficult-to-treat cases, has a team of nurses who are specialist in the care of infected and contaminated wounds. Standard sterility measures and a strict policy of hand-washing are applied (Fig 3). There is also a double-door system in each room that helps to maintain the isolation of patients colonized by multi-resistant microorganisms, such as MRSA, multi-resistant *P. aeruginosa* or ESBL-producing *Enterobacteriaceae* (Fig 4).

Cases commonly admitted to the unit include septic arthritis, infectious spondylodiscitis, extra-axial osteomyelitis, complicated skin and soft tissue wounds and orthopedic device-related infections, including osteo-synthesis-associated infections and prosthetic joint infections.

Fig 2 - The doctoral candidate and his mentor in the Bone & Joint Infection Unit

Cases commonly admitted to the unit include septic arthritis, infectious spondylodiscitis, extra-axial osteomyelitis, complicated skin and soft tissue wounds and orthopedic device-related infections, including osteo-synthesis-associated infections and prosthetic joint infections.

Since 2003, data of patients with PJI admitted to the unit have been prospectively recorded in a database. The study protocol includes data on patients' baseline features, the type of prosthesis, clinical presentation and diagnosis of the PJI episode, microbiological details, surgical management and antibiotic treatment, and follow-up (Annexe I).

1.1.2. The Spanish Network for Research into Infectious Diseases

Clinical studies of PJI are commonly limited by a small sample sizes. Some studies have recruited large but include patients with very different pathologies (i.e. studies analyzing cases of PJI along with cases of vertebral osteomyelitis and diabetic foot infection), etiologies (cases due to staphylococci along with cases by streptococci and

GNB) and/or treatments (i.e. outcome of patients undergoing DAIR along with cases undergoing prosthesis removal). Therefore, the conclusions drawn from these studies are usually difficult to interpret. Multicenter stduies allow the recruitment of larger samples with more statistical power, without losing the homogeneity of the patholofy studied. These studies may also increase the external validity and extrapolability of the observations.

The Infectious Diseases Department at the Hospital Universitario de Bellvitge is part of the Spanish Network for Research into Infectious Diseases (REIPI, www.reipi.org), funded by the Instituto de Salud Carlos III. The Work-Package "Optimizing the management of prosthetic joint infection by MDR bacteria" is one of the main lines of research. The Network includes the Spanish Group for the Study of Pathogenesis and Antimicrobial Treatment of Prosthetic Joint Infections, which brings together researchers from 13 hospitals and is led by Prof. Javier Ariza, who is on of the directors of this thesis.

Fig 3 (left) – Double-door system.

Fig 4 (above) – Clorhexidine and antiseptic solutions for wash-hand and hand-desinfection

This group has published diagnostic and therapeutic guidelines and protocols with the aim of homogenizing clinical practice at Spanish hospitals (7, 131). What is more, between 2003 and 2006 a common on-line database allowed prospective inclusion of cases of PJI; eventually, more than 450 episodes were recorded. These data were stduied in a series of publications: on early post-surgical infections (128), hematogenous cases (129) and late-chronic episodes (132).

This network has given the doctoral candidate the opportunity to take part in multicenter studies involving other hospitals and researchers following common practices. The network has also provided the tools for collecting multicenter data in the form of on-line databases.

1.2. Clinical approach to PJI and definitions

1.2.1. Clinical and microbiological diagnosis of PJI

The diagnosis of prosthetic joint infection is based on a compatible clinical picture plus positive cultures from sterile sites, fundamentally surgical samples, joint aspirates or blood (57). The clinical expression depends on the type of prosthetic joint infection. For the purposes of our studies, we followed the classification of Tsukayama *et al* (35), mentioned above:

- **Early post-surgical**: cases with symptoms and signs of infection beginning within the first 30 days after the placement of the prostheses.
- **Hematogenous**: for acute-onset cases, any time after the placement of the prosthesis, in the context of a suspected or documented bloodstream infection.
- **Late-chronic**: for cases with insidious symptoms beginning beyond the first 30 days after the prosthesis placement – typically months later, and sometimes years.
- **Positive intra-operative cultures (PIOC)**: for cases with no evidence of infection, but the diagnosis is established on the bases of positive intraoperative cultures during a one-step prosthetic revision procedure.

Acute cases (i.e. early post-surgical and hematogenous) usually present with acute inflammatory signs of the surgical wound, and may also present with systemic signs such as fever, a high leukocyte count and high levels of CRP. Chronic infections usually present with chronic joint pain, and are difficult to distinguish from aseptic loosening of the prosthesis. The presence of a sinus tract is very characteristic. In any case, the presence of purulence surrounding the prosthesis at the time of surgery is highly suggestive of infection.

Surgical samples may include synovial tissue, peri-prosthetic bone and soft-tissue, synovial fluid and purulent material, and prosthetic cement when available. At our hospital, sonication of prosthetic components is not routinely performed. Samples are seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate and MacConkey agar) and incubated for 10 days. Liquid cultures are routinely re-seeded in solid media every 48 hours or whenever they become turbid. Microorganisms are identified according to standard criteria (133). In addition, samples are also seeded in specific media in order to grow anaerobic microorganisms, fungi and mycobacteria.

In an acute setting of PJI, a single valuable sample isolating a virulent pyogenic microorganism, such as *S. aureus*, *P. aeruginosa*, *Streptococcus* or *Enterobacteriaecae* is enough to consider the particular microorganism as relevant. In late-chronic infections, where the microorganisms usually responsible may also be potential skin-flora contaminants (i.e. CNS, *P. acnes*), five to six aerobic samples are taken from tissues surrounding the prosthesis, and two or more positive samples yielding the same microorganism are needed to consider it as responsible for the infection (47). Comparison of microorganisms growing in these samples is made phenotypically: the microbiological species and the antibiotic susceptibility pattern must coincide. Routine determination of the MIC is made by microdilution, following the CLSI guidelines (134).

1.2.2. Surgical and medical management of PJI

Surgical and medical management of PJI cases is based on current knowledge and guidelines (7, 8, 34, 56), but each case is evaluated separately according to its

particular presentation and conditions. In general, patients with acute presentation (i.e. early post-surgical and hematogenous cases), stable prosthesis and periprosthetic soft tissue without significant damage undergo debridement and implant retention, followed by a long course of antimicrobials. Ideally, debridement needs to be performed as soon as diagnosis is made; it consists in the excision of the wound margins, followed by removal of necrotic soft tissue, debris, hematoma and/or collection of pus from around the prosthesis (51). In the same procedure, all the removable components of the prosthesis (i.e. the polyethylene liner or the femoral head of a modular hip prosthesis) should be removed and replaced by new pieces (Fig 5).

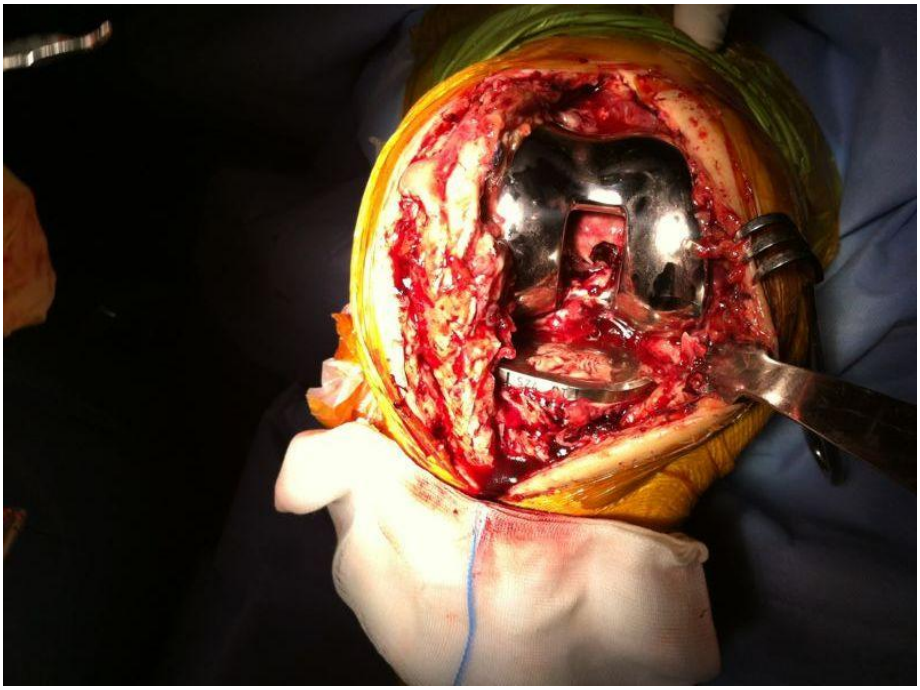

Fig. 5 – Knee-prosthetic joint infection by *S. aureus* submitted to DAIR. The polyethylene liner has been removed in order to perform a thorough debridement

Antibiotics are usually withheld until valuable surgical samples have been taken, provided that the patient's condition allows this delay. Then, empirical broad-spectrum antimicrobials (i.e. a combination of vancomycin plus ceftazidime) are started until the microorganisms responsible and their antimicrobial susceptibility profile are identified. Next, a tailored antimicrobial therapy is administered for a variable time period, usually around eight weeks. Staphylococci are usually treated with a rifampin-based

combination, levofloxacin plus rifampin being the treatment of choice. Streptococci and enterococci are usually treated with beta-lactams. GNB are treated with ciprofloxacin if they are susceptible, and otherwise with beta-lactams or co-trimoxazole. In the case of PJI by *P. aerguinosa*, an initial combined therapy is preferred (i.e. ceftazidime plus ciprofloxacin) for a variable period (i.e. 14 days), followed by monotherapy with ciprofloxacin.

In contrast, late-chronic infections are ideally submitted to a two-step prosthesis exchange procedure. In the first operation, the prosthesis and cement are removed and a thorough debridement of the surgical site is performed. In this same procedure a cement spacer is placed, with a double goal: first, to avoid the joint collapse so that the placement of a new prosthesis in the future will be technically easier, as well as providing some mobility to the joint to aid the patient recovery; and second, to provide local antimicrobial therapy, as these spacers may be loaded with antibiotics (vancomycin and/or gentamycin as a rule) which locally diffuse high concentrations of antimicrobials. Systemic antimicrobial therapy is then administered for a scheduled duration of six weeks. The specific antibiotic depends on the etiology and its susceptibility profile; treatment is chosen in the same way as in acute infections. The administration of rifampin for staphylococcal infections may not be necessary since the foreign body has been removed: the final decision depends on the type of *Staphylococcus*, the reason for removing the prosthesis (first therapy *versus* salvage therapy) and the grade of residual osteomyelitis.

The performance of the second step allows the placement of a new revision arthroplasty. Ideally, the patient should be discharged before this surgery and should be at home. An antibiotic-free period after the six weeks of therapy is preferred to allow the reconstitution of the normal skin flora of the patient. In this regard, the antibiotic prophylaxis for the new surgery should take into account the etiology of the previous infection and the antibiotic pressure received; the antibiotic spectrum usually needs to be wider than for standard prosthetic placement.

During the second step surgery, new samples are taken in order to confirm the sterility of the surgical site. These samples are processed and their results interpreted in a

similar way as the first-step surgery (60). Patients usually receive antibiotics for several days until confirmation that the cultures are negative. In the case of positive culures, patients receive a new course of a tailored antimicrobial therapy..

Some patients do not follow this standard two-step exchange procedure but undergo a one-step revision, ideally if the pathogen is known preoperatively and there are no signs of systemic infection (8). Other patients in whom the placement of a new revision arthroplasty is not feasible, undergo resection arthroplasty or even limb amputation.

1.2.3. Outcome and follow-up.

After surgery patients remain under antimicrobial therapy and are followed up by the unit's medical team, both surgeons and clinicians. Patients are followed-up in the outpatient clinic for at least two years after the end of therapy.

The goal of the treatment of a patient with PJI is to cure the infection and restore the patient's functionality (34). The possible reasons for failure are numerous and diverse: persistence of, or relapse due to, the same organism that caused the original infection; superinfection by new organisms at some point during the healing process; death caused by the infection, its treatment or its complications; failure to achieve the orthopedic goal (retention of the prosthesis in a DAIR management, or impossibility of reimplanting a new prosthesis in a one-step or two-step exchange strategy).

Therefore, in clinical studies evaluating the efficacy of DAIR, clinical failure is considered when

- the patient died due to causes related t the infection
- there were signs or symptoms of persistence, relapse or superinfection requiring unplanned salvage or supplementary therapies, such as
    - prosthesis removal
    - need for supplementary debridements beyond a defined period of time
    - new courses of antibiotics after the initial scheduled treatment

- long-term antimicrobial suppressive therapy

- early prosthetic aseptic loosening leading to prosthesis removal or exchange within the first two years after the debridement surgery.

In some studies, microbiological failure has also been analyzed, in order to better assess the impact of a particular antibiotic regime in the outcome.Microbiological failure has been considered among patients developing clinical failure as defined above, plus the re-isolation of the same microorganism causing the original infection.

1.3. Study design and statistical analysis

The clinical studies included in this thesis comprise four observational studies and two clinical trials. The observational studies are retrospective analyses of prospectively gathered data. As mentioned, the data on PJI are collected following a defined protocol (Annexe I). This information is critically reviewed and introduced in a Microsoft Access database. For multicenter observational studies, a common protocol and database have been used to introduce data since 2007 (Fig 6). All centers had a copy of the database, and the number of episodes identifying each case being restricted for each center. The data collected in these databases were added to the pre-existing REIPI database (active during the period 2003-2006).

For the prospective multicenter clinical trials, an *ad-hoc* website was built with an on-line database in which the information was recorded. For the randomized clinical trial, this website also had a randomization program for assigning patients to one or other arm of the study. The randomized clinical trial was registered prior to initiation (Annexe II). In both studies approval was obtained from the Ethical Committee at each participating center, and written informed consent was obtained from all subjects (Annex III).

Statistical analysis was made with the SPSS (Statistical Package for the Social Sciences) software (version 15.0 or higher). In general, categorical parameters were expressed as absolute numbers and percentage, and compared with the X^2-test or the Fisher exact

test, as appropriate. Continuous variables were expressed preferably as median and range or inter-quartile range (IQR), and compared with the *t* test or the Mann-Whitney *U* test, as appropriate. A sound evaluation of outcomes in the setting of bone and joint infection requires a long follow-up period. Thus, parameters associated with the outcome were identified with univariate analysis with Kaplan-Meier curves (long-rank test) and univariate Cox-regression, and with multivariate analysis with Cox-regression.

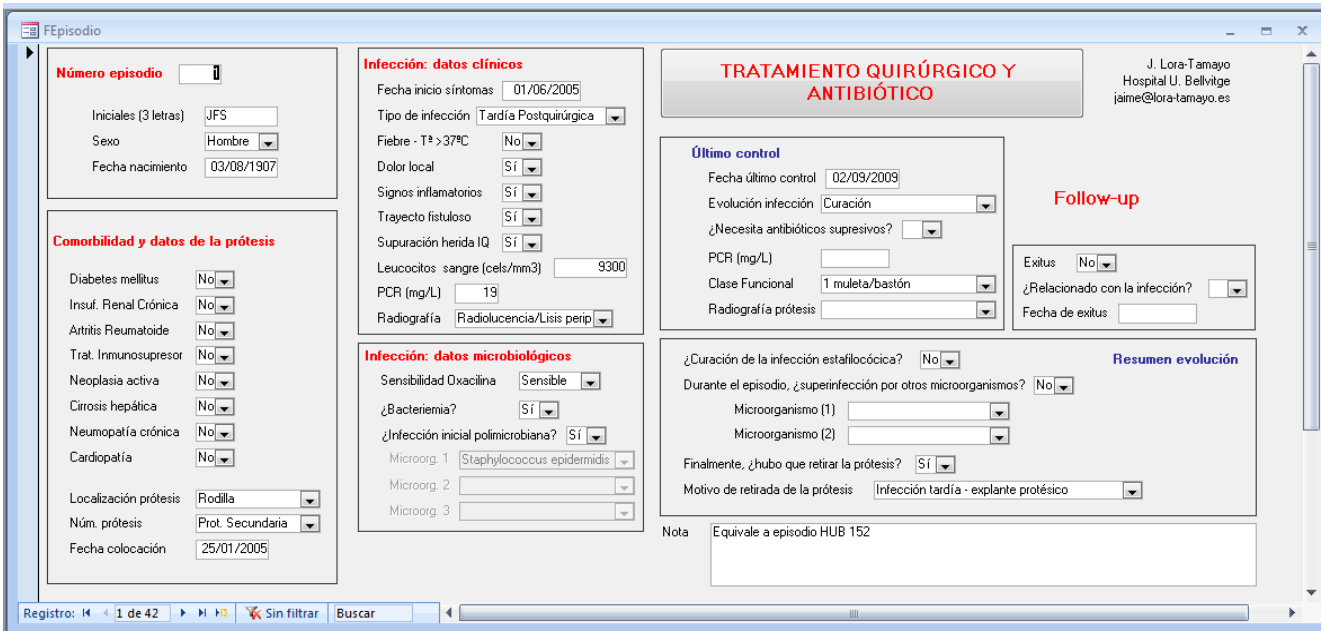

Fig 6 – Access database for the multicenter cohort of PJI by *S. aureus*

2. Experimental Research

2.1. Setting

2.1.1. The Experimental Infection Laboratory, Universidad de Barcelona

The Experimental Infection Laboratory, which is linked to the Department of Infectious Diseases (Hospital Universitario de Bellvitge), belongs to the Department of Clinical Sciences of the Faculty of Medicine of the University of Barcelona. It is located in the Campus Bellvitge, next to the Hospital. Among other projects, in 2005 our research group launched an experimental animal model (in rats) of foreign body associated infection caused by methicillin–susceptible and –resistant *S. aureus*. The results of several experiments have already been published in high impact factor journals (31, 76, 77, 135, 136). This field of research is still active, and is part of the Experimental

Research Platform of REIPI. The work conducted in the lab has a direct relation with the clinical research summarized in this thesis, on a bench-to-bed basis, and so it is complementary to the doctoral candidate's clinical research. The candidate has collaborated in the performance of various projects related with the experimental model, including killing-curves for planktonic and stationary bacteria, at both standard and high inocula. Preliminary static experiments were also developed with the CDC Biofilm Reactor (see below).

Fig 7. The doctoral candidate with researchers of D4 at the Monash Institute of Pharmaceutical Sciences, Melbourne, Victoria, Australia

2.1.2. Stage *abroad: the Monash Institute of Pharmaceutical Sciences*

The Monash Institute of Pharmaceutical Sciences (Monash University, Melbourne, Australia, www.monash.edu.au) is a world-leading institution in pharmacy education and research. Scientists in the department of Drug Delivery, Disposition and Dynamics (D4), led by Dr. Roger L. Nation and Dr. Jian Li, have filled an important gap in the

literature regarding our current knowledge on polymyxins. Among other milestones, alone and in collaboration with other researchers and clinicians, they have reported a simple method for assaying CMS and colistin by high-performance liquid chromatography (137, 138), have described the pharmacokinetics of CMS and colistin in patients with cystic fibrosis (109) and in critically ill patients (107), and have published relevant PK/PD papers on colistin and polymyxin B, alone and in combination with other antimicrobials, against several multi-resistant Gram-negative microorganisms (103-105, 137-140).

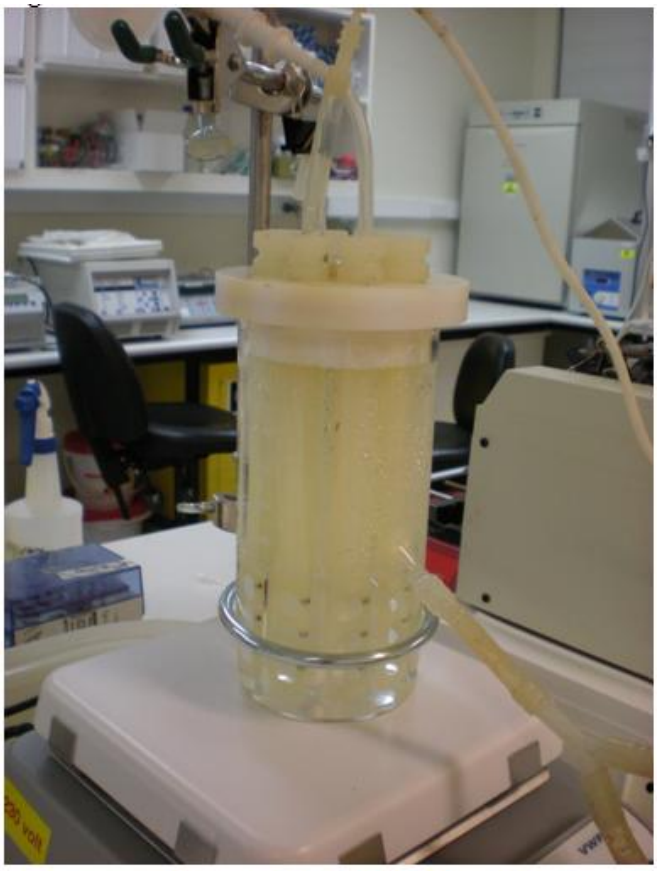

Fig 8 – The CDC Biofilm Reactor

For the study of the activity of colistin against multi-resistant *P. aeruginosa* biofilm, the doctoral candidate moved to Melbourne (Australia), and under the supervision of Dr. Li set an *in vitro* dynamic model in the laboratories of the Monash Institute of Pharmaceutical Sciences (Fig 7).

2.2 Dynamic in vitro biofilm model.

The model was based on the CDC Biofilm Reactor (CBR) (Fig 8) and the studies published by previous researchers (141-143). Briefly, this dynamic model consisted of a 1-L glass reactor connected to a 10-L carboy containing sterile drug-free media. The broth was pumped through the model with mixing and shear generated by a magnetic stir bar operating at 130 r.p.m. The volume of broth in the model was maintained at 350 mL and a waste vessel was connected. Eight polypropylene coupon holders were suspended from the lid, each containing three removable Teflon coupons (diameter 12.7 mm) on which biofilm formed (Fig 8, 9 and 10).

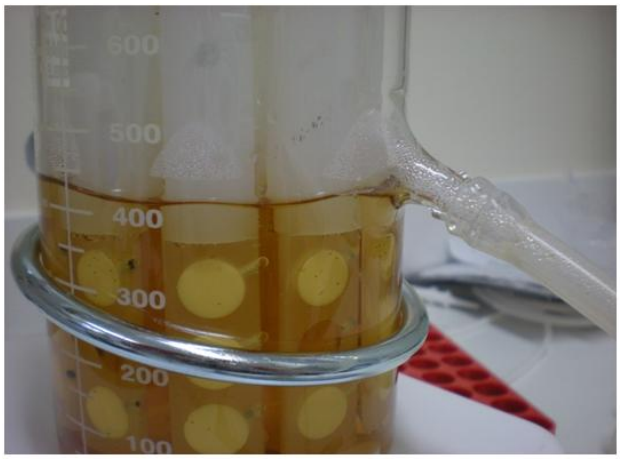

Fig 9 – The CDC Biofilm Reactor, detail: teflon coupons (biofilm growing surfaces) immersed in broth

Prior to each experiment, isolates were subcultured onto nutrient agar and incubated at 35˚C for 24 h. One colony was then selected and grown overnight in 10 mL of tryptic soy broth (TSB), from which early log-phase growth was obtained. A 1-mL aliquot of this early log-phase bacterial suspension was inoculated into the model at 37˚C, and a 28-h conditioning phase commenced. This consisted of 24 h incubation in drug-free nutrient-restricted media [cation-adjusted 1%-TSB (CA-1%TSB)], after which the model was emptied and fresh sterile CA-1%TSB media was pumped into the model at a flow rate of 11.67 mL/min for 4 h. After these initial 28 hours of biofilm growth, the antibiotic regime was begun and the experiment lasted for 72 more hours. The reactor was maintained at 37ºC and a stirring blade spun at 130 r.p.m.

Three different strains of *P. aeruginosa* were used in the experiments: a reference strain (PAO1) and two clonally unrelated clinical multi-resistant (carbapenem-resistant but colistin-susceptible) strains (HUB1 and HUB2), which had been responsible for clinical outbreaks in the Hospital Universitario de Bellvitge (144, 145). Population analyses profiles were performed in all three strains: heteroresistance was demonstrated for all three.

Colistin at two different clinically-relevant concentrations (1.25 mg/L and 3.50 mg/L), alone and in combination with doripenem, were examined. Colistin was administered by bolus administration at 0 h to achieve the desired concentration and then by spiking the CA-1%TSB media in the carboy with colistin. This approach mimicked the 'flat' plasma concentration-time profiles of colistin formed at steady state observed in critically-ill patients receiving CMS (107). Doripenem regimens were administered as a bolus dose every 8 h (target C_{max} 25 mg/L) with a flow rate of 4 mL/min in order to simulate a half-life ($t_{1/2}$) of 1 h. Flow rates were calibrated prior to each experiment

and monitored through the experiment to ensure the system was performing optimally.

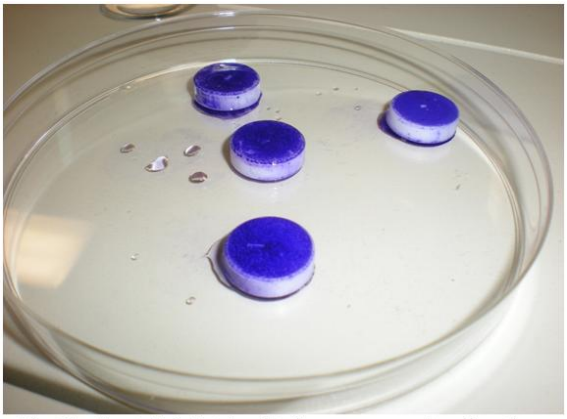

Fig 10 – Crystal violet dyed teflon coupons showing the surfaces of attachement of *P. aeruginosa* biofilm

For viable counting and examination of the emergence of colistin resistance in the biofilm-embedded cells, at 0 (prior to treatment), and at 4, 8, 24, 32, 48, 56 and 72 h three coupons were aseptically removed, rinsed twice in a phosphate buffered saline solution (PBS) to remove excess planktonic cells, and placed in sterile tubes containing 10 mL of PBS. Biofilm-embedded cells were recovered by three alternating 1-min cycles of vortexing and sonication at 43 kHz (Soniclean, Therbaton, Australia) followed by a final 1 min of vortexing. Media (1 mL) were also removed from the model at each time point for viable counting and examination of emergence of colistin resistance in planktonic cells.

Fig 11 – the doctoral candidate and the *in vitro* dynamic model for studying the activity of colistin and doripenem against biofilm-embedded *P. aeruginosa*

For enumeration of biofilm-embedded and planktonic viable cells, the respective samples were serially diluted with sterile saline and 50 µL was spirally plated onto drug-free nutrient agar (Media Preparation Unit) using an automatic spiral plater (WASP, Don Whitley Scientific, West Yorkshire, UK). Serial 10-fold dilutions and spiral plating, which further diluted the samples, minimized the antibiotic carryover. Colonies were counted using a ProtoCOL automated colony counter (Symbiosis, Cambridge, UK) after 24 h of incubation at 35°C and 48 h for plates with small colonies. In order to evaluate the emergence of colistin resistance (i.e. colonies able to grow in the presence of ≥4 mg/L colistin), both biofilm-embedded and planktonic (broth) samples were additionally plated in a similar manner onto nutrient agar containing colistin at 4 mg/L (Media Preparation Unit).

3. Funding and grants

The doctoral candidate received the following grants and fundings during his research process.

- Post-Residence grant from the Hospital Universitario de Bellvitge.
- Grant P-FIS from the Instituto de Salud Carlos III [PI09/00943].
- Travel grant from the Universidad de Barcelona.

In addition, some of the stduies were financed with the following grants:

- For the study “Safety and efficacy of a short schedule treatment of levofloxacin plus rifampin for staphylococcal PJI managed with DAIR, as compared with standard long schedules: a randomized clinical trial”
    - Support from REIPI
    - Support from Instituto de Salud Carlos III – Convocatoria 2008 del subprograma de proyectos de investigación clínica no comercial con medicamentos de uso humano (Expte EC08/00113)
    - Support from the Plataforma Española de Ensayos Clínicos (CAIBER), Instituto de Salud Carlos III (Convocatoria 2010).

- For the study “Linezolid in late-chronic prosthetic joint infection caused by Gram-positive bacteria”
    - Support from REIPI
    - Support from an unrestricted educational grant from Pfizer Spain.

- **RESULTS**

A. ANTIMICROBIAL THERAPY IN PROSTHETIC JOINT INFECTION MANAGED WITH IMPLANT RETENTION

A.1 Infection by *Staphylococcus* spp.

Aim 1 – To measure the impact of rifampin in the outcome of a large cohort of PJI by *S. aureus*, including MRSA.

Article 1 – *A Large Multicenter Study of Methicillin-Susceptible and Methicillin-Resistant* Staphylococcus aureus *Prosthetic Joint Infections Managed with Implant Retention*. **J. Lora-Tamayo**, O. Murillo, J. A. Iribarren, A. Soriano, M. Sánchez-Somolinos, J. M. Baraia-Etxaburu, A. Rico, J. Palomino, D. Rodríguez-Pardo, J. P. Horcajada, N. Benito, A. Bahamonde, A. Granados, M. D. del Toro, J. Cobo, M. Riera, A. Ramos, A. Jover-Sáenz, J. Ariza. Clinical Infectious Diseases 2013; 56: 182-194.

Book chapter – *Systemic treatment options for medical device-associated* infection. O. Murillo, **J. Lora-Tamayo**, J. Ariza. In: *Biomaterials associated infection*. T. F. Moriarty, S. A. J. Zaat, H. J. Busscher (eds). New York: Ed. Springer; 2013.

S. aureus is the microorganism most frequently responsible for PJI, especially in acute cases where DAIR may be attempted (7, 8, 56). *S. aureus* has been reported to increase the likelihood of clinical failure compared with other etiologies (51, 146), and a great deal of uncertainty still surrounds its management. The case series reported to date comprise only a low number of cases, sometimes mixed with infections caused by CNS, and a very low number of episodes due to MRSA (Table 1.1).

The role of rifampin was established in a randomized clinical trial (58). However, the study sample was not very large (n=33) and did not include cases due to MRSA. While the results significantly favoured the rifampin-based regime in the per-protocol analysis, there was a non-statistically significant trend in the intention-to-treat analysis (58). Therefore, the role of rifampin needs to be validated in large cohorts of patients, and it would be interesting to know whether benefits of rifampin also apply to MRSA.

Table 1.1 – Comparison of previous cohorts of PJI including cases of *S. aureus*.

Reference	Present work	Marculescu (146)	Byren (51)	Brandt (57)	Vilchez (52)	Barberan (161)	Senneville (149)	Aboltins (160)
Only SA-DAIR cases?*	Yes	No – 32% SA	No – 42% SA	Yes	Yes	No – 35% SA	No – 42% DAIR	No – 95% SA
DAIR cases (MSSA+MRSA)	345 (264+81)	32 (30+2)	48 (39+9)	33 (32+1)	53 (49+4)	21 (14+7)	41 (35+6)	20 (8+11)
Age (years)	73	74	70	70	70	75	66-70	76
Immunosuppressive therapy	6%	NA	NA	18%	NA	NA	11%	20%
Revision prosthesis	19%	NA	23%	36%	4%	NA	NA	5%
Hematogenous cases	Yes (15%)	NA	NA	NA	No	NA	Yes	20%
Bacteremia	16%	6%	NA	36%	11%	NA	18%	NA
Polymicrobial infection	10%	8%	<1%	NA	28%	NA	28%	0%[a]
Sinus tract	15%	15%	NA	15%	NA	≥ 12%	38%	0%
Time to infection†	73% < 30days	63% < 90 days	30% < 30days	62% <25days	100% < 2months	0.9 ±1.1 months	NA	20d (100% <3m)
Debridement delay‡	<3d 18%	NA	<3d in 63%	<3d 55%	<3d 34%	<1 month in 43%	NA	16
Extra-debridements	≥2 in 9%	≥2 in 21%	>2 in 18%	>2 in 15%	≥2 in 15%	NA	NA	Mean 2.2
Exchange of removable components	73%	48%	87%	NA	100%	NA	NA	NA
Success (DAIR-SA)	55%	13%	73%	36%	75%	62%	78%	90%

SA: *S. aureus*. DAIR: debridement, antibiotics and implant retention. †Time from prosthesis placement to onset of symptoms (in post-surgical cases). ‡Time from onset of symptoms to surgical debridement. NA: data non-available. * Some series do not only include prosthetic joint infections by *S.*

aureus managed with DAIR; unless otherwise specified, some of the data included in this summary (such as age, presence of sinus tract, etc) may refer to all patients in the series. [a]2 cases of polymicrobial staphylococcal PJI.

We therefore undertook a large retrospective observational multicenter study, including 17 Spanish hospitals from REIPI, between 2003 and 2010. Eligible patients were those with a PJI caused by *S. aureus* and managed with DAIR as the first-line treatment. We performed univariate and multivariate analyses with Cox Regression to identify the parameters associated with Overall Failure. We also used the adjusted hazard ratios from this multivariate analysis to calculate the composited relative risks for specific patients' profiles.

Length of therapy was defined as days of treatment with a particular antibiotic, counted from the day of debridement until the day of scheduled antibiotic withdrawal or the day of failure. This implies that antibiotic therapy could be shortened in cases failing prematurely and would not actually be the cause of failure but its consequence. Therefore, antimicrobial therapy parameters were only analyzed when the groups under comparison had had the same possibilities of receiving antibiotics. For this, besides an Overall Failure analysis, we performed a separate analysis of parameters predicting failure depending on the moment when it occurred:

- Early Failure: failure within 30 days of debridement surgery.
- Late Failure: failure while the patients was still under antimicrobial therapy, but happening beyond the first 30 days after debridement – in this cohort of patients, the impact of the antibiotics received in the first 30 days could be analyzed.
- Failure After Therapy: failure after the end of antimicrobial therapy – in this cohort of patients, the impact of antibiotics received during treatment could be analyzed.

Three hundred and forty-five cases of PJI due to *S. aureus* in 342 patients managed with DAIR were included in the analysis. One hundred and forty patients (41%)

Table 1.2 - Case series descriptions and comparative analysis of MSSA & MRSA cases, and Hematogenous & Post-surgical infections

			All cases (n=345)	MSSA (n=264)	MRSA (n=81)	p	Post-surgical (n=293)	Hematogenous (n=52)	p
Baseline features	Sex (men)		140 (41%)	112 (42%)	28 (35%)	0.208	119 (41%)	21 (40%)	0.975
	Age (years)		73 (64-79)	71 (63-77)	78 (71-82)	**<0.001**	72 (64-78)	74 (65-79)	0.337
	Diabetes mellitus		68 (19%)	47 (18%)	21 (26%)	0.097	60 (20%)	8 (15%)	0.389
	Chronic renal impairment		19 (6%)	7 (3%)	12 (15%)	**<0.001**	16 (5%)	3 (7%)	1.000
	Rheumatoid arthritis		30 (9%)	26 (10%)	4 (5%)	0.187	23 (8%)	7 (13%)	0.188
	Immunosuppressive therapy		22 (6%)	18 (7%)	4 (5%)	0.576	14 (5%)	8 (15%)	**0.010**
	Revision prosthesis		67 (19%)	46 (17%)	21 (26%)	0.091	58 (20%)	9 (17%)	0.676
	Prosthesis location	Knee	195 (57%)	166 (63%)	29 (36%)		157 (54%)	38 (73%)	
		Hip	146 (42%)	97 (37%)	49 (60%)	**<0.001**	133 (45%)	13 (25%)	**0.022**
		Others	4 (1.2%)	1 (0.4%)	3 (3.7%)		3 (1%)	1 (2%)	
Clinical presentation	Type of infection	Hematogenous	52 (15%)	46 (17%)	6 (7%)		-	-	
		Post-surgical[‡] <30d	215 (62%)	157 (59%)	58 (72%)	**0.057**	-	-	-
		Post-surgical[‡] >30d	78 (23%)	61 (23%)	17 (21%)		-	-	

	Time to infection (days) ‡	19 (11-31)	19 (11-31)	18 (10-29)	0.237	-	-	-
	Polymicrobial infection	64 (19%)	49 (19%)	15 (19%)	0.992	63 (22%)	1 (2%)	**0.001**
	MRSA infection	81 (23%)	-	-	-	75 (26%)	6 (12%)	**0.028**
	Bacteremia	54 (16%)	44 (17%)	10 (12%)	0.349	25 (9%)	29 (56%)	**<0.001**
	Temperature >37ºC	154 (45%)	127 (48%)	27 (33%)	**0.029**	113 (39%)	41 (79%)	**<0.001**
	Joint pain	272 (79%)	214 (81%)	58 (72%)	**0.064**	221 (75%)	51 (98%)	**<0.001**
	Sinus tract	50 (14%)	38 (14%)	12 (15%)	0.942	48 (16%)	2 (4%)	**0.016**
	Supuration	189 (56%)	132 (50%)	57 (70%)	**0.001**	187 (64%)	2 (4%)	**<0.001**
	Leukocytes (x10E9/l)	9.4 (6.6-13.4)	9.7 (6.9-13.8)	7.9 (5.1-11.2)	**0.014**	9.0 (6.3-12.6)	11.9 (8.5-16.0)	**<0.001**
	C-reactive protein (mg/l)	63 (20-172)	55 (20-177)	82 (21-167)	0.355	53 (12-132)	225 (48-353)	**<0.001**
Treatment & Outcome	Debridement delay (days) ¶	7 (4-14)	7 (4-14)	9 (4-16)	0.107	8 (4-16)	6 (3-11)	**0.031**
	≥2 debridements	30 (9%)	22 (8%)	8 (10%)	0.666	24 (8%)	6 (12%)	0.425
	Polyethylene exchange§	221 (73%)	171 (75%)	50 (68%)	0.249	194 (75%)	27 (63%)	0.080
	Global failure*	146 (45%)	112 (44%)	34 (46%)	0.778	114 (41%)	32 (65%)	**0.001**
	Early failure during therapy**	42 (12%)	31 (12%)	11 (14%)	0.573	33 (11%)	9 (18%)	0.220
	Late failure during therapy*	47 (14%)	28 (11%)	19 (26%)	**0.001**	40 (14%)	7 (14%)	0.861
	Failure after therapy*	57 (17%)	53 (21%)	4 (5%)	**0.012**	41 (15%)	16 (33%)	**<0.001**

MSSA: methicillin-susceptible *S. aureus*. MRSA: methicillin-resistant *S. aureus*. Categorical variables expressed in absolute number and (percentage); continuous variables expressed in median and (interquartile range). ‡Time to infection: time from prosthesis placement to onset of symptoms (excluding hematogenous infections). ¶Debridement delay: time from onset of symptoms to debridement surgery. Analysis excludes *17 patients (10 MSSA-PJI + 7 MRSA-PJI; 14 post-surgical cases + 3 hematogenous-PJI) and **7 (3 MSSA-PJI + 4 MRSA-PJI; 6 post-surgical cases and 1 hematogenous-PJI) with unknown outcome. §44 patients (36 MSSA-PJI + 8 MRSA-PJI; 35 post-surgical cases + 9 hematogenous-PJI) with no information regarding polyethylene exchange.

were men, and median age was 73 years (range 27-95). MRSA caused 81 (23%) episodes, occurring in older patients with more frequent comorbidity, especially chronic renal impairment (Table 1.2). MRSA episodes were mainly post-surgical and were more frequently in hip prosthesis. The surgical approach was the same as methicillin-susceptible *S. aureus* (MSSA) cases and, interestingly, both MSSA and MRSA cases were equally treated with rifampin-based combinations. Of course, the antibiotic accompanying rifampin was different: for MSSA cases, the most frequent combination was that of a beta-lactam plus rifampin, followed by fluoroquinolones plus rifampin;

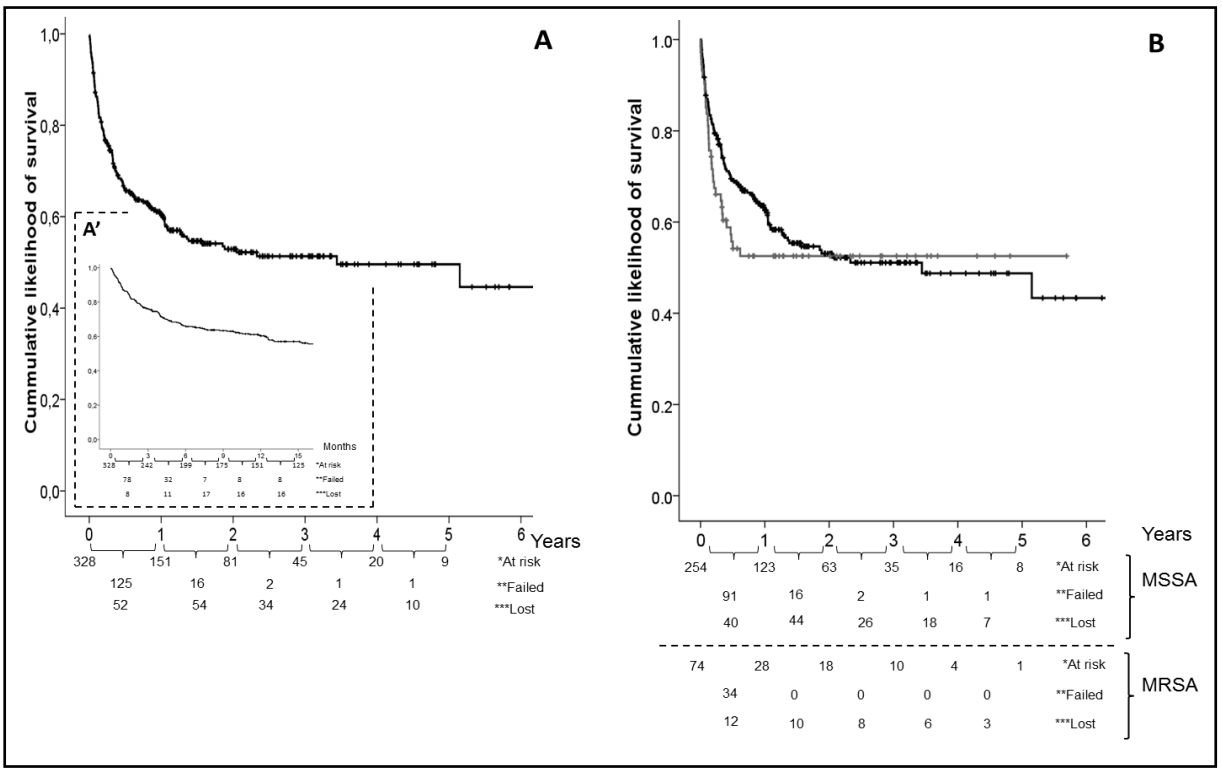

Fig 12 – Kaplan-Meier survival diagram of patients with PJI by *S. aureus*. A – Overall survival curve. A' – survival curve during the first 15 months of follow-up. B – Survival curve for methicillin-susceptible (black curve) and methicillin-resistant (grey curve) *S. aureus*. Log-rank test, p=0.374. *Number of patients at risk for failure at the beginning of the period. **Patients failing during the period. * Number of patients lost to follow-up during the period.**

for MRSA, an initial combination of a glycopeptide (namely vancomycin) plus rifampin was followed by an oral combination of rifampin plus co-trimoxazol, clindamycin or linezolid.

Overall failure evaluated in 328 patients was 45%, with no significant differences between MRSA and MSSA (rates of failure 44% and 46%, respectively; p = 0.778) (Fig 12). However, the dynamics of failure were very different: MRSA cases failed mainly while still on therapy, whereas MSSA cases failed once antimicrobial therapy had been withdrawn (Fig 13). Indeed, MRSA infection was an independent predictor of Late Failure [HR 2.33 (95%CI 1.25-4.33)], whereas it had a protective meaning in the analysis of Failure After Therapy [HR 0.33 (95%CI 0.12-0.92)] (Table 1.5).

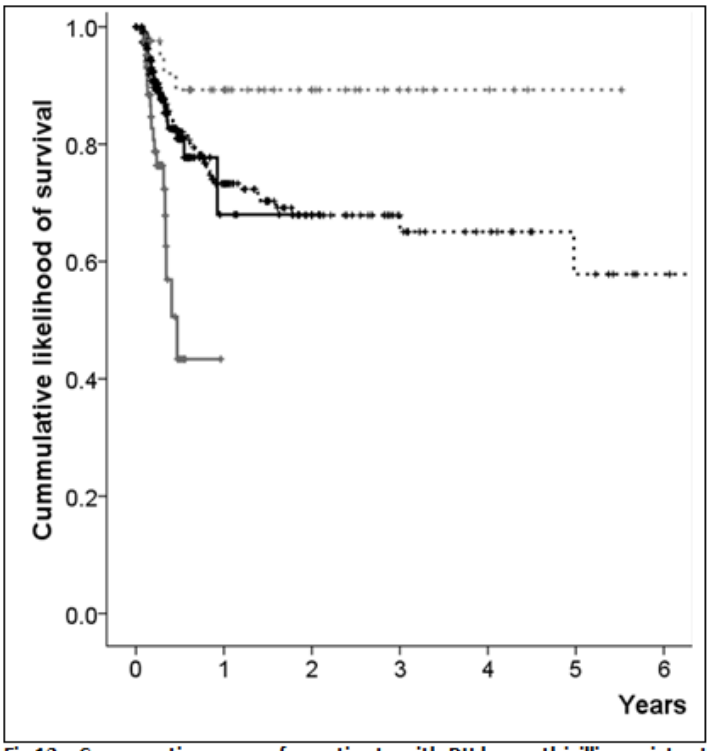

Fig 13 – Comparative curves for patients with PJI by methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *S. aureus*, during and after treatment. Patients with Early failure are excluded. Black lines: MSSA PJI while on therapy (continuous line) and after therapy (discontinuous line); long-rank test, p = 0.996. Grey lines: MRSA PJI while on therapy (continuous line) and after therapy (discontinuous line); long-rank test, p<0.001. Similar results were found when considering only post-surgical cases.

The multivariate Cox regression model for the analysis of independent predictors of Overall Failure is summarized in table 1.3. It shows that complex infections with a marked inflammatory pattern had a higher likelihood of failure: bacteremia, polymicrobial infection, a high CRP level at diagnosis and the need for two or more debridements in order to control the infection were independently associated with Overall Failure. In addition, patients under immunosuppressive therapy or patients in whom the removable components of

Table 1.3 Univariate and multivariate analysis of parameters predicting overall failure

	Categories (n)	Percentage of failure	Unadjusted analysis		Adjusted analysis	
			HR (95%CI)	p	HR (95%CI)	p
Sex (male)	Male (131)	42%	0.90 (0.64-1.26)	NS	-	-
	Female (197)	46%				
Age (years)	-	-	1.01 (0.99-1.02)	NS	-	-
Diabetes mellitus	Yes (62)	47%	1.10 (0.73-1.66)	NS	-	-
	No (266)	44%				
Renal chronic impairment	Yes (15)	67%	2.03 (1.07-3.87)	.051	-	-
	No (313)	44%				
Rheumatoid arthritis	Yes (29)	66%	1.84 (1.14-2.99)	.021	-	-
	No (297)	42%				
Immunosuppressive therapy	Yes (21)	71%	2.31 (1.35-3.94)	.006	2.23 (1.18-4.20)	.013
	No (307)	43%				
Revision prosthesis	Yes (64)	53%	1.41 (0.96 – 2.07)	.092	-	-
	No (264)	42%				
Prosthesis location (hip)	Hip (137)	42%	0.98 (0.70-1.37)	NS	-	-
	Other (191)	46%				
Hematogenous infection	Yes (49)	65%	1.83 (1.24-2.72)	.004	-	-
	No (279)	41%				
Infection by MRSA	Yes (74)	46%	1.19 (0.81-1.75)	NS	-	-
	No (254)	44%				
Bacteremia	Yes (52)	65%	2.29 (1.54-3.42)	<.001	1.81 (1.12-2.92)	.015
	No (276)	41%				
Polymicrobial infection	Yes (61)	59%	1.76 (1.21-2.57)	.005	1.77 (1.17-2.70)	.007
	No (267)	41%				

CRP at diagnosis (per 100 mg/l)	-	-	1.29 (1.13-1.48)	<.001	1.22 (1.03-1.43)	.021
Temperature > 37ºC	Yes (148)	51%	1.54 (1.10-2.14)	.011	-	-
	No (180)	39%				
Sinus tract	Yes (47)	47%	1.27 (0.81-2.01)	NS	-	-
	No (281)	44%				
Abnormal radiography	Yes (40)	60%	1.66 (1.07-2.57)	.033	-	-
	No (288)	42%				
Debridement delay > 10 days[a]	Yes (117)	50%	1.39 (1.00-1.94)	.050	-	-
	No (211)	42%				
Polyethylene exchange	Yes (212)	41%	0.56 (0.39 – 0.82)	.004	0.65 (0.44-0.95)	.026
	No (75)	56%				
Need for ≥ 2 debridements	Yes (38)	71%	1.98 (1.30-3.01)	.003	1.63 (1.03-2.59)	.039
	No (290)	41%				

For the multivariate analysis, variable with a *p* value <0.10 in the univariate analysis were including in a stepwise backward selection process (p-in <0.05 and p-out <0.10 were used in each step). Abbreviations: CI, confidence interval; CRP: C-reactive protein; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; NS, non-significant (p>0.10). [a]Debridement delay: time from onset of symtoms to debridement

the prosthesis were not exchanged during debridement also had a higher likelihood of failure. Table 1.4 shows a comparison of composited HR of patients with a specific profile taking into account these parameters – i.e., a patient under immunosuppressive therapy, with a bacteremic polymicrobial infection, presenting with a CRP level > 200 mg/L and managed with more than one debridement but without exchange of removable components would have a risk of failure 17 times higher than a patient in the opposite situation.

Table 1.4 - Comparative patterns of composed Hazard Ratio of Overall Failure

				Monomicrobial infection CRP at diagnosis (mg/L)			Polymicrobial infection CRP at diagnosis (mg/L)		
		PLE-ex	≥ 2 debrid.	0-100	101-200	>200	0-100	101-200	>200
No IS therapy	No Bact.	No	No	1,55	1,88	2,29	2,75	3,34	4,06
			Yes	2,53	3,07	3,73	4,48	5,44	6,62
		Yes	No	**1,00**	1,22	1,48	1,77	2,16	2,62
			Yes	1,63	1,98	2,41	2,89	3,51	4,27
	With Bact.	No	No	2,80	3,40	4,14	4,96	6,03	7,34
			Yes	4,56	5,55	6,74	8,09	9,83	11,96
		Yes	No	1,81	2,20	2,67	3,20	3,89	4,73
			Yes	2,94	3,58	4,35	5,22	6,35	7,71
Under IS Therapy	No Bact.	No	No	3,45	4,19	5,10	6,12	7,44	9,04
			Yes	5,62	6,84	8,31	9,97	12,12	14,73
		Yes	No	2,23	2,71	3,29	3,95	4,80	5,83
			Yes	3,63	4,41	5,36	6,43	7,82	9,51
	With Bact.	No	No	6,23	7,58	9,21	11,05	13,44	16,33
			Yes	10,16	12,35	15,02	18,01	21,90	26,62
		Yes	No	4,02	4,89	5,95	7,13	8,67	10,54
			Yes	6,56	7,97	9,69	11,62	14,13	17,17

HR = 1 is assigned to the lowest risk pattern (framed cell). IS: immunosuppressive therapy. PLE-ex: exchange of removable components of the prosthesis during debridement (i.e the polyethylene component). CRP: C-reactive protein. Bact., bacteremia. Debrid., debridement

Table 1.5 - Univariate and multivariate analysis of parameters predicting Early failure, Late failure and Failure after Therapy

	Early Failure (n=338; faiure=42)				Late Failure (n=284; failure=47)				Failure after Therapy (n=231; failure=57)			
	Unadjust. OR (95%CI)	p	Adjusted OR (95%CI)	p	Unadjust. HR (95%CI)	p	Adjusted HR (95%CI)	p	Unadjust. HR (95%CI)	p	Adjusted HR (95%CI)	p
Sex (male)	1.78 (0.93-3.41)	.081	2.48 (1.19-5.19)	.016	0.70 (0.37-1.31)	NS	-	-	0.68 (0.39-1.19)	NS	-	-
Age (years)	1.02 (0.99-1.06)	NS	-	-	1.03 (1.00-1.06)	.032	1.03 (1.00-1.07)	.052	0.98 (0.96-1.00)	NS	-	-
Diabetes mellitus	0.67 (0.27-1.66)	NS	-	-	1.46 (0.74-2.88)	NS	-	-	1.29 (0.68-2.45)	NS	-	-
Renal chronic impairment	1.44 (0.40-5.19)	NS	-	-	2.54 (1.00-6.45)	.081	-	-	1.69 (0.41-6.95)	NS	-	-
Rheumatoid arthritis	2.91 (1.20-7.04)	.018	3.88 (1.44-10.4)	.007	1.49 (0.63-3.52)	NS	-	-	1.39 (0.55-3.48)	NS	-	-
Immunosuppr. therapy	2.20 (0.77-6.32)	NS	-	-	2.41 (1.07-5.42)	.054	3.05 (1.30-7.14)	.010	1.86 (.58-5.98)	NS	-	-
Revision prosthesis	1.56 (0.74-3.28)	NS	-	-	2.00 (1.08-3.70)	.036	-	-	0.89 (0.42-1.88)	NS	-	-
Hip prosthesis	1.06 (0.55-2.03)	NS	-	-	1.72 (0.95-3.13)	.080	-	-	0.81 (0.46-1.40)	NS	-	-
Hematogenous infection	1.65 (0.74-3.69)	NS	-	-	0.85 (0.38-1.91)	NS	-	-	2.93 (1.64-5.25)	.001	2.46 (1.35-4.48)	.003
Infection by MRSA	1.24 (0.59-2.59)	NS	-	-	2.75 (1.53-4.94)	.001	2.33 (1.25-4.33)	.008	0.33 (0.12-0.91)	.012	0.33 (0.12-0.92)	-
Bacteremia	4.18 (2.06-8.50)	<.001	5.03 (2.11-12.0)	<.001	1.26 (0.57-2.76)	NS	-	-	1.97 (0.97-4.01)	0.078	-	-
Polymicrobial infection	3.65 (1.83-7.29)	<.001	7.50 (3.23-17.4)	<.001	2.56 (1.31-5.01)	.011	-	-	0.75 (0.34-1.67)	NS	-	-

CRP at diagnosis (100 mg/l)	1.45 (1.11-1.89)	0.007	1.52 (1.11-2.09)	.010	1.08 (0.84-1.40)	NS	-	-	-	-	-	-
Temperature >37ºC	1.71 (0.89-3.29)	NS	-	-	0.98 (0.55-1.74)	NS	-	-	-	-	-	-
Sinus tract	1.05 (0.42-2.66)	NS	-	-	2.18 (1.13-4.21)	.029	1.88 (0.94-3.77)	.076	0.69 (0.25-1.92)	NS	-	-
Abnormal radiography	0.98 (.36-2.64)	NS	-	-	2.58 (1.34-4.99)	.010	2.28 (1.14-4.54)	.019	1.49 (0.67-3.29)	NS	-	-
Debridement delay*†	0.97 (0.78-1.21)*	NS	-	-	2.00 (1.13-3.54)*	.019	-	-	1.002 (1.001-1.004)†	0.062	1.004 (1.001-1.006)†	.028
Polyethylene exchange‡	0.59 (0.29-1.20)	NS	-	-	0.40 (0.21-0.77)	.008	-	-	0.63 (0.33-1.20)	NS	-	-
Need for ≥ 2 debridements	1.04 (0.38-2.83)	NS	-	-	2.13 (1.08-4.18)	.042	2.25 (1.11-4.56)	.025	2.58 (1.33-4.99)	.011	2.51 (1.27-4.98)	.008
§ Rifampin	-	-	-	-	0.56 (0.31-1.01)	.062	0.49 (0.26-0.91)	.024	0.60 (0.34-1.07)	.095	-	-
§ Levofloxacin + Rifampin	-	-	-	-	0.33 (0.12 – 0.92)	.014	-	-	1.00 (0.56-1.77)	NS	-	-
§ Vancomycin + Rifampin	-	-	-	-	0.82 (0.25-2.66)	NS	-	-	0.36 (0.09-1.46)	NS	-	-

For the multivariate analysis, variables with a p value <0.10 in the univariate analysis were included in a stepwise backward selection process for all multivariate analyses (p-in<0.05 and p-out<0.10 were used in each step). OR: odds ratio. HR: hazard ratio. 95%CI: 95% confidence interval. NS: non-significant (p>0.10). Abbreviations: Immunosupr., immunosuppressant; MRSA, methicillin-resistant *S. aureus*; CRP, C-reactive protein ; Debridement delay, time from onset of symptoms to debridement (*more than10 days; †days to debridement). § Data regarding antibiotics refers to antimicrobials administered for more than 14 days during the first 30 days after therapy. ‡Multivariate analyses do not include Polyethylene Exchange due to significant lack of data.

Table 1.6. Parameters influencing failure in post-surgical infections after the first 30 days of therapy

	All post-surgical episodes (n=244; failures=81)				MSSA post-surgical episodes (n=185; failures=60)				MRSA post-surgical episodes (n=59; failures=21)			
	Unadjusted HR (95%CI)	p	Adjusted HR (95%CI)	p	Unadjusted HR (95%CI)	p	Adjusted HR (95%CI)	p	Unadjusted HR (95%CI)	p	Adjusted ¶ HR (95%CI)	p
Sex (male)	0.73 (0.46-1.17)	NS	-	-	0.75 (0.43-1.28)	NS	-	-	0.72 (0.28-1.87)	NS	-	-
Age (years)	1.00 (0.98-1.02)	NS	-	-	0.99 (0.97-1.02)	NS	-	-	1.00 (0.96-1.04)	NS	-	-
Diabetes mellitus	1.35 (0.80-2.26)	NS	-	-	1.24 (0.66-2.34)	NS	-	-	1.51 (0.61-3.75)	NS	-	-
Renal chronic impairment	2.87 (1.24-6.63)	**.032**	-	-	3.24 (0.78-13.5)	NS	-	-	2.08 (0.70-6.18)	NS	-	-
Rheumatoid arthritis	1.60 (0.80-3.19)	NS	-	-	1.70 (0.81-3.59)	NS	-	-	1.70 (0.23-12.8)	NS	-	-
Immunosup. therapy	2.46 (1.13-5.36)	**.045**	-	-	3.30 (1.41-7.74)	**.018**	3.40 (1.39-8.37)	**.008**	1.05 (0.14-7.83)	NS	-	-
Revision prosthesis	1.66 (1.01-2.74)	**.056**	-	-	1.97 (1.08-3.61)	**.038**	-	-	1.09 (0.44-2.69)	NS	-	-
Hip prosthesis	1.08 (0.69-1.68)	NS	-	-	0.93 (0.55-1.59)	NS	-	-	1.26 (0.51-3.12)	NS	-	-
†Time to infection >90 d	2.19 (1.18-4.05)	**.013**	-	-	1.84 (0.98-3.45)	NS	2.18 (1.04-4.56)	**.039**	7.48 (2.01-27.8)	.013	-	-
Infection by MRSA	1.32 (0.80-2.18)	NS	-	-	-	-	-	-	-	-	-	-
Bacteremia	1.70 (0.77-3.73)	NS	-	-	2.21 (0.99-4.95)	**.078**	2.35 (1.04-5.36)	**.040**	-	-	-	-
Polymicrobial infection	1.47 (0.88-2.47)	NS	-	-	1.19 (0.64-2.21)	NS	-	-	2.81 (1.07-7.39)	.052	-	-

CRP diagnosis (100 mg/l)	1.28 (1.02-1.60)	**.047**	1.32 (1.05-1.66)	**.018**	1.22 (0.94-1.59)	NS	-	-	1.95 (1.02-3.75)	.052	-	-
Temperature > 37ºC	1.30 (0.83-2.04)	NS	-	-	1.23 (0.73-2.08)	NS	-	-	1.89 (0.75-4.75)	NS	-	-
Sinus tract	1.62 (0.93-2.82)	**.086**	-	-	1.49 (0.77-2.89)	NS	-	-	2.15 (0.78-5.92)	NS	-	-
Abnormal radiography	2.24 (1.31-3.85)	**.007**	2.22 (1.30-3.81)	**.004**	1.77 (0.92-3.42)	NS	-	-	3.60 (1.37-9.45)	.019	4.49 (1.68-12.0)	**.003**
‡Debridement delay >10d	1.57 (1.01-2.45)	**.049**	1.68 (1.07-2.64)	**.024**	1.85 (0.91-3.77)	**.089**	-	-	1.50 (0.63-3.58)	NS	-	-
Polyethylene exchange*	0.57 (0.34-0.97)	**.045**	-	-	0.70 (0.36-1.37)	NS	-	-	0.46 (0.19-1.13)	.096	-	-
Need ≥ 2 debridements	3.15 (1.88-5.28)	**<.001**	3.82 (2.24-6.51)	**<.001**	4.34 (2.39-7.89)	**<.001**	5.36 (2.88-9.98)	**<.001**	1.62 (0.54-4.81)	NS	-	-
§Rifampin	0.55 (0.34-0.87)	**.011**	0.52 (0.32-0.83)	**.006**	0.67 (0.39-1.17)	NS	-	-	0.27 (0.11-0.65)	.007	-	-
§Levofloxacin + Rifampin	0.48 (0.27-0.88)	**.010**	-	-	0.50 (0.27-0.92)	**.019**	0.42 (0.22-0.80)	**.008**	-	NS	-	-
§Vancomycin + Rifampin	0.45 (0.17-1.24)	**.081**	-	-	-	-	-	-	0.34 (0.11-1.01)	.032	0.29 (0.10-0.87)	**.027**

For the multivariate analysis, variables with a p value <0.10 in the univariate analysis were included in a stepwise backward selection process for all multivariate analyses (p-in<0.05 and p-out<0.10 were used in each step, except ¶, where p-out was <0.05). Patients with Early Failure are excluded from this analysis. MSSA: methicillin-susceptible *S. aureus*. MRSA: methicillin-resistant *S. aureus*. HR (95%CI): Hazard Ratio (95% confidence interval).NS: non-significant (p>0.10). Immunosup.: immunosuppressive. *Polyethylene exchange not included in multivariate analysis due to significant lack of data. †Time to infection: time from prosthesis placement to the onset of symptoms. CRP: C-reactive protein. ‡Debridement delay: time from onset of symptoms to debridement. §All antimicrobial data refers to antibiotics received during more than 14 days within the first 30 days after debridement.

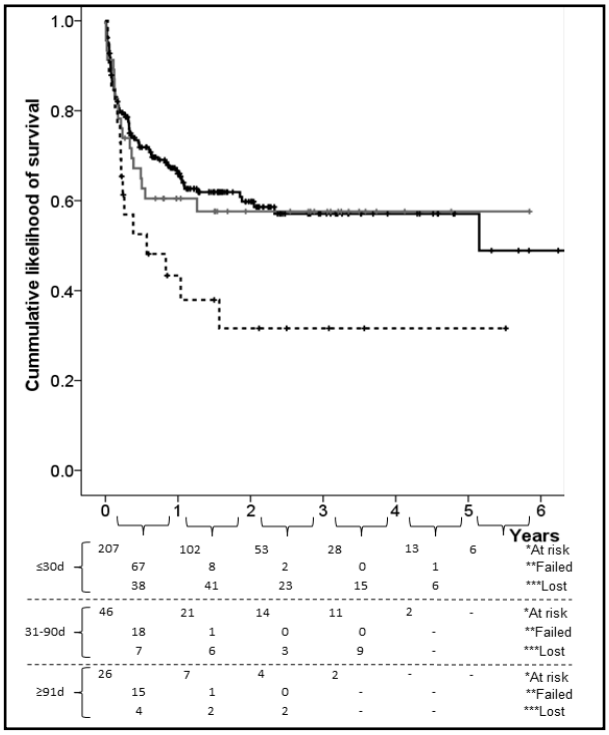

Fig 14 – Influence of the time from prosthesis placement to the beginning of symtoms among post-surgical cases. Black continuous line: symptoms beginning ≤30 days after the placement of the prosthesis (n=207, 38% failures). Grey continuous line: symptoms beginning 31-90 days (n=46; 41% failures). Black discontinuous line: symtoms beginning ≥91 days (n=26, 62% failures). Long-rank test, pp = 0.052. *Patients ar risk for failure at the beginning of the period. **Patients failing during the period. * Patients lost to follow-up during the period. Six patients with unknown outcome were excluded from this analysis.**

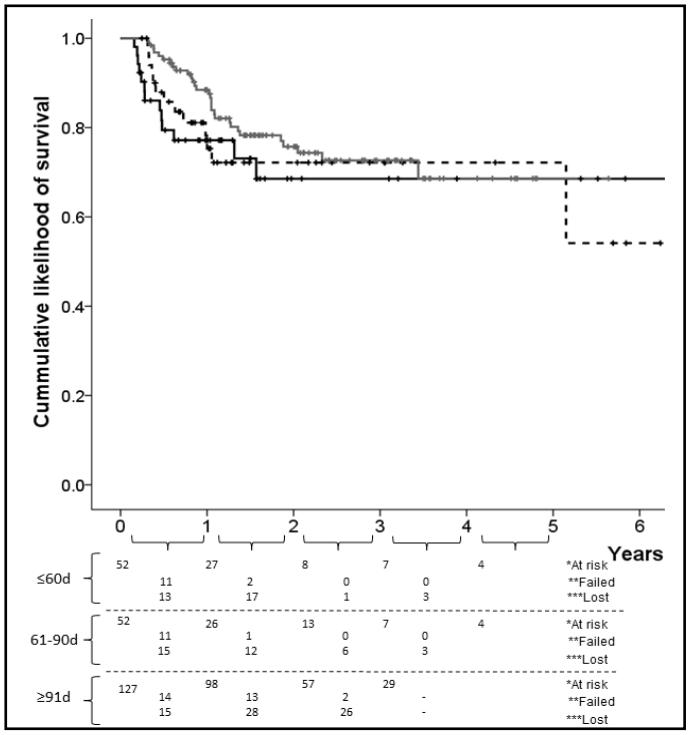

Fig 15 – Comparative survival curves of different lenghts for treatment among patients who finished the scheduled antimicrobial therapy without failing. Continuous black line: patients treated with antimicrobial therapy for 60 days or less (n=52); discontinuous black line: patients treated for 61 to 90 days (n=52); grey line: patients treated for more than 90 days (n=127); long-rank test, p = 0.434. *Patients at risk for failure at the beginning of the period. ** Patients failing during the period. * Patients lost to follow-up during the period.**

Table 1.5 shows that the use of a rifampin-combination for at least 15 days during the first 30 days after debridement was independently associated with a lower likelihood of Late Failure [HR 0.49 (95%CI 0.26-0.91)]. A non-significant trend was observed for Failure After Therapy [HR 0.60 (95%CI 0.34-107)]. The independent influence of rifampin-based treatments early after debridement was also observed for post-surgical cases, both when combined with levofloxacin for MSSA cases and with vancomycin for MRSA cases (Table 1.6).

Fig 14 shows that patients undergoing DAIR with a prosthesis age ranging between 30 and 90 days had a similar prognosis to those with a prosthesis age of less than 30 days,

whereas cases with a prosthesis age >90 days had a worse prognosis. Fig 15 shows that the length of therapy was not associated with the prognosis.

In summary, this is the largest cohort ever reported of patients with PJI by *S. aureus* managed with DAIR. We observed an overall likelihood of success of 55%. MSSA and MRSA were both treated with rifampin-based combinations. Although their overall prognoses were also alike, the dynamics of failure were very different: MSSA cases failed after withdrawal of the antimicrobials, whereas MRSA cases failed in spite of being still under therapy. Early treatment with rifampin was independently associated with a better prognosis.

Aim 2 – To assess the efficacy of a short schedule of levofloxacin plus rifampin in staphylococcal PJI

Oral communication 1 - *Short vs long duration of levofloxacin plus rifampin for acute staphylococcal prosthetic joint infection managed with implant retention: preliminary results of a clinical trial.* **J. Lora-Tamayo,** G. Euba, J. Cobo, J. Horcajada, A. Soriano, E. Sandoval, N. Benito, D. Rodríguez-Pardo, L. Falguera, M. del Toro, J. Palomino, J. Iribarren, A. Jover-Sáenz, M. Sánchez-Somolinos, A. Ramos, J. Baraia-Etxaburu, M. Fernández, M. Riera, C. Pigrau, J. Ariza. 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy. Denver, USA, 10th-13th September 2013. Oral communication (H-1005) of the preliminary results of the clinical trial – recruitment of patients has been completed, the follow-up is currently ongoing, and the manuscript is under preparation.

In the setting of staphylococcal PJI managed with DAIR, current guidelines recommend the use of rifampin plus a quinolone (i.e. levofloxacin) for long periods (8, 56). Although it is accepted that biofilm-associated infections need longer therapies than planktonic infections, the precise length of therapy is unknwon. Current recommendations set the treatment of hip and knee prosthetic infections at 3 and 6 months respectively (8, 56). Although the rationale for these lengths is exclusively empirical and not evidence-based, the need for such long treatments is widely accepted and barely questioned.

Nevertheless, long antimicrobial therapies may present several drawbacks: they may carry toxic adverse effects (75, 130, 147), they increase the costs, and they may select resistant bacteria (125, 126). Shorter courses of the fluoroquinolone-rifampin combination have shown similar results (52, 127-130, 148), and a retrospective study observed that extending the antimicrobial therapy was not associated with a better outcome (51). These studies raise the possibility that a short course of antibiotics may not be inferior to the standard long course, but prospective controlled trials demonstrating this are lacking.

We therefore undertook a multicenter open randomized clinical trial in 17 Spanish hospitals belonging to the REIPI. Eligible patients were those with a hematogenous or early post-surgical prosthetic joint infection (onset of symptoms within the first 30 days after the placement of the prosthesis) caused by *Staphylococcus*, either *S. aureus* or CNS, managed with DAIR. After debridement, patients were randomized to receive either a short schedule of treatment of 8 weeks (both hip and knee prosthesis) or a standard long schedule of 3 or 6 months (for hip or knee prosthesis respectively). Treatment consisted in the combination of rifampin (600 mg in a single fasting daily dose) and levofloxacin (750 mg once daily). Patients were followed up for at least one year after the end of therapy. The primary end point was the cure rate. This was analyzed as an intention-to-treat (ITT; all randomized patients) and a per-protocol analysis (PP; randomized patients who did not abandon the study for toxicity or other reasons).

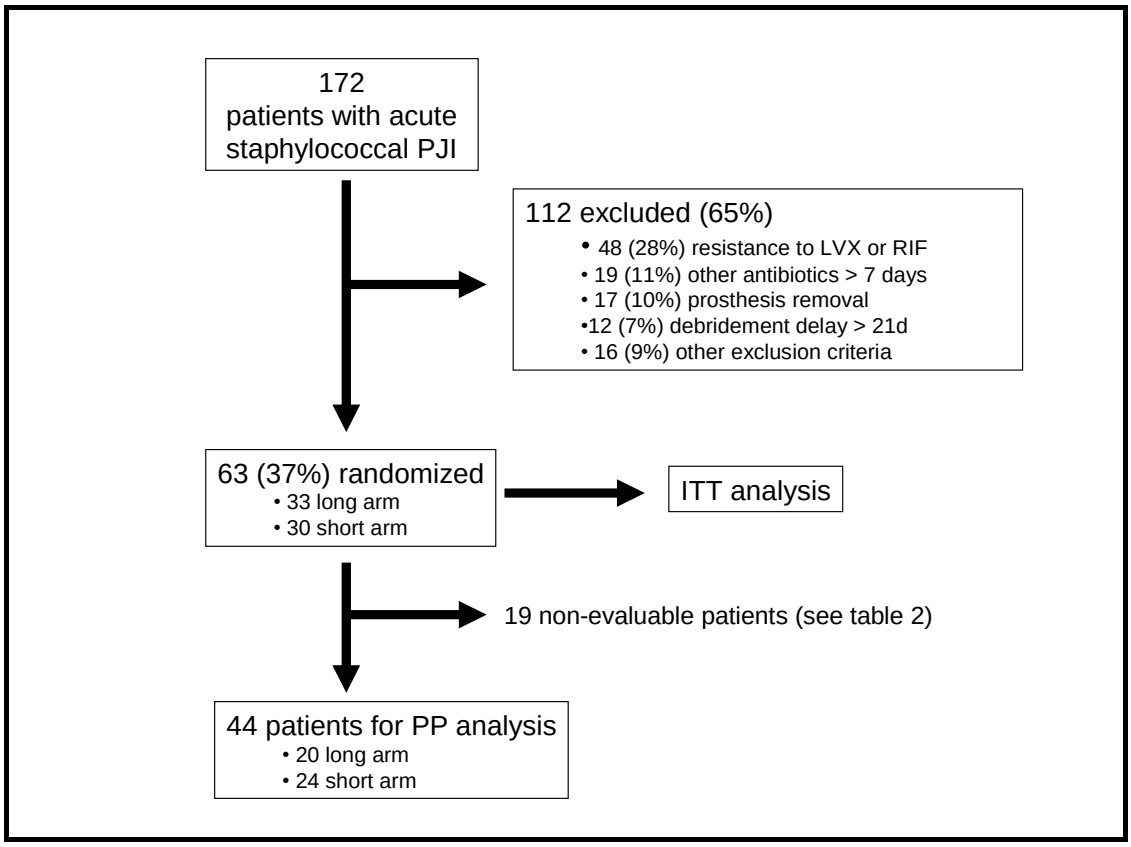

Figure 16 – Chartflow. PJI: prosthetic joint infection. LVX: levofloxacin. RIF: rifampin. ITT: intention-to-treat analysis. PP: per-protocol analysis.

Table 2.1 – Baseline features, clinical presentation and surgical treatment of all patients randomized

	Long arm (n=33)	Short arm (n=30)	p
Baseline features			
Sex (men)	19 (58%)	11 (37%)	0.097
Age (years)	74 (65-80)	70 (61-79)	0.175
Diabetes	10 (30%)	5 (16%)	0.204
Chronic heart disease	3 (9%)	4 (13%)	0.700
Liver chronic disease	3 (9%)	4 (13$)	0.700
Lung chronic disease	4 (12%)	9 (30%)	0.080
Renal chronic impairment	1 (3%)	0 (0%)	1.000
Cancer	2 (6%)	1 (3%)	1.000
Dementia	6 (18%)	1(3%)	0.107
Charlson Index ≥2	10 (30%)	9 (30%)	0.979
Clinical presentation			
Prosthesis location (hip)	18 (54%)	11 (37%)	0.155
Revision prosthesis	5 (15%)	4 (13%)	1.000
Hematogenous infection	5 (15%)	9 (30%)	0.157
Time to infection*	14 (10-22)	18 (11-23)	0.494
Temperature >37ºC	7 (22%)	6 (23%)	0.913
Sinus tract	16 (50%)	9 (32%)	0.162
Leukocyte count (x10E9/L)	10.7 (6.8-12.0)	9.0 (7.2-11.2)	0.573
Microbiological data			
Polymicrobial infection†	9 (27%)	2 (7%)	0.046
Infection by *S. aureus*	27 (82%)	21 (70%)	0.376

Infection by CNS	8 (24%)	10 (33%)	0.578
Infection by other microorganisms‡	8 (24%)	1 (3%)	0.028
Surgical data			
Time to debridement**	6 (2-9)	4 (3-8)	0.571
Need for >1 debridement	8 (24%)	5 (17%)	0.458
Exchange of removable components	21 (66%)	24 (80%)	0.205

CNS: coagulase-negative staphylococci. *Time from prosthesis placement to beginning of symptoms (hematogenous cases excluded). ** Time from onset of symptoms to debridement. † 3 infections with *S. aureus* and CNS. ‡ 3 *Proteus mirabilis*, 2 *Propionibacterium acnes*, 1 *Escherichia coli*, 1 *Pseudomonas aeruginosa*, 1 *E. faecalis*, 1 *Streptococcus* beta-heameolyticus,

Case series of staphylococcal PJI managed with DAIR have established a success rate with standard therapy of 75% (long arm) (51, 149). Assuming a maximum difference of 15% between the short and long arm of the study, a power of 80% and an α-error of 0.025 (non-inferiority hypothesis), 89 patients per group would be required. Assuming a loss rate of 10%, the total number of cases required would be 195. The study began in 2009 and the recruitment period ended in 2013. Follow-up is still on-going and the results are preliminary. The study was registered in the International Standars Randomised Controlles Trial Number (ISRCTN) with the number 35285839 (Annexe II), and also received the EudraCT number 2007-001863-31.

A hundred and seventy-two patients were eligible for the study, although only 63 (37%) met the inclusion criteria, gave their informed consent and were randomized (Fig 16): 33 (52%) were assigned to the long arm and 30 (48%) to the short arm. Although the number of patients initially targeted was not achieved, the recruitment continued for four years; it was stopped due to the low rate of inclsuion and because of the report of a a decrease in the incidence of acute PJI by *Staphylococcus* in our media (REIPI on-going analysis of epidemiological data) (36).

Baseline features, clinical presentation, microbiological data and surgical management of all randomized patients are summarized in table 2.1. No significant differences were

found among patients in the long and short arms, except for a higher rate of polymicrobial infection in the long arm, as well as a trend towards the need to perform more than one debridement (35% vs 8%, p=0.057). A non-significant higher rate of knee-prosthesis was also observed in the short arm. The comparison of patients evaluable for the per-protocol analysis is similar (data not shown).

Per-protocol analysis

Forty-four patients were evaluable per-protocol (20 in the long arm and 24 in the short arm). Overall success was observed in 41 patients (93%) after a median follow up of 355 days (IQR 193-697). Figure 17 summarizes the Kaplan-Meier survival curve of patients included in both arms, and show no differences between the two schedules of the study: the mean survival time since the end of therapy was 45 months (95CI 41-50 months in the long arm), and 39 months (95CI 34-43 months) in the short arm (Long-rank test, p=0.848). One patient failed in the long arm (5%) and two (8%) in the short arm (p=1.0). All three patients had monomicrobial infections by *S. aureus*. The patient failing in the long-arm carried a hip prosthesis and presented failure 54 days after the end of therapy, requiring prosthesis removal to control the infection. Both patients failing in the short arm carried knee prostheses and failed 64 and 204 days after the end of therapy respectively; both required prosthesis removal. Similar results were observed when sub-analyses for hips and knees were performed. The statistical power of this analysis, maintaining the parameters above specified, is 29%.

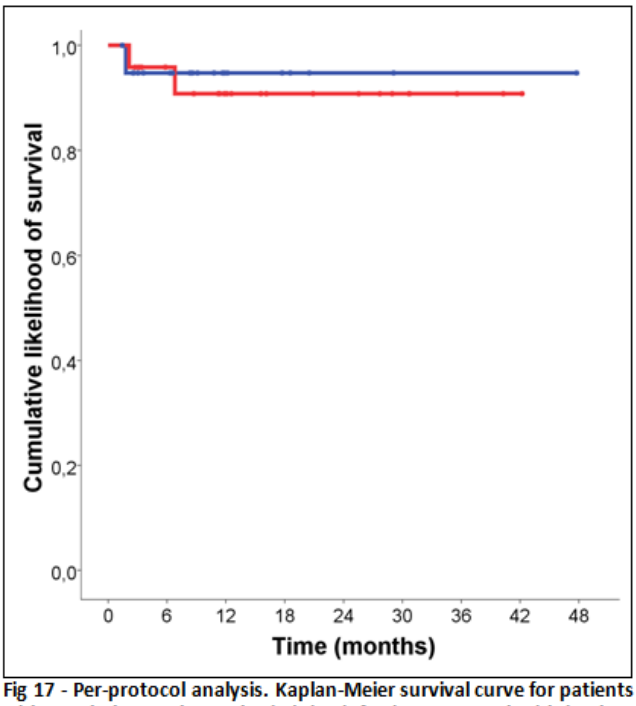

Fig 17 - Per-protocol analysis. Kaplan-Meier survival curve for patients with staphylococcal prosthetic joint infection managed with implant retention and a long schedule (blue line, n=20) or a short schedule (red line, n=24) of levofloxacino plus rifampin. Long rank test, p=0.763.

Intention-to-treat analysis

Nineteen patients (30%) were not evaluable per-protocol (Table 2.2): 13 (39%) in the long arm vs 6 (20%) in the short arm (p=0.094). Interestingly, ten (16%) patients needed antibiotic withdrawal due to toxicity, presenting adverse events a median of 20 days after debridement (IQR 14-24). In one patient (10%) in the long arm the adverse event happened beyond the first 8 weeks of treatment (at 110 days). Two patients (3%) were treated for a period longer than scheduled by randomization due to a delay in the subsidence of inflammatory signs. Valuable samples from these patients could be obtained some time after stoppage of antibiotics, and did not yield bacterial growth; one of these patients in the long arm, carried a hip prosthesis and was treated for 4 months, and the other in the short arm had a knee prosthesis and was treated for more than 2 months; they were considered non-evaluable in the per-protocol analysis.

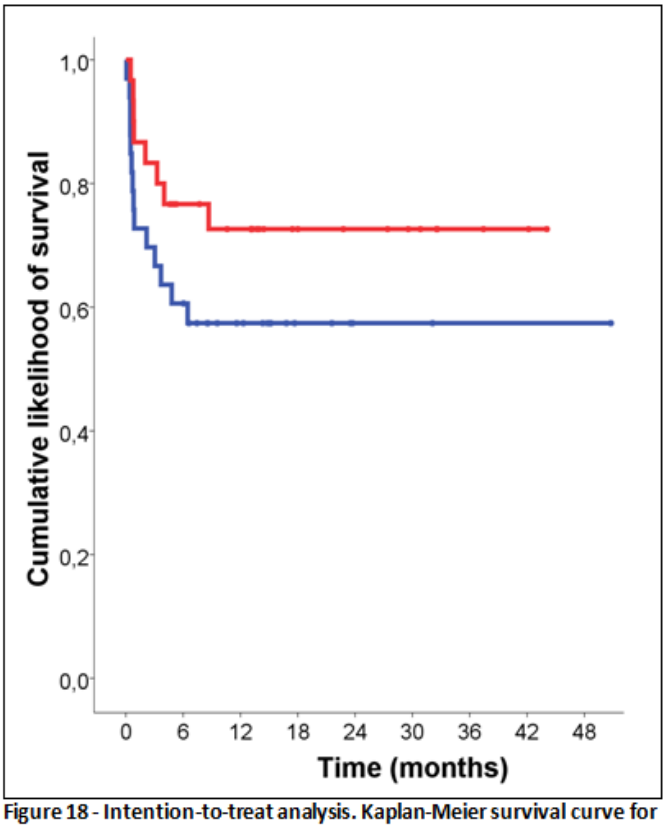

Figure 18 - Intention-to-treat analysis. Kaplan-Meier survival curve for patients with staphylococcal prosthetic joint infection managed with implant retention and a long schedule (blue line, n=33) or a short schedule (red line, n=30) of levofloxacino plus rifampin. Long rank test, p=0.156.

Success among all 63 patients randomized was observed in 41 (65%) patients: 19 (58%) in the long arm and 22 (73%) in the short arm (p=0.190). Figure 18 summarizes the Kaplan-Meier survival curve of these patients, which showed no statistically significant differences between the two schedules: the mean survival time since the surgery of debridement was 30 months (95CI 22-38 months) in the long arm and 33 months (95CI 26-39 months) in the short arm (Long-rank test, p = 0.156). Similar results were observed when sub-analyses for hips and knees were performed. The statistical power of this analysis, maintaining the same specified parameters, is 39%.

In summary, we presented the first clinical trial comparing the results of two different durations of the same treatment with levofloxacin plus rifampin in the setting of acute staphylococcal prosthetic joint infection managed with implant retention. Although this analysis has a low statistical power, these results show that a short schedule of 8 weeks was as effective as a long standard course of antibiotics of 3 or 6 months.

Table 2.2 – Patients non-evaluable for the per-protocol analysis

	Long arm (n=13)	Short arm (n=6)	P
Toxicity to antibiotics	6 (46%)	4 (67%)	0.628
Toxicity to rifampin*	5 (39%)	2 (33%)	1.000
Toxicity to levofloxacin**	1 (8%)	2 (33%)	0.222
Early prosthesis removal for orthopaedic reasons	2 (15%)	0 (0%)	1.000
Lost of follow-up	4 (31%)	1 (17%)	1.000
Protocol violation	1 (8%)	1 (18%)	1.000

*digestive intolerance in 4, cholestatic hepatitis in 1, cutaneous rash in 1, and interaction with methadone in 1. ** long QT segment in 1, impairment of a myastenic syndrome in 1, and arthralgias and myalgias in 1.

Aim 3 – To evaluate the combination daptomycin plus rifampin for fluoroquinolone-resistant staphylococcal PJI

Article 2 – *Efficacy and Safety of High Doses of Daptomycin (10 mg/kg/d) plus Rifampin for the Treatment of Staphylococcal Prosthetic Joint Infection Managed with Implant Retention*. **J. Lora-Tamayo**, J. Parra-Ruiz, D. Rodríguez-Pardo, J. Barberán, A. Ribera, E. Tornero, C. Pigrau, J. Mensa, J. Ariza, A. Soriano. Submitted for publication.

The antimicrobial therapy of choice in the setting of an acute staphylococcal prosthetic joint infection managed with DAIR is a rifampin-based combination (7, 8, 56, 58). However, in the setting of MRSA infection or other fluoroquinolone-resistant staphylococci, the choice of the best rifampin-based combination is still controversial. The combination of high doses of daptomycin plus rifampin has been shown to be the most active treatment in animal experimental models of foreign body and prosthetic joint infection (69, 76). However, clinical experience is scarce. Cases treated with daptomycin received either low doses or were not given rifampin (82, 83, 150). Our aim was to assess the efficacy and safety of the combination of daptomycin at high doses (10 mg/kg/d) plus rifampin for the treatment of acute staphylococcal prosthetic joint infection managed with debridement and implant retention.

We undertook a retrospective, observational, multicenter study (5 hospitals in the framework of REIPI) from 2010 to 2012. Eligible patients were those with acute PJI caused by fluoroquinolone-resistant *Staphylococcus* (either *S. aureus* or CNS) managed with DAIR and the combination of daptomycin at high doses (10 mg/kg/d) plus rifampin (600 mg/d) as first-line therapy, for a scheduled time of 6 weeks. Supplementary oral antibiotics after this period could be received at the discretion of the assisting medical team. A minimal period of 15 days with this treatment was needed to evaluate efficacy. We compared the rates of clinical and microbiological failure with two historical cohorts of PJI caused by *S. aureus* and CNS treated with implant retention and alternative rifampin-based combinations.

Table 3.1 – Characteristics of patients with staphylococcal PJI managed with DAIR and daptomycin (10 mg/kg/d) plus rifampin

Sex/ Age (years)	Comor-bidity	Prosthesis Location*	Time to Infection[§]	Etiology	MIC for Dapto/Vanco (mg/L)	Time to Debrid[#]/ Nº Debrid	Daptomycin Dose[†]/ Length[‡]	Rifampin Length[‡]	Suppl.ATB (length[‡])	Clinical Cure / Follow-up[‡]	Microorg. in failure
F / 63	None	THA*	11	MRSA	0.25 / 2	2 / 1	10.0 / 41	41	No	No / 82	MRSA
F / 60	Cancer	THA	13	CNS	1 / 4	1 / 1	10.0 / 43	43	No	Yes / 387	-
F / 85	None	THA	8	CNS + *K. pneumoniae*	0.38 / 3	5 / 2	10.0 /42	44	No	Yes / 785	-
M / 79	DM	THA	30	MRSA	0.19 / 2	10 / 2	10.0 / 56	56	No	No / 0	*E. coli*, *Klebsiella*, Anaerobes
F / 90	Dementia	HHA	19	CNS	1 / 3	5 /1	10.0 / 39	39	No	Yes / 15	-
F / 84	Dementia	HHA	48	MRSA	0.25 / 2	4 /1	12.5 / 39	39	No	Yes / 970	-
F / 85	DM, Dementia	HHA	5	MRSA + *E. coli*	0.25 / 2	3 /2	8.8 /39	42	No	No / 20	Negative cultures
F / 70	Corticoids	THA	20	MRSA, *Proteus*, *P. aeruginosa*	0.25 / 2	14 / 2	11.0 / 42	42	No	No / 288	*E. coli*

M / 78	DM, COPD, Dementia	HHA	13	MRSA + *Enterobacter*	0.19 / 3	3 / 2	10.8 / 42	45	LNZ+RIF (14)	Yes / 8	-
F / 80	None	THA*	50	MRSA	0.19 / 2	3/ 2	10.0 / 37	51	LNZ+RIF(14)	Yes / 21	-
F / 69	None	Knee	28	MRSA	0.125/1	2 / 1	9.2 /47	41	No	No / 91	MRSA
M / 81	DM	Knee	17	MRSA	0.125/1.5	5 / 1	9.3 / 46	74	CMX+RIF (41)	No / 113	MRSA
F / 84	None	THA*	14	CNS + *E. faecalis*	≤1* / ≤4*	1 / 1	8.3 / 44	44	No	No / 483	*E. faecalis*
M / 58	DM	Knee	28	MSSA	0.5/1.0	1 / 1	9.5 / 44	42	No	Yes / 747	-
M / 80	DM	Knee	22	CNS	0.5/1.0	6 / 1	9.7 / 48	45	No	Yes / 751	-
F / 63	DM	Knee	26	CNS	0.5/1.0	9 / 2	9.7 / 51	49	No	No / 76	CNS
F / 72	None	Knee	9	MRSA	≤1* / ≤4*	6 / 1	10.5 / 35	35	No	Yes / 731	-
F / 68	None	Knee*	12	CNS	≤1* / ≤4*	9 / 1	9.9 / 42	42	No	No / 0	CNS

All cases were early post-surgical. M: male; F: female; DM: diabetes mellitus. Cort: chronic treatment with corticosteroids. COPD: chronic obstructive pulmonary disease. [§]Revision prosthesis. THA: total hip artrhoplasty; HHA: hip hemiarthroplasty. [Ø]Time to infection: time from prosthesis placement to beginning of symptoms (in days). MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *S. aureus*. CNS: coagulase-negative *Staphylococcus*. MIC for Dapto/Vanco: minimal inhibitory concentration for daptomycin and vancomycin, respectively; values obtained by the E-test method or *microdilution. [#]Time to debridement: time from beginning of symptoms to debridement. Nº debrid: number of debridements within the first 15 days after the first debridement. †Dose of daptomycin expressed in mg/kg/d. ‡Length of antimicrobial therapy and follow-up are expressed in days. LNZ: linezolid; RIF: Rifampin; CMX: co-trimoxazole.

Table 3.2 – Outcome of patients with staphylococcal PJI submitted to debridement and treatment with daptomycin (10mg/kg/d) plus rifampin.

	All (n=18)	CNS (n=7)	*S. aureus* (n=11)	p
Clinical failure	9 (50%)	3 (43%)	6 (55%)	1.00
Clinical failure while on treatment	2/9 (22%)	1/3 (33%)	1/6 (17%)	1.00
Microbiological failure	5 (28%)	2 (29%)	3 (27%)	1.00
Microbiological failure while on treatment	1/5 (20%)	1/2 (50%)	0/3 (0%)	0.40

See definitions in the text – microbiological failure are failures due to the same Staphylococcus originally causing the infection.

Twenty patients met the inclusion criteria. Two patients (10%) needed to be withdrawn shortly after the beginning of therapy due to toxicity (one caused by daptomycin), thus leaving 18 patients evaluable for efficacy (Table 3.1): 13 (72%) were women, and median age was 79 years (range 58-90). All presented with early-post surgical infection, with symptoms starting a median of 18 days (range 5-50) after the placement of the prosthesis. *S. aureus* was the causing microorganism in 11 (61%) cases, and CNS in seven (39%). Patients were treated with rifampin plus daptomycin at a median dose of 10.0 mg/kg/d (range 8.33-12.5) for a median of 42 days (range 35-56) with no significant adverse effects or significant rise in CPK levels.

Nine (50%) cases were considered to be clinically cured (Table 3.2) after a median follow-up of 749 days (range 387-970) (these data do not include 2 very old and fragile patients died early after the end of therapy, due to causes unrelated with the infection). Thus, nine (50%) cases showed clinical failure, with the same *Staphylococcus* being recovered in 5 cases (28% of microbiological failure). No rise in the daptomycin or rifampin MIC was observed in these cases.

These rates of clinical and microbiological failure were comparable to those observed in the historical cohort (Table 3.3). However, an important difference was observed regarding the moment of failure: while 73% of cases failing in the historical cohort did

Table 3.3 – Comparison of the present series of PJI with a historical cohort of PJI by staphylococci also managed with DAIR, exchange of removable components and ≥15 days of an alternative rifampin-based combination.

	Historical series (n=44)	Present Series (n=18)	p
Infection by *S. aureus*	32 (73%)	11 (61%)	0.368
Sex (women)	24 (55%)	13 (72%)	0.198
Age (years)	74 (66-79)	79 (67-84)	0.274
Diabetes	10 (23%)	7 (39%)	0.214
Renal chronic failure	4 (9%)	0 (0%)	0.310
Immunosuppressive therapy	2 (5%)	1 (6%)	1.000
Any comorbidity	18 (42%)	11 (61%)	0.170
Knee prosthesis	22 (50%)	7 (39%)	0.426
Non-cemented hip hemiarthroplasty	4 (9%)	4 (22%)	0.214
Revision prosthesis	11 (25%)	4 (22%)	1.000
Hematogenous infection	2 (5%)	0 (0%)	1.000
Bacteremia	3 (8%)	2 (11%)	0.646
Polymicrobial infection	9 (21%)	5 (28%)	0.524
CRP at diagnosis (mg/L)	37 (8-111)	60 (37-173)	0.174
Time to debridement (days)*	6 (3-13)	5 (2-7)	0.113
Exchange of removable components	44 (100%)	18 (100%)	-
Clinical failure	15 (34%)	9 (50%)	0.265
Clinical failure while on therapy	11/15 (73%)	2/9 (22%)	**0.033**
Microbiological failure	13 (30%)	5 (29%)	1.000
Microbiological failure while on therapy	9/13 (69%)	1/5 (20%)	0.118

Continuous variables expressed in median (and interquartile range); categorical variables expressed in absolute number (and percentage). *Time from onset of symptoms to surgery of debridement. CRP: C-reactive protein.

so while they were still under therapy, in the daptomycin-rifampin series only 22% failed while on therapy (p=0.033), with failure happening some weeks after the end of therapy in most cases. A trend towards this difference could also be seen for microbiological failure, which was significant only when analyzed for *S. aureus*.

In summary, in the setting of fluoroquinolone-resistant staphylococcal PJI managed with implant retention, the combination of daptomycin at high doses (10 mg/kg/d) plus rifampin obtained similar results to other rifampin-based combinations, but treatment failure was less frequent while patients were still under antibiotic treatment.

A.2. Infection by Gram-negative bacilli

Aim 4 – To assess the impact of fluoroquinolones in the outcome of a large cohort of PJI by Gram-negative bacilli.

Article 3 – *Gram-negative prosthetic joint infections: outcome of debridement, antibiotics and implant retention approach. A large multicenter study.* D. Rodríguez-Pardo, C. Pigrau, **J. Lora-Tamayo**, A. Soriano, M. D. del Toro, J. Cobo, J. Palomino, G. Euba, M. Riera, M. Sánchez-Somolinos, N. Benito, M. Fernández-Sampedro, L. Sorli, L. Guio, J. A. Iribarren, J. M. Baraia-Etxaburu, A. Ramos, A. Bahamonde, X. Flores-Sánchez, P. S. Corona, J. Ariza. Submitted for publication.

Gram-negative bacilli are less common cause of PJI than Gram-positive cocci. Still, they are responsible for 10-23% of these infections (35, 36, 39), and as they are often acute (7, 35, 151), management involving implant retention is frequently attempted. The odds of curing a PJI by with implant retention range from 26 to 88%, but studies addressing this question often include mixed infections and small samples (39, 97, 98). While some of these papers note the importance of quinolones for the treatment of PJI by GNB (97-99), larger samples are needed to validate this observation, and there is uncertainty regarding specific Gram-negative microorganisms.

We conducted a retrospective observational multicenter study involving 16 Spanish hospitals in the framework of the REIPI. Eligible patients were those with a PJI caused by GNB. Polymicrobial cases were included, provided they did not include Gram-positive microorganisms. Univariate and multivariate analyses of outcome predictors was performed on cases managed with DAIR.

Two hundred and forty-two episodes of PJI in 242 patients originally caused by GNB were recorded (Table 4.1). DAIR was the most common management, performed in 174 (72%) patients, most of them with acute post-surgical infection: 24 (14%) hematogenous PJI and 130 (75%) early post-surgical (onset of symptoms within the first 30 days after the placement of the prosthesis); there were 20 (11%) patients with

Table 4.1 - Demographic Data, Comorbid Conditions, and Symptoms at Presentation in 242 Gram-Negative Prosthetic Joint Infections Sorted by Surgical Approach

Variables		All cases (n=242)	Patients treated with DAIR N=174 (72%)	Patients not treated with DAIR N=68 (28%)	*P*
Baseline Features	Age (years) †	76 (68-81)	76 (69-81)	77 (65-81)	0.96
	Sex (male)	81 (34)	59 (34)	22 (32)	0.82
	Diabetes mellitus	52 (22)	37 (21)	15 (22)	0.89
	Chronic renal impairment	23 (10)	15 (9)	8 (12)	0.45
	Use of steroids	21 (9)	16 (9)	5 (7)	0.65
	Rheumatoid arthritis	19 (8)	12 (7)	7 (10)	0.37
	Malignancy	16 (7)	13 (7)	3 (4)	0.57
	Revision prosthesis	69 (29)	49 (28)	20 (29)	0.85
	Prosthesis location (hip)	150 (62)	115 (66)	35 (41)	0.03
Clinical Presentation	Type of infection				
	Hematogenous PJI	37 (15)	24 (14)	13 (19)	0.30
	Early postoperative PJI ≤ 30 days	152 (63)	130 (75)	22 (34)	<0.001
	Late chronic PJI >30 days	51 (21)	20 (11)	31 (46)	<0.001
	Positive intraoperative culture	2 (1)	-	2 (1)	-
	Time to infection, days*†	16 (9-38)	14 (8-24)	349 (90-1307)	<0.001
	Bacteremia	17 (7)	11 (6)	6 (9)	0.28
	Pain	182 (75)	130 (75)	52 (76)	0.83
	Inflammatory signs	172 (71)	130 (75)	42 (62)	0.046
	Purulence drainage	139 (57)	113 (65)	26 (38)	<0.001
	Temperature ≥38 Cº	81 (34)	62 (36)	19 (28)	0.25
Microbiol. and Laborartory Data	Leukocytes(x10^9/L) †	8.5 (6.5-11)	8.5 (6.1-11)	8.7 (7.0-11)	0.73
	CRP, mg/L**†	23 (7-55)	21.8 (7-49)	36 (13-94)	0.14
	Ciprofloxacin-susceptible isolates	200 (83)	139 (80)	61 (90)	0.03
	Pseudomonas spp. infection	68 (28)	43 (25)	25 (37)	0.06
	ESBL-GNB Infection	19 (8)	16 (9)	3 (4)	0.22
	Infection caused by ≥2 GNB	40 (17)	33 (19)	7 (10)	0.10
Treatment	Debridement delay (days)*** †	6.5 (1-21)	5 (1-14)	24 (3-111)	<0.001

	≥2 debridements at any time	21 (8)	21 (12)	-	-
	Polyethylene exchange[#]	96 (40)	96 (55)	-	-
	Patients treated with ciprofloxacin	177 (73)	125 (71)	53 (78)	0.29
Outcome	Overall mortality	43 (18)	33 (19)	10 (15)	0.49
	Mortality due to the infection[≠]	12 (5)	5 (3)	7 (10)	0.12

Categorical data are expressed as absolute number (percentage) and †continuous variables as median (interquartile range).Abbreviations: CP, ciprofloxacin; DAIR, debridement, antibiotics, and implant retention; ESBL-GNB, extended-spectrum beta-lactamase-producing gram-negative bacteria; GNB, gram-negative bacteria. *Time to infection: time from prosthesis placement to onset of symptoms, excluding hematogenous infections ** CRP: C-reactive protein value was available in 151 of 242 (62%) patients: 114 patients treated with DAIR and 37 not treated with DAIR ***Debridement delay: time from onset of symptoms to surgery, excluding 7 cases in which surgery was not performed [#]Information on polyethylene exchange was only investigated in patients treated with DAIR: in 96 of 174 cases it was changed, in 47 it was not changed, and in 31 cases this information was not available.[≠] Deaths attributed to PJI. All related deaths occurred within 30 days from the diagnosis

symptoms beginning after 30 days, of whom 12 began within the first 90 days after the prosthesis placement. The most frequent microorganism was *E. coli* (63 isolates) (Table 4.2). There were 34 polymicrobial infections (20%), *Pseudomonas* spp was present in 43 isolates (20%); the rate of polymicrobial infection higher in these cases.

Overall, 68% patients were considered to be cured after a follow-up of 25 months (IQR 15-39). The univariate and multivariate analyses for identifying parameters predicting failure are summarized in Table 4.3. Chronic renal failure was independently associated with failed treatment [HR 2.56 (95CI 1.14-5.77)], and treatment with ciprofloxacin was an independent predictor of success [HR for failure 0.23 (95CI 0.13-0.40)] (Fig 19). In fact, the use of quinolones was also associated with a good outcome when analyzed for the cohort of patients with pseudomonal infection: success was observed in 33/42 (79%) cases, with a higher use of quinolones among cases with a favorable outcome than among cases that failed (88% vs 45%; p=0.013). The success rate among ESBL-producing GNB was 8/15 (53%), with a non-significantly better prognosis in the two patients who were able to receive ciprofloxacin (100% success) as compared with fluoroquinolone-resistant cases (46% success) (p=0.467).

Of interest, although there were vey few post-surgical cases with a prosthesis age longer than 30 days when the symptoms of infection began, we found no difference in prognosis according to this parameter (Fig 20).

Table 4.2 - Microbiological Findings in 174 Patients with Gram-negative Prosthetic Joint Infections Treated with DAIR

Microorganisms	**N=174 episodes with 211 isolates (100%)***
Enterobacteriaceae	162 (77)
Escherichia coli	63 (30)
Proteus spp.	31 (15)
Enterobacter spp.	29 (14)
Klebsiella spp.	14 (7)
Morganella morganii	10 (5)
Serratia marcescens	8 (4)
Salmonella spp.	5 (2)
Citrobacter spp.	2 (1)
Pseudomonas spp.[a]	43 (20)
Other gram-negative bacteria	6 (2) **

Abbreviations: DAIR, debridement, antibiotics and implant retention; GNB, gram-negative bacteria; GN-PJI, gram-negative prosthetic joint infection.* Among 174 episodes of GN-PJIs treated with DAIR, 34 were polymicrobial infections caused by more than one GNB, accounting for a total of 211 isolates.

[a] *P. aeruginosa* in all but 3 cases, in which *P. stuzeri* was identified **Other GNB include: 3 *Bacteroides fragilis, 1 Pasteurella multocida 1 Alcaligenes xylosoxidans, 1 Rahnella aquatilis*

In summary, we analyzed predictors of failure in a very large cohort of patients with PJI by GNB managed with DAIR. Among other parameters, the use of ciprofloxacin was an independent predictor of success.

Table 5 - Univariate and Multivariate Analysis of Parameters Predicting Overall Failure in 173 patients treated with DAIR and known outcome

	Unadjusted Analysis		Adjusted Analysis	
	HR (95%CI)	*P*	aHR (95%CI)	*P*
Male Sex	.99 (0.56-1.73)	*.9613*	-	-
Age (years)	1.03 (1.00-1.05)	.0685	1.01 (0.13-1.04)	.6000
Diabetes mellitus	1.28 (0.69-2.38)	.4407	-	-
Chronic renal failure	2.14 (0.97-4.76)	.0604	2.56 (1,14-5.77)	.0232
Rheumatoid arthritis	1.37 (0.55-3.45)	.4988	-	-
Use of steroids	1.32 (0.57-3.09)	.5189	-	-
Revision prosthesis	1.04 (0.59-1.84)	.8922	-	-
Prosthesis location, hip	1.52 (0.85-2.73)	.1612	-	-
Hematogenous PJIs	0.90 (0.40-2.02)	.8170	-	-
Late chronic PJI	1.23 (0.58-2.64)	.8170	-	-
Bacteremia due to GNB	1.30 (0.46-3.62)	.6205	-	-
Fever	1.02 (0.59-1.79)	.9321	-	-
Local pain	0.84 (0.46-1.55)	.5780	-	-
External inflammatory signs	1.11 (0.60-2.07)	.7411	-	-
Purulence	1.49 (0.83-2.67)	.1796	1.64 (0.91-2.98)	.1002
Polymicrobial PJI	1.18 (0.61-2.29)	.6201	-	-
Pseudomonas spp. PJI	0.59 (0.29- 1.20)	.1440	-	-
GNB susceptible to CP	0.31 (0.18-0.54)	.0000	-	--
ESBL-GNB PJI	1.73 (0.78-3.82)	.1773	-	-
CRP at diagnosis, per 100mg/L*	1.00 (1.001-1.007)	.016	-	-
Leukocyte count (10^9/L)	1,005 (0,951-1,061)	.8684	-	-
Need for $\geq$ 2 debridements**	2.15 (1.11-4.18)	.0237	-	-
Debridement delay (days)***	1.004 (0.996-1.013)	.2835	-	-
Polyethylene exchange*	0.73 (0.35-1.51)	.3994	-	-
Treatment with CP	0.22 (0.13-0.37)	.0000	0.23 (0.13-0.40)	.0000
Combined antibiotic therapy	0.42 (0.21-0.87)	.0189	0.52 (0.25-1.06)	.0735

Abbreviations: CI, confidence interval; CP, ciprofloxacin; CPR, C-reactive protein; ESBL-GNB, extended-spectrum beta-lactamase-producing gram-negative bacteria; HR, hazard ratio; aHR, adjusted hazard ratio; GNB, gram-negative bacilli; PJI, prosthetic joint infection.

* Multivariate analyses do not include CPR at diagnosis or polyethylene exchange, due to significant lack of data

** Need for $\geq$2 debridements at any time since diagnosis

***Debridement delay: days from onset of symptoms to debridement

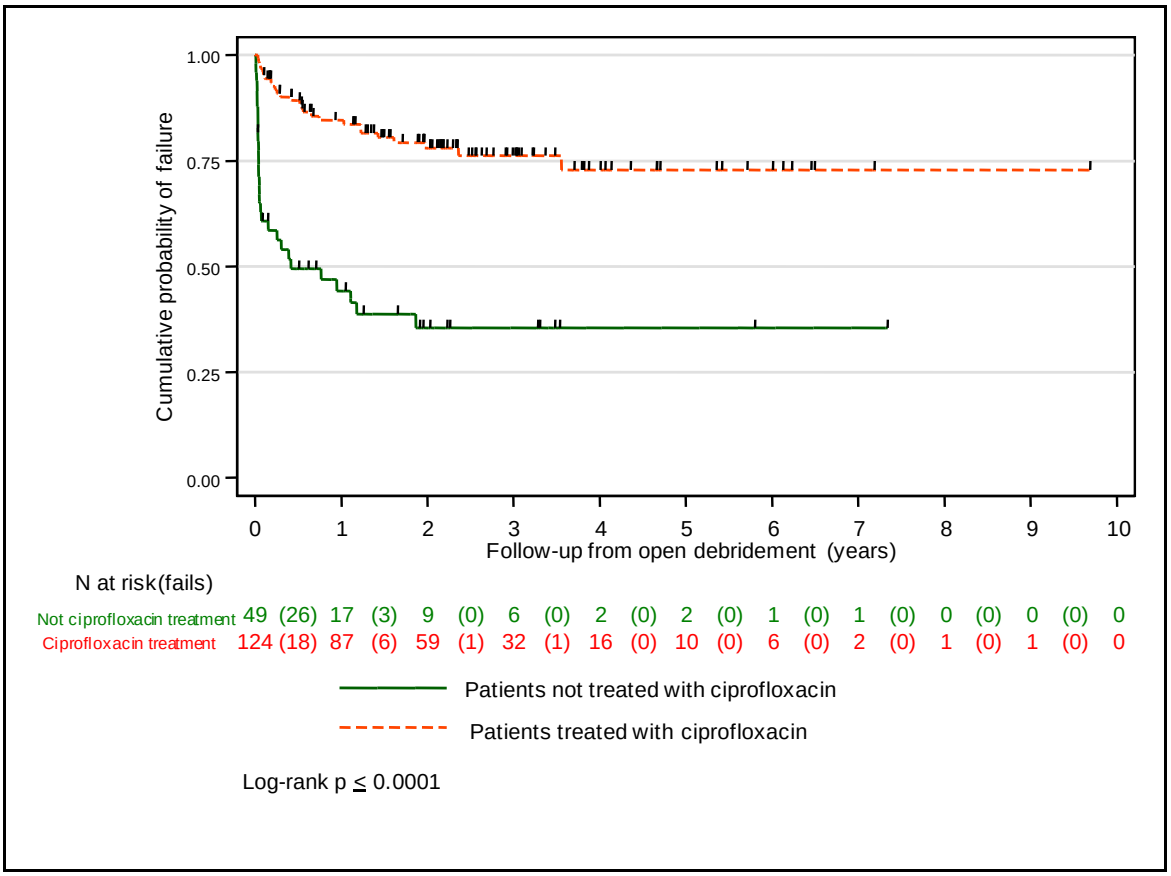

Fig 19 - Kaplan-Meier survival curves por patients treated with ciprofloxacin or other treatments

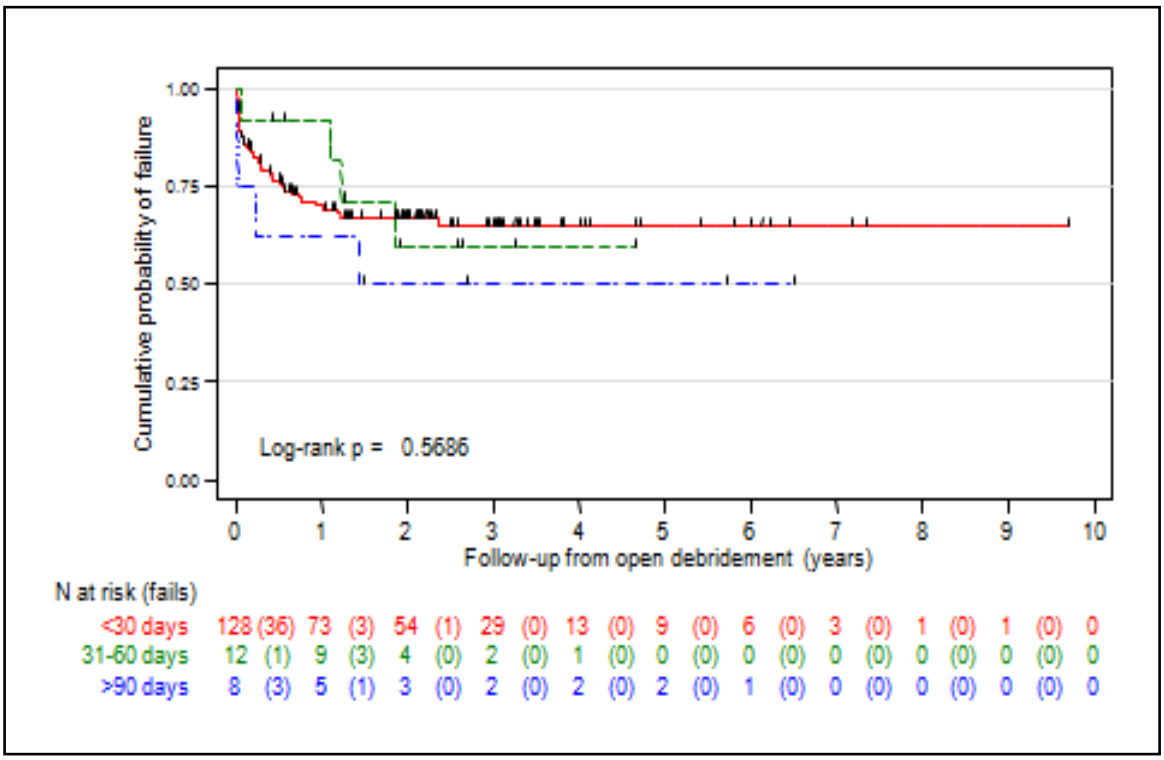

Fig 20 - Comparative survival Kaplan-Meier curves of patients with post-surgical infection, sorted by the age of the prosthesis at the time of onset of symptoms

A.3. Infection in the elderly.

Aim 5 – Comparative evaluation of antibiotic efficacy in patients carrying total hip prosthesis or hip hemiarthroplasties.

Article 4 – *Infected Hip Hemiarthroplasties and Total Hip Arthroplasties: Differential Findings and Prognosis*. **J. Lora-Tamayo**, G. Euba, A. Ribera, O. Murillo, S. Pedrero, D. García-Somoza, M. Pujol, X. Cabo, J. Ariza. Journal of Infection 2013; 67: 536-544.

Patients carrying hip devices are quite heterogeneous. Although total hip arthroplasties (THA) are sometimes used to treat hip fracture in non-elderly patients, they are usually placed during elective surgery for non-traumatic reasons, such as degenerative joint disease (i.e. arthrosis), rheumatoid arthritis or aseptic necrosis of the femoral head. By contrast, hip hemiarthroplasties (HHA), which substitute only the femoral part of the hip joint, are usually placed in the setting of an emergency procedure for the treatment of hip fracture in the elderly (152). The main goal after a hip fracture is to restore the patient's previous condition (1, 152). In this regard, HHA may be further subdivided in cemented (C-HHA) and non-cemented (NC-HHA). The former involve a more sophisticated surgery, and are usually reserved for patients with acceptable previous mobility (153, 154).

Thus, patients carrying a THA or a HHA differ widely, as do the conditions under which the prosthesis is placed. However, in the setting of infection, the surgical and medical management is similar, and the literature tends either to ignore HHA or to include them together with the analysis of THA (8, 34, 35, 51). Our hypothesis is that patients with infected HHA or THA may present different clinical and microbiological characteristics and thus need different regimes of antibiotic treatment and have a different prognosis.

In order to assess these differences, we undertook an observational retrospective study, including all patients attended for hip PJI in our Bone & Joint Infection Unit between 2003 and 2011. A comparative analysis of cases with THA and HHA was performed, and also of patients with C-HHA and NC-HHA. Since clinical signs,

Table 4.1- Baseline characteristics of all episodes of hip prosthetic joint infection

		All epsiodes (n=210)	All HHA (n=62)	THA (n=148)	p	NC-HHA (n=29)	C-HHA (n=33)	p
Age (years)		74	80 (64-80)	74	<0.001	84	77	<0.001
Sex (women)		135 (64%)	42 (68%)	93 (63%)	0.499	23 (79%)	19 (58%)	0.065
Rheumatoid arthritis		13 (6%)	0 (0%)	13 (9%)	0.012	0 (0%)	0 (0%)	-
Diabetes mellitus		46 (22%)	20 (32%)	26 (18%)	0.019	5 (17%)	15 (46%)	0.018
Liver cirrhosis		12 (6%)	4 (7%)	8 (5%)	0.766	0 (0%)	4 (12%)	0.116
Heart disease		48 (23%)	25 (40%)	23 (16%)	<0.001	14 (48%)	11 (33%)	0.231
Lung chronic disease		23 (11%)	8 (13%)	15 (10%)	0.558	2 (7%)	6 (18%)	0.264
Immunosuppressant therapy		24 (11%)	4 (7%)	20 (14%)	0.142	0 (0%)	4 (4%)	0.116
Any comorbidity		111 (53%)	41 (66%)	70 (47%)	0.013	17 (59%)	24 (73%)	0.242
Revision prosthesis		52 (25%)	1 (2%)	51 (35%)	<0.001	1 (3%)	0 (0%)	0.475
Type of infection*	Early	119 (57%)	52 (84%)	67 (45%)	<0.001	27 (93%)	25 (76%)	0.301
	Late-chronic	57 (27%)	6 (10%)	51 (35%)		2 (7%)	4 (12%)	
	Hematogenous	17 (8%)	2 (3%)	15 (10%)		0 (0%)	2 (6%)	
	PIOC	17 *8%)	2 (3%)	15 (10%)		0 (0%)	2 (6%)	

Continous variables expressed in absolute number (and percentage); continuous variables expressed in median (and interquartil range). HHA: hip hemiartrhplasties. THA: total hip arthroplasties. NC-HHA: non-cemented hip hemiarthroplasty. C-HHA: cemented hip hemiarthroplasty. *Type of infection according to Tsukayama; PIOC: positive intraoperative cultures

symptoms and microorganisms responsible for the infection differ widely according to the type of PJI, a further comparative analysis of etiologies, clinical presentation and outcome was performed for cases managed with DAIR. At the time of debridement, NC-HHA are not usually osteo-integrated, since they are non-cemented and have usually puit in place only a short time before. Thus, at our institution treatment of an early infection of NC-HHA does not follow standard DAIR, but the device is easily removed and exchanged for another non-cemented prosthesis in the same procedure. For the purposes of this study, these patients are compared along with THA and C-HHA patients who underwent DAIR. In addition, univariate and multivariate Cox regression

Table 4.2 – Comparative analysis of infected HHA and infected THA managed with DAIR

	All episodes (n=123)	HHA (n=51)	THA (n=72)	p	NC-HHA (n=24)	C-HAA (n=27)	p	p#
Revision prosthesis	24 (20%)	0 (0%)	24 (34%)	<0.01	0 (0%)	0 (0%)	-	<0.01
Type of infection*								
Early	112 (91%)	49 (96%)	63 (88%)		24 (100%)	25 (93%)		
Late-chronic	3 (2%)	1 (2%)	2 (3%)	0.23	0 (0%)	1 (4%)	1.00	0.63
Hematogenous	8 (7%)	1 (2%)	7 (10%)		0 (0%)	1 (4%)		
Time to infection**	12 (7-18)	13 (7-18)	12 (7-17)	0.77	12 (7-17)	14 (8-19)	0.66	0.59
Pain	57 (46%)	16 (31%)	41 (57%)	0.01	9 (38%)	7 (26%)	0.37	0.01
Inflammatory signs	82 (67%)	34 (67%)	48 (67%)	1.00	15 (63%)	19 (70%)	0.55	0.73
Suppuration	71 (58%)	29 (57%)	42 (58%)	1.00	16 (67%)	13 (48%)	0.18	0.36
Fistula	25 (21%)	11 (22%)	14 (20%)	0.80	4 (17%)	7 (26%)	0.42	0.50
Temperature > 37˚C	52 (43%)	18 (35%)	34 (49%)	0.15	9 (38%)	9 (33%)	0.76	0.18
ESR at diagnosis (mm/h)	46 (33-64)	48 (29-65)	46 (34-63)	0.91	58 (28-67)	46 (26-60)	0.44	0.95
CRP at diagnosis (mg/l)	57 (21-132)	69 (33-161)	54 (12-126)	0.37	95 (13-226)	69 (35-142)	0.85	0.39
Leukocyte at diagnosis (x10E^9/l)	9.2 (7.1-11.4)	9.4 (8.4-12)	9.0 (6.4-12)	0.19	9.7 (8.6-12)	9.4 (5.7-12)	0.40	0.78
Rx signs of infection	9 (8%)	1 (2%)	8 (11%)	0.08	1 (4%)	0 (0%)	1.00	0.20
Bacteremia	7 (6%)	4 (8%)	3 (4%)	0.45	4 (17%)	0 (0%)	0.04	0.560
Polymicrobial infection	56 (46%)	26 (51%)	30 (42%)	0.31	11 (46%)	15 (56%)	0.49	0.216
Infection by *S. aureus*	45 (37%)	13 (26%)	32 (44%)	0.03	9 (38%)	4 (15%)	0.06	0.006
MSSA¶	36/45 (80%)	9 (69%)	27 (84%)	0.41	6/9 (67%)	3/4 (75%)	1.00	0.535
MRSA¶	9/45 (20%)	4/13 (31%)	5/32 (16%)	0.41	3/9 (33%)	1/4 (25%)	1.00	0.535
Infection by *P. aeruginosa*	33 (27%)	17 (33%)	16 (22%)	0.17	8 (33%)	9 (33%)	1.00	0.257
FQ-R *P. aeruginosa* ¶	2/33 (6%)	2/17 (12%)	0/16 (0%)	0.49	0/8 (0%)	2/9 (22%)	0.47	0.120
Infection by *Enterobacteriaceae*	56 (46%)	27 (53%)	29 (40%)	0.17	12 (50%)	15 (56%)	0.69	0.173
FQ-R *Enterobacteriaceae*¶	20/56 (36%)	12/27 (44%)	8/29 (28%)	0.27	3/12 (25%)	9/15 (33%)	0.07	0.053
ESBL-P *Enterobacteriacae*¶	6/56 (11%)	3/27 (11%)	3/29 (10%)	1.00	1/12 (8%)	2/15 (13%)	1.00	1.000
Infection by Gram-negative bacilli	74 (60%)	37 (73%)	37 (51%)	0.02	16 (67%)	21 (78%)	0.38	0.018
FQ-R Gram-negative bacilli¶	21/74 (28%)	13/37 (35%)	8/37 (22%)	0.20	3/16 (19%)	10/21 (48%)	0.07	0.040

Infection by *Enterococcus*	13 (11%)	4 (8%)	9 (13%)	0.41	1 (4%)	3 (11%)	0.61	1.000
Days of antimicrobial therapy†	58 (51-63)	56 (44-60)	60 (54-68)	0.02	56 (41-60)	55 (48-62)	1.00	0.104
Need for ≥2 debridements	24 (20%)	7 (14%)	17 (24%)	0.17	2 (8%)	5 (19%)	0.43	0.587
Exchange of removable components	77 (63%)	47 (92%)	30 (42%)	<0.01	24 (100%)	23 (85%)	0.11	<0.001
Time to debridement (days)‡	5.0 (3.0-10.0)	5.0 (3.0-8.0)	6.5 (4.0-13)	0.08	4 (3-6)	6 (3-11)	0.20	0.684
Overall failure§	44 (37%)	15 (31%)	29 (41%)	0.26	6 (26%)	9 (36%)	0.46	0.634
Failure while on therapy§ Ø	24/44 (55%)	11/15 (73%)	13/29 (45%)	0.07	4/6 (67%)	7/9 (78%)	1.00	0.130
Failure after therapy§ Ø	20/44 (46%)	4/15 (27%)	16/29 (55%)		2/6 (33%)	2/9 (22%)		
Overall mortality§	26 (22%)	17 (35%)	9 (13%)	<0.01	4 (17%)	13 (52%)	0.01	<0.001
Mortality related to infection§	13 (11%)	10 (21%)	3 (4%)	0.01	2 (9%)	8 (32%)	0.08	0.001

Categorical variables expressed in absolute number (and percentage); continuous variables expressed in median (and interquartile range). *Type of infection according to Tsukayama.** Time to infection: time from prosthesis placement to beginning of symptoms (8 hematogenous cases excluded). ¶Percentages and comparisons referred to resistant strains in each etiologic group. †For patients finishing the scheduled treatment without failing (n=91). ‡Time to debridement: time from beginning of symptoms to surgery of debridement. §5 patients excluded, with unknown outcome. ØPercentages given in rapport to total of failures. FQ-R: fluoroquinolone-resistant. ESBL-P: extended spectrum beta-lactamase producing. #Comparison between THA and C-HHA. The number of infections by Gram-negative bacilli is less than the simple sum of episodes by Gram-negative bacilli and episodes by Enterobacteriaceae, since there are polymicrobial infections caused by several Gram-negative bacilli.

analyses were performed to identify parameters independently associated with Failure.

There were 210 cases of hip prosthetic joint infection, occurring in 197 patients with a median age of 74 years (IQR 64-80 years) and of whom 124 (63%) were women (Table 4.1). One hundred and forty-eight (61%) carried a THA, and 62 (39%) patients carried a HHA – of these, 29 (48%) were NC-HHA, and 33 (53%) were C-HHA. As expected, patients carrying THA were younger and had fewer baseline conditions (except for a higher frequency of rheumatoid arthritis). The majority of infections among HHA were early post-surgical, whereas the type of PJI was more varied among THA.

A total of 123 (59%) patients underwent DAIR (72 THA and 51 HHA). Clinical presentation was similar in these patients, but the etiology responsible for the

infection was different (Table 4.2): infection by MSSA was more frequent among patients carrying a THA, while GNB were more frequent in HHA, with a higher prevalence of fluoroquinolone resistance in the C-HHA group. DAIR management was similar in the groups, except for a higher rate of removable components exchange among patients carrying a C-HHA.

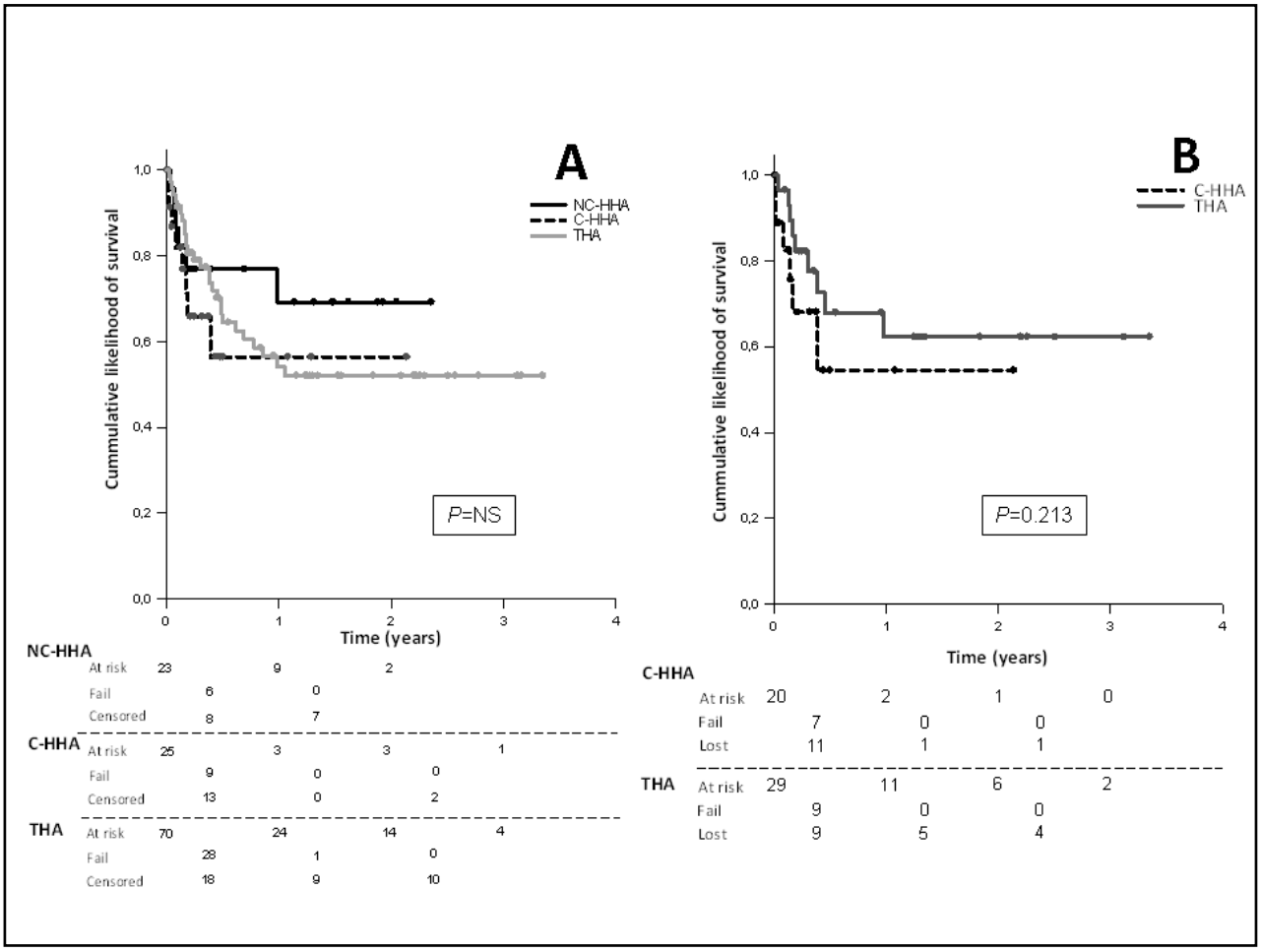

Fig 21 – Cumulative likelihood of survival of THA, CHHA and NC-HHA after DAIR.

THA: total hip arthroplasty (grey continuous line); C-HHA: cemented hip hemiarthroplasty (black discontinuous line); NC-HHA: non-cemented hip hemiarthroplasty (black continuous line). Labels: at risk denotes the number of patients at risk of failing at the beginning of the period; Fail denotes the patients actually failing during the period; Lost denotes the number of patients lost for follow-up during the period (censored times). A: all cases submitted to DAIR with known outcome: n = 118; Long-rank test, p = 0.333. B: subanalysis of post-surgical cemented hip device infection in which removable components were excluded during debridement: THA, n=29; C-HHA, n=20; long-rank test, p=0.213.

Overall failure of DAIR management was 37%, with no significant differences between the specific hip devices (Fig 21-A). Independent predictors of failure were related with a higher inflammatory pattern of the infection (hematogenous infection, high level of leukocytes, and need for two or more debridements) and etiology (infection by MRSA and infection by *Enterococcus* sp) (Table 4.3). Since NC-HHA are not really submitted to an implant retention, and the rate of removable component exchange was higher in C-HHA cases than in THA cases, we performed a sub-analysis of post-surgical cases of C-HHA and THA cases where the removable exchange of components had been performed, and found a trend towards a better prognosis among patients carrying a THA (Fig 21-B).

Finally, an important difference among patients carrying one or other hip device was found regarding mortality. Crude mortality was 21% among HHA patients and 4% among THA cases (p=0.005). Mortality related to the infection was also higher among HHA episodes (35% vs 13%; p=0.004). Mortality was particularly higher among patients with C-HHA as compared with NC-HHA: 52% vs 17% for crude mortality respectively (p<0.001); and 32% vs 9% for mortality related infection respectively (p=0.001).

Table 4.3 – Multivariate analysis of parameters predicting failure

	HR (CI95%)	p
Hematogenous infection	3.87 (1.52-9.83)	0.005
Leukocytes (x10E9/l)	1.10 (1.03-1.18)	0.006
Need for 2 debridements or more	2.47 (1.24-4.94)	0.010
Infection by MRSA	3.75 (1.66-8.50)	0.002
Infection by Enterococcus	4.83 (1.98-11.9)	0.001

The following parameters were included in an initial model of multivariate analysis: diabetes, immunosuppressant therapy, C-HHA vs other hip devices, hematogenous infection, radiographic signs of infection, leukocyte count, bacteremia, infection by MRSA, infection by fluoroquinolone-resistant Gram-negative bacilli, infection by *Enterococci,* exchange of removable components during debridement and need for 2 debridements or more.

In summary, we confirmed our hypothesis that patients carrying THA and HHA differ in terms of baseline features and type and etiology of PJI. The specific microorganism was an important parameter predicting failure, and obviously conditioned the antibiotic treatment that the patients received. The specific device does not perform as a predictor of failure *per se*, but as an identifier of a particular type of infection in a specific host, and probably with a higher likelihood of failure.

B. ANTIMICROBIAL THERAPY IN PROSTHETIC JOINT INFECTION MANAGED WITH PROSTHESIS REMOVAL

Aim 6 – To evaluate linezolid in PJI by Gram-positive microorganisms managed with a two-step exchange procedure.

Article 5 – *Linezolid in Late-Chronic Prosthetic Joint Infection Caused by Gram-Positive Bacteria*. J. Cobo, **J. Lora-Tamayo**, G. Euba, A. Jover-Sáenz, J. Palomino, M. D. del Toro, D. Rodríguez-Pardo, M. Riera, J. Ariza. Diagnostic Microbiology and Infection 2013; 76: 93-98.

The standard treatment of chronic PJI is a two-step exchange procedure (7, 8). The rationale for such a complex management is to provide a sterile surgical site before the placement of a new prosthesis to prevent its recolonization by remaining microorganisms. The success rate of this management is approximately 90% (7, 8).

However, cultures systematically performed at prosthesis reimplantation have shown positive results in 6-20% (59-63), suggesting that surgical site sterility is not always guaranteed. In most of these cases, the isolates are CNS, frequently resistant to the antimicrobials used during the previous weeks, either systemically or in the cement spacer placed during the first surgery of revision (63). The origin of these resistant CNS is uncertain. One hypothesis is that these microorganisms have been selected by the antibiotics from an originally underdiagnosed polyclonal CNS infection. Another hypothesis is that they belong to the patient's skin flora, modified by the antimicrobial treatment, and have superinfected the surgical site at some point during the healing process. Any of these two possibilities would suggest that antibiotics with extended anti-staphylococcal spectrum could be useful for avoiding persistence/superinfection by resistant CNS (60, 63).

In this context, linezolid is an attractive alternative: it possesses a wide anti-Gram positive bacteria spectrum, and has 100% bioavailability and good diffussion in bone

tissue (84, 85). However, clinical experience with linezolid in this setting is scarce (88, 157) and toxicity is a matter of concern (86-88, 156).

Table 6.1 - Etiology of 25 cases* of PJI by Gram-positive bacteria

Microorganism		**n (%)**
Staphylococcus aureus[†]		4 (15)
Coagulase-negative *Staphylococci* (CNS)[‡]		18 (66)
	S. epidermidis 13	
	S. lugdunensis 1	
	S. capitis 1	
	S. hominis 1	
	CNS sp. 2	
Streptococci		3 (11)
	S. intermedius 1	
	S. viridans 1	
	S. agalactiae 1	
Propionibacterium acnes		1 (4)
Corynebacterium striatum		1 (4)

*Two cases of polymicrobial infection. [†]No strains methicillin-resistant. [‡]6 strains (33%) methicillin-resistant.

To assess the efficacy and safety of linezolid over six weeks, we undertook a prospective, open-label, non-randomized, non-comparative, multicenter clinical trial in seven teaching hospitals in Spain. Eligible patients were those undergoing a two-step prosthesis revision for the treatment of PJI. After the first surgery (and placement of a cement spacer that could not be loaded with vancomycin) and after patients had given their written consent (Annexe II), they were treated with linezolid 600 mg/12h *per os* during a scheduled duration of six weeks. Afterwards patients underwent a second

surgery in order to place a new prosthesis. Before this, samples from surgical site were taken in order to assess the sterility of the surgical site.

A per-protocol analysis was performed, which considered both clinical and microbiological cure. Toxicity was assessed during treatment by weekly interview with the patient, as well as by hemogram and biochemistry profiles. In addition, linezolid-induced thrombocytopenia was assessed by means of a case-control study comparing patients treated with linezolid (cases) and 25 historical controls, who were patients with chronic PJI managed at two of the participating hospitals, also treated with a two-step exchange procedure and six weeks of antimicrobial therapy other than linezolid. Matching between cases and historical controls was made by age and sex.

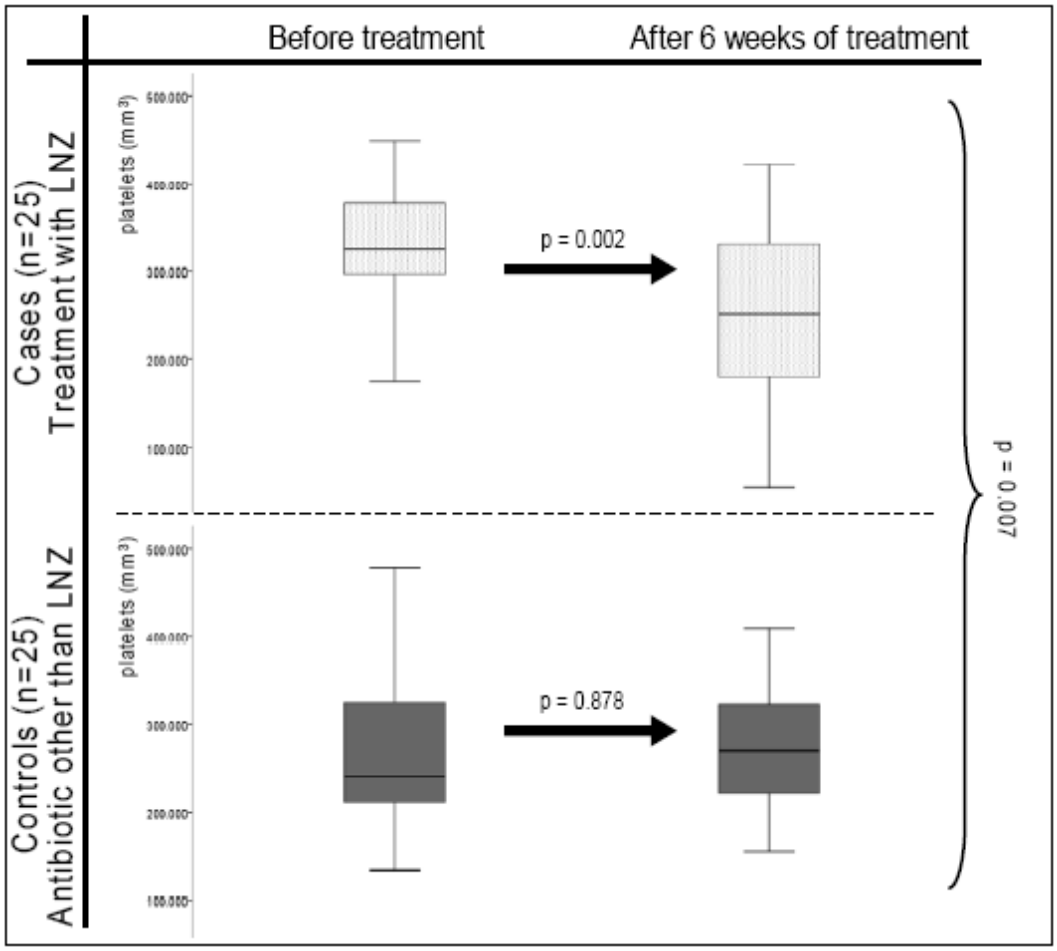

Fig 22 – Comparative count evolution after 6 weeks of treatment with linezolid vs. 6 weeks of treatment with an alternative therapy

Twenty-five patients were recruited [20 (80%) women, with a median age of 73 years (range 59-89 years)]. Sixteen (64%) had knee prosthesis and nine (32%) carried hip prosthesis. Table 6.1 summarizes the microorganisms responsible for the infections, which were mostly caused by *Staphylococcus*. Table 6.2 summarizes the adverse events occurring while on therapy, most of which appeared during the 2nd or 3rd week of treatment with linezolid. Most symptoms were mild and linezolid could be continued, but in three cases the antibiotic had to be withdrawn. In all three cases toxicity reversed after stopping linezolid. One patient developed significant thrombocytopenia <100,000/mm^3 and bleeding. In addition, a significant overall decrease was observed among all patients (mean decrease of –82,417 ± 117,901 platelets/mm^3), though this was not observed among the historical cases (Fig 22).

The outcome of patients is summarized in Figure 23. Of the 22 patients who could be administered linezolid for six weeks, there were two (9%) clinical failures (also with positive cultures at surgical site) and 20 (91%) were considered clinically cured. Of these, one (5%) had positive cultures yielding microorganisms other than the original CNS causing the infection. Overall cure (clinical and microbiological) was observed in 19/22 patients (86%).

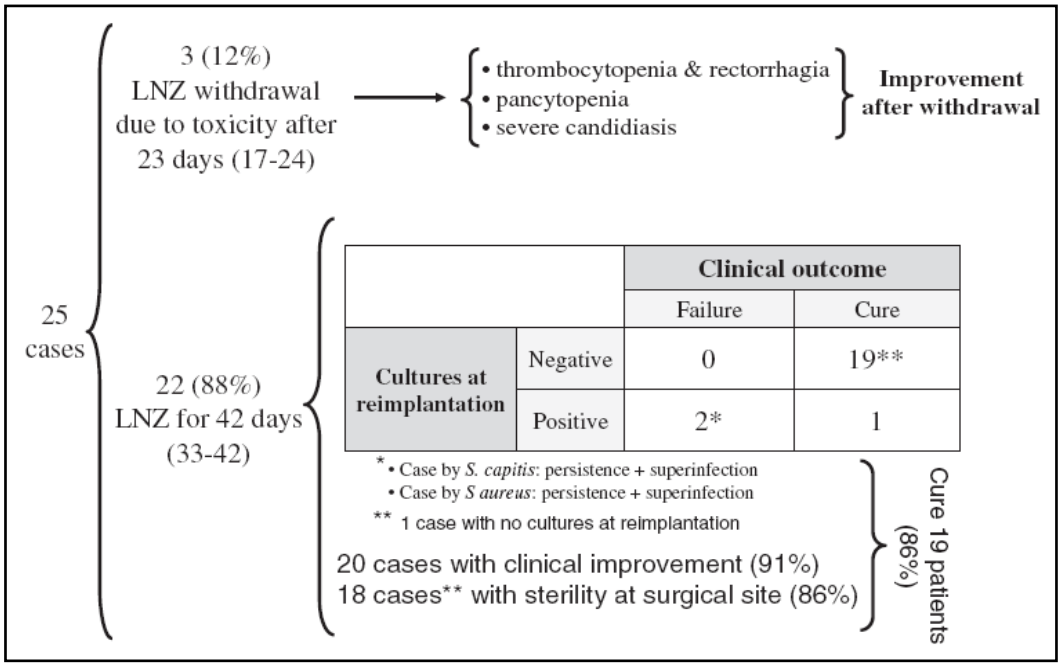

Fig 23 - Outcome

Table 6.2 - Clinical adverse effects (AE) of Linezolid during therapy

Adverse effect	Mild Toxicity	Antibiotic withdrawal	Median time to Develop AE (weeks)
Nausea	10 (40%)	None	3
Vomiting	7 (28%)	None	2
Abdominal pain	8 (32%)	None	3
Diarrhea	4 (16%)	None	2.5
Headache	3 (12%)	None	5
Dizziness	6 (24%)	None	3
Neuropathy	-	-	-
Drowsiness	2 (8%)	None	5
Paresthesia	1 (4%)	None	2
Blurred vision	1 (4%)	None	6
Hearing loss	-	-	-
Tinnitus	-	-	-
Unspecific taste distortion	6 (24%)	None	3
Metallic taste	3 (12%)	None	4
Insomnia	5 (20%)	None	1
Anxiety	5 (20%)	None	2
Behavior disorders	2 (8%)	None	4
Mood disorders	6 (24%)	None	2.5
Cough	1 (4%)	None	3
Dyspnea	1 (4%)	None	1
Chest pain	-	-	-
Palpitations	1 (4%)	None	6
Arthralgias	2 (8%)	None	5
Myalgias	2 (8%)	None	5
Exanthema	1 (4%)	1 (4%)	3.5

Pruritus	5 (20%)	None	3
Candidiasis	5 (16%)	1 (4%)	3
Thrombocytopenia*	19 (76%)	1 (4%)	4
Anemia**	9 (36%)	None	4

***** Platelet count below 100,000/mm^3 or below75% of the baseline count. **Haemoglobin below 90 g/l or below 75% of haemoglobin 1 week after surgery.

In summary, treatment with linezolid during six weeks was efficacious in the two-step revision procedure for infected joint prosthesis by Gram-positive bacteria. The rate of non-sterile surgical site at the time of reimplantation was lower than in previous reports. Tolerance to the antibiotic seems acceptable, but close surveillance is required, especially after the first two or three weeks of treatment.

C. ANTIMICROBIAL ACTIVITY ON BIOFILMS OF MULTI-RESISTANT GRAM-NEGATIVE BACILLI

Aim 7 – To study the activity of colistin against multi-resistant *P. aeruginosa* biofilm in an *in vitro* experimental model.

Article 6 – *Activity of Colistin Combined with Doripenem at Clinically Relevant Concentrations Against Multidrug-Resistant* Pseudomonas aeruginosa *in an* In Vitro *Dynamic Biofilm Model*. **J. Lora-Tamayo**, O. Murillo, P. J. Bergen, R. L. Nation, A. Poudyal, X. Luo, H. Y. Yu, J. Ariza, J. li. Submitted for publication to the Journal of Antimicrobial Chemotherapy (October 2013) and returned for minor revision (January 2014).

Article 7 – *PK/PD Models in Antibacterial Development*. T. Velkov, P. J. Bergen, **J. Lora-Tamayo**, C. B. Landersdorfer, J. Li. Current Opinion in Microbiology, 2013 Jul 18 [Epub ahead of print].

The incidence of multi-resistant *Pseudomonas aeruginosa* infections is increasing worldwide (100). Colistin, among other long-forgotten antimicrobial drugs, is being used as a last-line therapy for these infections (101, 102). The activity of colistin and its potential synergy with carbapenems have not been tested for biofilm-embedded *P. aeruginosa*. In order to study this, we set up an in vitro PK/PD dynamic model, based upon the CDC biofilm reactor (CBR). Two different clinically relevant concentrations of colistin, doripenem and their combinations were tested on three different strains of *P. aeruginosa* (one carbapenem-susceptible referral strain and two carbapenem-resistant clinical strains). Population analysis profiles were performed in these three strains in order to detect heteroresistant subpopulations (Fig 24). MIC values to doripenem and colistin are summarized in Table 7.1.

The activity of the regimes was measured as the change in $\log_{10}$ CFU/cm^2 at any given sampling time and the starting point of the experiment. Treatments were considered

to be bactericidal (99.9% kill) when they led to a ≥3 log_{10} CFU/cm^2 reduction compared to the corresponding bacterial count at zero time. Monotherapy or combination regimes causing a reduction of ≥1 log_{10} CFU/cm^2 at a specified time were considered active. Synergy was defined as ≥2 log_{10} CFU/cm^2 for the combination relative to the most active corresponding monotherapy at a specified time. The emergence of colistin resistance, defined as the ability to grow on plates with a colistin concentration of 4 mg/L, was also measured.

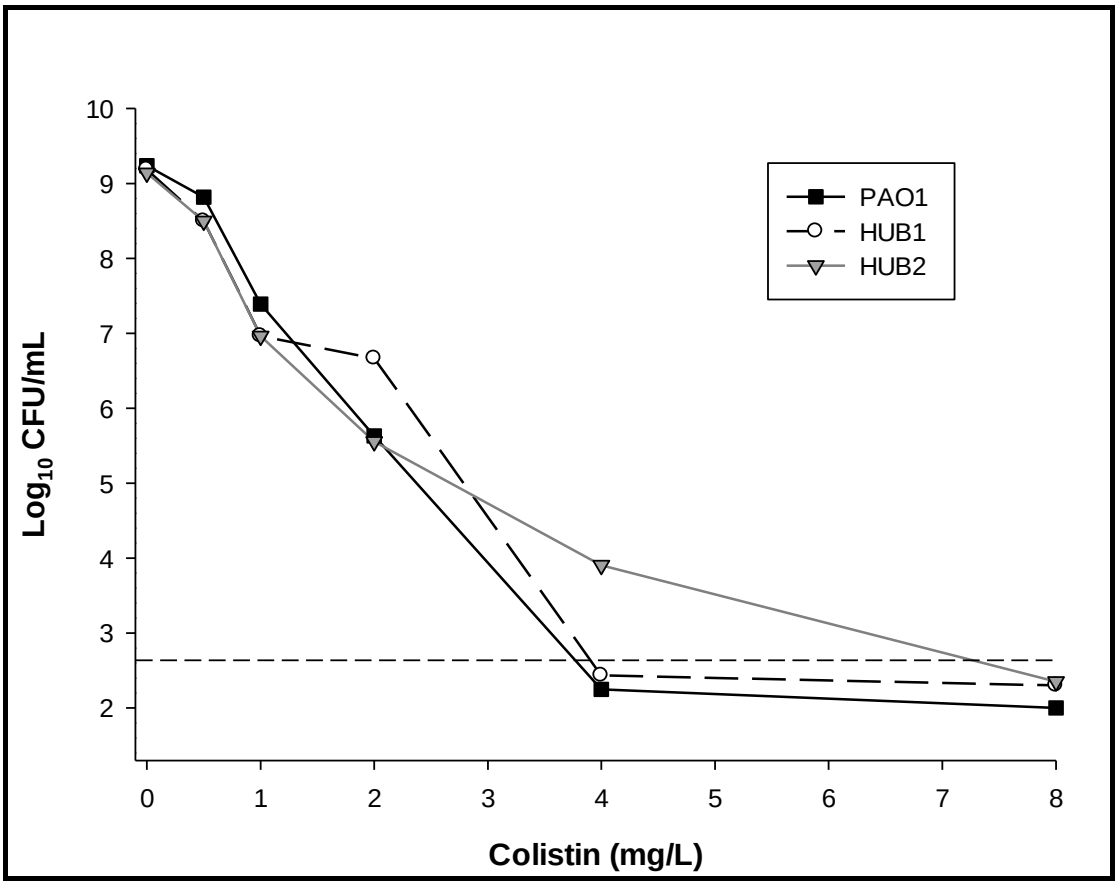

Fig 24- Baseline PAPs of the reference strain PAO1 and clinical isolates HUB1 and HUB2 at an initial inoculum of ~10^9 CFU/mL. The *y* axis starts from the limit of detection, and the limit of quantification is indicated by the horizontal broken line.

Absolute counts of bacterial-embedded cells over time with no antibiotics may be seen in Fig 25. Results of the activity of antibiotic on biofilm-embedded cells are summarized in Fig 26 and Table 7.2. Colistin at 1.25 mg/L achieved modest activity ≈ -1 - -2 log against the clinical strains, and none against PAO1. Higher concentrations (3.50 mg/L) showed increased activity: ≈ -2 log against PAO1, bactericidal activity against

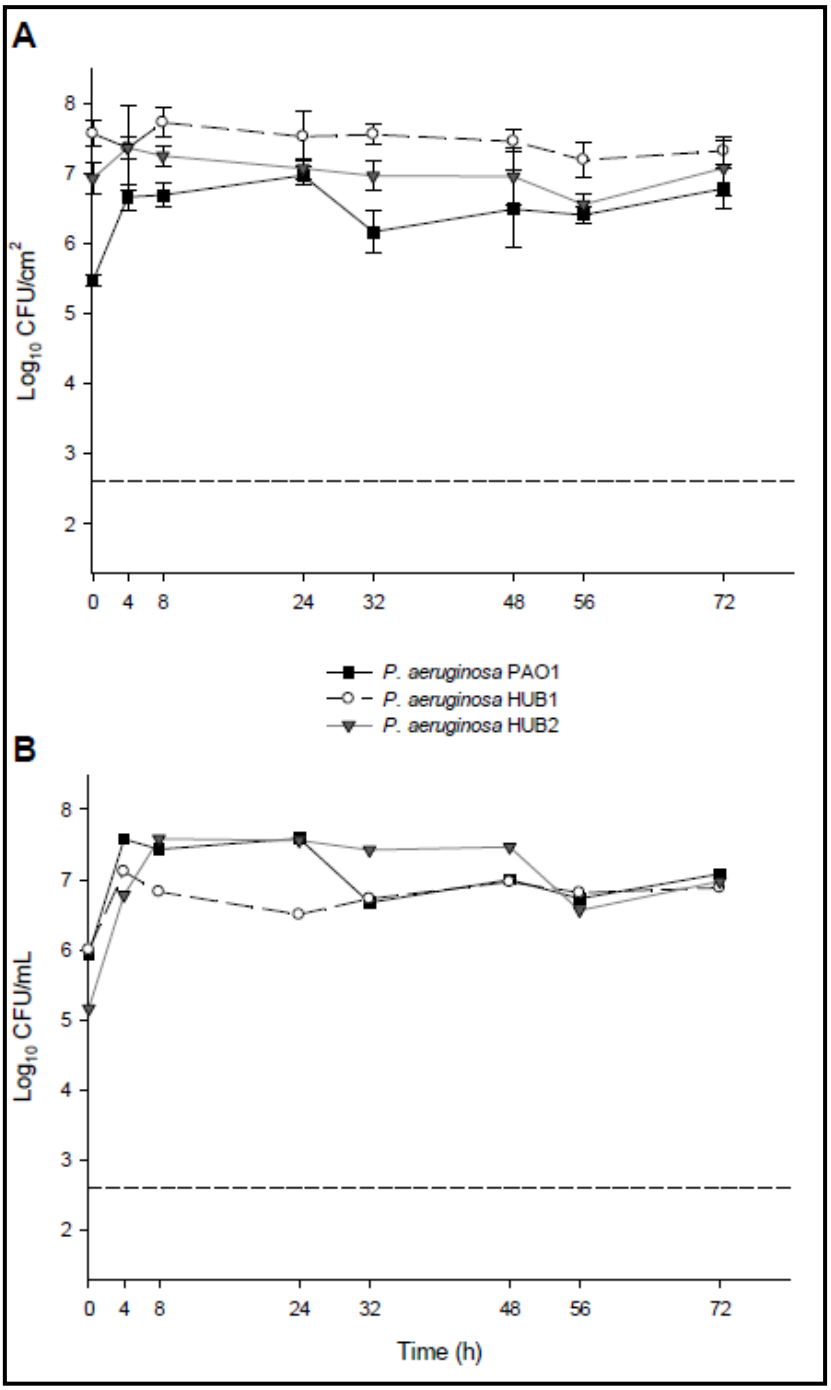

Fig 25 – Bacterial growth in the absence of colistin and doripenem (i.e. growth controls) for biofilm-embedded (panel A) and planktonic (panel B) bacteria for the three strains of *P. aeruginosa*. Time on the *x* axis begins immediately after the 28 h-conditioning phase. The *y* axis starts from the limit of detection, and the limit of quantification is indicated by the horizontal broken line. Data are presented as means ± standard deviation of the mean (panel A) or as mean (panel B)

HUB1 and ≈ -2.5 log against HUB2; however, regrowth was observed in all three strains, and in the case of PAO1 and HUB1 emergence of colistin resistance could be observed. As expected, doripenem had no effect on the clinical carbapenem-resistant

clinical strains, being active only against PAO1; however, its activity was not bactericidal.

Doripenem enhanced the activity of both concentrations of colistin. For PAO1 additivity was observed when combined with colistin 1.25 mg/L, with synergy at several time points when combined with colistin 3.50 mg/L. Although to a lesser degree, these enhanced effects were also observed against the carbapenem-resistant strains. Notably, the rate of regrowth was less, and no emergence of colistin-resistant biofilm-embedded bacteria was observed with the combination therapies.

Table. 7.1 - MICs (mg/L)* for the *P. aeruginosa* isolates examined in this study

	Colistin		Doripenem	
	CAMHB	**CA-1%TSB**	**CAMHB**	**CA-1%TSB**
PAO1	1	2	1	<0.125
HUB1	2	2	>128	128
HUB2	1	2	16	8

* CLSI breakpoints for colistin were ≤2 mg/L for susceptibility, 4 mg/L for intermediate, and ≥8 mg/L for resistance. For doripenem, the breakpoints were ≤2 mg/L for susceptibility and >2 mg/L for resistance.[48]

The activity of the antibiotics was less pronounced when tested for the broth-floating bacteria (Fig 27). It is likely that these bacteria were not really planktonic, but had recently been released from the biofilm and so had not still recovered the planktonic properties.

In summary, we showed an enhanced activity of the combination of colistin plus doripenem as compared with monotherapies. This improved activity was still observed in the two clinical carbapenem-resistant strains. These observations support the current recommendation of using colistin in combination, especially in demanding scenarios such as biofilm-associated infections.

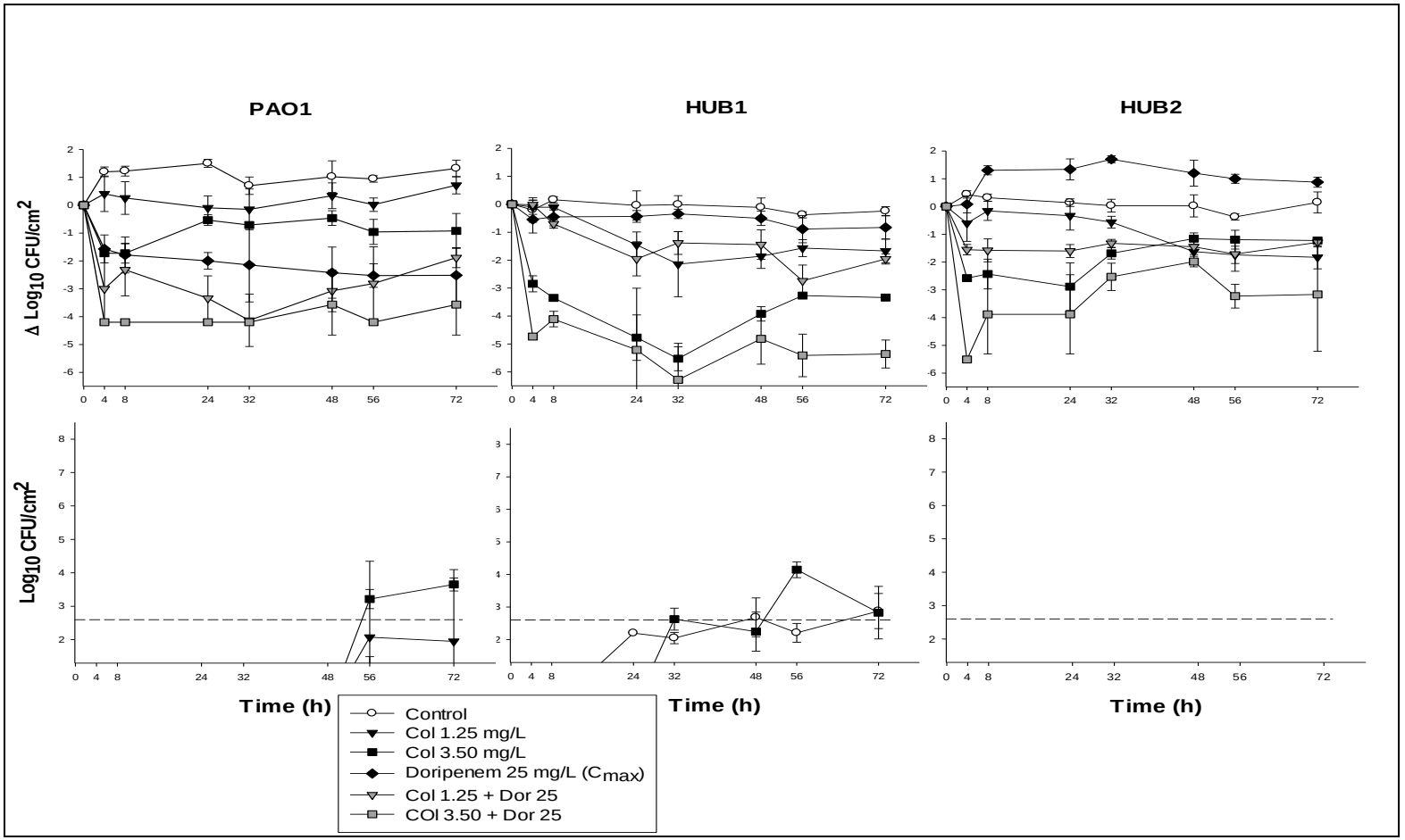

Figure 26- <u>Upper panels</u>: Bacterial killing by two different concentration of colistin (Col), doripenem (Dor), and its combinations against biofilm-embedded cells of three different *P. aeruginosa* strains; results expressed using the log change method. <u>Lower panels</u>: Emergence of colistin resistance (i.e. colonies able to grow in the presence of ≥4 mg/L colistin) among biofilm-embedded *P. aeruginosa*; results expressed as the absolute number of recovered bacteria. For the lower panels, the limit of quantification is indicated by the horizontal broken line. Data are presented as means ± standard deviation of the mean.

Table 7.2 – Log change biofilm-embedded cell counts

		Monotherapies			Combinations	
Strain	**Hour**	Col 1.25	Col 3.50	Dori	Col1.25+Dori	Col3.50+Dori
PAO-1	**4**	+0.41	-1.72	-1.57	-3.00	-4.20
	8	+0.26	-1.72	-1.78	-2.32	-4.20
	24	-0.09	-0.53	-2.00	-3.34	-4.20
	32	-0.15	-0.71	-2.15	-4.13	-4.20
	48	0.34	-0.46	-2.42	-3.07	-3.56
	56	0.02	-0.96	-2.52	-2.81	-4.20
	72	0.72	-0.92	-2.51	-1.88	-3.56
HUB-1	**4**	-0.11	-2.85	-0.54	-0.02	-4.73
	8	-0.11	-3.35	-0.45	-0.70	-4.11
	24	-1.46	-4.77	-0.44	-1.95	-5.21
	32	-2.14	-5.53	-0.34	-1.38	-6.28
	48	-1.86	-3.92	-0.50	-1.44	-4.82
	56	-1.57	-3.27	-0.89	-2.74	-5.41
	72	-1.67	-3.34	-0.83	-1.96	-5.36
HUB-2	**4**	-0.60	-2.58	+0.08	-1.55	-5.51
	8	-0.15	-2.43	+1.31	-1.58	-3.88
	24	-0.33	-2.89	+1.34	-1.60	-3.88
	32	-0.56	-1.69	+1.70	-1.33	-2.53
	48	-1.62	-1.15	+1.21	-1.45	-1.98
	56	-1.74	-1.19	+1.00	-1.71	-3.23
	72	-1.83	-1.22	+0.88	-1.29	-3.17

Results expressed as mean log changes of biofilm-embedded bacteria (cfu/ cm^2) throughout the experiment. Among monotherapies, a grey background denotes a decrease ≥ 1 log cfu/cm^2. Among combinations, orange and green backgrounds denote additivity (decrease >1 and <2 log cfu/cm^2 with the combination compared to its most active component) or synergy (decrease ≥ 2 log cfu/cm^2 with the combination compared to its most active component), respectively.

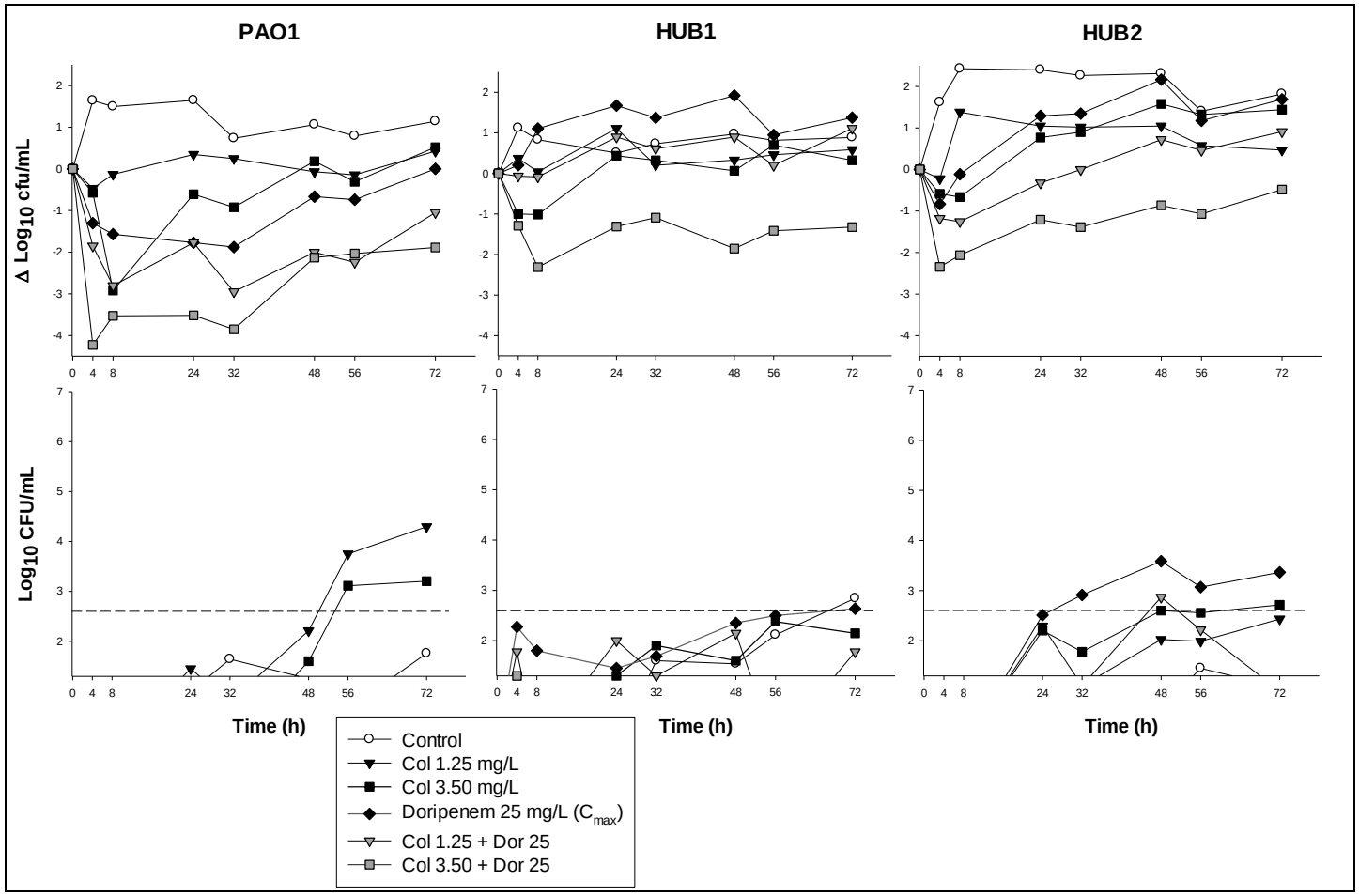

Figure 27 - <u>Upper panels</u>: Bacterial killing by colistin (Col) alone at two different clinically relevant concentrations, doripenem (Dor) alone, and in combination against three different strains of *P. aeruginosa* recovered from the media within the reactor (i.e. planktonic cells); results expressed using the log change method. <u>Lower panels</u>: Emergence of colistin resistance (i.e. colonies able to grow in the presence of ≥4 mg/L colistin) among planktonic *P. aeruginosa*; results expressed as the absolute number of recovered bacteria. For the lower panels, the limit of quantification is indicated by the horizontal broken line

- **DISCUSSION**

1. Antimicrobial therapy in PJI managed with implant retention.

Infection associated with a foreign body usually requires its removal in order to achieve cure. This happens frequently in the setting of PJI, and is the rule in other foreign body-associated infections such as cerebro-spinal fluid shunt infections, pace-maker-infections or most vascular catheter infections (157-159). However, and as mentioned earlier, acute PJI may be treated with retention of the orthopedic device, which is desirable for both the patient and the health-care system (7, 8, 54). Obviously, the management of the infection without removing the foreign body is a much more demanding scenario in which treatment needs to be maximally optimized. This involves not only early and thorough debridement, but also the best antimicrobial treatment available.

1.1. The importance of antibiotic treatment in prognosis

The first study included in this thesis is the largest case series on *S. aureus* PJI managed with DAIR published to date. The overall likelihood of curing and retaining a prosthetic device when infected by *S. aureus* and managed with DAIR was 55%. As expected, treatment with rifampin was independently associated with a longer period without treatment failure. Our results are in agreement with previous studies with fewer patients (58), and support the treatment of staphylococcal PJI with a rifampin-based combination.

The cases of Gram-negative PJI presented in this thesis also comprise the largest series ever reported of Gram-negative PJI managed with DAIR. The absence of Gram-positive microorganisms in this cohort increases its homogeneity. The overall success rate was 68%, and treatment with ciprofloxacin, when feasible, showed to be key for increasing the likelihood of success: the risk of failure for patients treated with this antibiotic was halved compared with patients who did not receive a fluoroquinolone.

In both retrospective studies we demonstrated the importance of optimized antimicrobial treatment. Both rifampin and ciprofloxacin have shown an independent influence on the outcome of staphylococcal and Gram-negative PJI, respectively.

These two large studies support the idea that DAIR may still be performed in infections caused by resistant microorganisms, as long as an antibiotic agent with activity against biofilm microorganisms is available. For instance, in our series the cure rate of PJI by *P. aeruginosa* was 79%, reflecting the high rate of ciprofloxacin-susceptible *P. aeruginosa* included. Although there were very few cases, this good prognosis was also observed among ESBL-producing microorganisms when treated with ciprofloxacin. In our series, fluoroquinolone susceptibility and tolerance was far more important than other considerations, such as the specific etiology of the infection.

In the case of staphylococcal infection, little experience of MRSA PJI managed with DAIR has been reported (Table 1.1) (51, 52, 57, 123, 148, 160, 161). Prognosis is expected to be unfavorable due to the limited choice of antibiotics. However, in our study the rate of success was similar for MSSA and MRSA cases. We believe that the wide use of rifampin in both scenarios helped to homogenize the prognosis of these two infections. Notwithstanding, we found a significant difference in the time of failure: while MSSA PJI – which had been mainly treated with levofloxacin plus rifampin – did not fail until the antibiotics were stopped, MRSA cases that failed did so in spite of being under antimicrobial therapy. These different dynamics were still observed after excluding hematogenous cases (namely those due to MSSA). Apart from this, MSSA and MRSA cases were similar in most characteristics, including surgical management and use of rifampin, and so this would suggest that the combination of fluoroquinolones plus rifampin was more effective (as it avoided failure while it was being used) than alternative rifampin-based combinations used for MRSA cases (as they did not avoid failure).

1.2. Alternative rifampin-based combinations for staphylococcal PJI

Thus, although the use of rifampin significantly improved prognosis and homogenized MRSA and MSSA cases, the dynamics of failure observed suggest that not all rifampin-combinations are the same. As previously mentioned, in the setting of staphylococcal PJI the combination of choice is rifampin plus a fluoroquinolone (7, 8, 58). However, when quinolones cannot be used (as is commonly the case in the setting of MRSA infection) the best alternative is still to be established.

Not including fluoroquinolones, several experimental animal models have shown that the combination of high doses of daptomycin plus rifampin is more active than alternative rifampin-based combinations for the treatment of prosthetic joint infection or other foreign body-related infections (69, 76, 77). However, the efficacy of high doses of daptomycin plus rifampin has not been proved on clinical grounds. Although there is some experience with daptomycin for prosthetic joint infections, it has been tested either alone or at low doses (82), in combination with rifampin but in settings other than DAIR (162) or with very few patients (83, 150).

We presented a homogenous cohort of patients with staphylococcal PJI managed with DAIR and treated with high doses of daptmycin plus rifampin. This treatment achieved similar results to those obtained with alternative rifampin-based combinations, but interestingly patients who failed only did so when the antibiotics were withdrawn. This contrasted significantly with the historical cohort with which we compared our results, and would suggest that daptomycin plus rifampin would be more active than non-fluoroquinolone rifampin-based alternative combinations, in a similar way as we observed in the case of MSSA with the levofloxacin plus rifampin combination.

A significant limitation of this study is the short length of therapy with which most of our patients were treated, similar to that used in classical case series in which all treatment was administered intravenously (57). Indeed, the intravenous route precluded extending the length of therapy in our patients, and it is not clear whether the rates of failure might not have been lower had these patients been treated with a

supplementary course of oral antibiotics. In fact, three patients were treated orally after the completion of six weeks of daptomycin plus rifampin, but we cannot rule out the possibility that this choice was made due to a poorer prognosis of these patients. Also, the sample finally recruited was not very large. In any case, our results suggest that high doses of daptomycin plus rifampin could be used as the treatment of choice after debridement during a variable period of time (i.e. the first weeks), followed by an oral combination which also includes rifampin.

1.3. Rate of success with DAIR and the importance of Zimmerli's algorithm

The success rates in the two large cohorts described are in a intermediate point as compared with previous reports (39, 51, 52, 57, 97-99, 123, 148, 160, 161). These previous studies presented much smaller and less homogenous samples; our two cohorts probably reflect better the real likelihood of curing the infection and retaining the prosthesis.

Furthermore, two important remarks may help to interpret our results. First, our definition of failure was quite wide, and included not only microbiological failure of the original infection (either by staphylococci or by a GNB respectively), but also any other contingency that may have occurred in the complex process of healing, such as superinfection by other microorganisms, persistence of bacteria other than the original etiology in the setting of a polymicrobial infection, or poor functional prosthetic status in spite of microbiological eradication leading to prosthesis removal. Briefly, failure in our studies included entities beyond strict microbiological causes, and so it may have been overestimated as compared with previous reports.

In addition, our studies included all patients submitted to DAIR, thus reflecting common clinical practice and differing from other studies with highly selected cases due to restricted inclusion criteria and wide exclusion criteria. Although the decision of whether to submit a patient to DAIR is usually made according to current guidelines (8, 56), every case has its own particularities and the decision is taken on an individual

basis (56). There is no doubt that Zimmerli's algorithm for identifying candidates who will most likely benefit from DAIR is most helpful (8), but deciding how to manage a patient not meet Zimmerli's criteria is not straightforward. Importantly, the removal of a recently cemented prosthesis should be balanced against the risks of a complex surgical procedure with high levels of bleeding, which may not be advisable in older and fragile patients, in whom the performance of surgical debridement may control the infection (56). Furthermore, the decision to apply DAIR does not necessarily preclude the possibility of further prosthesis removal as salvage therapy, if finally the outcome is not favorable.

In this regard, it is worth noting that 48% of patients with staphylococcal PJI who not meetZimmerli's criteria were still cured. A similar observation was made in the Gram-negative PJI cohort: 53% of patients who did not meet Zimmerli's criteria did well with DAIR. In summary, while our results in both studies are in agreement with Zimmerli's algorithm for identifying patients that will benefit from DAIR, we believe that patients not meeting Zimmerli's criteria should be evaluated individually, since DAIR may still be appropriate for them.

An important criterion when deciding to submit a patient to DAIR is how 'acute' the infection is. This is related with the biofilm maturity: the older the biofilm, the less effective its surgical and medical treatment will be (111, 112, 142, 163). This is why chronic-late infections are usually submitted to a one- or two-step exchange procedures, whereas in acute cases the possibility of DAIR is considered. While some authors include in the 'acute infections' group all post-surgical cases where the symptoms start within the first three months after the prosthesis placement (8), some others are more strict and reduce this window to the first 30 days (35, 56). Our results, in both the staphylococcal and the GNB cohort, show a similar prognosis for cases with symptoms starting in the first month after the prosthesis placement or within the first three months (Fig 14 and Fig 20). Thus, our studies argue against restricting DAIR to patients with symptomonset within the first month, and support extending this period to the first three months after the prosthesis placement.

1.4. The efficacy of shorter schedules of treatment

This thesis also addresses the controversial theme of the length of therapy in patients submitted to DAIR. The rationale for long treatments is based on the difficulties for treating PJI managed with implant retention, mainly due to the presence of bacterial biofilm attached to the prosthesis surface. This has led to the recommendation of administering high doses of antimicrobials during long periods of time, which is facilitated by the good bioavailability and reasonable tolerance of antibiotics such as fluoroquinolones and rifampin (8, 56).

However, while it is reasonable to treat biofilm-related infections with longer therapies than planktonic infections, the exact length of therapy remains unknown. Current recommendations for treating during 3 to 6 months are not evidence-based, but have been empirically established (147). In this regard, it is likely that shorter therapies (but still longer than those used for standard planktonic infections) may be as efficacious as current schedules. In this regard, Byren et al showed that the higher likelihood of relapse observed after antibiotic withdrawal did not depend on the length of therapy (51). Another retrospective observational study with a pre-post design also observed no differences in the failure rate after reducing the length of therapy from 6 or 3 months to 3 or 2 months for knee and hip prostheses respectively (130). Indeed, in the staphylococcal cohort presented here, we also showed that longer therapies were not associated with better outcomes (Fig 15).

The clinical trial presented in this thesis is the first randomized study showing that, among patients with acute staphylococcal prosthetic joint infection managed with DAIR, a short course of levofloxacin plus rifampin is not inferior to a long standard treatment. Therefore, our results suggest that patients may be safely treated with a shorter course of antibiotics without decreasing the odds of curing the infection and retaining the prosthesis. These results are in agreement with previous retrospective observational studies and also support the idea that patients who will not finally be cured by DAIR will not benefit from extending the length of therapy, as the time of relapse is simply postponed (51). While the minimal duration of the antimicrobial

therapy for prosthetic joint infection remains uncertain, our results support the use of shorter courses than those recommended.

The shortening of the length of therapy is desirable in order to reduce potential toxicity, the ecological impact of antibiotics and the economic costs of the infection. Indeed, there is potential drug-related toxicity, especially with long therapies. Schindler *et al* reported a higher rate of adverse events among patients with bone and joint infection treated with long courses of antibiotics (147). Valour *et al* recently reported a large cohort of patients with bone and joint infection among whom 15% suffered from serious adverse effects (75), a percentage similar to our rate. In our study only one patient presented a serious adverse effect beyond the first 8 weeks of therapy, but in Valour's cohort 25% of patients who developed a serious adverse event did so after 61 days of treatment (75).

In addition, the use of antibiotics has an ecological impact and may be responsible for the increasing emergence of resistance. Hospital and community consumption of fluoroquinolones has been associated with higher rates of MRSA and fluoroquinolone-resistant *E. coli* and *P. aeruginosa* hospital isolations (125, 126). Finally, and regardless of the cost of drug-related adverse events, treatments have an economic burden which is proportional to the length of therapy.

The major limitation of our clinical trial is the small sample size and therefore the lack of power to detect differences between the two schedules, if indeed therea are differences. Although the amount of eligible patients (n=174) approached the number of patients previously calculated (n=195), only one third of these patients could be randomized. This, along with the high number of patients lost during treatment or follow-up and not included in the per-protocol analysis, is illustrative of the difficulties involved in performing these unique studies and in including the whole spectrum of clinical presentation of acute staphylococcal PJI. Indeed, patients with severe bacteremic infection needing long courses of intravenous beta-lactam antibiotics could not be included. Nor could patients who did not tolerate the treatment with rifampin be analyzed per-protocol. Thus, a supplementary benefit from long standard treatments in these patients cannot be ruled out.

However, we performed a randomized clinical trial, including a homogeneous prospective cohort of 24 patients following a short schedule in whom the rate of success was >90%. Furthermore, taking into account the percentages of failure in the two arms (5% in the long arm and 8.3% in the short arm) and hypothesizing that these differences were significant, the number of patients needed to be treated with the long schedule in order to benefit just one patient would be 31. This should be balanced with the drawbacks of long therapies mentioned above.

1.5 The influence of antimicrobial treatment in special cohorts of patients

Antibiotic treatment must be tailored according to the particularities of biofilm-associated infection and the specific microorganism responsible for the episode of infection. The etiology of PJI is determined by many different parameters, among them the host of the infection and the particularities of the infected device.

We have shown that hip hemiarthroplasty infection is different from total hip arthroplasty infection. The comparison of patients carrying THA and HHA confirms that they belong to different populations: patients carrying a HHA are older and consequently they have more age-related conditions. This is consistent with the indications of the two devices: HHA are placed in emergency procedures for the treatment of hip fracture in the elderly, while THA are commonly used to treat joint degenerative diseases in younger patients; although they may also be placed after a hip fracture, patients are usually younger and have better previous mobility (1, 152).

The microbiology of the episode of infection in patients carrying a HHA is probably conditioned by the urgent nature of the procedure and the age of the patient: it is not uncommon for surgical wounds to become contaminated by urinary or fecal material. Thus, it was not surprising that the rate of infection by GNB among these patients was higher. What is more, the rate of resistant microorganisms was higher among patients carrying HHA (i.e. MRSA and fluoroquinolone-resistant GNB), something that is probably related to the age and the closer relationship of these patients with long-term care facilities and the health-care system (164, 165).

The observation of a similar overall prognosis for THA and HHA infections was somewhat surprising, since older age, more frequent comorbidities and more frequent resistant microorganisms would imply a poorer prognosis for patients carrying a HHA (98). It is likely that these variables have been balanced by some other adverse parameters that are more frequently observed in the THA group, either independently associated with a worse outcome in this study (i.e. hematogenous infection) or not, but known to imply a worse prognosis according to other studies, such as the lower rate of removable component exchange, or the higher frequency of *S. aureus* infection (51, 129, 146, 166). In this regard, the sub-analysis performed only with patients with 'true' implant retention (i.e. C-HHA and THA), post-surgical infection and exchange of removable components showed a trend towards poorer prognosis of patients carrying a HHA.

Notably, patients with an infected C-HHA showed a significantly higher rate of crude mortality and mortality related to the infection. It is known that elderly patients suffering from hip fracture are at an increased risk of death (167, 168). In our series, these patients had more baseline conditions, and in some cases the appearance of infection may represent the last step on the way to a fatal outcome. However, this mortality rate remains higher than that showed by patients carrying a NC-HHA, in spite of the latter's more advanced age. We believe that there are two main reasons. First, NC-HHA patients were indeed older than C-HHA patients, but presented fewer baseline conditions. Second, the debridement in NC-HHA is probably better than the one performed in C-HHA: as mentioned, patients with infected NC-HHA undergo debridement plus a one-step exchange procedure of the hip hemiarthroplasty, while patients with C-HHA retain the cemented components of the prosthesis. Thus, it is likely that the infection may be controlled more effectively in NC-HHA patients, therefore reducing the rate of infection-related deaths. We do not believe that the conclusion to be drawn from this observation is that all patients with acute C-HHA infection should undergo prosthesis removal, because these devices are cemented and their removal is not easy – in some cases it may require a longer and bleeding surgery (i.e. transfemoral osteotomy), which may jeopardize the life of the patient.

2. Antimicrobial therapy in prosthetic joint infection managed with implant removal

2.1 Lower rate of positive cultures at reimplantation with oral treatment

Our study supports the use of linezolid for the treatment of prosthetic joint infections caused by Gram-positive microorganisms managed with a two-step exchange procedure. The rate of success (86%) is similar to the rates reported in other series (8, 59, 169, 170).

Surgical site cultures taken during reimplantation surgery were positive in only one patient with clinical cure (5%), a rate lower than that reported by previous case series in which wide anti-Gram-positive bacteria antibiotics were not systematically used (59-63). Although the interpretation of cultures taken in reimplantation surgery is controversial and non-standardized, monitoring the sterility of the surgical site seems to be important. Relapse is more likely in the case of positive cultures (62), and additional prolonged courses of antibiotics may be needed (59).

The possible finding of CNS at the surgical site, which is often resistant to the antibiotics used after prosthesis removal during the first-step surgery, either locally (in the cement spacer) or systemically, suggests that chronic prosthetic joint infections may be due not only to one clone of CNS, but instead to several clones (60, 63). This is consistent with the pathogenesis of these late-chronic post-surgical infections, in which microorganisms coming from the patient's skin flora colonize the orthopedic device during the surgery of placement (7, 8). In this regard, it may well be that not just one but several clones of CNS from the patient's skin flora are capable of reaching the prosthesis. These CNS clones do not necessarily share the same antibiotic susceptibility profile, and so the antibiotic pressure of a particular antimicrobial may select the resistant clones, which may finally be found at the time of reimplanting the prosthesis. An alternative hypothesis suggests that new CNS from the patient's skin flora which were not responsible for the original infection, are able to colonize the cement spacer (which is also a foreign body) or the surgical site at some point during this long and complex medico-surgical management (i.e. during the removal of the prosthesis, or via the surgical wound drainages, or via the surgical wound itself). In a

similar manner as previously mentioned, these new colonizers would be CNS clones selected by the specific antibiotics the patient is being administered. This would argue in favor of using wide anti-Gram positive bacteria antimicrobials, regardless of the specific antibiotic susceptibility profile of the CNS isolated from cultures, in order to target all the possible clones responsible for the infection, or at risk of superinfecting the surgical site. Recently, Cabo *et al* showed that the likelihood of positive cultures at reimplantation was lower when there was a spacer loaded with vancomycin and/or the patient had received ≥ 14 days of systemic glycopeptides or linezolid (20% vs 43%, p=0.13) (60).

The advantage of linezolid over other antibiotics with universal activity against Gram-positive bacteria, such as glycopeptides or daptomycin, relies in its excellent bioavailability which allows its administration *per os*. This avoids the use of intravenous catheters and their potential complications, and also allows treatment to be administered on an out-patient basis. Our experience puts in perspective the real need for intravenous therapies if active bioavailable antibiotics may be used.

However, there is concern regarding linezolid's potential toxicity. Overall, in our study this antibiotic was reasonably tolerated, with the exception of three patients (12%) needing its withdrawal. Also, a general progressive thrombocytopenia was observed, although it was clinically relevant in only one patient. This, and the fact that most adverse effects happened after two-three weeks of therapy, suggests that linezolid toxicity is accumulative and dose-related, an so may be acceptable acceptable in schedules during less than six weeks. Also, the toxicity of linezolid may be reduced when combined with rifampin (171) which is known to decrease its serum levels (172), or if shorter courses of antibiotics are considered (173, 174).

3. Antimicrobial activity on biofilms of multi-resistant Gram-negative baclli

3.1. Colistin enhances doripenem activity against biofilm-embedded carbapenem-resistant *P. aeruginosa*

Multi-resistant microorganisms are not uncommon as the cause of biofilm-associated infections, including prosthetic joint infections (100). Frequently, the only antimicrobial available is colistin, and thus more data on the activity of this antibiotic in a biofilm setting is urgently needed (101).

The PK/PD problems of colistin have already been discussed (see introduction). Briefly, colistin's activity depends on the AUC/MIC ratio, and so high concentrations are needed (103). However, colistin concentration after administration of its pro-drug CMS is usually suboptimal, even after optimizing CMS dosage (106-108), and increasing CMS doses further is precluded by its potential nephroxicity (101, 110). In addition, the phenomenon of colistin heteroresistance has been described for multiple strains of various GNB (104, 105, 113), showing that it is not an infrequent problem. Suboptimal concentrations of colistin may potentially amplify resistant subpopulations, leading to therapy failure.

This has led current opinion to support the use of colistin in combination with a second antimicrobial. Previous *in vitro* studies had indeed shown a synergistic effect of colistin with imipenem or doripenem against planktonic cells (113, 118). What is more, some in vitro studies on biofilm have shown the benefits of a combined therapy with colistin plus a second antimicrobial (i.e. aminoglucosides or ciprofloxacin) based on different targets within the biofilm structure of *P. aeruginosa*: while colistin acts in the deeper layers on metabolically less active bacteria, the other antibiotics have shown to target metabolically more active cells located in more superficial layers (120, 121). More recently, in a foreign body infection animal model Corvec *et al* showed an enhanced result of colistin-based combinations than colistin alone (122).

In vitro PK/PD models allow the evaluation of the activity of antimicrobials by simulating the pharmacokinetic profiles of the drugs. Conditions under which the

experiments are performed are easily controlled, and the reproducibility of the observations is adequate to improve our understanding of the activity and effects of antimicrobials in a particular scenario of infection. The absence of an immune system allows the evaluation of the antibiotics without the interference of other antimicrobial circumstances. The CBR is a validated tool for the study of the activity of antibiotics on bacterial biofilm (141, 142).

To the best of our knowledge, our study is the first to investigate the activity of colistin/carbapenem combinations against biofilm-embedded *P. aeruginosa*. In our model using the CBR, we employed 1%TSB – this is a nutrient-restricted media favoring the growth of biofilm-embedded bacteria over planktonic cells, and has been used in the standardization of the CBR with *P. aeruginosa* (142). The media was adjusted with Ca^{2+} and Mg^{2+}, as the concentrations of these cations may affect colistin's antibacterial activity.

As expected, in our experiments we observed a higher killing of biofilm-embedded bacteria with the highest concentration of colistin used (111). Interestingly, and in agreement with previous planktonic experiments (113, 118, 175-177), regrowth of biofilm-embedded bacteria was generally observed with colistin monotherapy. Amplification of previously existing resistant subpopulations (Fig 24) may explain in part these observations. Doripenem monotherapy presented non-bactericidal activity against the carbapenem-susceptible strain, and obviously no activity against the two carbapenem-resistant strains.

It was interesting that the addition of doripenem to colistin resulted synergistic over the corresponding monotherapy against all three isolates, especially with the highest concentration of colistin (3.50 mg/L). Notably, combined regimes avoided the emergence of resistant subpopulations of biofilm-embedded bacteria.

The PK/PD difficulties described above are increased in the biofilm setting. In a recently published study in a mouse lung infection biofilm model, a colistin concentration of 64 x MIC (i.e. 128 mg/L) was required to achieve a 1 $\log_{10}$ decrease in

CFU/lung (112). These concentrations are far above the upper limits of clinically achievable concentrations of colistin following intravenous administration of CMS (≈ 10 mg/L) (106-109). Indeed, our results show poor killing with colistin monotherapy at clinically relevant concentrations, but the combination of colistin (3.50 mg/L) plus doripenem produced greater and more sustained killing than either antibiotic alone. We believe that these findings favor the use of colistin in combination in front of *P. aeruginosa* biofilm infections.

Importantly, the enhanced activity of the combination was also observed in the two carbapenem-resistant strains. This is of particular interest, and suggests that the synergistic effect observed may be due not only to the killing of the subpopulation which is resistant to the second antibiotic in the combination (and vice versa) (113), but also to other mechanisms based on the modification of the permeability of the bacterial cell. Indeed, polymyxins are known to target the outer membrane of the bacteria and significantly modify its permeability (114). This might recover the activity of some antibiotics to which the bacteria had become resistant, especially if they are related to a decreased permeability (178).

The performance of these experiments with three different strains of *P. aeruginosa* increases the value of our observations. There are several possible reasons for the strain-to-strain differences in biofilm-embedded and planktonic killing observed. First, biofilm-forming ability and particular biofilm characteristic are strain-dependent (20, 142); the three strains used in this investigation established biofilms with varying initial cell densities following the same conditioning phase (Fig 25). Second, the different PAPs observed for the three strains prior to antibiotic treatment (Fig 24) indicate slightly different frequencies of pre-existing colistin-resistant subpopulations at the beginning of therapy. Finally, the two clinical isolated had a different mechanism of carbapenem resistance: a carbapenemase in one strain, and an efflux-pump plus a β-lactamase in the other).

In summary, our experiments show for the first time that clinically relevant concentrations of colistin plus doripenem increase the bacterial killing of biofilm-

embedded *P. aeruginosa*, including carbapenem-resistant isolates, with negligible emergence of colistin resistance. This supports the use of colistin in combination against biofilm infections. Further investigations using validated animal biofilm models are warranted.

- **CONCLUSIONS (according to aims)**

A. Antimicrobial therapy in prosthetic joint infection managed with implant retention.

A.1. Infection by *Staphylococcus* spp.

Aim 1 – To measure the impact of rifampin in the outcome of a large cohort of PJI by *S. aureus*, including MRSA

1.1. The use of rifampin independently improves the prognosis of patients with staphylococcal PJI managed with implant retention

1.2. Cases due to MRSA also benefit from treatment with a rifampin-based combination, its prognosis being similar to that of MSSA

1.3. Dynamics of failure were differed depending on the specific rifampin-based combination, suggesting better activity for quinolones plus rifampin

Aim 2 – To assess the efficacy of a short schedule of levofloxacin plus rifampin in staphylococcal PJI

2.1. An 8-week treatment regime with the combination of levofloxacin plus rifampin in the setting of acute staphylococcal PJI managed with implant retention is not inferior to standard long treatments of 3 or 6 months.

Aim 3 – To evaluate the combination daptomycin plus rifampin for fluoroquinolone-resistant staphylococcal PJI

3.1 The use of high doses of daptomycin plus rifampin in the setting of acute fluoroquinolone-resistant staphylococcal PJI managed with implant retention may be an alternative to standard therapy

3.2 The rate of failure during treatment with this combination was low compared with previous reports, suggesting better activity than other non-fluoroquinolone rifampin-based combinations

A.2. Infection by Gram-negative bacilli

Aim 4 – To assess the impact of fluoroquinolones in the outcome of a large cohort of PJI by Gram-negative bacilli

4.1. The use of ciprofloxacin independently improves the prognosis of patients with acute PJI by GNB managed with implant retention

4.2 This benefit extends to different types of specific GNB, including *Pseudomonas aeruginosa*.

A.3. Infection in the elderly

Aim 5 – Comparative evaluation of the antibiotic efficacy in patients carrying total hip prosthesis or hip hemiarthroplasties.

5.1. Patients with hip hemiarthroplasty infection or total hip arthroplasty infection differ not only in the type of hip device, but also in the patient's baselines features, clinical presentation and etiology of the infection.

5.2. Overall outcome of DAIR management is similar for both the two kind of patients, probably due to a balanced distribution of parameters of bad prognosis in the two grops.

5.3. Infection of hip hemiarthroplasty is a complication with a bad prognosis and leading to a high rate of mortality, especially among cemented-devices.

B. Antimicrobial therapy in prosthetic joint infection managed with implant removal.

Aim 6 – Evaluation of linezolid in PJI by Gram-positive microorganisms managed with a two-step exchange procedure

6.1. Oral administration of linezolid for six weeks provides effective antibiotic treatment for the management of these infections

6.2. The rate of positive cultures at the surgical site at the time of reimplantation was 5% among patients with clinical cure, a rate lower than previously reported

6.3. Safety and tolerance are acceptable, with reversible toxicity after linezolid withdrawal, but close surveillance is required, especially after 2-3 weeks of therapy.

6.4. Linezolid may be considered as the treatment of choice in this type of infections, but further comparative clinical studies are warranted.

C. Antimicrobial activity on biofilms of multi-resistant Gram-negative bacilli.

Aim 7 – Evaluation of colistin against multi-resistant *P. aeruginosa* biofilm in an *in vitro* experimental model.

7.1. Clinically relevant concentrations of colistin have a strain-dependent activity on biofilm-embedded *P. aeruginosa*, but regrowth is usually observed when used in monotherapy

7.2. Colistin activity is enhanced by combination with doripenem, which achieves a synergistic effect when high concentrations of colistin are used. This improved activity is also observed in the setting of carbapenem-resistant *P. aeruginosa*

7.3. Regrowth was less frequent with the combination of colistin and doripenem, it avoiding the emergence of colistin-resitant biofilm-embedded cells.

7.4. Our observations support the use of the combination of colistin plus doripenem in the setting of multi-resistant *P. aeruginosa* biofilm. These results need to be validated in animal experimental models.

- **REFERENCES**

References

(1) Zuckerman JD. Hip fracture. N Engl J Med 1996; 334: 1519-25.

(2) Bernardino S. Total elbow arthroplasty: history, current concepts and future. Clin Rheumatol 2010; 29: 1217-21.

(3) Easley ME, Adams SB Jr, Hembree WC, DeOrio JK. Results of a total ankle arthroplasty. J Bone Joint Surg Am 2011; 93: 1455-68.

(4) Watts AC, Hearnden AJ, Trail IA, Hayton MJ, Nuttal D, Stanley JK. Pyrocarbon proximal interphalangeal joint arthroplasry: minimum two-year follow-up. J Hand Surg Am 2012; 37: 882-8.

(5) Kumar CS, Holt G. Hallux metatarsophalangeal arthroplasry in the rheumatoid forefoot. Foot Ankel Clin N Am 2007; 12: 405-16.

(6) Chancelot C. Les prothèses du poignet. Chir Main 2006; 25: 271-279.

(7) Ariza J, Euba G, Murillo O. Orthopedic device-related infections. Enferm Infecc Microbiol Clin 2008; 26: 380-90.

(8) Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645-54.

(9) Del Pozo JL, Patel R. Infection associated with prosthetic joints. N Engl J Med 2009; 361: 787-94.

(10) Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. J Arthropl 2012; 27:61-5.

(11) Peel TN, Dowsey MM, Daffy JR, Stanley PA, Choong PFM, Buising KL. Risk factors for prosthetic hip and knee infections according to arthroplasty site. J Hosp Infect 2011; 79: 129-33.

(12) Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J. Periprosthetic joint infection. The incidence, timing, and predisposing factors. Clin Orthop Rel Res 2008; 466: 1710-5.

(13) Steckelberg JM, Osmonm DR. Prosthetic joint infections. In: Waldvogel FA, Bisno AL, editors. Infections associated with indwelling medical devices, 3rd ed. Washington: ASM Press; 2000; p. 173-209.

(14) Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998: 27: 1247-54.

(15) Murdoch DR, Roberts SA, Fowler Jr VG, Shah MA, Taylor SL, Morris AJ ; Corey GR. Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. Clin Infect Dis 2001; 32: 647-9.

(16) Sendi P, Banderet F, Graber P, Zimmerli W. Periprosthetic joint infection following *Staphylococcus aureus* bacteremia. J Infect 2011; 63: 17-22.

(17) Romanò CL, Romanò D, Logoluso N, Meani E. Septic versus aseptic hip revision: how different? J Orthopaed Truamtol 2010; 11: 167-74.

(18) De Man FHR, Sendi P, Zimmerli W, Maurer TB, Ochsner PE, Ilchmann T. Infectiological, functional, and radiographic outcome after revision for prosthetic hip infection according to a strict algorithm. Acta Orthop 2011; 82: 27-34.

(19) Donaldson AD, Jalaludin BB, Chan RC. Patient perceptions of osteomyelitis, septic arthritis and prosthetic joint infection: the psychological influence of methicillin-resistant *Staphylococcus aureus*. Intern Med J 2007; 37: 536-42.

(20) Costerton JE, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999; 284: 1318-22.

(21) Stewart PS, Costerton JW. Antibiotic resistance of bacterial biofilms. Lancet 2001; 358: 135-38.

(22) Pasmore M, Costerton JW. Biofilms, bacterial signaling, and their ties to marine biology. J Ind Microbiol Biotechnol 2003; 30: 407-13.

(23) Bardouniotis E, Ceri H, Olson M. Biofilm formation and biocide susceptibility testing of *Mycobacterium fortuitum* and *Micobacterium marinum*. Curr Microbiol 2003; 48: 28-32.

(24) Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010; 35: 322-32.

(25) Gilbert P, Collier PJ, Brown MRW. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrob Agents Chemother 1990; 34: 1865-8.

(26) Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. Antimicrob Agents Chemother 2000; 44: 1818-24.

(27) Gilbert DN, Leggett JE. Aminoglycosides. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia: Churchill Livingston and Elsevier; 2010; p. 359-84.

(28) Driffield K, Miller K, Bostock JM, O'Neill AJ, Chopra I. Increased mutability of *Pseudomonas aeruginosa* in biofilms. J Antimicrob Chemother 2008; 61: 1053-6.

(29) García-Castillo M, del Campo R, Baquero F, Morosini MI, Turrientes MC, Zamora J, Cantón R. Stationary biofilm growth normalizes mutation frequencies and mutant prevention concentrations in *Pseduomonas aeruginosa* from cystic fibrosis patients. Clin Microbiol Infect 2011; 17: 704-11.

(30) Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. *Staphylococcus aureus* small colony variants in prosthetic joint infection. Clin Infect Dis 2006; 43: 961-7.

(31) Murillo O, Doménech A, García A, Tubau F, Cabellos C, Gudiol F, Ariza J. Efficacy of high doses of levofloxacin in experimental foreign body infection by methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother 2006; 50: 4011-7.

(32) Marin M, Raoult D. Intracellular organisms. Int J Antimicrobiol Agents 1997; 9: 61-70.

(33) Proctor RA, Peters G. Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. Clin Infect Dis 1998; 27: 419-23.

(34) Cobo J, Del Pozo JL. Prosthetic joint infection : diagnosis and management. Expert Rev Anti Infect Ther 2011 ; 9: 787-802.

(35) Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg [Am] 1996;78-A: 512-23.

(36) Benito N, Franco M, Ribera A, Rodríguez-Pardo D, del Toro D, Fresco G, Fernández-Sampredro M, Guío L, Sánchez-Ribas E, Sorlí L, Soriano A, Marinescu C, Jover A, Ramos A, Sobrino B, Baraia-Etxaburu J, Pigrau C, Ariza J. Current etiology of prosthetic joint infections. Poster K-728 presented at the 53rd Interscience Conceference of Antimicrobial Agents and Chemotherapy, Chicago (USA), 2013.

(37) Rupp ME, Fey PD. *Staphylococcus epidermidis* and other coagulase-negative staphylococci. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia: Churchill Livingston and Elsevier; 2010; p. 2579-89.

(38) Zeller V, Lavigne M, Leclerc P, Lhotellier L, Graff W, Ziza JM, Desplaces N, Mamoudy P. Group B streptococcal prosthetic joint infections: a retrospective study of 30 cases. Presse Med 2009; 38: 1577-84.

(39) Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis 2009; 49: 1036-43.

(40) Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, Gullerud R, Osmon DR. Culture-negative prosthetic joint infection. Clin Infect Dis 2007; 45: 1113-9.

(41) Zappe B, Graf S, Ochsner PE, Zimmerli W, Sendi P. *Propionibacterium* spp in prosthetic joint infections: a diagnostic challenge. Arch Orthop Trauma Surg 2008; 128: 1039-46.

(42) Zeller V, Ghorbani A, Strady C, Leonard P, Mamoudy P, Desplaces N. *Propionibacterium acnes*: an agent of prosthetic joint infection and colonization. J Infect 2007; 55: 119-24.

(43) Könönen E. Anaerobic Gram-postive nonsporulating bacilli. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia: Churchill Livingston and Elsevier; 2010; p. 3125-8.

(44) Della Valle CJ, Sporer SM, Jacobs JJ, Berger RA, Rosenber AG, Paprosky WG. Preoperative testing for sepsis before revision total knee arthroplasty. J Arthroplasty 2007; 22 (Suppl 2): 90-2.

(45) Spanghel MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 1999; 81: 672-83.

(46) Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelber JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med 2004; 117: 556-62.

(47) Atkins BL, Atanasou N, Deeks JJ, Crook DWM, Simpson H, Peto TEA, McLardy-Smith P, Berendt AR. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. J Clin Microbiol 1998; 36: 2932-9.

(48) Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, Mandrekar JN, Cockerill FR, Steckelberg JM, Greenleaf JF, Patel R. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007; 357: 654-63.

(49) Schäfer P, Fing B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis 2008; 47: 1403-9.

(50) Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. J Bone Joint Surg Br 1995; 77: 28-33.

(51) Byren I, Bejon P, Atkins BL, Angus B, Masters S, McLardy-Smith P, Gundle R, Berendt A. One hundred and twelve infected arhtroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother 2009; 63: 1264-71.

(52) Vilchez F, Martínez-Pastor JC, García-Ramiro S, Bori G, Maculé F, Sierra J, Font L, Mensa J, Soriano A. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infection due to *Staphylococcus aureus* treated with debridement. Clin Microbiol Infect 2011; 17: 439-44.

(53) Sia IG, Berbari EF, Karchmer AW. Prosthetic joint infections. Infec Dis North Am 2005; 19: 885-914.

(54) Fisman DN, Reilly DT, Karchmer AW, Goldie SJ. Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. Clin Infect Dis 2001; 32: 419-30.

(55) El Helou OC, Berbari EF, Lahr BD, Eckel-Passow JE, Razonable RR, Sia IG, Virk A, Walker RC, Steckelberg JM, Wilson WE, Hanssen AD, Osmon DR. Efficacy and safety of

rifampin containing regimen for staphylococcal prosthetic joint infections treated with debridement and retention. Eu J Clin Microbiol Infect Dis 2010; 29: 961-7.

(56) Osmon DR; Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2013; 56: e1-e25.

(57) Brandt CM, Sistrunk WW, Duffy MC, Hanssen AD, Steckelberg JM, Listrup DM, Osmon DR. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis 1997; 24: 914-9.

(58) Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections. A randomized controlled trial. JAMA 1998; 279: 1537-41.

(59) Bejon PA, Berendt A, Atkins BL, Green N, Parry H, Masters S, Mclardy-Smith P, Gundle R, Byren I. Two-stage revision for prosthetic joint infection: predictors of outcome and the role of reimplantation microbiology. J Antimicrob Chemother 2010; 65: 569-75.

(60) Cabo J, Euba G, Saborido A, González-Panisello M, Domínguez MA, Agulló JL, Murillo O, Verdaguer R, Ariza A. Clinical outcome and microbiological findings using antibiotic-loaded spacers in two-stage revisión of prosthetic joint infections. J Infect 2011; 63: 23-31.

(61) Della Valle CJ, Bogner E, Desai P, Lonner JH, Adle E, Zuckerman JD. Analysis of frozen sections of intraoperative specimens obtained at the time of reoperation after hip or knee resection arthroplasry for the treatment of infection. J Bone Joint Surg Am 1999; 81: 684-90.

(62) Mont MA, Walkman BJ, Hungerford DS. Evaluation of preoperative cultures before second-stage reimplantation of a total knee prosthesis complicated by infection. J Bone Joint Surg Am 2000; 82: 1552-7.

(63) Murillo O, Euba G, Calatayud L, Domínguez MA, Verdaguer R, Pérez A, Cabo J, Ariza J. The role of intraoperatibe cultures at the time of reimplantation in the management of infected total joint arthroplasty. Eur J Clin Microbiol Infect Dis 2008; 27: 805-11.

(64) Widmer AF, Frei R, Rajacic Z, Zimmerli W. Correlation between *in vivo* and *in vitro* efficacy of antimicrobial agents against foreign body infections. J Infect Dis 1990; 162: 96-102.

(65) Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 1999; 37: 1771-6.

(66) Landersdorfer C, Bulitta JB, Kinzig M, Holzgrabe U, Sörgel F. Penetration of antibacterials into bone. Pharmacokinetic, pharmacodynamic and bioanalytical considerations. Clin Pharmacokinet 2009; 48: 89-124.

(67) Sendi P, Zimmerli W. Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect 2012; 18: 1176-84.

(68) Vergidis P, Rouse MS, Euba G, Karau MJ, Schmidy SM, Mandrekar JN, Steckelberg JM, Patel R. Treatment with linezolid or vancomycin in combination with rifampin is effective in an animal model of methicillin-resistant *Staphylococcus aureus* foreign body osteomyelitis. Antimicrob Agents Chemother 2011; 55: 1182-6.

(69) Saleh-Mghir A, Muller-Serieys C, Dinh A, Massias L, Crémieux AC. Adjunctive rifampin is crucial to optimizing daptomycin-efficacy against rabbit prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2011; 55: 4589-93.

(70) Kucers A, Croew S, Grayson ML, Hoy J. The use of antibiotics: a clinical review of antibacterial, antifungal and antiviral drugs. 5th ed. Oxford: Butterworth Heinemann; 1997.

(71) San Juan R, García-Reyne A, Caba P, Chaves F, Resines C, Llanos F, López-Medrano F, Lizasoain M, Aguado JM. Safety and efficacy of moxifloxacin monotherapy for treatment orthopedic implant-related staphylococcal infections. Antimicrob Agents Chemother 2010; 54: 5161-6.

(72) Van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, Gillespie SH, Boeree MJ. Why do we use 600 mg of rifampin in tuberculosis treatment? Clin Infect Dis 2011; 52: e194-9.

(73) Kenny MT, Strates B. Metabolism and pharmacokinetics of the antibiotic rifampin. Drug Metab Rev 1981; 12: 159-218.

(74) Guillaume M, Garraffo R, Bensalem M, Janssen C, Bland S, Gaillat J, Bru JP. Pharmacokinetic and dynamic study of levofloxacin and rifampicin in bone and joint infections. Med Mal Infect 2012; 49: 414-20.

(75) Valour F, Karsenty J, Bouaziz A, Ader F, Tod M, Lustig S, Laurent F, Ecochard R, Chidiac C, Ferry T. Antimicrobial-related severe adverse events during treatment of methicillin-susceptible *Staphylococcus aureus* bone and joint infection. Antimicrob Agents Chemother 2013 [Epub ahead of print].

(76) Garrigós C, Murillo O, Euba G, Verdaguer R, Tubau F, Cabellos C, Cabo J, Ariza J. Efficacy of usual and high doses of daptomycin in combination with rifampin versus alternative therapies in experimental foreign body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2010; 54: 5251-6.

(77) Garrigós C, Murillo O, Lora-Tamayo J, Verdaguer R, Tubau F, Cabellos C, Cabo J, Ariza J. Fosfomycin-daptomycin and other fosfomycin combinations as alternative therapies in experimental and foreign body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2013, 57: 606-10.

(78) Ryback MJ. The efficacy and safety of daptomycin: first in a new class of antibiotics for Gram-positive bacteria. Clin Microbiol Infect 2006; 12 (suppl.1): 24-32.

(79) Stewart PS, Davison WM, Steenbergen JN. Daptomycin rapidly penetrates a *Staphylococcus epidermidis* biofilm. Antimicrob Agents Chemother 2009; 53: 3505-7.

(80) Traunmüller F, Schintler MV, Metzler J, Spendel S, Mauric O, Popovic M, Konz KH, Scharnagl E, Joukhadar C. Soft tissue and bone penetration abilities of daptomycin in diabetic patients with bacterial foot infections. J Antimicrob Chemother 210; 65: 1252-7.

(81) Mélard A, Garcia LG, Das D, Rozenberg R, Tulkens PM, Van Bambeke F, Lemaire S. Activity of ceftaroline against extracellular (broth) and intracellular (THP-1 monocytes) of methicillin-resistant *Staphylococcus aureus*: comparison with vancomycin, linezolid and daptomycin. J Antimicrob Chemother 2013; 68: 648-58.

(82) Rao N, Regalla DM. Uncertain efficacy of daptomycin for prosthetic joint infections. Clin Orthop Rel Res 2006; 451: 34-7.

(83) Corona Pérez Cardona PS, Barro Ojeda V, Rodríguez Pardo D, Pigrau Serrallach C, Guerra Farfán E, Amat Mateu C, Flores Sánchez X. Clinical experience with daptomycin for the treatment of patients with knee and hip periprosthetic joint infections. J Antimicrob Chemother 2012; 67: 1749-54.

(84) Clemmet D, Markham A. Linezolid. Drugs 2000; 59: 815-27.

(85) Rana B, Butcher I, Grigoris P, Murnaghan C, Seaton RA, Tobin CM. Linezolid penetration into osteo-articular tissues. J Antimicrob Chemother 2002; 50: 747-50.

(86) Legout L, Valette M, Dezeque H, Nguyen S, Lemaire X, Loïez C, Caillaux M, Beltrand E, Dubreuil L, Yazdanpanah Y, Migaud H, Senneville E. Tolerability of prolonged linezolid therapy in bone and joint infection: prospective effect of rifampicin on the occurrence of anemia? J Antimicrob Chemother 2010; 65: 2224-30.

(87) Rayner CR, Baddour LM, Birmingham MC, Norden C, Meagher AK, Schentag JJ. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. Infection 2004; 32: 8-14.

(88) Senneville E, Legout I, Valette M, Yazdanpanah Y, Beltrand E, Caillaux M, Migaud H, Mouton Y. Effectiveness and tolerability of proloned linezolid treatment for chronic osteomyelitis : a retrospective study. Clin Ther 2006; 28: 1155-63.

(89) Meehan AM, Osmon DR, Duffy MCT, Hanssen AD, Keating MR. Outcome of penicillin-susceptible streptococcal prosthetic joint infection treated with debridement and retention of the prosthesis. Clin Infect Dis 2003; 36: 845-9.

(90) Fiaux E, Beltrand E, Legout L, Senneville E. Streptococcal prosthetic joint infection : outcome at 2-year follow-up and risk factors of failure. Poster K-246 presented at the 51st Interscience Conference of Antimicrobial Agents and Chemotherapy, Chicago (USA), 2011.

(91) Arias CA, Murray BE. *Enterococcus* species, *Streptococcus bovis* group, and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia: Churchill Livingston and Elsevier; 2010; p. 2643-53.

(92) El Helou OC, Berbari EF, Marculescu CE, El Atrouni WI, Razonable RR, Steckelberg JM, Hanssen AD, Osmon DR. Outcome of enterococcal prosthetic joint infection: is combination systemic therapy superior to monotherapy? Clin Infect Dis 2008; 47: 903-9.

(93) Euba G, Lora-Tamayo J, Murillo O, Pedrero S, Cabo J, Verdaguer R, Ariza J. Pilot study of ampicillin-ceftriaxone combination for treatment of orthopedic infectious due to *Enterococcus faecalis*. Antimicrob Agents Chemother 2009; 53: 4305-10.

(94) Fernández-Hidalgo N, Almirante B, Gavaldà J, Gurgui M, Peña C, de Alarcón A, Ruiz J, Vilacosta I, Montejo M, Vallejo N, López-Medrano F, Plata A, López J, Hidalgo-Tenorio C, Gálvez J, Sáez C, Lomas JM, Falcone M, de la Torre J, Matínez-Lacasa X, Pahissa A. Ampicillin plus ceftriaxone is as effective as ampicillin plus gentamicina for treating *Enterococcus faecalis* infective endocarditis. Clin Infect Dis 2013; 56: 1261-8.

(95) Fiaux E, Beltrand E, Legout L, Loïez C, Senneville E. Rifampin combination therapy for enterococcal prosthetic joint infection. Poster K-240 presented at the 51st Interscience Conference of Antimicrobial Agents and Chemotherapy, Chicago (USA), 2011.

(96) Tanaka G, Shigeta M, Komatsuzawa H, Sugai M, Suginaka H, Usui T. Effect of the growth rate of *Pseudomonas aeruginosa* biofilms on the susceptibility to antimicrobial agents: beta-lactams and fluoroquinolones. Chemotherapy 1999; 45: 28-36.

(97) Aboltins CA, Dowsey MM, Buising KL, Peel TN, Daffy JR, Choong PFM, Stanley PA. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. Clin Microbiol Infect 2011; 17:862-7.

(98) Martínez-Pastor JC, Muñoz-Mahamud E, Vilchez F, García-Ramiro S, Bori G, Sierra J, Martínez JA, Font L, Mensa J, Soriano A. Outcome of acute prosthetic Joint infections due to Gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother 2009; 53: 4772-7.

(99) Jaén N, Martínez-Pastor JC, Muñoz-Mahamud E, García-Ramiro S, Bosch J, Mensa J, Soriano A. Long-term outcome of acute prosthetic joint infections due to gram-negative bacilli treated with retention of prosthesis. Rev Esp Quimioter 2012; 25: 194-8.

(100) Boucher HW, Talbot GH, Bradley JS, Edwards JE, .Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009; 48: 1-12.

(101) Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, Paterson DL. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006; 6: 589-601.

(102) Nation RL, Li J. Colistin in the 21st century. Curr Opin Infect Dis 2009; 22: 535-43.

(103) Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL. Pharmacokinetic/Pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an *in vitro* model. Antimicrob Agents Chemother 2010; 54: 3783-9.

(104) Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, Liolios L. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006; 50: 2946-50.

(105) Poudyal A, Howden BP, Bell JM, Gao W, Owen RJ. *In vitro* pharmacodynamics of colistin against multidrug-resistant *Klebsiella pneumoniae*. J Antimicrob Chemother 2008; 62: 1311-8.

(106) Couet W, Grégoire N, Gobin P, Saulnier PJ, Frasca D, Marchand S, Mimoz O. Pharmacokinetics of colistin and colistimethate sodium after a single 80-mg intravenous dose of CMS in young healthy volunteers. Clin Pharmacol Ther 2011; 89: 875-9.

(107) Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira FP, Forrest A, Nation RL. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestion for various categories of patients. Antimicrob Agents Chemother 2011; 55: 3284-94.

(108) Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, Karaiskos I, Poulakou G, Kontopidou F, Armaganidis A, Cars O, Giamarellou H. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infection caused by Gram-negative bacteria. Antimicrob Agents Chemother 2009; 53: 3430-6.

(109) Li J, Coulthard K, Milne R, Nation RL, Conway S, Peckham D, Etherington C, Turnidge J. Steady-state pharmacokinetics of intravenous colistin methanesulphonate in patients with cystic fibrosis. J Antimicrob Chemother 2003; 52: 987-92.

(110) Dalfino L, Puntillo F, Mosca A, Monno R, Spada ML, Coppolecchia S, Miragliotta G, Bruno F, Brienza N. High-dose, extended-interval colistin admnistration in critically ill patients: is this the right dosing strategy? A preliminary study. Clin Infect Dis 2012; 43: 1720-6.

(111) Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 2011; 55: 4469-74.

(112) Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N. *In vivo* pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. Antimicrob Agents Chemother 2012; 56: 2683-90.

(113) Bergen PJ, Forrest A, Bulitta JB, Tsuji BT, Sidjabat HE, Paterson DL, Li J, Nation RL. Clinically relevant plasma concentrations of colistin in combination with imipenem enhance pharmacodynamic activity against multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula. Antimicrob Agents Chemother 2011; 55: 5134-42.

(114) Hancock RE, Wong PG. Compounds which increase the permeability of the *Pseudomonas aeruginosa* outer membrane. Antimicrob Agents Chemother 1984; 26: 48-52.

(115) Hancock RE. Peptide antibiotics. Lancet 1997; 349: 418-22.

(116) Koike M, Iida K, Matsuo T. Electron microscopic studies on mode of action of polymyxin. J Bacteriol 1969; 97:448-52.

(117) Lorian V. The mode of action of antibiotics on Gram-negative bacilli. Arch Intern Med 1971; 128: 623-32.

(118) Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, Paterson DL, Nation RL, Li J. Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an *in vitro* pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother 2011; 55: 5685-95.

(119) Zusman O, Avni T, Leibovici L, Adler A, Friberg L, Stergiopoulou T, Carmeli Y, Paul M. Systematic review and meta-analysis of *in vitro* synergy of polymyxins and carbapenems. Antimicrob Agents Chemother 2013; 57: 5104-11.

(120) Herrmann G, Yang L, Wu H, Song Z, Wang H, Høiby N, Ulrich M, Molin S, Riethmüller J, Döring G. Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*. J Infect Dis 2010; 202: 1585-1592.

(121) Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to the metabolically active cells, and depends on the *pmr* and *mexAB-oprM* genes. Molec Microbiol 2008; 68: 223-240.

(122) Corvec S, Furustrand U, Betrisey B, Borens O, Trampuz A. Activities of fosfomycin, tigecyclin, colistin, and gentamicin against extended-spectrum-β-lactamase-producing *Escherichia coli* in a foreign body infection model. Antimicrob Agents Chemother 2013; 57: 1421-7.

(123) Bradbury T, Fehring TK, Taunton M, Hanssen A, Azzam K, Parvizi J, Odum SM. The fate of acute methicillin-resistant *Staphylococcus aureus* periprosthetic knee infections treated with open debridement and retention of components. J Arthroplasty 2009; 24: 101-4.

(124) Meropol SB, Chan KA, Chen Z, Finkelstein JA, Hennessy S, Lautenbach E, Platt R, Schech SD, Shatin D, Metlay JP. Adverse events associated with prolonged antibiotic use. Pharmacoepidemiol Drug Saf 2008; 17: 523-32.

(125) MacDougall C, Powell JP, Johnson CK, Edmond MB, Polk RE. Hospital and community fluoroquinolone use and resistance in *Staphylococcus aureus* and *Escherichia coli* in 17 US hospitals. Clin Infect Dis 2005; 41: 435-40.

(126) Polk RE, Johnson CK, McClish D, Wenzel RP, Edmond MB. Predicting hospital rates of fluoroquinolone-resistant *Pseudomonas aeruginosa* from fluoroquinolone use in US hospitals and their surrounding communities. Clin Infect Dis 2004; 39: 497-503.

(127) Bernard L, Legout L, Zürcher-Pfund L, Stern R, Rohner P, Peter R, Assal M, Lew D, Hoffmeyer P, Uçkay I. Six weeks of antibiotic treatment is sufficient following surgery for septic arthroplasty. J Infect 2010; 61: 125-32.

(128) Cobo J, García San Miguel L, Euba G, Rodríguez D, García-Lechuz JM, Riera M, Falgueras L, Palomino N, del Toro MD, Pigrau C, Ariza J. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. Clin Microbiol Infect 2011; 17: 1632-37.

(129) Rodríguez D, Pigrau C, Euba G, Cobo J, García-Lechuz J, Palomino J, Riera M, del Toro MD, Granados A, Ariza J. Acute haematogenous prosthetic joint infection: prospective evaluation of medical and surgical Management. Clin Microbiol Infect 2010; 16: 1789-95.

(130) Puhto AP, Puhto T, Surjala H. Short-course antibiotics for prosthetic joint infections treated with prosthesis retention. Clin Microbiol Infect 2012; 18: 1143-8.

(131) Guerrero A, Ariza J, Gomis M, Barberán J, Sáncez C, Barros C. Infecciones osteoarticulares y de partes blandas. In: Aguado JM, Almirante B, Fortún J. Protocolos Clínicos SEIMC. Spain: Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica; 2000.

(132) Toro MD, Rodríguez-Baño J, Euba G, Pigrau C, García-Lechuz J, Palomino J, Cobo J, Riera M, Benito N, Ariza J. Chronic prosthetic Joint infections: epidemiology, microbiology and management. Poster K-545 presented at the 48th Interscience Conference of Antimicrobial Agents and Chemotherapy, Washington (USA), 2008.

(133) Murray PR, Baron EJ, Jorgesen JH, Landry ML, Pfaller MA, editors. Manual of clinical microbiology. 9th ed. Washington, D.C.: American Society for Microbiology; 2007.

(134) Clinical and Laboratory Standards Institute. Performance Standards of Antimicrobial Susceptibility Testing: Twenty-Third Informational Supplement (M100-S23). CLSI. Wayne (PA): 2013.

(135) Murillo O, Pachón ME, Euba G, Verdaguer R, Tubau F, Cabellos C, Cabo J, Gudiol F, Ariza J. Antagonistic effect of rifampin on the efficacy of high-dose levofloxacin in staphylococcal experimental foreign body infection. Antimicrob Agents Chemother 2008; 52: 3681-6.

(136) Murillo O, Pachón ME, Euba G, Verdaguer R, Carreras M, Cabellos C, Cabo J, Gudiol F, Ariza J. Intracellular antimicrobial activity appearing as a relevant factor in antibiotic efficacy against an experimental foreign body infection caused by *Staphylococcus aureus*. J Antimicrobiol Chemother 2009; 64: 1062-6.

(137) Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K, Valentine J. Simple method for assaying colistin methanesulfonate in plasma and urine using high-performance liquid chromatography. Antimicrob Agents Chemother 2002; 46: 3304-7.

(138) Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K. Stability of colistin and colistin methanesulfonate in aqueous media and plasma determined by high-performance liquid chromatography. Antimicrob Agents Chemother 2003; 47: 1364-70.

(139) Zavascki AP, Goldani LZ, Cao G, superti SV, Lutz L, arth AL, Ramos F, Boniatti MM, Nation RL, Li J. Pharmacokinetics of intravenous polymyxin B in critically ill patients. Clin Infect Dis 2008; 47: 1298-304

(140) Lee HJ, Bergen PJ, Bulitta JB, Tsuji B, Forrest A, Nation RL, Li J. Synergistic activity of colistin and rifampin combination against multidrug-resistant *Acinetobacter baumanii* in an *in vitro* pharmacokinetic/pharmacodynamic model. Antimicro Agents Chemother 2013; 57: 3738-45.

(141) Donlan RM, Piede JA, Heyes CD, Sanii L, Murga R, Edmonds P, El-Sayed I, El-Sayed MA. Model system for growing and quantifying *Streptococcus pneumoniae* biofilms *in situ* and in real time. Appl Environ Microbiol 2004; 70: 4980-8.

(142) Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. Statistical assessment of a laboratory method for growing biofilms. Microbiol 2005; 151: 757-62.

(143) Parra-Ruiz J, Vidaillac C, Rose WE, Rybak MJ. Activities of high-dose daptomycin, vancomycin, and moxifloxacin alone or in combination with clarithromycin or rifampin in a novel *in vitro* model of *Staphylococcus aureus* biofilm. Antimicrob Agents Chemother 2010; 54: 5329-34.

(144) Peña C, Suárez C, Tubau F, Gutiérrez O, Domínguez A, Oliver A, Pujol M, Gudiol F, Ariza J. Nosocomial spread of *Pseudomonas aeruginosa* producing the metallo-beta-lactamase VIM-2 in a Spanish hospital: clinical and epidemiological implications. Clin Microbiol Infect 2007; 13: 1026-9.

(145) Peña C, Suárez C, Tubau F, Juan C, Moya B, Domínguez MA, Oliver A, Pujol M, Ariza J. Nosocomial outbreak of a non-cefepime-susceptible cefatizidme-susceptible *Pseudomonas aeruginosa* strain overexpressing MexXY-OprM and producing an integron-borne PSE-1 β-lactamase. J Clin Microbiol 2009; 47: 2381-7.

(146) Marculescu CE, Berbari EF, Hanssen AD, Steckelberg JM, Harmsen SW, Mandrekar JN, Osmon DR. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis 2006; 42: 471-8.

(147) Schindler M, Bernard L, Belaieff W, Gamulin A, Racloz G, Emonet S, Lew D, Hoffmeyer P, Uçkay I. Epidemiology of adverse events and *Clostridium difficile*-associated diarrhea during long-term antibiotic therapy for osteoarticular infections. J Infect 2013; 67: 433-8.

(148) Soriano A, García S, Bori G, Almela M, Gallart X, Maculé F, Sierra J, Martínez JA, Suso S, Mensa J. Treatment of acute post-surgical infection of joint arthroplasty. Clin Microbiol Infect 2006; 12: 930-33.

(149) Senneville E, Joulie D, Legout L, Valette M, Dezèque H, Beltrand E, Roselé B, d'Escrivan T, Loïez C, Caillaux M, Yazdanpanah Y, Maynou C, Migaud H. Outcome and predictors of treatment failure in total hip/knee prosthetic joint infections due to *Staphylococcus aureus*. Clin Infect Dis 2011; 53: 334-40.

(150) Jogun K, Vaudaux P, Garbino J, Pagani L, Hoffmeyer P, Lew D, Uçkay I. The safety and efficacy of high dose daptomycin combined with rifampin for the treatment of Gram-positive osteoarticular infections. Int Orthop 2013; 37: 1375-80.

(151) Tattevin P, Crémieux AC, Pottier P, Huten D, Carbon C. Prosthetic joint infection : when can prosthesis salvage be considered? Clin Infect Dis 1999; 29: 292-5.

(152) Hopley C, Stengel D, Ekkernkamp A, Wich M. Primary total hip arthroplasty versus hemiarthroplasty for displaced intracapsular hip fractures in older patients: systematic review. BMJ 2010; 340: c2332.

(153) Luo X, He S, Li Z, Huang D. Systematic review of cemented versus uncemented hemiarthroplasty for displaced femoral neck fractures in older patients. Arch Orthop Trauma Surg 2012; 132: 455-63.

(154) Parker MI, Pryor G, Gurusamy K. Cemented versus uncemented hemiarthroplasry for intracapsular hip fractures: a randomized controlled trial in 400 patients. J Bone Jt Surg Br 2010; 92: 116-22.

(155) Rao N, Hamilton CW. Efficacy and safety of linezolid for Gram-positive orthopedic infections: a prospective case series. Diagn Microbiol Infect Dis 2007; 59: 173-9.

(156) Vinh DC, Rubinstein E. Linezolid: a review of safety and tolerability. J Infect 2009; 59: S59-74.

(157) Jiménez-Mejías ME, García-Cabrera E. Infecciones relacionadas con los sistemas de drenaje de líquido cegalorraquídeo. Enferm Infecc Microbiol Clin 2008; 26: 240-51

(158) Fortún J. Infecciones asociadas a dispositivos intravasculares utilizados para la terapia de infusión. Enferm Infecc Microbiol Clin 2008; 26: 168-74.

(159) Almirante B, Miró JM. Infecciones asociadas a las válvulas protésicas cardíacas, las prótesis vasculares y los dispositivos de electroestimulación cardíacos. Enferm Infec Microbiol Clin 2008; 26: 647-64.

(160) Aboltins CA, Page MA, Buising KL, Jenney AWJ, Daffy JR, Choong PFM, Stanley PA. Treatment of staphylococcal prosthetic joint infections with debridement, prosthesis retention and oral rifampicin and fusidic acid. Clin Microbiol Infect 2007; 13: 586-91.

(161) Barberán J, Aguilar L, Carroquino G, Giménez MJ, Sánchez B, Martínez D, Prieto J. Conservative treatment of staphylococcal prosthetic joint infections in elderly patients. Am J Med 2006; 119: 993.e7-10.

(162) Byren I, Rege S, Campanaro E, Yankelev S, Anastasiou D, Kuropatkin G, Evans R. Randomized controlled trial of the safety and efficacy of daptomycin versus standard-of-care therapy for management of patients with osteomyelitis associated with prosthetic devices undergoing two-stage revision arthroplasty. Antimicrob Agents Chemother 2012; 56: 5626-32.

(163) Bardouniotis E, Ceri H, Olson M. Biofilm formation and biocide susceptibility testing of *Mycobacterium fortuitum* and *Micobacterium marinum*. Curr Microbiol 2003; 48: 28-32.

(164) Manzur A, Gudiol F. Methicillin-resistant *Staphylococcus aureus* in long-term care facilities. Clin Microbiol Infect 2009; 15: 26-30.

(165) Lautenbach E, Fisman NO, Bilker WB, Castiglioni A, Metlay JP, Edelstein PH, Strom BL. Risk factors for fluoroquinolone resistance in nosocomial *Escherichia coli* and *Klebsiella pneumoniae* infections. Arch Intern Med 2002; 162: 2469-77.

(166) Choi HR, von Knoch F, Zurakowski D, Nelson SB, Malchau H. Can implant retention be recommended for treatment of infected TKA? Clin Orthop Relat Res 2011; 469: 961-9.

(167) Petersen MB, Jørgensen HL, Hansen K, Duus BR. Factors affecting postoperative mortality of patients with displaced neck fracture. Injury 2006; 37: 705-11.

(168) Kesmezacar H, Ayhan E, Unlu MC, Seker A, Karaca S. Predictors of mortality in elderly patients with an intertrochanteric or a femoral neck fracture. J Trauma 2010; 68: 153-8.

(169) Jämsen E, Stogiannidis I, Malmivaara A, Pajamäki J, Puolakka T, Konttinen Y. Outcome of prosthesis exchange for infected knee arthroplasty: the effect of treatment approach. A systematic review of the literature. Acta Orthop 2009; 80: 67-77.

(170) Mittal Y, Fehring TK, Hanssen A, Marculescu C, Odum SM, Osmon D. Two-stage reimplantation for periprosthetic knee infection involving resistant organisms. J Bone Joint Surg Am 2007; 89: 1552-7.

(171) Soriano A, Ortega M, García S, Peñarroja G, Bové A, Marcos M, Martínez JC, Martínez JA, Mensa J. Comparative study of the effects of pyridoxine, rifampin and renal function on hematological adverse events induced by linezolid. Antimicrob Agents Chemother 2007; 51: 2559-63.

(172) Egle H, Trittler R, Kümmerer K, Lemmen SW. Linezolid and rifampin: drug interaction contrary to expectations? Clin Pharmacol Ther 2005; 77: 451-3.

(173) Hsieh PH, Huang KC, Lee PC, Lee MS. Two-stage revision of infected hip arthroplasty using an antibiotic-loaded spacer: retrospective comparison between short-term and prolonged antibiotic therapy. J Antimicrob Chemother 2009; 64: 392-7.

(174) McKenna PB, O'Shea K, Masterson EL. Two-stage revision of infected hip arthroplasty using a shortened post-operative course of antibiotics. Arch Orthop Trauma Surg 2009; 129: 489-94.

(175) Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic model. J Antimicrob Chemother 2008; 61: 636-42.

(176) Gunderson BW, Ibrahim KH, Hovde LB, Fromm TL, Reed MD, Rotschafer JC. Synergistic activity of colistina and ceftazidime against multianbiotic-resistant *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic model. Antimicrob Agents Chemother 2003; 47: 905-9.

(177) Bulitta JB, Yang JC, Yohonn L, Ly NS, Brown SV, D'Hondt RE, Jusko WJ, Forrest A, Tsuji BT. Attenuation of colistin bactericidal activity by high inoculum of *Pseudomonas aeruginosa* characterized by a new mechanism-based population pharmacodynamic model. Antimicrob Agents Chemother 2010; 54: 2051-62.

(178) Sánchez Gómez S. Potenciación de antibióticos mediante péptidos catiónicos permeabilizantes: aplicación al desarrollo de terapias frente a la sepsis debida a *Pseudomonas aeruginosa* multirresistente [Tesis doctoral]. Pamplona: Universidad de Navarra ; 2008.

- **ANNEXES**

Annexe I

Standardized protocol for
gathering data of PJI in the
Unit of Bone and Joint Infection
(Hospital Universitario de Bellvitge)

Episodi

RESUM PACIENT

Nom i cognoms

NHC

Pròtesi

Tipus infecció

Ingrés (mes/any)

Micro

Infecció

Recidiva (s/n)

Superinfecció / Reinfecció

IQ

ATB

DATOS DEL PACIENTE

Nombre | Apellidos

Iniciales | Sexo

NHC

F. Nacimiento: | Teléfono:

Hospital:

E. Base 1: | E. Base 2:

E. Base 3:

Clasificación:

aguda postq | hematógena | postquir tardía | cultivo IO +

Tipo de prótesis:

PTC | PTR | HAC | Osteosínt | Codo | Hombro

Fecha de colocación prótesis:

CIRUGIA

Prótesis:

primaria | secundaria | terciaria | cementada con ATB | cementada sin ATB

híbrida

Material prótesis:

cromocobalto | ac. inox | titanio | cerámica | polietileno

hidroxiapatita | otros

Cirugía:

profilaxis ATB | ASA | Duración (min):

DIAGNOSTICO

Fecha Diagnóstico:

Duración clínica (días): (fecha inicio de síntomas)
(días desde diagnóstico hasta el día del tratamiento-quirúrgico o ATB)

Duración ingreso (días):
(suma total de días de todos los ingresos relacionados con el episodio)

Evento previo (en 1 año):

Artroscopia	Administración intracavitaria de fármaco
Bursitis prerotuliana	Infección superficial
Infección previa de articulación	Bacteriemia mismo gèrmen
Maniobras bacteriémicas	Endocarditis
Infección respiratoria	Infección GI
Infección urinaria	Otras infecciones

Clínica:

Dolor	S. Inflamatorios	Supuración
Fístula	Fiebre	
Merle A	Knee society	Leucocitos

Rx simple:

Osteolisi geodas	Lisis periprótesis lineal
Reacción perióstica	Aflojamiento protésico

AP:

Pus macroscópico		Bx peroperatoria >10 leucos/c
Bx sinovial:	PMN	Reacc cuerpo extraño MS
Bx ósea:	PMN	Reacc cuerpo extraño MS

Líquido articular

Gluc (mg/dl)	Proteínas (g/l)	Nº céls	Tipo céls

PCR

Fecha	Valor

VSG

Fecha	Valor

Exploracion de imagen

Fecha	Infección (S/N)

MICROORGANISMO

Nombre:

Papel: Infección / Recidiva/Persistencia / Superinfección

Fecha muestra quirúrgica:

ATB previo (s/n):

	Realizado (nº)	Positivo (nº)
Frotis 1		
Frotis 2		
M. sinovial		
Cemento		
Prótesis		
BH cótilo		
BH fémur		
BH tibia		
M. periprót		
L. articular		

Muestra no quirúrgica 1: Fecha 1

Muestra no quirúrgica 2: Fecha 2

Muestra no quirúrgica 3: Fecha 3

Sensibilidad ATB

Penicilina	Oxacilina	Gentamicina	Cotrimoxazol
Clindamicina	Rifampicina	Ciprofloxacino	Vancomicina
Teicoplanina	Linezolid	Amoxi-clavul	Cefotaxima
Ampicilina	Imipenem	Eritromicina	Aztreonam
Piperacilina	Ceftazidima		

TRATAMIENTO

TRAT QUIRÚRGICO:

Fecha	Tipo

Fecha	Tipo

Opciones: Desbridamiento, Rec 1T, Rec 2T 1º, Rec 2T 2º, Girldstone, Artrodesis, Fij ext, Retirada material + fij ext, Amputación, Cir plástical, Rec 1T cótilo, Rec 1T vástago Desbridamiento + rosario genta, Desbridamiento + retirada material

Espaciador (s/n): ATB espaciador:

Cemento (s/n)): ATB cemento:

Hueso de banco (s/n):

Cultivo en el 2º tiempo (s/n):

Microorganismo en 2º tiempo:

Material protésico

Tipo de tto quirúrgico: Desbridam Recambio 1T

Recambio 2T Artrodesis

Retirada + implante misma prótesis

TRAT ANTIBIÓTICO:

ATB (solo/combinación)	Inicio	Final

Efectos secundarios:

Tipo Ef. secundario	Leve/Grave	ATB	Fecha

EVOLUCION

(Desde la finalización del tratamiento ATB)

6 meses:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

1 año:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

1,5 años:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

2 años:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

Exitus | Causa | Relacionada / No relacionada

NOTAS

Annexe II

ISRCTN – Register of the trial
"Comparative study of the efficacy of 'short' and 'long'
duration levofloxacin-rifampin combination in the treatment
of early postoperative and haematogenous
staphylococcal prosthetic joint infection"

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Comparative study of the efficacy of "short" and "long" duration levofloxacin-rifampicin combination therapy in the treatment of early postoperative and haemotogenous staphylococcal prosthetic joint infection

ISRCTN	ISRCTN35285839
ClinicalTrials.gov identifier	
Public title	Comparative study of the efficacy of "short" and "long" duration levofloxacin-rifampicin combination therapy in the treatment of early postoperative and haemotogenous staphylococcal prosthetic joint infection
Scientific title	Comparative study of the efficacy of "short" and "long" duration levofloxacin-rifampicin combination therapy in the treatment of early postoperative and haemotogenous staphylococcal prosthetic joint infection: a phase IV, multicentre, open trial
Acronym	N/A
Serial number at source	LR-07
Study hypothesis	In the early postoperative and haematogenous staphylococcal prosthetic joint infection with stable implant, treated with surgical debridement and the antibiotic combination of rifampicin and levofloxacin, a short length of therapy of 8 weeks is non inferior to a longer standard therapy of 3 to 6 months (3 in hip prosthesis, and 6 in knee prosthesis)
Lay summary	Not provided at time of registration
Ethics approval	Ethic Committee for Clinical Research (CEIC - Comité Ético de Investigación Clínica. Hospital Universitario de Bellvtige. c/ Feixa Llarga s/n. 08907 L'Hospitalet de Llobregat - Barcelona, Spain) approved on 6th November 2008
Study design	Phase IV multicentre open trial
Countries of recruitment	Spain
Disease/condition /study domain	Prosthetic joint infection
Participants - inclusion criteria	1. Diagnosis of prosthesis joint infection: fever, local pain, inflammatory signs or purulent exudate in the surgical wound and/or purulent macroscopic exudate during the debridement surgery. Prosthesis joint infection will be considered early-postoperative if symptoms and signs begin in the first 30 days after the placement of the prosthesis. It will be considered haematogenous when the clinical picture is acute and/or it develops in the setting of bacteremia or concomitant to other distant infection. 2. Diagnosis of staphylococcal etiology: Staphylococcus sp must be isolated from reliable samples, such as blood cultures or purulent exudate obtained during surgery or by arthrocentesis. Polymicrobial cases will be accepted if it is not necessary to add more antibiotics with anti-staphylococcal activity to the oral combination of rifampicin and levofloxacin.
Participants - exclusion criteria	1. Age less than 18 years 2. Pregnancy or breastfeeding 3. Women who may become pregnant in whom methods of contraception cannot be guaranteed during the period of antibiotic therapy 4. Life-expectancy less than 6 months 5. Unwillingness to parcipate in the study or to give written-informed consent 6. Unwillingness to avoid the use of contact lenses during the period of antibiotic therapy 7. Reasonable doubts about the patient’s treatment observance 8. Allergy or intolerance to quinolones and/or rifampicin which lead to the antimicrobial(s) withdrawal. Prosthesis joint infection by quinolones and/or rifampicin resistance 9. Administration of antibiotics with anti-staphylococcal activity different from rifampicin or levofloxacin for more than 7 days, during the period of study or during the follow-up 10. Delay in performing the surgical debridement of the prosthesis infection of 21 or more days, counting from the beginning of symptoms and signs of infection 11. Radiographic signs of prosthesis loosening in simple X-ray 12. Prosthesis removal during surgery
Anticipated start date	13/04/2009
Anticipated end date	13/04/2013
Status of trial	Ongoing

Patient information material	Not available in web format, please use the contact details below to request a patient information sheet
Target number of participants	195
Interventions	In the same clinical setting of early postoperative or haematogenous staphylococcal prosthetic joint infection treated with surgical debridement. The intervention consists of administering the same antimicrobial therapy for different lengths of therapy: short duration of 8 weeks vs longer therapy of 3-6 months
Primary outcome measure(s)	To assess the efficacy of a treatment consisting in early surgical debridement and antimicrobial therapy with an oral combination of rifampin and levofloxacin during either 8 weeks (“Short” schedule group) or 3 (hip prosthesis) to 6 (knee prosthesis) months (“Long” schedule group; standard schedule), in the early-postoperative and haematogenous prosthesis joint infection of staphylococcal etiology (Staphylococcus aureus and Coagulase-negative Staphylococcus)
Secondary outcome measure(s)	1. Success of therapy: absence of fever, inflammatory signs or fistula and absence of radiographic prosthesis loosening during the follow-up (12 months) 2. Failure, defined as: 2.1. Persistence of the infection either during treatment (persistence of inflammatory symptoms and signs which lead to the removal of the prosthesis) or at the end of treatment [(symptoms and signs suggestive of infection, with positive cultures (either from surgical or clinically significant samples]). A high value of C-reactive protein at the end of treatment, without clinical signs of relapse or persistence, is not considered criteria of failure by itself. 2.2. Relapse of the infection: initial remission of inflammatory symptoms and signs with posterior reappearance and positive cultures of the same microorganism responsible of the infection from surgical or clinically significant samples. 2.3. Reinfection: initial remission of inflammatory symptoms and signs with posterior reappearance and positive cultures of a different microorganism from surgical or clinically significant samples. In cases of persistence or relapse, evaluation of possible development of resistance to either rifampicin or quinolones will be performed. 3. Aseptic prosthesis loosening during follow-up, with no clinical evidence of infection and negative cultures 4. Adverse events. All adverse events will be collected, and the possible relation with the antibiotics will be evaluated. Serious adverse events will be reported to authorities, according to the law (Real Decreto 223/2004). Especial attention will be given to the following adverse events: 4.1. Gastrointestinal adverse events: vomiting, nausea, etc 4.2. Rise in liver enzymes 4.3. Flu-like syndrome secondary to rifampicin (head-ache, chills or rigors, arthralgias, myalgias…) 4.4. Lupus-like syndrome secondary to rifampicin 4.5. Myopathy or tendinitis secondary to levofloxacin
Sources of funding	Carlos III Health Institute (Instituto de Salud Carlos III) (Spain) - Health Research Fund (Fondo de Investigaciones Sanitarias [FIS]) - Ministry of Health ref: Expte EC/08/00113
Trial website	
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Annexe III

Informed consent form for the study “Safety and efficacy of a short schedule treatment of levofloxacin plus rifampin for staphylococcal PJI managed with DAIR, as compared with standard long schedules: a randomized clinical trial”

INFORMACIÓN AL PACIENTE

Estudio Comparativo de la Eficacia de Pautas "Cortas" y "Largas" de la Combinación Rifampicina – Levofloxacino en la Infección Estafilocócica Postquirúrgica Precoz y Hematógena de Prótesis Articular

Promotor: Dr. J. Ariza Cardenal (Hospital Universitario de Bellvitge)

Usted tiene una infección de su prótesis articular producida por una bacteria. El tratamiento más indicado en su caso es una cirugía de limpieza seguida de una pauta prolongada de antibióticos. Debe saber que la curación de estas infecciones no es fácil: en algunas personas puede ser necesario realizar varias operaciones y, si el tratamiento no resulta eficaz, eventualmente, puede requerirse la retirada de la prótesis.

Con los conocimientos actuales en Medicina, existe una idea razonable de la duración que debe tener el tratamiento antibiótico para su infección, pero no es posible determinarla con total exactitud. Los antibióticos son fármacos para el tratamiento de las infecciones producidas por bacterias y, como todos los medicamentos, pueden tener sus efectos adversos. Este estudio, con la participación de varios hospitales del país, ayudará a establecer la duración más apropiada del tratamiento antibiótico, con el fin de que sea eficaz y tenga los mínimos efectos adversos.

Se le van a administrar dos antibióticos (*levofloxacino* y *rifampicina*) que ya se utilizan habitualmente en este tipo de infecciones y que, en general, son bien tolerados. Entre los efectos secundarios que pueden producir se encuentran la alteración de la función del hígado, que se irá controlando mientras tome la medicación, y la interacción de *rifampicina* con otros fármacos, como anticoagulantes orales o antiepilépticos. *Rifampicina* tiñe las secreciones corporales (orina, lágrimas, etc.) de color anaranjado; si utiliza lentes de contacto debe advertírselo a su médico antes de participar en el estudio.

La duración de su tratamiento vendrá determinada por el grupo que le sea asignado al azar: grupo de tratamiento "corto", 8 semanas; grupo de tratamiento "largo", 3 meses si su prótesis es de cadera, 6 meses si su prótesis es de rodilla. Estos períodos se han elegido en base a pautas que ya han demostrado su eficacia y que se utilizan en hospitales de todo el mundo. Si no acepta participar en el estudio se le administrarán estos mismos antibióticos por la duración que habitualmente se utiliza en nuestro hospital en este tipo de infección: 8 semanas (salvo complicaciones). Los controles periódicos clínicos y analíticos necesarios para comprobar la buena evolución de la infección serán los mismos que se practican habitualmente en este tipo de infecciones. Participar en el estudio no supone un número adicional de visitas, análisis de sangre u otras exploraciones complementarias.

La participación en este estudio puede proporcionar a los pacientes beneficios derivados de la posibilidad de evitar un ingreso hospitalario prolongado, puesto que el tratamiento se administrará predominantemente por vía oral. Por otro lado, la posible constatación de que un período menos prolongado de tratamiento antibiótico con rifampicina y levofloxacino en este tipo de infecciones pueda proporcionar resultados satisfactorios, supone para los pacientes reducir los efectos secundarios derivados del consumo de fármacos y una oferta más confortable, y para el conjunto del sistema una disminución notable del gasto sanitario. Por otro lado, la participación en este estudio no comporta riesgos añadidos a los derivados de los efectos adversos de la antibioterapia, previamente mencionados.

Su participación en este proyecto es libre. Si acepta participar y apareciera algún problema incompatible con la continuidad del estudio, será atendido igualmente fuera del estudio. En el caso improbable de que sufriera daños derivados de la medicación, debe saber que el promotor ha contratado una póliza de responsabilidad civil que cubre los posibles daños o perjuicios derivados de su participación en el estudio, de acuerdo con los términos del RD 223/2004 (Compañía aseguradora ……; nº de póliza ……).

Así mismo, puede retirarse voluntariamente del estudio sin que eso afecte a la atención que requiera, ni a la relación con su médico.

Sólo aquellos datos de la historia clínica que estén relacionados con el estudio serán objeto de comprobación. Esta comprobación se hará en la medida de lo posible en presencia del Investigador Principal / Investigadores Colaboradores, responsables de garantizar la confidencialidad de todos los datos de las historias clínicas pertenecientes a los sujetos participantes en el ensayo clínico. Los datos recogidos para el estudio estarán identificados mediante un código y sólo el investigador principal / colaboradores podrá relacionar dichos datos con usted y con su historia clínica. El tratamiento de los datos se hará con las medidas de seguridad establecidas en cumplimiento de la Ley Orgánica 15/1999 de Protección de Datos de carácter personal y si además se transmiten datos a terceros se hará según lo establecido en la mencionada Normativa. El paciente tiene derecho de acceso, rectificación y cancelación u oposición de sus datos en cualquier momento.

Si tiene dudas sobre lo que ha leído, debe preguntar a su equipo médico. Si desea participar, deberá firmar la hoja de consentimiento informado que le adjuntamos. Para cualquier duda o consulta durante el periodo de duración del estudio, puede dirigirse al siguiente investigador de su hospital: Dr./Dra…… Teléfono…….. Le agradecemos de antemano su atención por haber leído esta información.

CONSENTIMIENTO INFORMADO

Por la presente hago constar que:

Yo, **(nombre y apellidos del paciente)** _____,

O

Yo, **(nombre y apellidos del familiar/tutor)** _____ en calidad de **(relación con el paciente)** _____ y responsable de **(nombre y apellidos del paciente)** _____,

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.
He sido informado por el/la Dr./Dra. **(nombre y apellidos del investigador)** _____.

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:
1º Cuando me parezca oportuno
2º Sin tener que justificar mis motivos
3º Sin que esto repercuta en mis cuidados médicos

- Presto libremente mi conformidad para participar en el estudio y doy mi consentimiento para el acceso y utilización de mis datos en las condiciones detalladas en la hoja de información.

- Accedo a que las muestras de sangre o tejidos obtenidas para el estudio puedan ser utilizadas en el futuro para nuevos análisis relacionados con la enfermedad o fármacos del estudio no previstos en el protocolo actual (quedando excluidos los análisis genéticos).

□ **SI** □ **NO**

Firma del paciente o familiar/tutor:

Nombre:

Fecha:

Firma del investigador:

Nombre:

Fecha:

COPIA PARA EL PACIENTE

CONSENTIMIENTO INFORMADO

Por la presente hago constar que:

Yo, **(nombre y apellidos del paciente)** _____,

O

Yo, **(nombre y apellidos del familiar/tutor)** _____ en calidad de **(relación con el paciente)** _____ y responsable de **(nombre y apellidos del paciente)** _____,

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.
He sido informado por el/la Dr./Dra. **(nombre y apellidos del investigador)** _____.

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:
1º Cuando me parezca oportuno
2º Sin tener que justificar mis motivos
3º Sin que esto repercuta en mis cuidados médicos

- Presto libremente mi conformidad para participar en el estudio y doy mi consentimiento para el acceso y utilización de mis datos en las condiciones detalladas en la hoja de información.

- Accedo a que las muestras de sangre o tejidos obtenidas para el estudio puedan ser utilizadas en el futuro para nuevos análisis relacionados con la enfermedad o fármacos del estudio no previstos en el protocolo actual (quedando excluidos los análisis genéticos).

□ **SI** □ **NO**

Firma del paciente o familiar/tutor:

Nombre:

Fecha:

Firma del investigador:

Nombre:

Fecha:

COPIA PARA EL INVESTIGADOR

Annexe IV
Articles

MAJOR ARTICLE

A Large Multicenter Study of Methicillin–Susceptible and Methicillin–Resistant *Staphylococcus aureus* Prosthetic Joint Infections Managed With Implant Retention

Jaime Lora-Tamayo,[1] Oscar Murillo,[1] José Antonio Iribarren,[6] Alex Soriano,[2] Mar Sánchez-Somolinos,[7] Josu Miren Baraia-Etxaburu,[11] Alicia Rico,[8] Julián Palomino,[12] Dolors Rodríguez-Pardo,[3] Juan Pablo Horcajada,[4] Natividad Benito,[5] Alberto Bahamonde,[14] Ana Granados,[15] María Dolores del Toro,[13] Javier Cobo,[11] Melchor Riera,[16] Antonio Ramos,[10] Alfredo Jover-Sáenz,[17] and Javier Ariza,[1] on behalf of the REIPI Group for the Study of Prosthetic Infection

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Background. Several series predicting the prognosis of staphylococcal prosthetic joint infection (PJI) managed with debridement, antibiotics, and implant retention (DAIR) have been published, but some of their conclusions are controversial. At present, little is known regarding the efficacy of the different antibiotics that are used or their ability to eliminate methicillin-resistant *S. aureus* (MRSA) infection.

Methods. This was a retrospective, multicenter, observational study of cases of PJI by *S. aureus* that were managed with DAIR (2003–2010). Cases were classified as failures when infection persistence/relapse, death, need for salvage therapy, or prosthesis removal occurred. The parameters that predicted failure were analyzed with logistic and Cox regression.

Results. Out of 345 episodes (41% men, 73 years), 81 episodes were caused by MRSA. Fifty-two were hematogenous, with poorer prognoses, and 88% were caused by methicillin-susceptible *S. aureus* (MSSA). Antibiotics were used for a median of 93 days, with similar use of rifampin-based combinations in MSSA- and MRSA-PJI. Failure occurred in 45% of episodes, often early after debridement. The median survival time was 1257 days. There were no overall prognostic differences between MSSA- and MRSA-PJI, but there was a higher incidence of MRSA-PJI treatment failure during the period of treatment (HR 2.34), while there was a higher incidence of MSSA-PJI treatment failure after therapy. Rifampin-based combinations exhibited an independent protective effect. Other independent predictors of outcome were polymicrobial, inflammatory, and bacteremic infections requiring more than 1 debridement, immunosuppressive therapy, and the exchange of removable components of the prosthesis.

Conclusions. This is the largest series of PJI by *S. aureus* managed with DAIR reported to date. The success rate was 55%. The use of rifampin may have contributed to homogenizing MSSA and MRSA prognoses, although the specific rifampin combinations may have had different efficacies.

Keywords. prosthetic joint infection; *Staphylococcus aureus*; MRSA; rifampin; antibiotics and implant retention (DAIR).

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Staphylococcus aureus is the microorganism most frequently responsible for prosthetic joint infection (PJI), especially in acute cases where debridement, antibiotics, and implant retention (DAIR) may be attempted [1–3]. This approach may cure the patient, reduce costs, and prevent the loss of bone stock and the need for additional operations [3–6].

An increased risk of joint failure has been associated with *S. aureus* infection [3, 7, 8], as well as with delay in administration of debridement, older age of the implant, prosthesis loosening, or the presence of a sinus tract [1, 2, 9, 10]. However, clinical series of PJI by *S. aureus* addressing the efficacy of DAIR usually include small samples, frequently in combination with coagulase-negative *Staphylococci* (CNS) [4, 11]. Moreover, the efficacy of DAIR may have improved with the introduction of rifampin during the last decade [12].

Infection by methicillin-resistant *S. aureus* (MRSA) could worsen the prognosis [13–18], due to the more limited range of antibiotics available [19]. However, this poorer prognosis of MRSA-PJI is controversial because case series include small numbers of MRSA infections, and the outcome of some of these patients was improved when treated with rifampin [8, 9, 20].

We present a large, multicenter series of cases of PJI by *S. aureus* treated with DAIR. The aim of this study was to assess the efficacy of DAIR, to identify factors predicting failure, and to establish the impact of MRSA and the use of rifampin combinations on prognosis.

METHODS

Setting and Patients

A retrospective, observational study was carried out in 17 hospitals in Spain, in the framework of the Spanish Network for Research in Infectious Diseases between 2003–2010. All cases of PJI originally caused by *S. aureus* and managed with DAIR were included, regardless of the age of the implant at the time of symptom onset. Patients with an unstable prosthesis or with surrounding soft tissues badly damaged did not undergo DAIR. Polymicrobial infections were also included. Cases where *S. aureus* did not cause the original PJI but participated later as a superinfecting microorganism were excluded. The identification of cases with *S. aureus* PJI was made from previously registered databases of PJI or from the general archives in each hospital. The decision to undergo DAIR and antimicrobial therapy was made by the attending medical team, based upon current recommendations [2, 5].

PJI by *S. aureus* was defined by ≥2 surgical, joint-aspirated or blood cultures yielding *S. aureus*, or by 1 such positive culture plus the presence of typical clinical symptoms and signs, such as joint pain, erythema and other inflammatory signs, or the presence of a sinus tract or purulence around the prosthesis during surgery [9]. Microorganisms were identified according to standard criteria [21] after samples had been seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate, and MacConkey agar) and incubated for at least 7 days. PJI was subsequently classified as post-surgical or hematogenous, being the latter characterized by an acute clinical presentation associated with documented or suspected bloodstream infection [1, 2].

Data on clinical presentation, risk factors for PJI, and baseline characteristics were recorded. Rheumatoid arthritis was defined by diagnostic criteria [22]. Chronic renal impairment was defined as a level of creatinine>150 μmol/L. Diagnostic prosthesis radiography was considered to be abnormal if there were signs of loosening or infection. Information regarding surgical treatment, exchange of removable pieces of the prosthesis (in at least 1 debridement), and type and duration of antimicrobials were also recorded. A composite variable based upon Zimmerli's algorithm [2] was considered if the patient was submitted to debridement within the first 21 days after symptom onset, plus if the prosthesis radiography was normal, plus if the prosthesis had been placed less than 3 months after the beginning of symptoms (for post-surgical cases).

All information was introduced in a specifically designed Microsoft Access database. All cases were critically reviewed by 2 authors (J.L-T. & J.A.). Any controversy or contradiction found was double-checked by the investigator at each hospital.

Clinical and Surgical Management

DAIR management has been described elsewhere [8]. Standard procedure consists of checking the solid fixation of the prosthesis, and when possible, the prosthetic exchangeable components are removed. After debridement, intravenous antibiotics of wide antimicrobial-spectrum are administered. Once the antimicrobial susceptibility was available, antibiotics were adjusted according to current guidelines. The intravenous route was maintained for a variable period depending on each hospital protocol, usually followed by oral antibiotics also for a variable time.

DAIR was considered to initiate with the first debridement procedure. Cases initially treated with antibiotics for >7 days without evident signs of infection during debridement, and if samples taken yielded no microorganisms, were not included in this analysis.

Outcome and Follow-Up

Failure was defined as: a) death related to the infection; b) prosthesis removal within 2 years of the beginning of treatment, for any cause, or after 2 years due to persistence/relapse of the staphylococcal infection and/or caused by other superinfecting microorganisms; c) the need for extra debridements

30 days after the first; or d) the need for extra courses of antibiotic after the initial scheduled treatment, including long-term antibiotic suppressive therapy (AST). Although these last 2 criteria (extra debridements and AST in patients with a foreseeable bad outcome) are not well established reasons for failure, they were assumed as a consensus among the investigators.

We performed an *Overall Failure* analysis. In order to evaluate the impact of antimicrobial therapy, we took into account failure dynamics and also performed a separate analysis of parameters predicting failure depending on the moment when it occurred:

- *Early Failure*: failure within 30 days of debridement surgery.
- *Late Failure*: failure while the patient was still under antimicrobial therapy, but occurring after the first 30 days after debridement.
- *Failure After Therapy*: failure after the end of antimicrobial therapy.

Statistical Analysis

Comparative analyses were performed with X^2 or Fisher's test for categorical variables, and the Mann-Whitney *U*-test for continuous variables. Multivariate analysis of parameters predicting *Early Failure* was made by logistic regression. Univariate and multivariate analyses of parameters predicting *Overall Failure*, *Late Failure*, and *Failure After Therapy* were made with Cox-regression, considering failures as main events, while loss of follow-up, death unrelated to infection, a new episode of PJI, or prosthesis removal any time 2 years after the beginning of treatment for orthopedic reasons were considered censored cases.

The length of antibiotic therapy could be shortened in cases failing prematurely and would not actually be the cause of failure but its consequence, and therefore, the antimicrobial therapy parameters were only analyzed when the comparison groups had had the same possibilities of receiving antibiotics. For this reason, this influence was not analyzed in *Overall* and *Early Failures*. The influence of antibiotics administered during the first 30 days was analyzed for *Late Failure* and *Failure After Therapy*, and the influence of the whole length of treatment was analyzed for *Failure After Therapy*.

Data were analyzed using SPSS (Statistical Package for the Social Sciences) software (version 15.0). All analyses were 2-tailed, and a P value $< .05$ was considered statistically significant.

RESULTS

Description of the Series

A total of 561 cases of PJI by *S. aureus* were diagnosed. Among them, 349 (62%) were managed with DAIR. Four (1%) patients were removed from this set due to a significant lack of data. Therefore, our analysis was carried out with 345 episodes occurring in 342 patients, of whom 140 (41%) were men, with a median age of 73 years (range 27–95). MRSA caused 81 (23%) episodes.

Infection was polymicrobial in 64 cases (19%). The most frequent microorganisms accompanying *S. aureus* were *Enterobacteriaceae* (of 33 cases, 11 were *Proteus* spp and 9 were *E. coli*), followed by CNS (10 isolates), *Pseudomonas* spp (8 isolates), *Enterococci* (7 isolates) and *Streptococci* (6 isolates).

There were 78 (23%) post-surgical cases with symptoms that began more than 30 days after the placement of the prosthesis (median of 64 days, interquartile range [IQR]: 35–184). In 50 (64%) cases, symptoms began within the initial 90 days.

All patients received appropriate initial empirical antibiotic therapy, and further specific antibiotic regimens were double-checked with microbiological susceptibility tests.

Hematogenous Versus Post-Surgical PJI

Table 1 shows a comparative analysis between post-surgical and hematogenous infections. The latter occurred more frequently among immunosuppressed patients, was often located in knee prostheses, and was monomicrobial, being caused by methicillin-susceptible *S. aureus* (MSSA) in 88% of cases. Hematogenous infections presented a more inflammatory clinical picture and a poorer prognosis, in spite of undergoing earlier debridement.

MRSA- Versus MSSA-PJI

Table 1 also presents a comparative analysis of MRSA and MSSA cases. MRSA-PJIs were most often suppurative and post-surgical hip-PJIs, occurring in older patients with more frequent comorbidity, especially chronic renal impairment. However, when hematogenous infections were excluded, no significant differences in the clinical presentation were observed (data not shown).

The surgical approach was alike in the MRSA and MSSA groups. Also, the length of antimicrobial therapy was similar in patients who completed the scheduled treatment without failing (94 days [IQR 61–162] vs 91 days [IQR 74–120]; $P = .922$). As expected, there were major differences between MRSA and MSSA cases regarding the specific antibiotics administered (Table 2). However, in both scenarios, rifampin was extensively used and to a similar extent: 303 (88%) patients were treated at some point with rifampin. Among patients not presenting *Early Failure,* 222 (76%) had been treated for ≥2 weeks during the first month after debridement, and among the patients who did not fail during the scheduled treatment, 189 (80%) had received rifampin for ≥4 weeks. In addition, rifampin treatment was initiated very early [delay of 0 days after debridement (IQR: 0–5)]. In the case of MSSA

Table 1. Case Series Description and Comparative Analysis of Methicillin-Susceptible *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* Cases, and Hematogenous and Post-Surgical Infections

		All Cases (n = 345)	MSSA (n = 264)	MRSA (n = 81)	*P*	Post-Surgical (n = 293)	Hematogenous (n = 52)	*P*
Baseline features	Sex (men)	140 (41%)	112 (42%)	28 (35%)	.208	119 (41%)	21 (40%)	.975
	Age (years)	73 (64–79)	71 (63–77)	78 (71–82)	<.001	72 (64–78)	74 (65–79)	.337
	Diabetes mellitus	68 (19%)	47 (18%)	21 (26%)	.097	60 (20%)	8 (15%)	.389
	Chronic renal impairment	19 (6%)	7 (3%)	12 (15%)	<.001	16 (5%)	3 (7%)	1.000
	Rheumatoid arthritis	30 (9%)	26 (10%)	4 (5%)	.187	23 (8%)	7 (13%)	.188
	Immunosuppressive therapy	22 (6%)	18 (7%)	4 (5%)	.576	14 (5%)	8 (15%)	.010
	Revision prosthesis	67 (19%)	46 (17%)	21 (26%)	.091	58 (20%)	9 (17%)	.676
	Prosthesis location							
	Knee	195 (57%)	166 (63%)	29 (36%)	<.001	157 (54%)	38 (73%)	.022
	Hip	146 (42%)	97 (37%)	49 (60%)		133 (45%)	13 (25%)	
	Other	4 (1.2%)	1 (0.4%)	3 (3.7%)		3 (1%)	1 (2%)	
Clinical presentation	Type of infection							
	Hematogenous	52 (15%)	46 (17%)	6 (7%)	.057	–	–	–
	Post-surgical[a] <30 days	215 (62%)	157 (59%)	58 (72%)		–	–	
	Post-surgical[a] >30 days	78 (23%)	61 (23%)	17 (21%)		–	–	
	Time to infection (days)[a]	19 (11–31)	19 (11–31)	18 (10–29)	.237	–	–	–
	Polymicrobial infection	64 (19%)	49 (19%)	15 (19%)	.992	63 (22%)	1 (2%)	.001
	MRSA infection	81 (23%)	–	–	–	75 (26%)	6 (12%)	.028
	Bacteremia	54 (16%)	44 (17%)	10 (12%)	.349	25 (9%)	29 (56%)	<.001
	Temperature >37°C	154 (45%)	127 (48%)	27 (33%)	.029	113 (39%)	41 (79%)	<.001
	Joint pain	272 (79%)	214 (81%)	58 (72%)	.064	221 (75%)	51 (98%)	<.001
	Sinus tract	50 (14%)	38 (14%)	12 (15%)	.942	48 (16%)	2 (4%)	.016
	Suppuration	189 (56%)	132 (50%)	57 (70%)	.001	187 (64%)	2 (4%)	<.001
	Leukocytes (10^9/L)	9.4 (6.6–13.4)	9.7 (6.9–13.8)	7.9 (5.1–11.2)	.014	9.0 (6.3–12.6)	11.9 (8.5–16.0)	<.001
	C-reactive protein (mg/L)	63 (20–172)	55 (20–177)	82 (21–167)	.355	53 (12–132)	225 (48–353)	<.001
Treatment and outcome	Debridement delay (days)[b]	7 (4–14)	7 (4–14)	9 (4–16)	.107	8 (4–16)	6 (3–11)	.031
	≥2 debridements	30 (9%)	22 (8%)	8 (10%)	.666	24 (8%)	6 (12%)	.425
	Polyethylene exchange[c]	221 (73%)	171 (75%)	50 (68%)	.249	194 (75%)	27 (63%)	.080

Table 1 continued.

	All Cases (n = 345)	MSSA (n = 264)	MRSA (n = 81)	*P*	Post-Surgical (n = 293)	Hematogenous (n = 52)	*P*
Global failure[d]	146 (45%)	112 (44%)	34 (46%)	.778	114 (41%)	32 (65%)	.001
Early Failure during therapy[e]	42 (12%)	31 (12%)	11 (14%)	.573	33 (11%)	9 (18%)	.220
Late Failure during therapy[d]	47 (14%)	28 (11%)	19 (26%)	.001	40 (14%)	7 (14%)	.861
Failure After Therapy[d]	57 (17%)	53 (21%)	4 (5%)	.012	41 (15%)	16 (33%)	<.001

Categorical variables expressed in absolute number and (percentage); continuous variables expressed in median and (interquartile range).
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.
[a] Time to infection: time from prosthesis placement to onset of symptoms (excluding hematogenous infections).
[b] Debridement delay: time from onset of symptoms to debridement surgery.
Analysis excludes the following:
[c] 44 patients (36 MSSA–prosthetic joint infection [PJI] + 8 MRSA-PJI; 35 post-surgical cases + 9 hematogenous-PJI) with no information regarding polyethylene exchange.
[d] 17 patients with unknown outcome (10 MSSA-PJI + 7 MRSA-PJI; 14 post-surgical cases + 3 hematogenous-PJI).
[e] 7 patients with unknown outcome at this point (3 MSSA-PJI + 4 MRSA-PJI; 6 post-surgical cases and 1 hematogenous-PJI).

infections, combinations of rifampin were mostly made with beta-lactams (13%) or quinolones (75%, mainly levofloxacin), while MRSA cases were treated with combinations of rifampin and glycopeptides (18%, namely vancomycin), cotrimoxazole (46%), linezolid (24%), or clindamycin (10%).

Outcome

Seventeen patients (5%) were lost to follow-up and/or had an unknown outcome. Among the 328 remaining patients, failure was documented in 146 (45%): there were 10 (7%) related deaths; 114 (78%) patients required the removal of the prosthesis to control the infection (in 81% due to staphylococcal persistence or relapse); 14 (10%) patients needed further courses of antibiotics and/or debridements more than 30 days after the initial one; and 8 (5%) patients needed long-term AST.

Among 60 cases treated with rifampin and finally presenting staphylococcal persistence or relapse, development of resistance to this antibiotic was observed in 6 (10%). Three were MRSA-PJIs: 2 received vancomycin and 1 cotrimoxazole; and 3 were MSSA-PJI: 1 was treated with cotrimoxazole and 2 with fluoroquinolones (1 also developed resistance to levofloxacin). Among polymicrobial infections, no patient failed exclusively due to persistence or relapse of the initially accompanying microorganisms other than *S. aureus*. The median survival time without failure was 1257 days (95% confidence interval: 361–2153). The rate of failure was not related with any particular period of the study.

Dynamics of Failure

Figure 1*A* illustrates that the likelihood of failure was much higher during the first few weeks after debridement. Among all failures, 42 (29%) occurred within the first 30 days of surgery. Table 3 shows the parameters associated with *Early Failure*: patients with inflammatory, polymicrobial, and bloodstream infections, rheumatoid arthritis, or male sex were more likely to fail.

After the initial 30 days, 47 (32%) patients failed while still on therapy (*Late Failure*). Older immunosuppressed patients with the presence of a sinus tract and MRSA-PJIs were more likely to fail, as well as patients needing ≥2 debridements.

There were 57 (39%) *Failures After Therapy*. Independent predictors were hematogenous infection, PJI by MSSA, delayed debridement, and the need for ≥2 debridements to control the infection.

Table 4 summarizes the parameters related with *Overall Failure*. Immunosuppression and the degree of complexity of the infection (polymicrobial, bacteremic, or presenting with high CRP levels) were independent predictors of failure. The need for ≥2 debridements also increased the likelihood of

Table 2. Antimicrobial Treatment in Methicillin-Susceptible *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* Prosthetic Joint Infection

	Whole Treatment (n = 235)[a]					Treatment During the First 30 Days After Debridement (n = 296)[b]				
	MSSA		MRSA			MSSA		MRSA		
	Days[c]	>28 Days (%)	Days[c]	>28 Days (%)	*P*[d]	Days[c]	>14 Days (%)	Days[c]	>14 Days (%)	*P*[d]
Any antibiotic	117 ± 90	–	105 ± 58	–	.922	–	–	–	–	–
Rifampin	90 ± 90	78	93 ± 63	93	.263	21 ± 11	75	23 ± 11	77	.193
Quinolones	82 ± 84	76	9 ± 43	5	<.001	13 ± 10	50	0.6 ± 2.6	2	<.001
plus Rifampin	67 ± 85	64	9 ± 43	5	<.001	11 ± 10	42	0.4 ± 2.3	2	<.001
Beta-Lactams	16 ± 22	17	4 ± 14	2	<.001	13 ± 10	39	3 ± 7	8	<.001
plus Rifampin	10 ± 16	9	3 ± 14	2	<.001	8 ± 10	25	0.9 ± 4.1	3	<.001
Glycopeptides	2 ± 7	1	18 ± 17	12	<.001	2 ± 5	4	14 ± 11	49	<.001
plus Rifampin	1 ± 6	1	14 ± 16	10	<.001	1 ± 4	3	10 ± 11	36	<.001
Cotrimoxazole	15 ± 53	11	52 ± 54	60	<.001	1 ± 5	4	6 ± 10	17	<.001
plus Rifampin	8 ± 34	6	48 ± 53	57	<.001	1 ± 4	3	5 ± 9	15	<.001
Clindamycin	6 ± 25	6	11 ± 34	14	.081	0.5 ± 3.1	2	2 ± 6	8	.033
plus Rifampin	3 ± 20	3	9 ± 31	12	.029	0.2 ± 1.6	1	2 ± 6	8	.001
Linezolid	1 ± 8	2	15 ± 25	21	<.001	0.5 ± 3.2	2	4 ± 7	14	<.001
plus Rifampin	0.3 ± 3.5	0.5	12 ± 24	17	<.001	0.2 ± 2.1	0.4	4 ± 7	11	<.001

Common doses administered of these antibiotics were as follows: Rifampin 600 mg/d *per os* (oral administration; po) or intravenous (iv); Levofloxacin 750 mg/d po/iv; Ciprofloxacin 750–1000 mg/12 hours po or 200–400 mg/12 hours iv; Moxifloxacin 400 mg po; Cloxacillin 2 g/4 hours iv; Amoxicillin-clavulanate 1 g/8 hours iv; Cefepime 2 g/8–12 hours iv; Vancomycin 1 g/12 hours (and adjustment of doses depending on serum levels); Teicoplanin 400 mg/24 hours iv; Cotrimoxazole 800/160 mg /12 hours po/iv; Clindamycin 600 mg/6–8 hours po/iv; Linezolid 600 mg /12 hours iv/po.

Abbreviations: MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

[a] Analysis made in patients who completed the scheduled treatment without failure.

[b] Analysis made in patients who did not fail during the first 30 days of treatment.

[c] Duration of antibiotic treatment expressed in mean of days ± standard deviation.

[d] Compared with the Mann-Whitney *U*-test.

failure, and the exchange of the polyethylene component was an independent predictor of success.

Overall, there were 82 patients (25%) who were managed with DAIR even though they did not accomplish Zimmerli's algorithm [2]. Failure was slightly higher among these patients (52% vs 42%; $P = .095$) and it presented earlier (920 ± 113 vs 1440 ± 94 days; $P = .065$).

MRSA Versus MSSA Outcome – Influence of Antimicrobial Therapy

Overall, there were similar failure rates for MRSA- and MSSA-PJI (46 vs 44%; $P = .778$), but with different dynamics (Figure 1*B*). During the antimicrobial treatment and after the first 30 days, MRSA cases were more than twice as likely to fail as MSSA-PJIs. In contrast, after treatment, MSSA cases failed more than MRSA-PJIs (Figure 2). This was still observed after excluding hematogenous cases (data not shown).

Patients treated with rifampin during the first 30 days of treatment showed a lower likelihood of *Late Failure* (Table 3). An analysis of post-surgical PJIs, without the influence of hematogenous cases, is presented in Table 5, which shows that rifampin-based combinations exerted an independent favorable influence.

We were not able to demonstrate the influence of antibiotics administered after the first 30 days. Figure 3 also illustrates that longer treatments were not associated with better outcomes. Finally, post-surgical cases with onset of symptoms more than 90 days after the placement of the prosthesis had a tendency towards a worse outcome (Figure 4).

DISCUSSION

In this series of PJI by *S. aureus*, DAIR was able to save 55% of implants in the long-term. While this percentage is in the middle range of staphylococcal-PJI series managed with DAIR (13–75%) [7–11, 16, 18, 23], it was low when compared with other recent cohorts using rifampin-based regimens [10, 20, 23]. Our rate of success may have been lowered by a longer delay in the administration of debridement and the inclusion of hematogenous cases and prosthesis placed >90 days before the beginning of symptoms, as well as the criteria we used,

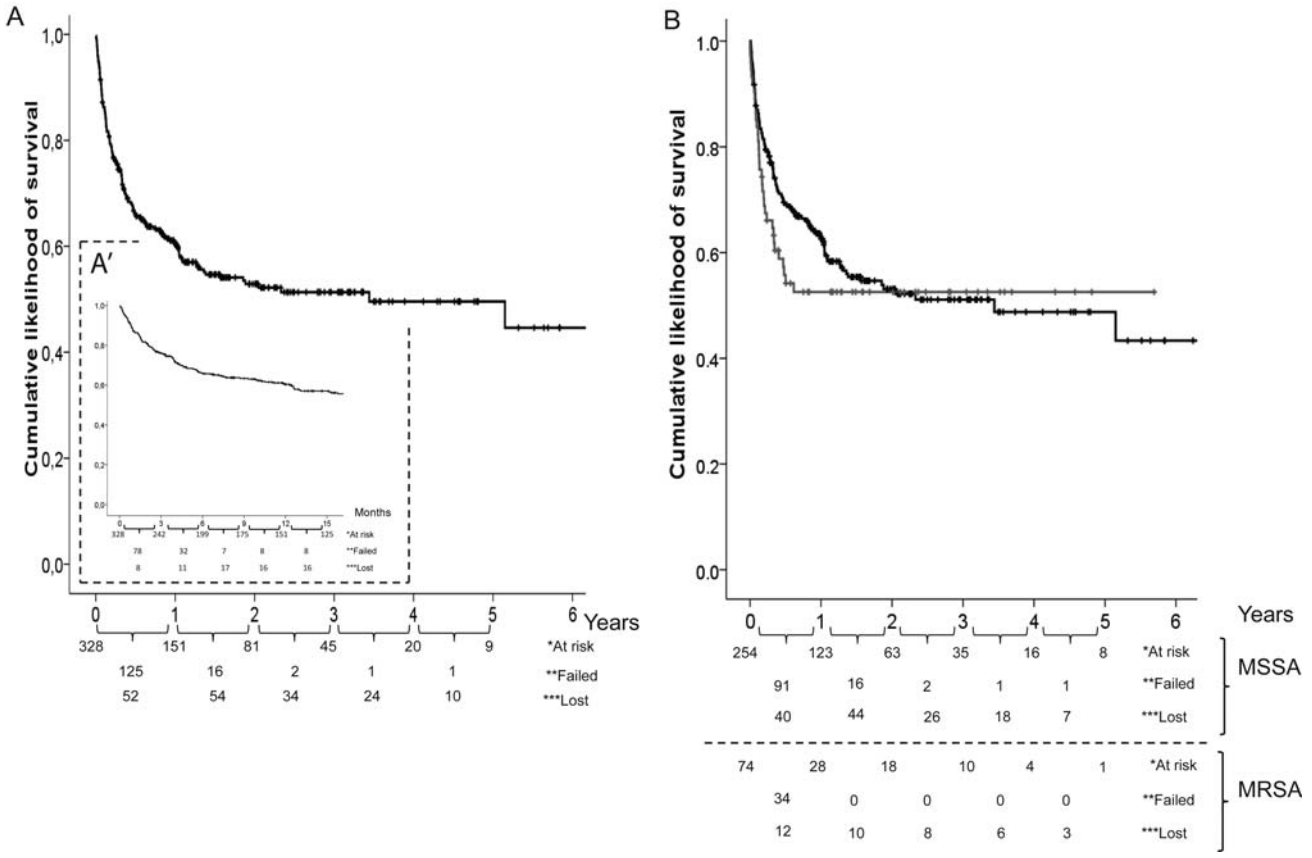

Figure 1. Kaplan-Meier survival diagram of patients with prosthetic joint infection by *Staphylococcus aureus*. *A*, Overall survival curve. *A′*, Survival curve during the first 15 months of follow-up. *B*, Survival curve for methicillin-susceptible *S. aureus* (black curve) and methicillin-resistant *S. aureus* (grey curve); log-rank test: $P = .374$; *Number of patients at risk for failure at the beginning of the period. **Patients failing during the period. ***Number of patients lost to follow-up during the period.

such as considering the patients as failures if they underwent long-term AST. However, because this is a multicenter study with a larger sample than previous analyses ($n = 21–53$), our data may be closest to the real likelihood of healing and retaining a joint prosthesis after staphylococcal PJI [7–11, 16].

Overall, the treatment of MRSA infections was not less successful than MSSA-PJI. This contrasts with previous reports of MRSA-PJI, which suggested poorer rates of success (16–35%) [11, 13, 16, 17]. Interestingly, most of our MRSA infections were early and extensively treated with rifampin. This antibiotic has been shown to maintain strong antimicrobial activity against clinical and experimental staphylococcal foreign body infections [12, 19, 24–26]. Indeed, modern series of PJI using rifampin for MRSA infections have shown better results than earlier ones, with success rates of 67%–100% [8, 10, 20].

Nevertheless, the behavior of MRSA- and MSSA-PJI was not the same: 88% of MRSA failures occurred during the first weeks after debridement while patients were still on therapy; in contrast, half of MSSA failures occurred once the antibiotic was withdrawn. When we excluded hematogenous patients, we again observed these different dynamics in failure, as well as a similar clinical presentation of MRSA- and MSSA-PJI. The surgical approach in the 2 groups was very similar, and so the main difference in treatment between MRSA and MSSA cases was the specific rifampin-based combination used in each group. Although a direct comparison between the specific combinations was not possible, this may suggest that not all treatments with rifampin are the same. The specific combination of quinolones plus rifampin has been considered the treatment of choice of MSSA-PJI [2, 23]. Thus, rifampin combinations for MRSA-PJI did not avoid failure as much as rifampin-fluoroquinolone combinations did among MSSA-cases, not failing until the withdrawal of the antibiotics. In addition, the development of resistance to rifampin among failures was less frequent when rifampin was combined with quinolones rather than other antimicrobials.

In our series, the type of antibiotic therapy administered during the first 30 days after debridement had an influence on

Table 3. Univariate and Multivariate Analysis of Parameters Predicting *Early Failure*, *Late Failure* and *Failure After Therapy*

	Early Failure (n = 338; failure = 42)				Late Failure (n = 284; failure = 47)				Failure After Therapy (n = 231; failure = 57)			
	Unadjusted OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*	Unadjusted HR (95%CI)	*P*	Adjusted HR (95%CI)	*P*	Unadjusted HR (95%CI)	*P*	Adjusted HR (95%CI)	*P*
Sex (male)	1.78 (.93–3.41)	.081	2.48 (1.19–5.19)	.016	.70 (.37–1.31)	NS	–	–	.68 (.39–1.19)	NS	–	–
Age (years)	1.02 (.99–1.06)	NS	–	–	1.03 (1.00–1.06)	.032	1.03 (1.00–1.07)	.052	.98 (.96–1.00)	NS	–	–
Diabetes mellitus	.67 (.27–1.66)	NS	–	–	1.46 (.74–2.88)	NS	–	–	1.29 (.68–2.45)	NS	–	–
Chronic renal impairment	1.44 (.40–5.19)	NS	–	–	2.54 (1.00–6.45)	.081	–	–	1.69 (.41–6.95)	NS	–	–
Rheumatoid arthritis	2.91 (1.20–7.04)	.018	3.88 (1.44–10.4)	.007	1.49 (.63–3.52)	NS	–	–	1.39 (.55–3.48)	NS	–	–
Immunosuppressive therapy	2.20 (.77–6.32)	NS	–	–	2.41 (1.07–5.42)	.054	3.05 (1.30–7.14)	.010	1.86 (.58–5.98)	NS	–	–
Revision prosthesis	1.56 (.74–3.28)	NS	–	–	2.00 (1.08–3.70)	.036	–	–	.89 (.42–1.88)	NS	–	–
Hip prosthesis	1.06 (.55–2.03)	NS	–	–	1.72 (.95–3.13)	.080	–	–	.81 (.46–1.40)	NS	–	–
Hematogenous infection	1.65 (.74–3.69)	NS	–	–	.85 (.38–1.91)	NS	–	–	2.93 (1.64–5.25)	.001	2.46 (1.35–4.48)	.003
Infection by MRSA	1.24 (.59–2.59)	NS	–	–	2.75 (1.53–4.94)	.001	2.33 (1.25–4.33)	.008	.33 (.12–.91)	.012	.33 (.12–.92)	–
Bacteremia	4.18 (2.06–8.50)	<.001	5.03 (2.11–12.0)	<.001	1.26 (.57–2.76)	NS	–	–	1.97 (.97–4.01)	.078	–	–
Polymicrobial infection	3.65 (1.83–7.29)	<.001	7.50 (3.23–17.4)	<.001	2.56 (1.31–5.01)	.011	–	–	.75 (.34–1.67)	NS	–	–
CRP at diagnosis (per 100 mg/L)	1.45 (1.11–1.89)	.007	1.52 (1.11–2.09)	.010	1.08 (.84–1.40)	NS	–	–	–	–	–	–
Temperature >37°C	1.71 (.89–3.29)	NS	–	–	.98 (.55–1.74)	NS	–	–	–	–	–	–
Sinus tract	1.05 (.42–2.66)	NS	–	–	2.18 (1.13–4.21)	.029	1.88 (0.94–3.77)	.076	.69 (.25–1.92)	NS	–	–
Abnormal radiography	.98 (.36–2.64)	NS	–	–	2.58 (1.34–4.99)	.010	2.28 (1.14–4.54)	.019	1.49 (.67–3.29)	NS	–	–
Debridement delay[a,b]	.97 (.78–1.21)[a]	NS	–	–	2.00 (1.13–3.54)[a]	.019	–	–	1.002 (1.001–1.004)[b]	.062	1.004 (1.001–1.006)[b]	.028
Polyethylene exchange[c]	.59 (.29–1.20)	NS	–	–	.40 (.21–.77)	.008	–	–	.63 (.33–1.20)	NS	–	–
Need for ≥ 2 debridements	1.04 (.38–2.83)	NS	–	–	2.13 (1.08–4.18)	.042	2.25 (1.11–4.56)	.025	2.58 (1.33–4.99)	.011	2.51 (1.27–4.98)	.008
Rifampin[d]	–	–	–	–	.56 (.31–1.01)	.062	0.49 (0.26–0.91)	.024	.60 (.34–1.07)	.095	–	–
Levofloxacin + Rifampin[d]	–	–	–	–	.33 (.12–0.92)	.014	–	–	1.00 (.56–1.77)	NS	–	–
Vancomycin + Rifampin[d]	–	–	–	–	.82 (.25–2.66)	NS	–	–	.36 (.09–1.46)	NS	–	–

For the multivariate analysis, variables with a *P* value < .10 in the univariate analysis were included in a stepwise backward selection process (*P*-in<.05 and *P*-out<.10 were used in each step).

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; NS, non-significant (*P* > .10); OR, odds ratio.

Debridement delay, time from onset of symptoms to debridement ([a]more than10 days; [b]days to debridement).

[c] Multivariate analyses do not include polyethylene exchange due to a significant lack of data.

[d] Data regarding antibiotics refer to antimicrobials administered for more than 14 days during the first 30 days after therapy.

Table 4. Univariate and Multivariate Analysis of Parameters Predicting Overall Failure

	Categories (n)	Days Without Failure[a]	Percentage of Failure (%)	Unadjusted Analysis		Adjusted Analysis	
				HR (95%CI)	*P*	HR (95%CI)	*P*
Sex (male)	Male (131)	1502 ± 117	42	.90 (.64–1.26)	NS	–	–
	Female (197)	1085 ± 80	46				
Age (years)	–	–	–	1.01 (.99–1.02)	NS	–	–
Diabetes mellitus	Yes (62)	1102 ± 140	47	1.10 (.73–1.66)	NS	–	–
	No (266)	1372 ± 93	44				
Chronic renal impairment	Yes (15)	553 ± 192	67	2.03 (1.07–3.87)	.051	–	–
	No (313)	1390 ± 84	44				
Rheumatoid arthritis	Yes (29)	732 ± 163	66	1.84 (1.14–2.99)	.021	–	–
	No (297)	1409 ± 88	42				
Immunosuppressive therapy	Yes (21)	278 ± 67	71	2.31 (1.35–3.94)	.006	2.23 (1.18–4.20)	.013
	No (307)	1416 ± 85	43				
Revision prosthesis	Yes (64)	968 ± 126	53	1.41 (.96–2.07)	.092	–	–
	No (264)	1412 ± 92	42				
Prosthesis location (hip)	Hip (137)	1375 ± 132	42	.98 (.70–1.37)	NS	–	–
	Other (191)	1147 ± 77	46				
Hematogenous infection	Yes (49)	689 ± 136	65	1.83 (1.24–2.72)	.004	–	–
	No (279)	1473 ± 89	41				
Infection by MRSA	Yes (74)	1126 ± 120	46	1.19 (.81–1.75)	NS	–	–
	No (254)	1364 ± 93	44				
Bacteremia	Yes (52)	650 ± 136	65	2.29 (1.54–3.42)	<.001	1.81 (1.12–2.92)	.015
	No (276)	1481 ± 89	41				
Polymicrobial infection	Yes (61)	1013 ± 173	59	1.76 (1.21–2.57)	.005	1.77 (1.17–2.70)	.007
	No (267)	1445 ± 86	41				
CRP at diagnosis (per 100 mg/L)	–	–	–	1.29 (1.13–1.48)	<.001	1.22 (1.03–1.43)	.021
Temperature >37°C	Yes (148)	982 ± 92	51	1.54 (1.10–2.14)	.011	–	–
	No (180)	1530 ± 112	39				
Sinus tract	Yes (47)	845 ± 122	47	1.27 (.81–2.01)	NS	–	–
	No (281)	1409 ± 88	44				
Abnormal radiography	Yes (40)	611 ± 118	60	1.66 (1.07–2.57)	.033	–	–
	No (288)	1430 ± 87	42				
Debridement							
delay >10 days[b]	Yes (117)	1475 ± 101	50	1.39 (1.00–1.94)	.050	–	–
	No (211)	1165 ± 149	42				
Polyethylene exchange	Yes (212)	1484 ± 98	41	.56 (.39–.82)	.004	0.65 (0.44–0.95)	.026
	No (75)	701 ± 99	56				
Need for ≥2 debridements	Yes (38)	649 ± 142	71	1.98 (1.30–3.01)	.003	1.63 (1.03–2.59)	.039
	No (290)	1452 ± 89	41				

For the multivariate analysis, variables with a *P* value < .10 in the univariate analysis were included in a stepwise backward selection process (*P*-in<.05 and *P*-out<.10 were used in each step).

According to this model, in a non-immunosuppressed patient with a monomicrobial prosthetic joint infection and no bacteremia, CRP less than 100 mg/L and the need for only 1 debridement with exchange of removable pieces, the likelihood of success at 6 months would be 77%, while for a patient with the opposite situation, it would be less than 1%.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; NS, non-significant (*P* > .10).

[a] Days without failure expressed in mean ± standard deviation.

[b] Debridement delay: time from onset of symptoms to debridement.

the outcome, indicating the importance of the initial antibiotics given just after surgery, when all efforts to remove the inoculum and the biofilm have been made.

Although our analysis was unable to show the influence of the therapy after the first few weeks, this does not mean that the antimicrobial therapy administered after this point was

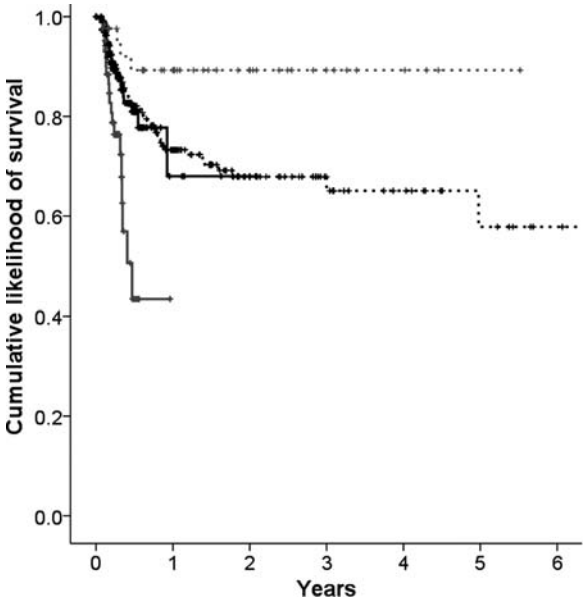

Figure 2. Comparative survival curves for patients with methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) prosthetic joint infection (PJI), during and after treatment. Patients with *Early Failure* are excluded. Black lines: MSSA PJI while on therapy (continuous line) and after therapy (discontinuous line); Log-rank test: $P = .996$. Grey lines: MRSA PJI while on therapy (continuous line) and after therapy (discontinuous line); Log-rank test: $P < .001$. Similar results were found when considering only post-surgical cases.

not important. In this regard, our results should be interpreted cautiously: most of our patients were treated for at least 60 days, meaning that a long course of antibiotics was needed; also, the retrospective nature of our work makes it difficult to compare different schedules. Furthermore, the length of treatment was the physician's choice, and so it is likely that longer therapies were applied in more severe cases, in a similar way as seen in Byren's work, which showed that prolonging therapy could delay relapse but not avoid it [8].

The literature mentions many other factors influencing the outcome of PJI that possess varying degrees of importance depending on the series. As noted by other authors [10], we found that the complexity and degree of inflammation of the infection were associated with the prognosis. Patients needing more than 1 debridement were also more likely to fail, probably because they had a more complex and highly inflammatory infection [10]. This inflammatory pattern was more frequent among hematogenous cases, carrying a worse prognosis [27–29].

Patients' baseline features and comorbidity also had an impact, especially in subjects under immunosuppressive therapy. Some studies report that revision prostheses have worse outcome than primary implants [8], but this issue is controversial [9], and we did not find statistical significance in our analysis.

In addition, because of the multicenter nature of our study, there may have been considerable surgical variability that likely influenced the outcome. We found that exchanging the polyethylene component of the prosthesis reduced the risk of failure by 33%, in spite of incomplete retrospective data.

In Brandt's study [9], patients delaying their debridement for >2 days had a poorer prognosis. This cutoff has not been confirmed by more recent studies [7, 8, 10], perhaps due to differences in patient selection or antibiotic management (ie, the addition of rifampin). Also, debridements may be performed earlier in more severe cases (ie, hematogenous infections), which would thus balance the real impact of an early debridement. In our study, we found that the time to debridement from the onset of symptoms was independently associated with prognosis when analyzing patients failing after treatment and also among post-surgical cases.

Recommendations pertaining to the age of the prosthesis at the time of attempting DAIR vary significantly between authors, ranging from less than 4 weeks [1, 3] to less than 90 days [2]. In our analysis, a similar prognosis was observed among these 2 groups of patients.

In summary, we present the largest series of staphylococcal PJIs managed with DAIR and assess the influence of different prognostic factors. A substantial number of patients fail early despite DAIR. Overall, the use of rifampin may contribute to homogenizing the prognosis for MRSA- and MSSA-PJI, although the differences we observed in their outcome may suggest a variable efficacy of the specific rifampin combination used. Further progress in the knowledge of these infections should come from prospective studies.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Table 5. Parameters Influencing Failure in Post-Surgical Infections After the First 30 Days of Therapy

	All Post-Surgical Episodes (n = 244; Failures = 81)				MSSA Post-Surgical Episodes (n = 185; Failures = 60)				MRSA Post-Surgical Episodes (n = 59; Failures = 21)			
	Unadjusted HR (95%CI)	*P*	Adjusted HR (95%CI)	*P*	Unadjusted HR (95%CI)	*P*	Adjusted HR (95%CI)	*P*	Unadjusted HR (95%CI)	*P*	Adjusted[a] HR (95%CI)	*P*
Sex (male)	.73 (.46–1.17)	NS	–	–	.75 (.43–1.28)	NS	–	–	.72 (.28–1.87)	NS	–	–
Age (years)	1.00 (.98–1.02)	NS	–	–	.99 (.97–1.02)	NS	–	–	1.00 (.96–1.04)	NS	–	–
Diabetes mellitus	1.35 (.80–2.26)	NS	–	–	1.24 (.66–2.34)	NS	–	–	1.51 (.61–3.75)	NS	–	–
Chronic renal impairment	2.87 (1.24–6.63)	.032	–	–	3.24 (.78–13.5)	NS	–	–	2.08 (.70–6.18)	NS	–	–
Rheumatoid arthritis	1.60 (.80–3.19)	NS	–	–	1.70 (.81–3.59)	NS	–	–	1.70 (.23–12.8)	NS	–	–
Immunosuppressive therapy	2.46 (1.13–5.36)	.045	–	–	3.30 (1.41–7.74)	.018	3.40 (1.39–8.37)	.008	1.05 (.14–7.83)	NS	–	–
Revision prosthesis	1.66 (1.01–2.74)	.056	–	–	1.97 (1.08–3.61)	.038	–	–	1.09 (.44–2.69)	NS	–	–
Hip prosthesis	1.08 (.69–1.68)	NS	–	–	.93 (.55–1.59)	NS	–	–	1.26 (.51–3.12)	NS	–	–
Time to infection >90 days[b]	2.19 (1.18–4.05)	.013	–	–	1.84 (.98–3.45)	.089	2.18 (1.04–4.56)	.039	7.48 (2.01–27.8)	.013	–	–
Infection by MRSA	1.32 (.80–2.18)	NS	–	–	–	–	–	–	–	–	–	–
Bacteremia	1.70 (.77–3.73)	NS	–	–	2.21 (.99–4.95)	.078	2.35 (1.04–5.36)	.040	–	–	–	–
Polymicrobial infection	1.47 (.88–2.47)	NS	–	–	1.19 (.64–2.21)	NS	–	–	2.81 (1.07–7.39)	.052	–	–
CRP diagnosis (100 mg/L)	1.28 (1.02–1.60)	.047	1.32 (1.05–1.66)	.018	1.22 (.94–1.59)	NS	–	–	1.95 (1.02–3.75)	.052	–	–
Temperature >37°C	1.30 (.83–2.04)	NS	–	–	1.23 (.73–2.08)	NS	–	–	1.89 (.75–4.75)	NS	–	–
Sinus tract	1.62 (.93–2.82)	.086	–	–	1.49 (.77–2.89)	NS	–	–	2.15 (.78–5.92)	NS	–	–
Abnormal radiography	2.24 (1.31–3.85)	.007	2.22 (1.30–3.81)	.004	1.77 (.92–3.42)	NS	–	–	3.60 (1.37–9.45)	.019	4.49 (1.68–12.0)	.003
Debridement delay >10 days[c]	1.57 (1.01–2.45)	.049	1.68 (1.07–2.64)	.024	1.85 (.91–3.77)	.089	–	–	1.50 (.63–3.58)	NS	–	–
Polyethylene exchange[d]	.57 (.34–.97)	.045	–	–	.70 (.36–1.37)	NS	–	–	.46 (.19–1.13)	.096	–	–
Need ≥2 debridements	3.15 (1.88–5.28)	<.001	3.82 (2.24–6.51)	<.001	4.34 (2.39–7.89)	<.001	5.36 (2.88–9.98)	<.001	1.62 (.54–4.81)	NS	–	–
Rifampin[e]	.55 (.34–0.87)	.011	.52 (.32–.83)	.006	.67 (.39–1.17)	NS	–	–	.27 (.11–.65)	.007	–	–
Levofloxacin + Rifampin[e]	.48 (.27–.88)	.010	–	–	.50 (.27–.92)	.019	.42 (.22–.80)	.008	–	NS	–	–
Vancomycin + Rifampin[e]	.45 (.17–1.24)	.081	–	–	–	–	–	–	.34 (.11–1.01)	.032	.29 (.10–.87)	.027

Patients with *Early Failure* were excluded from this analysis. For the multivariate analysis, variables with a *P* value < .10 in the univariate analysis were included in a stepwise backward selection process (*P*-in < .05 and *P*-out < .10 were used in each step, except [a], where *P*-out was <.05).

Abbreviations: CRP, C-reactive protein; HR (95%CI), hazard ratio (95% confidence interval); MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; NS, non-significant (*P* > .10).

[b] Time to infection: time from prosthesis placement to the onset of symptoms.

[c] Debridement delay: time from onset of symptoms to debridement.

[d] Polyethylene exchange not included in multivariate analysis due to a significant lack of data.

[e] All antimicrobial data refer to antibiotics received during more than 14 days within the first 30 days after debridement.

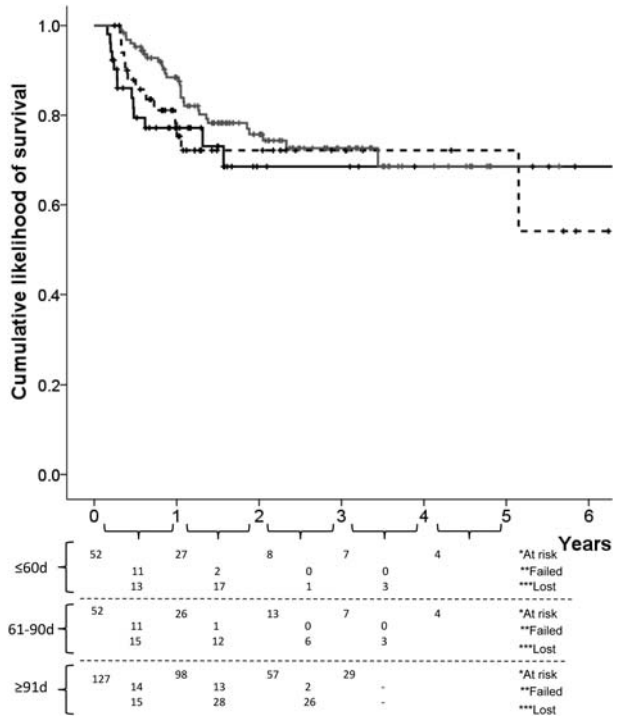

Figure 3. Comparative survival curves of different lengths for treatment among patients who finished the scheduled antimicrobial therapy without failing. Continuous black line: patients treated with antimicrobial therapy for 60 days or less (n = 52); discontinuous black line: patients treated for 61 to 90 days (n = 52); grey line: patients treated for more than 90 days (n = 127); log-rank-test: $P = .434$. *Number of patients at risk for failure at the beginning of the period. **Patients failing during the period. ***Number of patients lost to follow-up during the period.

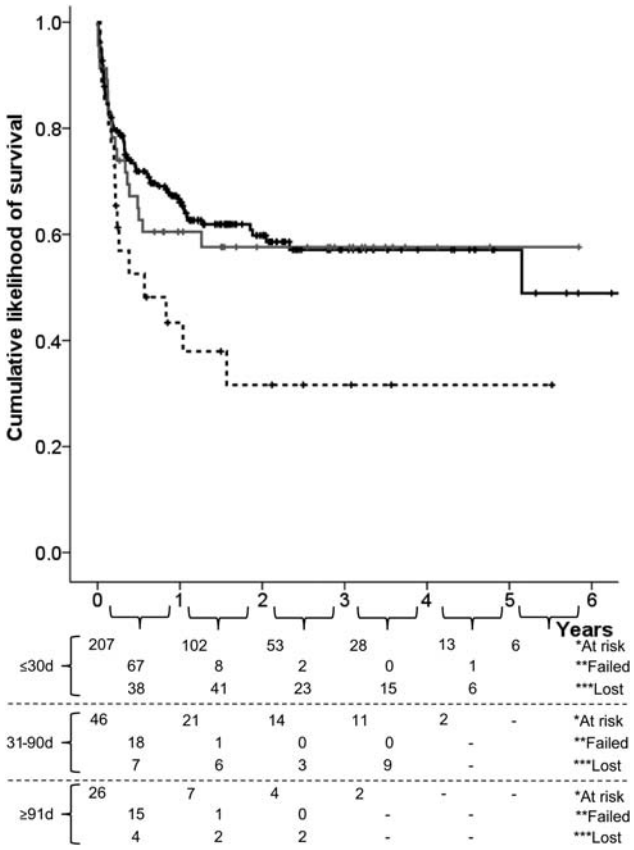

Figure 4. Influence of the time from prosthesis placement to the beginning of symptoms among post-surgical cases. Black continuous line: symptoms beginning less than 31 days after the placement of the prosthesis (n = 207; 38% failures). Grey continuous line: symptoms beginning 31 to 90 days after the placement of the prosthesis (n = 46; 41% failures). Black discontinuous line: symptoms beginning more than 90 days after the placement of the prosthesis (n = 26; 62% failures). Log-rank test: $P = .052$. *Number of patients at risk for failure at the beginning of the period. **Patients failing during the period. ***Number of patients lost to follow-up during the period. Six patients with unknown outcome were excluded from this analysis.

Potential conflicts of interest. All authors declare not to have any conflict of interest concerning this article. Regarding other activities outside this paper, A. S. declares having received honoraria from Pfizer and Novartis as payment for lectures; J. P. H. declares having received honoraria from Novartis, Pfizer, Astellas, MSD and Astra-Zeneca as payment for lectures; N. B. declares having received honoraria from Pfizer for consultancy tasks and development of educational presentations, and from MSD for consultancy tasks and also for the payment of travel/accommodation for scientific purposes; A. B. declares having received honoraria from Abbot, GlaxoSmithKline, Gilead, Novartis and Jansen for consultancy tasks, and also from Astra for lectures. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg Am **1996**; 78:512–23.
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic joint infections. N Negl J Med **2004**; 351:1645–54.
3. Sia IG, Berbari EF, Karchmer AW. Prosthetic joint infections. Infect Dis Clin N Am **2005**; 19:885–914.
4. El Helou OC, Berbari EF, Lahr BD, et al. Efficacy and safety of rifampin-containing regimen for staphylococcal prosthetic joint infections treated with debridement and retention. Eur J Clin Microbiol Infect Dis **2010**; 29:961–7.
5. Cobo J, Del Pozo JL. Prosthetic joint infection: diagnosis and management. Expert Rev Anti Infect Ther **2011**; 9:787–802.
6. Fisman DN, Reilly DT, Karchmer AW, Goldie SJ. Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. Clin Infect Dis **2001**; 32:419–30.
7. Marculescu CE, Berbari EF, Hanssen AD, et al. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis **2006**; 42:417–8.
8. Byren I, Bejon P, Atkins BL, et al. One hundred and twelve infected arthroplasties treated 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother **2009**; 63:1264–71.

9. Brandt CM, Sistrunk WW, Duffy MC, et al. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis **1997**; 24:914–9.
10. Vilchez F, Martínez-Pastor JC, García-Ramiro S, et al. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infection due to *Staphylococcus aureus* treated with debridement. Clin Microbiol Infect **2011**; 17:439–44.
11. Barberán J, Aguilar L, Carroquino G, et al. Conservative treatment of staphylococcal prosthetic joint infections in elderly patients. Am J Med **2006**; 119:993.e7–10.
12. Zimmerli W, Widmer AF, Blatter M, Frei R, Oschner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA **1998**; 279:1537–41.
13. Bradbury T, Fehring TK, Taunton M, et al. The fate of acute methicillin-resistant *Staphylococcus aureus* periprosthetic knee infections treated by open debridement and retention of components. J Arthroplasty **2009**; 24:101–4.
14. Kilgus DJ, Howe DJ, Strang BA. Results of periprosthetic hip and knee infections caused by resistant bacteria. Clin Orthop Relat Res **2002**; 404:116–24.
15. Soriano A, García S, Bori G, et al. Treatment of acute post-surgical infection of joint arthroplasty. Clin Microbiol Infect **2006**; 12: 930–3.
16. Salgado CD, Dash S, Cantey JR, Marculescu CE. Higher risk of failure of methicillin-resistant *Staphylococcus aureus* prosthetic joint infections. Clin Orthop Relat Res **2007**; 461:48–53.
17. Parvizi J, Pawasarat IM, Azzam KA, Joshi A, Hansen EN, Bozic KJ. Periprosthetic joint infection: the economic impact of methicillin-resistant infections. J Arthroplasty **2010**; 25:103–7.
18. Cobo J, Garcia San Miguel L, Euba G, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. Clin Microbiol Infect **2011**; 17:1632–7.
19. Ferry T, Uçkay I, Vaudaux P, et al. Risk factors for treatment failure in orthopedic device-related methicillin-resistant *Staphylococcus aureus* infection. Eur J Clin Microbiol Infect Dis **2010**; 29:171–80.
20. Aboltins CA, Page MA, Buising KL, et al. Treatment of staphylococcal prosthetic joint infections with debridement, prosthesis retention and oral rifampicin and fusidic acid. Clin Microbiol Infect **2007**; 13:586–91.
21. Koos WE, Lambe DW Jr. Staphylococcus. In: Balows A, Hausler WJ, Herrman KL, et al. Manual of Clinical Microbiology. 5th ed. Washinton, DC: American Society for Microbiology, **1991**:222–37.
22. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum **1988**; 31:315–24.
23. Senneville E, Joulie D, Legout L, et al. Outcome and predictors of treatment failure in total hip and knee prosthetic joint infections due to *Staphylococcus aureus*. Clin Infect Dis **2011**; 53:334–40.
24. Chuard C, Herrmann M, Vaudaux P, Waldvogel FA, Lew DP. Successful therapy of experimental chronic foreign-body infection due to methicillin-resistant *Staphylococcus aureus* by antimicrobial combinations. Antimicrob Agents Chemother **1991**; 35:2611–6.
25. Murillo O, Domenech A, Euba G, et al. Efficacy of linezolid alone and in combination with rifampin in staphylococcal experimental foreign-body infection. J Infect **2008**; 57:229–35.
26. Garrigós C, Murillo O, Euba G. Efficacy of usual and high doses of daptomycin in combination with rifampin versus alternative therapies in experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother **2010**; 54: 5251–6.
27. Vilchez F, Martínez-Pastor JC, García-Ramiro S, et al. Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. Int J Artif Organs **2011**; 34:863–9.
28. Rodríguez D, Pigrau C, Euba G, et al. Acute hematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. Clin Microbiol Infect **2010**; 16:1789–95.
29. Sendi P, Banderet F, Graber P, Zimmerli W. Clinical comparison between exogenous and hematogenous periprosthetic joint infections caused by *Staphylococcus aureus*. Clin Microbiol Infect **2011**; 17:1098–100.

Appendix

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EVALUATION OF HIGH DOSES OF DAPTOMYCIN (10 mg/kg/d) PLUS RIFAMPIN FOR THE TREATMENT OF STAPHYLOCOCCAL PROSTHETIC JOINT INFECTION MANAGED WITH IMPLANT RETENTION

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ABSTRACT

Background: In acute cases of prosthetic joint infection (PJI), debridement, antibiotics and implant retention (DAIR) may be attempted. The most appropriate treatment for fluoroquinolone-resistant staphylococci in this setting is uncertain. Daptomycin at high doses (10 mg/kg/d) plus rifampin (D10+R) have shown good results in experimental models, but clinical experience is scarce.

Methods: Retrospective, observational, multicenter study (2009-12). Patients with acute PJI by fluoroquinolone-resistant staphylococci managed with DAIR and D10+R as first-line therapy for six weeks were evaluated for safety and for clinical and microbiological failure. A comparison with matched historical cases of staphylococcal PJI managed with alternative rifampin-based combinations was also performed.

Results: 20 cases were initially included: two (10%) were withdrawn due to D10+R toxicity within the first 15 days of therapy, leaving 18 cases for efficacy evaluation: 13 (72%) women, age 79 yrs (58-90). PJI was caused by *S. aureus* in 11 cases (61%) and coagulase-negative staphylococci in 7 (39%). Clinical failure was observed in nine (50%) patients: in five cases staphylococci were recovered (28% of microbiological failures); no modification of daptomycin MIC was observed. Comparing the 18 cases with 44 matched historical cases, clinical and microbiological failure rates were similar, but whereas in the historical series failure occurred fundamentally during therapy, in the present series it was recorded mainly after discontinuation of antibiotics.

Interpretation: D10+R may be the initial treatment of choice for PJI by fluoroquinolone-resistant staphylococci managed with implant retention. Further studies are warranted to assess the optimal treatment for these infections.

INTRODUCTION

Prosthetic joint infection (PJI) is a fearsome complication after joint replacement (Zimmerli et al. 2004, Ariza et al. 2008, Del Pozo & Patel 2009, Moran et al. 2010). It frequently requires prosthesis removal and long courses of antimicrobials. However, in acute cases, a treatment comprising debridement, antibiotics and implant retention (DAIR) may be attempted (Zimmerli et al. 2004, Ariza et al. 2008, Byren et al. 2009), thus sparing the patient further surgery and loss of bone stock.

Staphylococcus aureus is the most likely etiology of acute PJI (Zimmerli et al. 2004, Ariza et al. 2008). In the DAIR setting, the antimicrobial treatment of choice is semi-synthetic penicillins followed by long courses of rifampin plus fluoroquinolones. However, methicillin-resistant *Staphylococcus aureus*, frequently resistant to fluoroquinolones, is not uncommon in this context (Peel et al. 2012, Lora-Tamayo et al. 2013). We recently observed that MRSA-PJI had a similar overall prognosis to that of methicillin-susceptible strains if rifampin was administered. However, we also observed that failure during treatment was more frequent in MRSA-PJI than in MSSA-PJI, suggesting that rifampin-combinations without fluroquinolones were less effective in avoiding relapse (Lora-Tamayo et al. 2013). Currently, the best treatment for fluoroquinolone-resistant strains remains uncertain.

Daptomycin is a recently introduced lipopeptide that has been approved for skin and soft-tissue infection and for bacteremia and endocarditis (Fenton et al. 2004, Enoch et al. 2007, Moise et al., 2009). Its high bactericidal activity against Gram-positive microorganisms, including both planktonic and biofilm-embedded bacteria (Enoch et al. 2004, Rybak 2006, Fenton et al. 2007, Murillo et al. 2009, Stewart et al. 2009), may

also make it suitable for treatment of PJI. However, its activity is concentration-dependent (Safdar et al. 2004, Ryback 2006, Enoch et al. 2007, Moise et al. 2009). The emergence of resistance along with clinical and microbiological failure has been reported with currently approved doses of 4-6 mg/kg/d (Manglini et al. 2005, Fowler Jr et al. 2006, Marty et al. 2006, Sharma et al. 2008, Dortet et al. 2013) and also in the setting of PJI (Rao & Regalla 2006). Current opinion supports the use of higher doses of daptoymicin (8-10 mg/kg/d) combined with a second drug in order to optimize its activity and avoid the emergence of resistance (Moise et al. 2009, Gould et al 2013).

RIfampin is key in the treatment of staphylococcal orthopedic device infections (Zimmerli et al. 1998, Zimmerli et al. 2004, Lora-Tamayo et al. 2013) and several experimental models have found the combination of high doses of daptomycin (equivalent to 8-10 mg/kg/d in humans) with rifampin to be the most effective treatment for foreign body-related infections caused by MRSA (Garrigos et al. 2010, Saleh-Mghir et al. 2011). Significantly, the combination also avoided the emergence of resistance (Garrigos et al. 2010, Saleh-Mghir et al. 2011).

There is little clinical information regarding the efficacy and safety of high doses of daptomycin in combination with rifampin in the setting of PJI due to methicillin-resistant *Staphylococcus* managed with DAIR (Corona et al. 2012, Jogun et al. 2013). We aimed to assess the efficacy and safety of daptomycin (10 mg/kg/d) plus rifampin in this clinical setting.

PATIENTS AND METHODS

Setting and patients

This retrospective observational study was performed in five Spanish hospitals between 2010 and 2012. All cases of fluoroquinolone-resistant staphylococcal post-surgical PJI managed with DAIR and high doses of daptomycin plus rifampin as first-line therapy were included in the analysis. A minimum of 15 days of treatment with the combination was required to assess its efficacy. The decision of treatment with daptomycin plus rifampin was taken at the discretion of the assisting medical team. Polymicrobial cases were not excluded. Cases where staphylococci were not the cause of the original infection, but participated later as superinfecting microorganisms, were not included. Cases were identified from prospective databases of cases with PJI.

Diagnosis of PJI was based on ≥2 positive surgical, joint-aspirated or blood positive cultures yielding the same microorganism with the same antimicrobial susceptibility. In the case of *S. aureus*, one such positive culture was considered sufficient, provided there were typical clinical symptoms and signs of PJI such as joint pain or local erythema, surgical wound discharge, the presence of a sinus tract or purulence surrounding the prosthesis during debridement (Brandt et al. 1997). Microorganisms were identified following standard criteria (Murray et al. 2007) after samples had been seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate and MacConkey agar) and incubated for at least 10 days. Vancomycin and daptomycin MIC values were determined by the E-test and microdilution methods.

Clinical and Surgical Management

The episode of PJI was classified as early, delayed or late, following Zimmerli et al (2004). The decision to use DAIR was taken in accordance with current guidelines (Zimmerli et al. 2004, Osmon et al. 2013). Broadly, there had to be an acute infection in a soundly fixed prosthesis and the periprosthetic soft tissue could not be badly damaged. DAIR has been described elsewhere (Byren et al. 2009, Vilchez et al. 2011). Briefly, it involves thorough surgical debridement of the prosthetic joint, including the exchange of its removable components. Non-cemented hemiarthroplasties (NC-HHA) usually undergo a 1-step prosthesis-exchange in these debridement surgeries; for the purposes of this study, these cases are also considered as DAIR-managed.

Antimicrobial therapy was usually withheld until microbiological samples had been taken, provided the patient was stable. After debridement, empirical broad spectrum antibiotics were administered. Once the microorganisms were identified and the antimicrobial susceptibilities determined, tailored antimicrobial therapy was initiated at the discretion of the medical team. In this case series, empirical treatment was administered for no longer than five days before switching to intravenous daptomycin at doses of 10 mg/kg/d. Rifampin was also administered (600 mg in a single fasting daily dose). Cases of polymicrobial PJI included in this review might also receive other antimicrobials, provided they did not have additional anti-Gram-positive activity.

Outcome and Follow-up.

Treatment with daptomycin plus rifampin was scheduled for a minimum of six weeks. A supplementary course of oral antibiotics might be administered at the discretion of the attending medical team. Close surveillance was performed during and after treatment focusing on clinical symptoms and signs suggesting relapse, including

determination of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). While on therapy, toxicity potentially related with the antimicrobial therapy was also assessed. Regarding daptomycin toxicity, patients were asked about muscle weakness or pain and creatine-phosphokinase (CPK) was routinely assessed.

Two primary outcomes were considered. By consensus among the investigators, **clinical failure** was defined as the persistence or reappearance of joint inflammatory signs during follow-up, usually requiring salvage therapy, such as extra debridements >15 days after the first one, prosthesis removal, or new courses of antimicrobial therapy. Clinical failure also included the need for suppressive antimicrobial therapy or the removal of the prosthesis for any cause (including orthopedic reasons within the first 2 years of follow-up). More specifically, **microbiological failure due to the original staphylococcal infection** was considered in any of the above cases when the *Staphylococcus* was again isolated from valuable samples, or if new cultures yielded no microorganisms in spite of persistent or reappearing purulent drainage from the surgical wound. Thus, cases with clinical failure and cultures yielding a microorganism other than *Staphylococcus* were not considered microbiological failure.

Cases were compared with matched historical controls with staphylococcal PJI. Matching was made by surgical approach (DAIR including exchange of removable components) and similar medical treatment with rifampin, defined as the use of a rifampin-based combination for at least 15 days [this minimal time under rifampin was a predictor of success in a previous study (Lora-Tamayo et al. 2013)]. Historical cases caused by *S. aureus* and coagulase-negative staphylococci (CNS) were searched in the REIPI's multicenter staphylococcal PJI database (32 of 81 cases due to MRSA met the

criteria) and the Hospital Clinic PJI database (12 of 43 episodes) respectively. Analyses of both databases have recently been published (Tornero et al. 2012, Lora-Tamayo et al, 2013).

Statistical analysis

Data were analyzed with SPSS (version 15.0). The Mann-Whitney *U* test and the X^2 test or the Fisher exact test were used to compare continuous and categorical variables respectively. All tests were 2-tailed, and a p value <0.05 was considered statistically significant.

RESULTS

Twenty patients presenting acute infection and undergoing DAIR were treated with daptomycin and rifampin as first line therapy. Of these, two (10%) did not complete the initially scheduled treatment with the combination due to persistent vomiting caused by rifampin within the first 15 days, finally leading to its withdrawal [one (5%) of them developed acute renal injury, and later rhabdomyolysis due to daptomycin].

Thus, 18 patients were analyzed for clinical and microbiological cure: 13 (72%) were women and median age was 79 years (range 58-90). Baseline characteristics and clinical data are summarized in Table 1. Infection was considered to be early in all patients, with symptoms beginning a median of 18 days (range 5-50) after prosthesis placement.

Infection was caused by *S. aureus* in 11 (61%) cases and CNS in seven (39%). All isolates but one (94%) were methicillin-resistant, and all were fluoroquinolone-resistant. Median daptomycin and vancomycin MICs were 0.25 mg/L (range 0.13 – 1) and 2 mg/L (range 1 – 4) respectively. There were five (28%) cases of polymicrobial infection. Nine (50%) presented with fever and in two (11%) bacteremia was documented (both PJI caused by *S. aureus*). Median leucocyte count and CRP level at diagnosis were $9.1 \cdot 10^9$/L (range 5.9-19.0) and 60 mg/L (range 27-190) respectively.

All patients underwent debridement with exchange of the removable components (and 1-step exchange in the four cases with NC-HHA) after a median time of 4.5 days since symptom onset (range 1-14).

Patients were treated with daptomycin at a median dose of 10.0 mg/kg/d (range 8.33-12.50) for a median of 42 days (range 35-56), with no significant adverse effects requiring discontinuation. CPK levels were measured while patients were on therapy in 13 cases (76%), and the normal upper limit was not exceeded. Rifampin was administered for a median of 42 days (range 35-74). Three (17%) cases were treated with supplementary antibiotics after daptomycin.

Outcome is summarized in Table 2. Nine (50%) cases were considered cured. Two very old and fragile patients died early after the end of therapy (both due to causes unrelated to the infection and without clinical signs of relapse) and one patient was followed up for only a short time. For the other six cases, median follow-up was 749 days (range 387-970).

Clinical failure was recorded in nine patients (50%): the same *Staphylococcus* spp was recovered in five (28% of microbiological failures). Neither daptomycin nor rifampin MIC showed any alterations in four of these cases (in one case this could not be tested). Four cases failed without evidence of staphylococcal persistence or relapse: one due to superinfection by Gram-negative and anaerobic bacteria; one polymicrobial infection (including *E. coli*) presenting a sinus tract nine months after the initial debridement, and with positive culture for *E. coli*; another polymicrobial staphylococcal-enterococcal infection presenting relapse of inflammatory signs in whom the prosthesis was finally removed and surgical cultures showed persistence of *E. faecalis*; and the fourth developed wound dehiscence without discharge and with negative cultures long after discontinuation of antimicrobial therapy. Interestingly, there was only one microbiological failure in a patient still under antimicrobial

treatment (caused by CNS). We identified no parameters associated with a higher likelihood of clinical or microbiological failure, including daptomycin MIC (data not shown).

The present series was compared with a historical cohort of 44 cases of staphylococcal PJI (32 by *S. aureus* and 12 by CNS). Those patients were given antimicrobials for a median of 82 days (IQR 52-111) with treatments that were mainly rifampin-based, usually with vancomycin for 1-2 weeks and then followed by an oral combination, commonly with co-trimoxazole. Table 3 summarizes this comparison. Baseline features, clinical presentation and surgical approach were similar, as were the rates of clinical and microbiological failure. In contrast, clinical failure in the historical series occurred mainly when patients were still under therapy. A similar trend was observed for microbiological failure, which was statistically significant when analyzed in the *S. aureus* episodes [microbiological failure while on therapy 7/8 (88%) in the historical cohort vs. 0/3 (0%) in the present case series (p=0.024)]. Microbiological failure during therapy in the historical cohort occurred a median of 38 days after debridement, and in three patients after 42 days (48, 86 and 123 days).

DISCUSSION

This is the first study addressing the efficacy and safety of a 6-week course of parenteral daptomycin at high doses plus rifampin as first-line therapy for acute staphylococcal PJI managed with DAIR. Clinical failure was recorded in 50%. In accordance with previous work by our group (Rodriguez et al. 2010, Cobo et al. 2011, Lora-Tamayo et al. 2013) our definition of clinical failure is quite broad, thus mirroring the complexity of patients with PJI. This definition may overestimate the actual failure rate due to staphylococcal persistence or relapse, since failure may be due to other causes such as superinfection by other microorganisms or orthopedic issues. To analyze the impact of the daptomycin-rifampin combination on the staphylococcal infection more precisely, we assessed microbiological failure due to the original staphylococcal infection, establishing a rate of 28% (and thus a rate of microbiological cure of 72%).

Our percentage of clinical failure is lower than that of the standard therapy against MRSA PJI based on glycopeptides [23%, according to Bradbury et al. (2009)]. It is uncertain if outcomes would have been worse if our patients had been treated with vancomycin, taking into account that median vancomycin MIC was 2 mg/L (obtained by the E-test method). Our percentage of clinical failure is also in the middle range compared with other case series of staphylococcal PJI (Brandt et al. 1997, Barberan et al. 2006, Aboltins et al. 2007, Vilchez et al. 2011, Senneville et al. 2011). However, most of those studies include low numbers of cases by methicillin-resistant staphylococci. Our group recently reported a large retrospective cohort of PJI by *S. aureus* managed with DAIR, including 81 cases by MRSA (Lora-Tamayo et al. 2013).

Most of these cases were treated with a rifampin-based regime, initially including vancomycin. Overall cure rate was 54% and, unlike methicillin-susceptible cases, most failures (88%) occurred while patients were still under therapy, suggesting that fluoroquinolone plus rifampin was more effective for avoiding failure than alternative rifampin-based regimes (Lora-Tamayo et al. 2013).

We also compared the present series with historical matched cases of staphylococcal PJI. Patients in both series had an *a priori* good prognosis: they had non-hematogenous infections with a low rate of bacteremia and underlying immunosuppressive therapy, and all had been treated with ≥15 days of rifampin. Interestingly, most failures in the historical cohort happened in spite of continued antimicrobial therapy, while most cases failing in the present series failed after antibiotic withdrawal. Taking into account the potential biases of this comparison, this suggests that the combination of daptomycin plus rifampin was more active and capable of delaying failure, as fluoroquinolones and rifampin do in the setting of methicillin-susceptible *S. aureus* infection (Lora-Tamayo et al. 2013).

Most of our patients were treated intravenously for six weeks, a period similar to that of classical case series when patients were given parenteral beta-lactams or glycopeptides (Brandt et al. 1997). The retrospective nature of our work makes it difficult to establish why three patients received oral supplementary therapy – this might indicate a difficulty in achieving clinical improvement, thus leading the medical team to maintain the antibiotics. In fact, the optimal duration of antimicrobial therapy is unknown, and long treatments have not been associated with better outcomes (Byren et al. 2009, Lora-Tamayo et al. 2013). It is not clear whether prolonging

antimicrobial therapy would have improved the results. In this regard, the intravenous administration of daptomycin precludes the use of longer treatments, but our results suggest that the combination of daptomycin at high doses plus rifampin could be used as initial therapy for several weeks, followed sequentially by an oral combination, ideally rifampin-based, for a supplementary period.

Daptomycin is attractive for the treatment of PJI by Gram-positive microorganisms: it is rapidly bactericidal, maintains its activity against adherent bacteria (Ryback 2006, Stewart et al. 2009) and has a rapid diffusion in biofilm (Stewart et al. 2009). Although active efflux transport significantly reduces its intracellular activity (Lemaire et al. 2007), this activity remains comparable to that of other antimicrobials (Mélard et al. 2013). Daptomycin is also active against adaptive forms such as small-colony variants (Baltch et al. 2008). However, there are few reports of its use in the setting of PJI managed with implant retention, fewer still with the combination of rifampin, and none with the doses we have used (Rao & Regalla 2006, Corona et al. 2012, Byren et al. 2012, Jogun et al. 2013).

The activity of daptomycin is concentration-dependent (Safdar et al. 2004, Ryback 2006, Moise et al. 2009). It is highly bound to proteins (Fenton et al. 2004, Enoch et al. 2007), and current concentrations at the infection site may not be sufficient. In the particular case of bone infections, the actual antimicrobial concentration is unclear due to the substantial variability between studies (Landersdorfer et al. 2009). Microdialysis studies in diabetic foot infection suggest that the concentration of daptomycin is similar to the protein-unbound fraction (Traunmüller et al. 2010), with a bone:serum concentration ratio comparable to glycopeptides or beta-lactams (Landersdorfer et al.

2009). In addition, heteroresistance to daptomycin has been described in several strains of *Staphylococci*, which makes it even more important to achieve high concentrations at the site of infection (Moise et al. 2009, Gould et al. 2013). The use of high doses of daptomycin plus its combination with a second antimicrobial (e.g. rifampin) may overcome the risk of clinical failure and the emergence of resistance (Moise et al. 2009, Gould et al 2013).

In this regard, in a tissue-cage experimental animal model Garrigós et al. (2010) showed that the combination of rifampin with daptomycin at high doses was significantly more active than other rifampin-based regimes. Interestingly, in these experiments, no daptomycin-resistance emergence was observed with the combination therapy (Garrigos et al. 2010). It may be argued that the concentrations of daptomycin achieved in the tissue cage of these experimental models may be higher than those actually obtained in bone tissue, thus overestimating the real effects of the antibiotic. However, similar results have been observed in another experimental animal model of PJI with rifampin plus daptomycin at doses equivalent to 8 mg/kg/d (Saleh-Mghir et al. 2011). Indeed, in our series, using high doses of daptomycin in combination with rifampin, we observed no cases of daptomycin or rifampin MIC modification among failures. Nor did these cases with a non-favorable outcome present with higher initial daptomycin MIC values.

The potential toxicity of high doses of daptomycin has been a cause for concern. However, doses of 12 mg/kg/d during 14 days have been well tolerated in healthy volunteers (Benvenuto et al. 2006). In our series, two patients (10%) on a 6-week course with the combination had to withdraw because of serious adverse effects,

daptomycin being the responsible drug in one case (5%). This percentage is lower than in other series of PJI using long courses of daptomycin (Rao & Regalla 2006, Moise et al. 2009, Corona et al. 2012, Byren et al. 2012, Gould et al. 2013).

Our study has several limitations. First, the total number of patients is relatively low; however, the cases included comprise a very homogenous cohort, and there is no previous information on the use of this antimicrobial combination in the setting of PJI managed with implant retention. Second, it is a retrospective study, and comparisons with historical cohorts may be biased. Further prospective studies are warranted, ideally comparing different regimes for MRSA PJI. Finally, some cases of non-cemented hemiarthroplasty infection were included – as these cases underwent a one-step prosthetic exchange, the quality of debridement may have been better than standard DAIR. Nevertheless, we recently showed that the overall prognosis for infected hip-hemiarthroplasties and total-hip arthroplasties managed with implant retention was similar (Lora-Tamayo et al. 2013).

In summary, we present the first case series of PJI treated with DAIR and the combination of daptomycin at high doses (10mg/kg/d) plus rifampin as first-line therapy. Treatment tolerance was good, and clinical and microbiological outcomes were comparable to previous regimes used for these infections. It is not clear whether prolonging antimicrobial therapy might have improved outcomes. Interestingly, the failure rate during therapy was lower than that previously reported, and no resistant microorganisms emerged in cases of failure. These results suggest that daptomycin at high doses plus rifampin could be considered as treatment of choice during the first weeks after debridement in the setting of fluoroquinolone-resistant staphylococcal PJI

managed with implant retention. This would be mandatory when the infection is due to strains with a high vancomycin MIC value.

AUTHOR CONTRIBUTION

JL-T, CP, JA and AS have participated in the conception and design of the study. JL-T, JP-R, DR-P, JB, AR and ET have been involved in the acquisition of the patients' data. JL-T and JA have performed the analysis of the data. JL-T, DR-P, CP, JM, JA and AS have participated in the drafting of the article and/or its critical revision. Finally, all authors have given their final approval of manuscript.

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CONFLICT OF INTEREST DISCLOSURE

All authors declare that no direct external support, grants or another ways of funding have been received for this study. They also declare that, non-related with the present article, JL-T has received honoraria and travel grants for scientific purposes for public speaking from Novartis and advisory board from Pfizer; JP-R is a speaker, consultant or received grant support from Astellas, Cubist, Bayer and Pfizer; DR-P declares having received honoraria from Pfizer and Novartis as payment for the development of educational lectures, and payment for travel for scientific purposes; J.B. has received speaking and advisory board honoraria from Novartis and Pfizer; CP has received speaking honoraria from Merk, Novartis and Pfizer; AS has received honoraria for public speaking for Pfizer and Novartis; and JM has received honoraria for public speaking for Pfizer and Novartis.

REFERENCES

Aboltins CA, Page MA, Buising KL, Jenney AW, Daffy JR, Choong PF, Stanley PA. Treatment of staphylococcal prosthetic joint infections with debridement, prosthesis retention and oral rifampicin and fusidic acid. Clin Microbiol Infect 2007; 13: 586-91.

Ariza J, Euba G, Murillo O. Orthopedic device-related infections. Enferm Infecc Microbiol Clin. 2008; 26: 380-90.

Baltch AL, Ritz WJ, Bopp LH, Michelsen P, Smith RP. Activities of daptomycin and comparative antimicrobials, singly and in combination, against extracellular and intracellular Staphylococcus aureus and its stable small-colony variant in human monocyte-derived macrophages in broth. Antimicrob Agents Chemother 2008; 52: 1829-33.

Barberán J, Aguilar L, Carroquino G, Giménez MJ, Sánchez B, Martínez D, Prieto J. Conservative treatment of staphylococcal prosthetic joint infections in elderly patients. Am J Med 2006; 119: 993.e7-10.

Benvenuto M, Benziger DP, Yankelev S, Vigliani G. Pharmacokinetics and tolerability of daptomycin at doses up to 12 milligrams per kilogram of body weight once daily in healthy volunteers. Antimicrob Agents Chemother 2006; 50: 3245-9.

Bradbury T, Fehring TK, Taunton M, Hanssen A, Azzam K, Parvizi J, Odum SM. The fate of acute methicillin-resistant Staphylococcus aureus periprosthetic knww infections treated by open debridement and retention of components. J Arthroplasty 2009; 24: 101-4.

Brandt CM, Sistrunk WW, Duffy MC, Hanssen AD, Steckelberg JM, Ilstrup DM, Osmon DR. Staphylococcus aureus prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis 1997; 24: 914-9.

Byren I, Bejon P, Atkins BL, Angus B, Masters S, McLardy-Smith P, Gundle R, Berendt A. One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother 2009; 63: 1264-71.

Byren I, Rege S, Campanaro E, Yankelev S, Anastasiou D, Kuropatkin G, Evans R. Randomized controlled trial of safety and efficacy of daptomycin versus standard-of-care therapy for management of patients with osteomyelitis associated with prosthetic devices undergoing two-stage revision arthroplasty. Antimicrob Agents Chemother 2012; 56: 5626-32.

Cobo J, Miguel LG, Euba G, Rodríguez D, García-Lechuz JM, Riera M, Falgueras L, Palomino J, Benito N, del Toro MD, Pigrau C, Ariza J. Early prosthetic joint infection : outcomes with debridement and implant retention followed by antibiotic therapy. Clin Microbiol Infect 2011; 17: 1632-7.

Corona Pérez Cardona PS, Barro Ojeda V, Rodríguez Pardo D, Pigrau Serrallach C, Guerra Farfán E, Amat Mateu C, Flores Sánchez X. Clinical experience with daptomycin for the treatment of patients with knee and hip perisprosthetic joint infections. J Antimicrob Chemother 2012 ; 67: 1749-54.

Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. N Engl J Med 2009; 361: 787-94.

Dortet L, Anguel N, Fortineau N, Richard C, Nordmann P. In vivo acquired daptomycin resistance during treatment for methicillin-resistant Staphylococcus aureus endocarditis. Int J Infect Dis 2013; 17: e.1976-6.

Enoch DA, Bygott JM, Daly ML, Karas JA. Daptomycin. J Infect 2007; 55: 205-13.

Fenton C, Keating GM, Curran MP. Daptomycin. Drugs 2004; 64: 445-55.

Fowler VG Jr, Boucher HW, Corey GR, Abrutyn E, Karchmer AW, Rupp ME, Levine DP, Chambers HF, Tally FP, Vigliani GA, Cabell CH, Link AS, DeMeyer I, Filler SG, Zervos M, Cook P, Parsonnet J, Bernstein JM, Price CS, Forrest GN, Fätkenheuer G, Gareca M, Rehm SJ, Brodt HR, Tice A, Cosgrove SE. Daptomycin versus standard therapy for bacteremia and endocarditis caused by Staphylococcus aureus. N Engl J Med 2006; 355: 653-65.

Garrigos C, Murillo O, Euba G, Verdaguer R, Tubau F, Cabellos C, Cabo J, Ariza J. Efficacy of usual and high doses of daptomycin in combination with rifampin versus alternative therapies in experimental foreign-body infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2010; 54: 5251-6.

Gould IM, Miró JM, Rybak MJ. Daptomycin: the role of high-dose and combination therapy for Gram-positive infections. Int J Antimicrob Agents 2013; 42: 202-10.

Jogun K, Vaudaux P, Garbino J, Pagani L, Hoffmeyer P, Lew D, Uçkay I. The safety and efficacy of high-dose daptomycin combined with rifampicin for the treatment of Gram-positive osteoarticular infections. Int Orthop 2013; 37: 1375-80.

Landersdorfer CB, Bulitta JB, Kinzig M, Holzgrabe U, Sörgel F. Penetration of antibacterials into bone. Pharmacokinetic, pharmacodynamic and bioanalytical considerations. Clin Pharmacokinet 2009; 48: 89-124.

Lemaire S, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM. Modulation of the cellular accumulation and intracellular activity of daptomycin towards phagocytized Staphylococcus aureus by the P-glycoprotein (MDR1) efflux transporter in human THP-1 macrophages and Madin-Darby canine kidney cells. Antimicrob Agents Chemother 2007; 51: 2748-57.

Lora-Tamayo J, Murillo O, Iribarren JA, Soriano A, Sánchez-Somolinos M, Baraia-Etxaburu JM, Rico A, Palomino J, Rodríguez-Pardo D, Horcajada JP, Benito N, Bahamonde A, Granados A, del Toro MD, Cobo J, Riera M, Ramos A, Jover-Sáenz A, Ariza J. A large multicenter study of methicillin-susceptible and methicillin-resistant Staphylococcus aureus prosthetic joint infections managed with implant retention. Clin Infect Dis 2013; 56: 182-94.

Lora-Tamayo J, Euba G, Ribera A, Murillo O, Pedrero S, García-Somoza D, Pujol M, Cabo X, Ariza J. Infected hip-hemiarthroplasties and total hip arthroplasties: differential findings and prognosis. J Infect 2013; 67: 536-44.

Mangilini A, Bica I, Snydman DR, Hamer DH. Daptomycin-resistant, methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis 2005; 40: 1058-60.

Marty FM, Yeh WW, Wennersten CB, Venkataraman L, Albano E, Alyea EP, Gold HS, Baden LR, Pillai SK. Emergence of a clinical daptomycin-resistant Staphylococcus

aureus isolate during treatment of methicillin-resistant Staphylococcus aureus bacteremia and osteomyelitis. J Clin Microbiol 2006; 44: 595-7.

Mélard A, Garcia LG, Das D, Rozenberg R, Tulkens PM, Van Bambeke F, Lemaire S. Activity of ceftaroline against extracellular (broth) and intracellular (THP-1 monocytes) of methicillin-resistant Staphylococcus aureus: comparison with vancomycin, linezolid and daptomycin. J Antimicrob Chemother 2013; 68: 648-58.

Moise PA, North D, Steenbergen JN, Sakoulas G. Susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus facts and assumptions. Lancet Infect Dis 2009; 9: 614-24.

Moise PA, Hershberger E, Amodio-Groton MI, Lamp KC. Safety and clinical outcomes when utilizing high-dose (≥8 mg/kg) daptomycin therapy. Ann Pharmacother 2009; 43: 1211-9.

Moran E, Byren I, Atkins BL. The diagnosis and management of prosthetic joint infections. J Antimicrob Chemother 2010; 65 Suppl 3: iii45-54.

Murillo O, Garrigós C, Pachón ME, Euba G, Verdaguer R, Cabellos C, Cabo J, Gudiol F, Ariza J. Efficacy of high doses of daptomycin versus alternative therapies against experimental foreign-body infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2009; 53: 4252-7.

Murray PR, Baron EJ, Jorgesen JH, Landry ML, Pfaller MA. Manual of Clinical Microbiology, 9th ed. ASM Press, Washington, DC 2007.

Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR. Diagnosis and management of prosthetic joint infection:

clinical practice guidleines by the Infectious Diseases Society of America. Clin Infect Dis 2013; 56: e1-25.

Peel TN, Cheng AC, Buising KL, Choong PF. Microbiological aetiology, epidemiology, and clinical profile of prosthetic joint infections: are current antibiotic prophylaxis guidelines effective? Antimicrob Agents Chemother 2012; 56: 2386-91.

Rao N, Regalla DM. Uncertain efficacy of daptomycin for prosthetic joint infections. Clin Orthop Rel Res 2006; 451: 34-7.

Rodríguez D, Pigrau C, Euba G, Cobo J, García-Lechuz J, Palomino J, Riera M, del Toro MD, Granados A, Ariza X. Acute hematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. Clin Microbiol Infect 2010; 16: 1789-95.

Ryback MJ. The efficacy and safety of daptomycin: first in a new class of antibiotics for Gram-positive bacteria. Clin Microbiol Infect 2006; 12 (Suppl. 1): 24-32.

Safdar N, Andes D, Craig WA. In vivo pharmacodynamic activity of daptomycin. Antimicrob Agents Chemother 2004; 48: 63-8.

Saleh-Mghir A, Muller-Serieys C, Dinh A, Massias L, Crémieux AC. Adjunctive rifampin is crucial to optimizing daptomycin efficacy against rabbit prosthetic joint infection due to methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2011; 55: 4589-93.

Senneville E, Joulie D, Legout L, Valette M, Dezèque H, Beltrand E, Roselé B, d'Escrivan T, Loïez C, Caillaux M, Yazdanpanah Y, Maynou C, Migaud H. Outcome and predictors of treatment failure in total hip and knee prosthetic joint infections due to Staphylococcus aureus. Clin Infect Dis 2011; 53: 334-40

Sharma M, Riederer K, Chase P, Khatib R. High rate of decreasing daptomycin susceptibility during the treatment of persistent Staphylococcus aureus bacteremia. Eur J Clin Microbiol Infect Dis 2008; 27: 433-7.

Stewart PS, Davison WM, Steenbergen JN. Daptomycin rapidly penetrates a Staphylococcus epidermidis biofilm. Antimicrob Agents Chemother 2009; 53: 3505-7.

Tornero E, García-Oltra E, García-Ramiro S, Martínez-Pastor JC, Bosch J, Climent C, Morata L, Camacho P, Mensa J, Soriano A. Prosthetic joint infections due to Staphylococcus aureus and coagulase-negative staphylococci. Int J Artif Organs 2012; 35: 884-92.

Traunmüller F, Schintler MV, Metzler J, Spendel S, Mauric O, Popovic M, Konz KH, Scharnagl E, Joukhadar C. Soft tissue and bone penetration abilities of daptomycin in diabetic patients with bacterial foot infections. J Antimicrob Chemother 2010; 65: 1252-7.

Vilchez F, Martínez-Pastor JC, García-Ramiro S, Bori G, Maculé F, Sierra J, Font L, Mensa J, Soriano A. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infection due to Staphylococcus aureus treated with debridement. Clin Microbiol Infect 2011; 17: 439-44.

Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. JAMA 1998; 279: 1537-41.

Zimmerli W, Trampuz A, Ochsner PE. Prosthetic joint infections. N Engl J Med 2004; 351: 1645-1654.

Table 1 – Characteristics of patients with staphylococcal PJI managed with DAIR and daptomycin (10 mg/kg/d) plus rifampin

Sex/ Age (years)	Comor-bidity	Prosthesis Location*	Time to Infection[§]	Etiology	MIC for Dapto/Vanco (mg/L)	Time to Debrid[#]/ Nº Debrid	Daptomycin Dose[†]/ Length[‡]	Rifampin Length[‡]	Suppl.ATB (length[‡])	Clinical Cure / Follow-up[‡]	Microorg. in failure
F / 63	None	THA*	11	MRSA	0.25 / 2	2 / 1	10.0 / 41	41	No	No / 82	MRSA
F / 60	Cancer	THA	13	CNS	1 / 4	1 / 1	10.0 / 43	43	No	Yes / 387	-
F / 85	None	THA	8	CNS + *K. pneumoniae*	0.38 / 3	5 / 2	10.0 /42	44	No	Yes / 785	-
M / 79	DM	THA	30	MRSA	0.19 / 2	10 / 2	10.0 / 56	56	No	No / 0	*E. coli*, *Klebsiella*, Anaerobes
F / 90	Dementia	HHA	19	CNS	1 / 3	5 /1	10.0 / 39	39	No	Yes / 15	-
F / 84	Dementia	HHA	48	MRSA	0.25 / 2	4 /1	12.5 / 39	39	No	Yes / 970	-
F / 85	DM, Dementia	HHA	5	MRSA + *E. coli*	0.25 / 2	3 /2	8.8 /39	42	No	No / 20	Negative cultures
F / 70	Corticoids	THA	20	MRSA, *Proteus*, *P. aeruginosa*	0.25 / 2	14 / 2	11.0 / 42	42	No	No / 288	*E. coli*
M / 78	DM, COPD, Dementia	HHA	13	MRSA + *Enterobacter*	0.19 / 3	3 / 2	10.8 / 42	45	LNZ+RIF (14)	Yes / 8	-
F / 80	None	THA*	50	MRSA	0.19 / 2	3/ 2	10.0 / 37	51	LNZ+RIF(14)	Yes / 21	-
F / 69	None	Knee	28	MRSA	0.125/1	2 / 1	9.2 /47	41	No	No / 91	MRSA
M / 81	DM	Knee	17	MRSA	0.125/1.5	5 / 1	9.3 / 46	74	CMX+RIF (41)	No / 113	MRSA
F / 84	None	THA*	14	CNS + *E. faecalis*	≤1* / ≤4*	1 / 1	8.3 / 44	44	No	No / 483	*E. faecalis*
M / 58	DM	Knee	28	MSSA	0.5/1.0	1 / 1	9.5 / 44	42	No	Yes / 747	-
M / 80	DM	Knee	22	CNS	0.5/1.0	6 / 1	9.7 / 48	45	No	Yes / 751	-
F / 63	DM	Knee	26	CNS	0.5/1.0	9 / 2	9.7 / 51	49	No	No / 76	SCN
F / 72	None	Knee	9	MRSA	≤1* / ≤4*	6 / 1	10.5 / 35	35	No	Yes / 731	-
F / 68	None	Knee*	12	CNS	≤1* / ≤4*	9 / 1	9.9 / 42	42	No	No / 0	SCN

All cases were early post-surgical. M: male; F: female; DM: diabetes mellitus. Cort: chronic treatment with corticosteroids. COPD: chronic obstructive pulmonary disease. [§]Revision prosthesis. THA: total hip artrhoplasty; HHA: hip hemiarthroplasty. [Ø]Time to infection: time from prosthesis placement to beginning of symptoms (in days). MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *S. aureus*. CNS: coagulase-negative *Staphylococcus*. MIC for Dapto/Vanco: minimal inhibitory concentration for daptomycin and vancomycin, respectively; values obtained by the E-test method or *microdilution. [#]Time to debridement: time from beginning of symptoms to debridement. Nº debrid: number of debridements within the first 15 days after the first debridement. †Dose of daptomycin expressed in mg/kg/d. ‡Length of antimicrobial therapy and follow-up are expressed in days. LNZ: linezolid; RIF: Rifampin; CMX: co-trimoxazole.

Table 2 – Outcome of patients with staphylococcal PJI submitted to debridement and treatment with daptomycin (10mg/kg/d) plus rifampin.

	All (n=18)	CNS (n=7)	S. aureus (n=11)	p
Clinical failure	9 (50%)	3 (43%)	6 (55%)	1.00
Clinical failure while on treatment	2/9 (22%)	1/3 (33%)	1/6 (17%)	1.00
Microbiological failure	5 (28%)	2 (29%)	3 (27%)	1.00
Microbiological failure while on treatment	1/5 (20%)	1/2 (50%)	0/3 (0%)	0.40

See definitions in the text – microbiological failure are failures due to the same Staphylococcus originally causing the infection.

Table 3 – Comparison of the present series of PJI with a historical cohort of PJI by staphylococci also managed with DAIR, exchange of removable components and ≥15 days of an alternative rifampin-based combination.

	Historical series (n=44)	Present Series (n=18)	p
Infection by *S. aureus*	32 (73%)	11 (61%)	0.368
Sex (women)	24 (55%)	13 (72%)	0.198
Age (years)	74 (66-79)	79 (67-84)	0.274
Diabetes	10 (23%)	7 (39%)	0.214
Renal chronic failure	4 (9%)	0 (0%)	0.310
Immunosuppressive therapy	2 (5%)	1 (6%)	1.000
Any comorbidity	18 (42%)	11 (61%)	0.170
Knee prosthesis	22 (50%)	7 (39%)	0.426
Non-cemented hip hemiarthroplasty	4 (9%)	4 (22%)	0.214
Revision prosthesis	11 (25%)	4 (22%)	1.000
Hematogenous infection	2 (5%)	0 (0%)	1.000
Bacteremia	3 (8%)	2 (11%)	0.646
Polymicrobial infection	9 (21%)	5 (28%)	0.524
CRP at diagnosis (mg/L)	37 (8-111)	60 (37-173)	0.174
Time to debridement (days)*	6 (3-13)	5 (2-7)	0.113
Exchange of removable components	44 (100%)	18 (100%)	-
Clinical failure	15 (34%)	9 (50%)	0.265
Clinical failure while on therapy	11/15 (73%)	2/9 (22%)	**0.033**
Microbiological failure	13 (30%)	5 (29%)	1.000
Microbiological failure while on therapy	9/13 (69%)	1/5 (20%)	0.118

Continuous variables expressed in median (and interquartile range); categorical variables expressed in absolute number (and percentage). *Time from onset of symptoms to surgery of debridement. CRP: C-reactive protein.

Running title: Ciprofloxacin use and Gram negative PJI

Gram-negative Prosthetic Joint Infection: Outcome of a Debridement, Antibiotics, and Implant Retention Approach. A Large Multicenter Study

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Key-Points: Outcome of gram-negative PJI treated with debridement, antibiotics, and implant retention was evaluated. Overall success rate was 68%, which increased to 79% when ciprofloxacin was used. Ciprofloxacin treatment exhibited a protective effect, while chronic renal impairment was predictive of failure.

Background. To evaluate the epidemiology and outcome of gram-negative prosthetic joint infection (GN-PJI) treated with debridement, antibiotics, and implant retention (DAIR), identify factors predictive of failure, and determine the impact of ciprofloxacin use on prognosis.

Methods. Retrospective, multicenter, observational study of GN-PJI diagnosed from 2003 through 2010 in 16 Spanish hospitals. Failure: persistence or reappearance of the inflammatory joint signs during follow-up, leading to unplanned surgery, related death, or suppressive antimicrobial therapy. Parameters predicting failure were analyzed with a Cox regression model.

Results. A total of 242 patients (33% men; median age 76 years, interquartile range [IQR] 68-81) with 242 episodes of GN-PJI were studied. The implants included 150 (62%) hip, 85 (35%) knee, 5 (2%) shoulder, and 2 (1%) elbow prostheses. There were 189 (78%) acute infections. Causative microorganisms were *Enterobacteriaceae* in 78%*, Pseudomonas* spp. in 20%, and other gram-negative bacilli in 2%. Overall, 19% of isolates were ciprofloxacin resistant. DAIR was used in 174 (72%) cases, with an overall success rate of 68%, which increased to 79% after a median of 25 months' follow-up in ciprofloxacin-susceptible GN-PJIs treated with ciprofloxacin. Ciprofloxacin treatment exhibited an independent protective effect (adjusted hazard ratio [aHR] 0.23, 95%CI, 0.13-0.40; *P*<0.001), whereas chronic renal impairment predicted failure (aHR, 2.56, 95%CI, 1.14-5.77; *P*=0.0232).

***Conclusions*.** Our results confirm a 79% success rate in ciprofloxacin-susceptible GN-PJI treated with debridement, ciprofloxacin, and implant retention. New therapeutic strategies are needed for ciprofloxacin-resistant PJI.

Prosthetic joint infection (PJI) is an uncommon complication (1%-2%) of joint replacement surgery that is associated with high morbidity and medical expenditure [1, 2]. The most frequently isolated microorganisms are gram-positive cocci. However, gram-negative bacteria (GNB) constitute 10% to 23% of all episodes, and these infections are often acute [3-5].

In patients with acute PJI and a stable implant, conservative management can be attempted, consisting of prompt debridement and implant retention, combined with prolonged pathogen-targeted therapy with antibiotics active against surface-adhering microorganisms [4-6]. This conservative approach has a success rate for staphylococcal infections ranging from 55% to over 75% [7-9]. In the case of gram-negative PJI (GN-PJI) there is little published experience, the data regarding treatment efficacy are inconsistent [3, 10-15] and often published series include mixed infections caused by both gram-positive and gram-negative bacteria; hence, it is difficult to know the true success rates in GN-PJI treated with debridement, antibiotics, and implant retention. Even so, in 2009, Martinez-Pastor *et al*. [10] reported a 74.5% success rate in their GN-PJI series, and identified C-reactive protein (CRP) concentration of $\leq$15 mg/dL at diagnosis and fluoroquinolone treatment as factors associated with a favorable prognosis. The growing resistance of GNBs to ciprofloxacin may increasingly complicate GN-PJI treatment and outcome.

We present a large multicenter series of GN-PJIs treated with debridement, antibiotics, and implant retention (DAIR). The aims of the study were to assess the efficacy of DAIR, identify predictive factors of failure, and establish the impact of ciprofloxacin use on the prognosis.

METHODS

Study Design

A retrospective observational cohort study performed in 16 Spanish hospitals in the framework of the Spanish Network for Research in Infectious Disease (REIPI).

Study Population

Cases were identified by searching in databases of previously recorded consecutive PJIs or the general archives of each participating hospital. All PJIs originally caused by GNB and diagnosed from January 2003 through December 2010 were examined. Polymicrobial infections caused by more than one GNB were included, but those caused by GNB and gram-positive cocci were excluded to assess the true impact of GNB in PJI. Patients in whom GNB did not cause the original PJI, but participated later as a superinfecting microorganism were also excluded.

Data Collection

The following data were recorded: demographics, comorbidities (presence or not of coronary disease, diabetes mellitus, malignancy, rheumatoid arthritis, liver cirrhosis, chronic renal failure, chronic obstructive pulmonary disease), site of implant, date of implantation, date of symptoms onset, clinical manifestations, leukocyte count, C-reactive protein (CRP) level at the diagnosis, preoperative radiology evaluation (classified as normal or pathological based on signs of prosthesis loosening or signs suggesting infection), microbiological data,

surgical treatment, antimicrobial therapy (treatment drug and length), and patient outcome.

All information was introduced in a Microsoft Access 2007 database. All cases were critically reviewed by two authors (D.R-P and C.P). All inconsistent data were checked by the investigator at each collaborating hospital. Institutional review board approval was not required because patients were treated according to local standards of care; no clinical interventions were made based on the data collection.

Microbiological Methods

Periprosthetic surgical cultures or joint fluid aspirates were inoculated onto blood agar enriched with 5% sterile bovine blood, chocolate agar and McConkey agar plates, and brain heart and thioglycolate broth for enriched and anaerobe culture, respectively. Microorganisms isolated were identified by conventional biochemical and metabolic tests, in most cases using an automatic system (Vitek or API System from bioMérieux Inc. or MicroScan WalkAway System from Siemens Healthcare Diagnostics). Antimicrobial susceptibility was assessed by methods used in each center (disk-diffusion, E-test, or microdilution technique), according to Clinical and Laboratory Standards Institute (CLSI) recommendations.

Definitions

The diagnosis of GN-PJI was established when two or more intraoperative cultures yielded the same GNB, a positive blood culture yielded GNB in the presence of clinical symptoms and signs of PJI, or there was evidence of

purulence surrounding the prosthesis and GNB growth in a single culture. PJI type was assigned according to the Tsukayama criteria, which classifies PJI based on the time from prosthesis implantation [16].

Clinical and Surgical Management

The decision to treat by debridement and the antibiotic therapy used was made by the attending medical team. DAIR management consists of prompt debridement with thorough removal of necrotic tissue, purulent collections, and debris around the implant, exchange of mobile arthroplasty parts when possible, and prosthesis retention. After obtaining tissue cultures, intravenous broad-spectrum antibiotics are administered, and treatment is adjusted according to susceptibility. Intravenous administration is followed by oral antibiotics according to published treatment recommendations [2, 5]. In all cases, a staff member of the infectious diseases department of each hospital participated in managing these patients. For the purposes of the present study, DAIR was considered to start with the first debridement surgery. Antimicrobials administered before this procedure were not considered a part of DAIR.

Outcome and Follow-up

We performed an overall failure analysis, in which failure was defined as persistence or reappearance of inflammatory joint signs during follow-up, leading to unplanned surgery. As established by consensus among the investigators, infection-related death, a second debridement >30 days after the first, prosthesis removal for any cause (including orthopedic reasons) within the

first 2 years of follow-up, and need for suppressive antimicrobial therapy were considered failure.

In addition, a subanalysis was performed to explore patient outcome based on whether or not they fulfilled Zimmerli's classification algorithm [2]. For this purpose, a composite variable was created for patients who underwent debridement within the first 21 days after symptoms onset, presented with infection within 3 months after implantation (in the absence of hematogenous PJI, which was managed as an acute infection), had a stable prosthesis, and received an agent with activity against biofilm microorganisms.

Statistical Analysis Categorical variables are expressed as count and percentage, and quantitative data as median and interquartile range (IQR). The chi-square test or Fisher exact test was used to compare distribution of categorical variables and the Student *t* test or Mann-Whitney *U* test for continuous variables, as appropriate. A *P*-value of <0.05 was considered statistically significant.

A Kaplan–Meier curve was performed to determine relationships between treatment failures after DAIR and treatment with ciprofloxacin in susceptible isolates.

A Cox regression model was applied to identify variables associated with overall failure using the DAIR approach. Variables with $P<0.1$ on univariate analysis were included in the multivariate models. In addition, variables with $P>0.1$ and considered clinically relevant based on experience and published data were forced into the multivariate model to investigate their effect. Ciprofloxacin treatment in susceptible cases was maintained in the final model

as a fixed variable. Because antibiotic therapy duration may have been shortened in cases failing prematurely, and this would not actually be the cause of failure but its consequence, this variable was not included in the model. Significant interactions between variables were ruled out. Statistical analyses were performed with SPSS, version 15.0 (SPSS, Chicago, IL, USA)

RESULTS

Study population and clinical presentation

Among 2015 PJIs occurring over the 8-year study period, 242 (12%) PJIs in 242 patients were originally caused by GNB (Figure 1). Median age of the study patients was 76 years (IQR, 68-81). The implants included 150 (62%) hip, 85 (35%) knee, 5 (2%) shoulder, and 2 (1%) elbow prostheses. Primary implants accounted for 173 (71%) and revision prostheses 69 (29%) cases. Demographic data, comorbid conditions, risk factors predisposing to PJI, and symptoms at presentation are shown in Table 1. DAIR was the most common surgical strategy, applied in 174 (72%) episodes (Figure 1). For the present study, analyses were carried out including the 174 PJIs treated with DAIR.

Analysis of patients treated with DAIR

Description of the series

Patients managed with DAIR had acute infection in 154 (88%) cases (130 [75%] early postoperative and 24 [14%] hematogenous) and late chronic infection in 20 (11%) cases although symptoms onset occurred between 31 and 90 days after implant placement in 12 of these 20 patients. The median time from prosthesis placement to symptoms onset was 13 days (IQR 7.2-18) in early infections and 65 days (IQR 46-1119) in late chronic infections.

Microbiologic findings

Microbiological findings are outlined in Table 2. Among 174 GN-PJIs, 34 were polymicrobial GNB infections (2 different GNB in 31 and 3 different GNB in 3 cases) accounting for a total of 211 isolates. Polymicrobial GNB infection was

more frequent in pseudomonal PJI (14 of 43, 33%) than in infections caused by other GNB (20 of 131, 14.5%) (*P*=0.013). Blood culture was positive in 11 GNB PJIs.

Overall, 41 of 211 (19%) GNB isolates were ciprofloxacin resistant, and the percentage was similar in pseudomonal PJI (7 of 43, 16%). Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* accounted for 16 of 211 (8%) isolates (11 *Escherichia coli,* 4 *Klebsiella pneumoniae,* and 1 *Enterobacter aerogenes),* among which 11 (69%) were ciprofloxacin resistant.

Medical treatment

Once tissue specimens had been obtained, intravenous broad-spectrum antibiotics were administered to all patients. Therapy was then adjusted according to the susceptibility pattern of the bacteria isolated from intraoperative cultures. Median duration of antibiotic treatment was 70 days (IQR 43-96): median intravenous treatment, 14 days (IQR 6-23) and oral antibiotics, 58 days (IQR 27-90). The intravenous antibiotic regimens used are summarized in Table 3. The oral antibiotics prescribed included ciprofloxacin in 111, cotrimoxazole in 8, and beta-lactams in 7 patients.

Among 139/174 (80%) cases of ciprofloxacin-susceptible GN-PJI, 124/139 (89%) were treated with ciprofloxacin for a median of 69 days (IQR 45-90).

Patients with *Pseudomonas* spp. PJI were treated for a median of 60 days (IQR 43-92). An initial combination of two antibiotics was used in 25/43 cases of pseudomonal PJI (carbapenem or other antipseudomonal beta-lactam plus ciprofloxacin in 21, antipseudomonal beta-lactam plus aminoglycoside in

4), an antipseudomonal beta-lactam in 9, a carbapenem in 6, and ciprofloxacin in 3. In 33/43 (77%) cases, intravenous therapy was followed by oral ciprofloxacin for a median of 43 days (IQR 26-79).

Patients with ESBL-producing strains were treated with a carbapenem in 13 cases, tigecycline in 2, and piperacillin-tazobactam in 1 patient with mixed infection due to ESBL-*Escherichia coli* and *Pseudomonas aeruginosa*. Ciprofloxacin was added to a carbapenem in 2 susceptible cases (combined therapy in 1 and sequential therapy in another patient). In patients who did not fail, median duration of antibiotic treatment in ESBL-producing GNB-PJI was 62 days (IQR 35-166). In those who failed, failures were detected within 30 days while antibiotics were ongoing in all except 1 case.

Outcome analysis

One patient with mixed infection by ESBL-producing *Escherichia coli* and *Pseudomonas aeruginosa* was lost to follow-up. Among the 173 remaining patients, failures were documented in 55/173 (32%): 39 (23%) required implant removal, 5 (3%) died due to infection-related causes (median time from diagnosis to death, 13 days [IQR 8-19]), 4 (2%) required long-course, suppressive antimicrobial therapy, 4 (2%) had a persistent sinus tract, and 3 (2%) needed a new debridement >30 days after the initial one.

Cases are classified according to ciprofloxacin susceptibility, treatment, and outcome (success or failure) in Table 4. Global success rate with DAIR was 68% (118 patients) after a median follow-up of 25 months (IQR 15-39). In patients with ciprofloxacin-susceptible GN-PJI treated with ciprofloxacin, success was 79% (98/124), whereas in those with susceptible infection not

treated with ciprofloxacin, success was 40% (6/15) (*P*=0.001). These two groups were comparable with regard to all variables analyzed except for age (data not shown). Median age of patients with ciprofloxacin-susceptible GN-PJI was 75 years (IQR 64-80) in those treated with ciprofloxacin and 80 years (IQR: 77-87) in cases not treated with ciprofloxacin (*P*=0.001). In ciprofloxacin-resistant cases, the efficacy of DAIR management was 41% (14/34).

The success rate in pseudomonal PJI was 79% (33 of 42 cases), which increased to 88% (29 of 33) when only pseudomonal PJIs treated with ciprofloxacin were considered. In infections caused by ESBL-producing *Enterobacteriaceae,* success was 53% (8 of 15).

The Kaplan-Meier time-to-failure curve showed an association with better outcome in patients treated with ciprofloxacin (log rank $\leq$ 0.0001) (Figure 2).

Potential risk factors in patients treated with DAIR who succeeded or failed are outlined in Table 5. For the multivariate analysis, a Cox regression model was fitted to assess whether ciprofloxacin treatment was predictive of DAIR success. C-reactive protein at diagnosis and polyethylene exchange were not included due to a significant lack of data. In susceptible GN-PJI, ciprofloxacin treatment exhibited an independent protective effect (adjusted hazard ratio [aHR] 0.23, 95%CI 0.13-0.40; *P*<0.001), whereas chronic renal impairment was predictive of failure (aHR 2.56, 95%CI 1.14-5.77; *P*=0.0232).

Regarding implementation of Zimmerli's algorithm, failure was significantly higher in patients who did not meet the criteria compared to those who did (35/75 [47%] vs. 20/98 [20%], *P*<0.001); Therefore, fulfillment of Zimmerli's algorithm was a protective factor on univariate analysis (HR 0.34 [0.20-0.59], *P*<0.0001). Focusing on patients who did not meet Zimmerli's

algorithm, the failure rate was higher in those with GNB-PJI due to ciprofloxacin-resistant GNB than in susceptible cases (29/49 [59%] vs.6/26 [23%], *P*=0.03).

DISCUSSION

To our knowledge, this is the largest reported case series of PJI caused by gram-negative bacteria, which accounted for 12% of all PJIs in our experience. The DAIR approach was used in 174 (72%) cases, with an overall success rate of 68% that increased to 79% in ciprofloxacin-susceptible GN-PJI treated with ciprofloxacin. Thus, our results suggest that the DAIR strategy would be a good initial surgical option in acute ciprofloxacin-susceptible GN-PJI.

As is stated in the IDSA guidelines [17], debridement without infected prosthesis removal is a feasible option for patients with well-fixed prostheses and acute infection. In recent studies, the efficacy of DAIR in GN-PJI has been investigated in limited series, and reported success rates vary considerably: some authors describe remission rates of only 27% [3], whereas others report rates of 70% or higher [10, 12, 13, 15, 18]. These differences in outcome have been attributed to several factors, such as inclusion of chronic infection or *Pseudomonas aeruginosa* infection (which might yield higher recurrence rates), and differences in ciprofloxacin use [3, 10, 13, 14, 18, 19]. Our results are consistent with those of Zmistowski *et al.* [15] and Martinez-Pastor *et al*. [10], who reported remission rates of 70% and 74%, respectively. Three years after that study, the same authors [19] reported a drop in the rate to 64% after long-term follow-up and considering aseptic loosening as failure, which again, concurs with our results.

Notably, we found an 88% success rate in pseudomonal PJI treated with ciprofloxacin. This finding supports the concept that it is not the causative microorganism, but rather, the susceptibility to ciprofloxacin and ciprofloxacin use which determines success in GN-PJI management. Therefore, ciprofloxacin treatment should be considered the cornerstone therapy for GN-PJI. The effectiveness of ciprofloxacin in these patients can be attributed to its good oral bioavailability, optimal diffusion into synovial fluid and bone, and activity against biofilms [20].

The increasing ciprofloxacin resistance rates among GNB is a cause for concern [21]. In our study, the efficacy of DAIR in ciprofloxacin-resistant cases dropped to 41%, a value similar to the 37% (12/19) reported in a previous study [19]. In this situation, other antibiotic options should be considered, but unfortunately, there is little available information regarding alternatives in this scenario [17, 21]. Rifampin in combination with antibiotics that permeabilize the bacterial membrane (eg, colistin) has demonstrated synergistic activity *in vitro* in GNB infection [22]. However, sufficient published evidence to recommend this combination is lacking [21]. In our study, 5 of 10 patients treated with or switched to cotrimoxazole without using ciprofloxacin were cured. Nonetheless, there is little published clinical data regarding cotrimoxazole use in GN-PJI. Further clinical studies are needed to clarify the value of drugs with good bone penetration such as cotrimoxazole or fosfomycin as ciprofloxacin alternatives.

Not only ciprofloxacin-resistant GNB, but also other multi-drug resistant GNB, such as ESBL-producing *Enterobacteriaceae,* may have high failure rates. In our experience, 16/174 GNB-PJIs treated with DAIR were caused by ESBL-producing *Enterobacteriaceae,* and the success rate was 53%, a

percentage higher than the previously 42.8% (3 out of 7 patients) reported value [11]. Only two of our cases were treated with ciprofloxacin; hence the use of this drug in susceptible ESBL-producing *Enterobacteriaceae* PJI could not be evaluated. Since ciprofloxacin resistance is common in ESBL-producing GNB (69% in our series), other combinations, such as carbapenems or colistin with fosfomycin, could be explored because of the high anti-biofilm activity and demonstrated synergistic effect of fosfomycin *in vitro* and in a foreign-body infection animal model [23, 24].

Repeat debridement was performed in our series when signs of infection persisted, and the need for two or more debridements was predictive of failure on univariate analysis. Although it is difficult to separate this factor from other risk variables, repeat debridement might indicate a more complicated infection; therefore, prosthesis removal should be considered.

Our analysis identified chronic renal insufficiency as a risk factor for failure, a finding consistent with the observation of other authors [25] that comorbidities can impact the patient's outcome. Based on our results, we recommend careful evaluation of the pros and cons of all surgical options in patients with chronic renal failure.

In accordance with previous studies [26], our results confirm the applicability of Zimmerli's algorithm, with a success rate of 80% in patients fulfilling the criteria. It is even more interesting that in patients who did not meet all the criteria, ciprofloxacin use in susceptible cases was associated with high success rates, again highlighting the favorable impact of ciprofloxacin in GN-PJI.

The observational retrospective nature of our study is an important limitation because of the potential drawbacks implicit in this type of study design. In addition, it is a multicenter study, which implies variability in the surgical criteria, which could have some influence on the patient's outcome. Nonetheless, all centers included had a specialized multidisciplinary team for the treatment of orthopedic infections, including infectious disease specialists, microbiologists, and specialized orthopedic surgeons, all of whom belong to the same national medical societies and use the same clinical and surgical criteria to evaluate patients.

In conclusion, we present the largest series of GN-PJI managed with DAIR. Our results confirm a 79% success rate in ciprofloxacin-susceptible GN-PJI treated with debridement, ciprofloxacin treatment, and implant retention. New therapeutic strategies are needed for ciprofloxacin-resistant infections.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Appendix

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References

1. Huo MH, Gilbert NF, Parvizi J. What's new in total hip arthroplasty. J Bone Joint Surg Am, **2007**; 89: 1874-1885.

2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med, **2004**; 351: 1645-1654.

3. Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis, **2009**; 49: 1036-1043.

4. Tattevin P, Cremieux AC, Pottier P, Huten D, Carbon C. Prosthetic joint infection: when can prosthesis salvage be considered? Clin Infect Dis, **1999**; 29: 292-295.

5. Zimmerli W, Ochsner PE. Management of infection associated with prosthetic joints. Infection, **2003**; 31: 99-108.

6 Marculescu CE, Berbari EF, Hanssen AD, *et al*. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis, **2006**; 42: 471-478.

7. Lora-Tamayo J, Murillo O, Iribarren JA, *et al*. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. Clin Infect Dis, **2013**; 56: 182-194.

8. Barberan J, Aguilar L, Carroquino G, *et al.* Conservative treatment of staphylococcal prosthetic joint infections in elderly patients. Am J Med, **2006**; 119: 993-1010.

9. Vilchez F, Martinez-Pastor JC, Garcia-Ramiro S, *et al*. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to S*taphylococcus aureus* treated with debridement. Clin Microbiol Infect, **2011**; 17: 439-444.

10. Martinez-Pastor JC, Munoz-Mahamud E, Vilchez F, *et al*. Outcome of acute prosthetic joint infections due to gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother, **2009**; 53: 4772-4777.

11. Martinez-Pastor JC, Vilchez F, Pitart C, Sierra JM, Soriano A. Antibiotic resistance in orthopaedic surgery: acute knee prosthetic joint infections due to extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. Eur J Clin Microbiol Infect Dis, **2010**; 29: 1039-1041.

12. Rodriguez D, Pigrau C, Euba G, *et al*. Acute haematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. Clin Microbiol Infect, **2010**; 16: 1789-1795.

13. Aboltins CA, Dowsey MM, Buising KL, *et al*. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. Clin Microbiol Infect , **2011**; 17: 862-867.

14. Legout L, Senneville E, Stern R, *et al.* Treatment of bone and joint infections caused by Gram-negative bacilli with a cefepime-fluoroquinolone combination. Clin Microbiol Infect , **2006**; 12:1030-1033.

15. Zmistowski B, Fedorka CJ, Sheehan E, Deirmengian G, Austin MS, Parvizi J. Prosthetic joint infection caused by gram-negative organisms. J Arthroplasty, **2011**; 26:104-108.

16. Tsukayama DT, Goldberg VM, Kyle R. Diagnosis and management of infection after total knee arthroplasty. J Bone Joint Surg Am, **2003**; 85-A Suppl 1:S75-S80.

17. Osmon DR, Berbari EF, Berendt AR,*et al*. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of america. Clin Infect Dis, **2013**; 56: e1-e25.

18. Uckay I, Bernard L. Gram-negative versus gram-positive prosthetic joint infections. Clin Infect Dis, **2010**; 50: 795.

19. Jaen N, Martinez-Pastor JC, Munoz-Mahamud E, *et al*. Long-term outcome of acute prosthetic joint infections due to gram-negative bacilli treated with retention of prosthesis. Rev Esp Quimioter, **2012**; 25:194-198.

20. Tanaka G, Shigeta M, Komatsuzawa H, Sugai M, Suginaka H, Usui T. Effect of the growth rate of P*seudomonas aeruginosa* biofilms on the susceptibility to antimicrobial agents: beta-lactams and fluoroquinolones. Chemotherapy, **1999**; 45: 28-36.

21. Sendi P, Zimmerli W. Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect, **2012**; 18: 1176-1184.

22. Drapeau CM, Grilli E, Petrosillo N. Rifampicin combined regimens for gram-negative infections: data from the literature. Int J Antimicrob Agents, **2010**; 35: 39-44.

23. Corvec S, Portillo ME, Pasticci BM, Borens O, Trampuz A. Epidemiology and new developments in the diagnosis of prosthetic joint infection. Int J Artif Organs, **2012**; 35: 923-934.

24. Samonis G, Maraki S, Karageorgopoulos DE, Vouloumanou EK, Falagas ME. Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* clinical isolates. Eur J Clin Microbiol Infect Dis, **2012**; 31: 695-701.

25. Ueng SW, Lee CY, Hu CC, Hsieh PH, Chang Y. What is the success of treatment of hip and knee candidal periprosthetic joint infection? Clin Orthop Relat Res, **2013**; 471: 3002-3009.

26. Martel-Laferriere V, Laflamme P, Ghannoum M, Fernandes J, Di ID, Lavergne V. Treatment of prosthetic joint infections: validation of a surgical algorithm and proposal of a simplified alternative. J Arthroplasty, **2013**; 28: 395-400.

Table 1. Demographic Data, Comorbid Conditions, and Symptoms at Presentation in 242 Gram-Negative Prosthetic Joint Infections Sorted by Surgical Approach

Variables		All patients N=242 (100%)	Patients treated with DAIR N=174 (72%)	Patients not treated with DAIR N=68 (28%)	*P*
Baseline features	Age, years; median (IQR range)	76 (68-81)	76 (69-81)	77 (65-81)	0.96
	Sex, male	81 (34)	59 (34)	22 (32)	0.82
	Diabetes mellitus	52 (22)	37 (21)	15 (22)	0.89
	Chronic renal impairment	23 (10)	15 (9)	8 (12)	0.45
	Use of steroids	21 (9)	16 (9)	5 (7)	0.65
	Rheumatoid arthritis	19 (8)	12 (7)	7 (10)	0.37
	Malignancy	16 (7)	13 (7)	3 (4)	0.57
	Revision prosthesis	69 (29)	49 (28)	20 (29)	0.85
	Prosthesis location				
	Hip	150 (62)	115 (66)	35 (51)	0.03
	Knee	85 (35)	57 (33)	28 (41)	0.22
	Other	7 (3)	2 (1)	5 (7)	0.02
Clinical presentation	Type of infection				
	Hematogenous PJI	37 (15)	24 (14)	13 (19)	0.30
	Early postoperative PJI $\leq$ 30 days	152 (63)	130 (75)	22 (34)	<0.001
	Late chronic PJI >30 days	51 (21)	20 (11)	31 (46)	<0.001
	Positive intraoperative culture	2 (1)	-	2 (1)	-
	Time to infection, days* median (IQR range)	16 (9-38)	14 (8-24)	349 (90-1307)	<0.001
	Bacteremia	17 (7)	11 (6)	6 (9)	0.28
	Pain	182 (75)	130 (75)	52 (76)	0.83
	Inflammatory signs	172 (71)	130 (75)	42 (62)	0.046
	Purulence drainage	139 (57)	113 (65)	26 (38)	<0.001
	Fever, temperature $\geq$38 C^{o}	81 (34)	62 (36)	19 (28)	0.25
Microbiological laboratory data	Leukocytes, 10^9/L median (IQR range)	8.5 (6.5-11.0)	8.5 (6.1-11.0)	8.7 (7.0-10.8)	0,73
	C-reactive protein, mg/L** median (IQR range)	23 (7-55)	21.8 (7-49)	36 (13-94)	0.14

	Ciprofloxacin-susceptible isolates	200 (83)	139 (80)	61 (90)	0.03
	Pseudomonas spp. infection	68 (28)	43 (25)	25 (37)	0.06
	ESBL-GNB Infection	19 (8)	16 (9)	3 (4)	0.22
	Infection caused by 2 or more GNB	40 (17)	33 (19)	7 (10)	0.10
Treatment	First surgical approach delay, days*** median (IQR range)	6.5 (1-21)	5 (1-14)	24 (3-111)	<0.001
	≥2 debridements at any time	21 (8)	21 (12)	-	-
	Polyethylene exchange[#]	96 (40)	96 (55)	-	-
	N. patients treated with CP when all isolated GNB were susceptible	177 (73)	125 (71)	53 (78)	0.29
Outcome	Overall mortality	43 (18)	33 (19)	10 (15)	0.49
	Mortality due to the infection[≠]	12 (5)	5 (3)	7 (10)	0.12

Categorical data are expressed as absolute number (percentage) and continuous variables as median (interquartile range).

Abbreviations: CP, ciprofloxacin; DAIR, debridement, antibiotics, and implant retention; ESBL-GNB, extended-spectrum beta-lactamase-producing gram-negative bacteria; GNB, gram-negative bacteria.

*Time to infection: time from prosthesis placement to onset of symptoms, excluding hematogenous infections

** C-reactive protein value was available in 151 of 242 (62%) patients: 114 patients treated with DAIR and 37 not treated with DAIR

***First surgical approach delay: time from onset of symptoms to surgery, excluding 7 cases in which surgery was not performed

[#]Information on polyethylene exchange was only investigated in patients treated with DAIR: in 96 of 174 cases it was changed, in 47 it was not changed, and in 31 cases this information was not available.

[≠] Deaths attributed to PJI. All related deaths occurred within 30 days from the diagnosis

Table 2. Microbiological Findings in 174 Patients with Gram-negative Prosthetic Joint Infections Treated with DAIR

Microorganisms	**N=174 episodes with 211 isolates (100%)***
Enterobacteriaceae	162 (77)
Escherichia coli	63 (30)
Proteus spp.	31 (15)
Enterobacter spp.	29 (14)
Klebsiella spp.	14 (7)
Morganella morganii	10 (5)
Serratia marcescens	8 (4)
Salmonella spp.	5 (2)
Citrobacter spp.	2 (1)
Pseudomonas spp.[a]	43 (20)
Other gram-negative bacteria	6 (2) **

Abbreviations: DAIR, debridement, antibiotics and implant retention; GNB, gram-negative bacteria; GN-PJI, gram-negative prosthetic joint infection

* Among 174 episodes of GN-PJIs treated with DAIR, 34 were polymicrobial infections caused by more than one GNB, accounting for a total of 211 isolates.

[a] *P. aeruginosa* in all but 3 cases, in which *P. stuzeri* was identified

** Other GNB include: 3 *Bacteroides fragilis, 1 Pasteurella multocida 1 Alcaligenes xylosoxidans, 1 Rahnella aquatilis*

Table 3. Intravenous Antimicrobial Therapy Used for 174 Episodes of Gram-Negative Prosthetic Joint Infections Treated with DAIR

Types of antimicrobial therapy (drugs)	GN-PJI treated with DAIR N=174 (100%)
Monotherapy (n=126)	
Non-carbapenem beta-lactam, without antipseudomonal activity	32 (18)
Carbapenem	31 (18)
Other beta-lactams with antipseudomonal activity	29 (17)
Fluoroquinolones	28 (16)
Aztreonam	3 (2)
Other monotherapies *	3 (2)
Combination therapy (n=48)	
Beta-lactam with antipseudomonal activity plus ciprofloxacin	24 (14)
Carbapenem plus ciprofloxacin	10 (6)
Beta-lactam without antipseudomonal activity plus ciprofloxacin	5 (3)
Beta-lactam with antipseudomonal activity plus aminoglycoside	6 (3)
Other combination therapies **	3 (2)

Abbreviations: DAIR, debridement, antibiotics, and implant retention; GN-PJI, gram-negative prosthetic joint infection**.**

*Other monotherapies included tigecycline in 2 cases and cotrimoxazole in 1 case

**Other combination therapies included ciprofloxacin plus cotrimoxazole in 2 cases and beta-lactam plus cotrimoxazole in 1 case

Table 4. Outcome of 173 cases of Gram-Negative Prosthetic Joint Infection (cases sorted by receiving or not ciprofloxacin treatment depending on ciprofloxacin susceptibility)

	N of failures (%)	N of successes (%)	Total cases (%)
GN-PJI susceptible to CP, treated with CP	26 (21)	98 (79)	124 (100)
GN-PJI susceptible to CP, not treated with CP	9 (60)	6 (40)	15 (100)
GN-PJI not susceptible to CP	20 (59)	14 (41)	34 (100)
Total	55 (32)	118 (68)	173 (100)

Abbreviations: GN-PJI, Gram-negative prosthetic joint infection, CP, ciprofloxacin, N= total number

Table 5. Univariate and Multivariate Analysis of Parameters Predicting Overall Failure in 173 patients treated with DAIR and known outcome

	Unadjusted Analysis		Adjusted Analysis	
	HR (95%CI)	*P*	aHR (95%CI)	*P*
Male Sex	.99 (0.56-1.73)	*.9613*	-	-
Age (years)	1.03 (1.00-1.05)	.0685	1.01 (0.13-1.04)	.6000
Diabetes mellitus	1.28 (0.69-2.38)	.4407	-	-
Chronic renal failure	2.14 (0.97-4.76)	.0604	2.56 (1,14-5.77)	.0232
Rheumatoid arthritis	1.37 (0.55-3.45)	.4988	-	-
Use of steroids	1.32 (0.57-3.09)	.5189	-	-
Revision prosthesis	1.04 (0.59-1.84)	.8922	-	-
Prosthesis location, hip	1.52 (0.85-2.73)	.1612	-	-
Prosthesis location, knee	0.69 (0.38-1.24)	.2162	-	-
Acute infection	0.80 (0.38-1.69)	.5563	-	-
Early postoperative PJI (reference)	1		-	-
Hematogenous PJIs	0.90 (0.40-2.02)	.8170	-	-
Late chronic PJI	1.23 (0.58-2.64)	.8170	-	-
Bacteremia due to GNB	1.30 (0.46-3.62)	.6205	-	-
Fever	1.02 (0.59-1.79)	.9321	-	-
Local pain	0.84 (0.46-1.55)	.5780	-	-
External inflammatory signs	1.11 (0.60-2.07)	.7411	-	-
Purulence	1.49 (0.83-2.67)	.1796	1.64 (0.91-2.98)	.1002
Polymicrobial PJI	1.18 (0.61-2.29)	.6201	-	-
Pseudomonas spp. PJI	0.59 (0.29- 1.20)	.1440	-	-
GNB susceptible to CP	0.31 (0.18-0.54)	.0000	-	--
ESBL-GNB PJI	1.73 (0.78-3.82)	.1773	-	-
CRP at diagnosis, per 100mg/L*	1.00 (1.001-1.007)	.016	-	-
Leukocytes count, 10^9/L	1,005 (0,951-1,061)	.8684	-	-
Need for $\geq$ 2 debridements**	2.15 (1.11-4.18)	.0237	-	-
Debridement delay, days***	1.004 (0.996-1.013)	.2835	-	-
Polyethylene exchange*	0.73 (0.35-1.51)	.3994	-	-
Treatment with CP	0.22 (0.13-0.37)	.0000	0.23 (0.13-0.40)	.0000
Combined antibiotic therapy	0.42 (0.21-0.87)	.0189	0.52 (0.25-1.06)	.0735

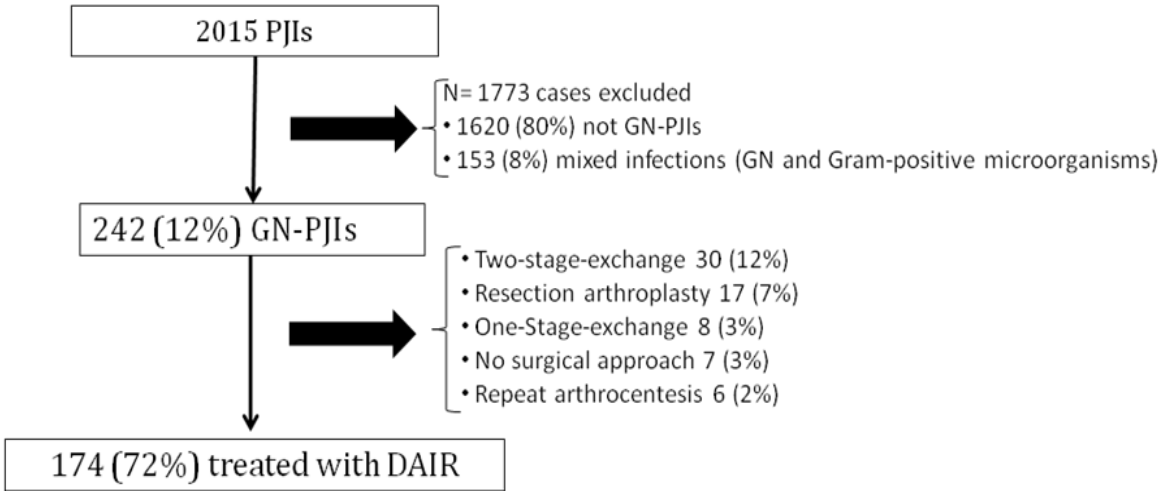
Abbreviations: CI, confidence interval; CP, ciprofloxacin; CPR, C-reactive protein (mg/L); ESB-GNB, extended-spectrum beta-lactamase-producing gram-negative bacteria; HR, hazard ratio; GNB, gram-negative bacilli; PJI, prosthetic joint infection.

* Multivariate analyses do not include CPR at diagnosis or polyethylene exchange, due to significant lack of data

** Need for $\geq$2 debridements at any time since diagnosis

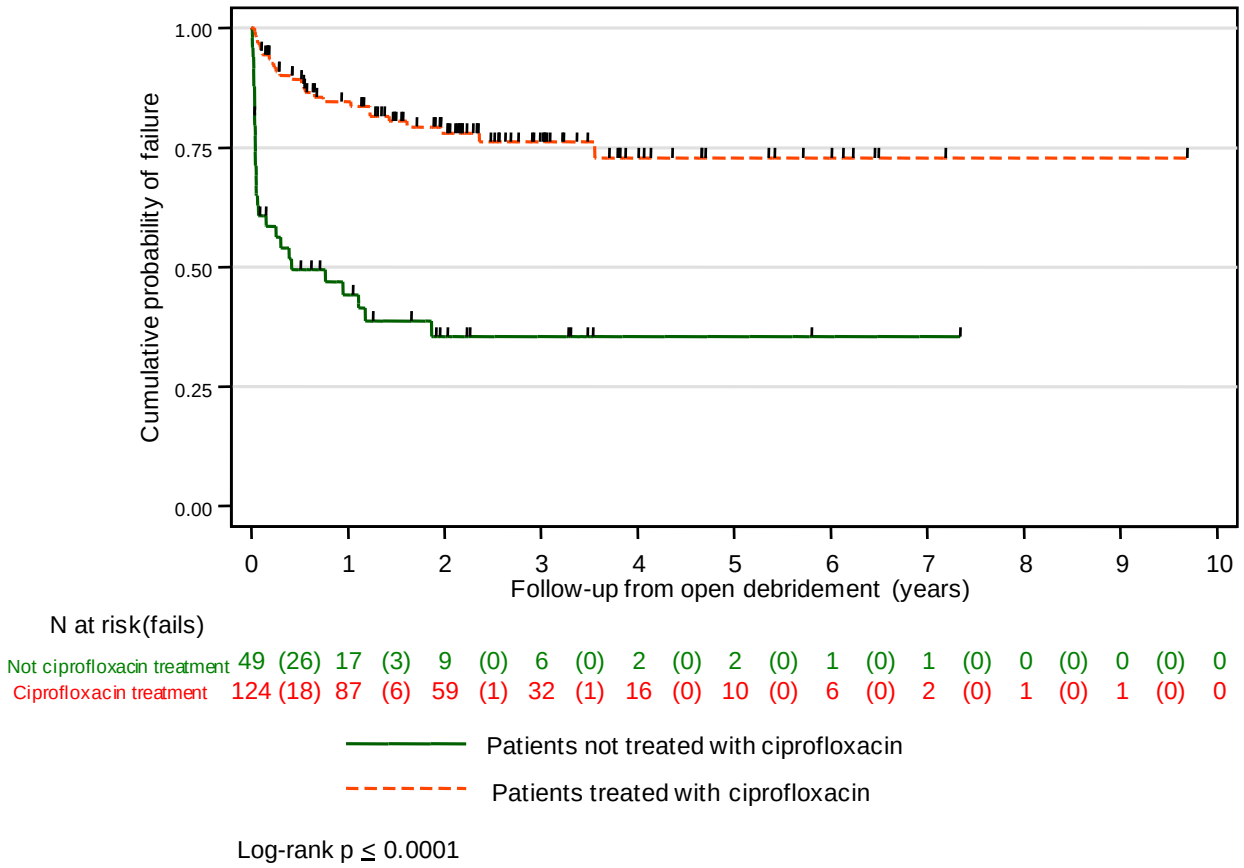
***Debridement delay: days from onset of symptoms to debridement

Figure 1. Flow Chart of GNB-PJI Cases Identified



Abbreviations: DAIR, debridement, antibiotics, and implant retention; GN, gram-negative bacilli; GN-PJI, gram-negative prosthetic joint infection; PJI, prosthetic joint infection

Figure 2. Kaplan-Meier Estimates of the Cumulative Risk of Failure-Free Survival in Patients Treated or not With Ciprofloxacin



N at risk(fails)

Not ciprofloxacin treatment	49	(26)	17	(3)	9	(0)	6	(0)	2	(0)	2	(0)	1	(0)	1	(0)	0	(0)	0	(0)	0
Ciprofloxacin treatment	124	(18)	87	(6)	59	(1)	32	(1)	16	(0)	10	(0)	6	(0)	2	(0)	1	(0)	1	(0)	0

Patients not treated with ciprofloxacin

Patients treated with ciprofloxacin

Log-rank p ≤ 0.0001

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Infected hip hemiarthroplasties and total hip arthroplasties: Differential findings and prognosis

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Debridement antibiotics and implant retention (DAIR);
Bone and joint infection

Summary *Objectives:* Infected hip hemiarthroplasties (HHA) are classically analyzed along with infected total hip arthroplasties (THA), but patients with either one or other device are different. We describe the clinical presentation, etiology and prognosis of infected HHA compared with infected THA.
Methods: Comparative study of patients with infected HHA and THA from a prospective database of prosthetic joint infection (PJI) cases in our hospital (2003–2011), focusing on patients managed with debridement, antibiotics and implant retention (DAIR).
Results: 210 episodes of hip-PJI (age 74 years, 63% women): 62 (39%) HHA and 148 (61%) THA. HHA-patients were older and had more comorbidities. Late-chronic and hematogenous infections were more frequent in THA. 123 (59%) patients were managed with DAIR: 72 THA and 51 HHA. *Staphylococcus aureus* was more frequent in THA (44% vs 26%, $p = 0.032$), while Gram-negative bacilli were more prevalent in HHA (73% vs 51%, $p = 0.018$), with a higher prevalence of fluoroquinolone-resistance in cemented-HHA. Overall failure was 37%, with no significant differences among groups. A higher mortality was observed in HHA cases (21% vs 4%, $p = 0.005$), particularly in cemented-HHA.

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Conclusion: Infected THA and HHA have different characteristics, etiology and prognosis. Overall failure was similar, probably balanced by different predictors among groups, but mortality was higher among cemented-HHA.

Introduction

Infection is a fearsome complication after placement of a hip prosthesis.[1,2] Although only occurring in 1–2% of implanted devices the absolute number of cases is high and will inevitably increase as a result of an ageing population and the total number of devices being implanted.[3] Recipients of and types of hip devices are quite heterogeneous: while hip hemiarthroplasties (HHA) are usually placed during an emergency procedure in elderly patients with femoral neck fracture, total hip arthroplasties (THA) are usually placed during elective surgery for degenerative joint diseases, such as rheumatoid arthritis, arthrosis or aseptic necrosis of the femoral head.[4,5] In addition, THA patients are usually younger, more stable and present with less comorbidities. Thus, patients carrying either one or other device are not the same, and the aseptic conditions under which the prosthesis is implanted may be not equally exhaustive. In consequence, microbiology responsible for the infection may be different, and so may also be its clinical presentation and prognosis. However, the management of infection is similar in both types of implants, and the literature tends either to ignore HHA or to include them together with the analysis of THA.[1,2,6,7] Our hypothesis is that patients with infected HHA or THA present with different clinical and microbiological characteristics, and that their prognosis may be different. The aim of this study is to assess these differences by means of a comparative analysis of patients with infected HHA and THA.

Patients and methods

Setting and patients

This study was conducted in the Bone and Joint Infection Unit of the Hospital Universitario de Bellvitge (Barcelona, Spain), an 800-bed teaching hospital that is reference for a population of 1 million. The Unit has a multidisciplinary team comprising orthopaedic surgeons, infectious diseases physicians and rheumatologists, and it is also a national reference for difficult-to-treat osteoarticular infections.

Information on patients with prosthetic joint infection (PJI) is prospectively gathered in an *ad hoc* database, including baseline characteristics of the patient and the prosthetic device, clinical presentation of the infection episode, surgical and medical treatment, microbiological data of the aetiology, follow-up and outcome. This study retrospectively analyses the data for all cases of hip PJI between 2003 and 2011.

As mentioned, two types of hip device were considered: THA and HHA. The placement of a THA is commonly an elective procedure, usually performed on patients with generative joint diseases, such as arthrosis, rheumatoid arthritis or aseptic necrosis of the femoral head. Sometimes

Table 1 Baseline characteristics of all episodes of hip prosthetic joint infection.

		All episodes (n = 210)	All HHA (n = 62)	THA (n = 148)	p	NC-HHA (n = 29)	C-HHA (n = 33)	p
Age (years)		74 (64–80)	80 (75–84)	70 (62–78)	<0.001	84 (79–88)	77 (72–81)	<0.001
Sex (women)		135 (64%)	42 (68%)	93 (63%)	0.499	23 (79%)	19 (58%)	0.065
Rheumatoid arthritis		13 (6%)	0 (0%)	13 (9%)	0.012	0 (0%)	0 (0%)	–
Diabetes mellitus		46 (22%)	20 (32%)	26 (18%)	0.019	5 (17%)	15 (46%)	0.018
Liver cirrhosis		12 (6%)	4 (7%)	8 (5%)	0.766	0 (0%)	4 (12%)	0.116
Heart disease		48 (23%)	25 (40%)	23 (16%)	<0.001	14 (48%)	11 (33%)	0.231
Lung chronic disease		23 (11%)	8 (13%)	15 (10%)	0.558	2 (7%)	6 (18%)	0.264
Immunosuppressant therapy		24 (11%)	4 (7%)	20 (14%)	0.142	0 (0%)	4 (4%)	0.116
Any comorbidity		111 (53%)	41 (66%)	70 (47%)	0.013	17 (59%)	24 (73%)	0.242
Revision prosthesis		52 (25%)	1 (2%)	51 (35%)	<0.001	1 (3%)	0 (0%)	0.475
Type of infection[a]	Early	119 (57%)	52 (84%)	67 (45%)	<0.001	27 (93%)	25 (76%)	0.301
	Late-chronic	57 (27%)	6 (10%)	51 (35%)		2 (7%)	4 (12%)	
	Hematogenous	17 (8%)	2 (3%)	15 (10%)		0 (0%)	2 (6%)	
	PIOC	17 (8%)	2 (3%)	15 (10%)		0 (0%)	2 (6%)	

Categorical variables expressed in absolute number (and percentage); continuous variables expressed in median (and interquartil range).
HHA: hip hemiartrhplasties; THA: total hip arthroplasties; NC-HHA: non-cemented hip hemiarthroplasty; C-HHA: cemented hip hemiarthroplasty; PIOC: positive intraoperative cultures.
[a] Type of infection according to Tsukayama.

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Table 2 Comparative analysis of infected hip hemiarthroplasties and infected total hip arthroplasties managed with DAIR.

	All episodes (n = 123)	HHA (n = 51)	THA (n = 72)	p	NC-HHA (n = 24)	C-HAA (n = 27)	p	p^h
Revision prosthesis	24 (20%)	0 (0%)	24 (34%)	<0.001	0 (0%)	0 (0%)	—	<0.001
Type of infection[a]								
Early	112 (91%)	49 (96%)	63 (88%)		24 (100%)	25 (93%)	1.000	0.634
Late-chronic	3 (2%)	1 (2%)	2 (3%)	0.226	0 (0%)	1 (4%)		
Hematogenous	8 (7%)	1 (2%)	7 (10%)		0 (0%)	1 (4%)		
Time to infection[b]	12 (7–18)	13 (7–18)	12 (7–17)	0.765	12 (7–17)	14 (8–19)	0.655	0.594
Pain	57 (46%)	16 (31%)	41 (57%)	0.005	9 (38%)	7 (26%)	0.374	0.006
Inflammatory signs	82 (67%)	34 (67%)	48 (67%)	1.000	15 (63%)	19 (70%)	0.552	0.726
Suppuration	71 (58%)	29 (57%)	42 (58%)	1.000	16 (67%)	13 (48%)	0.183	0.364
Fistula	25 (21%)	11 (22%)	14 (20%)	0.803	4 (17%)	7 (26%)	0.422	0.503
Temperature > 37 °C	52 (43%)	18 (35%)	34 (49%)	0.145	9 (38%)	9 (33%)	0.756	0.176
ESR at diagnosis (mm/h)	46 (33–64)	48 (29–65)	46 (34–63)	0.913	58 (28–67)	46 (26–60)	0.437	0.950
CRP at diagnosis (mg/l)	57 (21–132)	69 (33–161)	54 (12–126)	0.374	95 (13–226)	69 (35–142)	0.851	0.389
Leukocyte at diagnosis ($\times 10^9$/l)	9.2 (7.1–11.4)	9.4 (8.4–11.6)	9.0 (6.4–11.1)	0.191	9.7 (8.6–11.6)	9.4 (5.7–11.6)	0.404	0.782
Rx signs of infection	9 (8%)	1 (2%)	8 (11%)	0.082	1 (4%)	0 (0%)	1.000	0.195
Bacteremia	7 (6%)	4 (8%)	3 (4%)	0.447	4 (17%)	0 (0%)	0.043	0.560
Polymicrobial infection	56 (46%)	26 (51%)	30 (42%)	0.307	11 (46%)	15 (56%)	0.488	0.216
Infection by *S. aureus*	45 (37%)	13 (26%)	32 (44%)	0.032	9 (38%)	4 (15%)	0.064	0.006
MSSA[c]	36/45 (80%)	9 (69%)	27 (84%)	0.411	6/9 (67%)	3/4 (75%)	1.000	0.535
MRSA[c]	9/45 (20%)	4/13 (31%)	5/32 (16%)	0.411	3/9 (33%)	1/4 (25%)	1.000	0.535
Infection by *P. aeruginosa*	33 (27%)	17 (33%)	16 (22%)	0.171	8 (33%)	9 (33%)	1.000	0.257
FQ-R P *aeruginosa*[c]	2/33 (6%)	2/17 (12%)	0/16 (0%)	0.485	0/8 (0%)	2/9 (22%)	0.471	0.120
Infection by *Enterobacteriaceae*	56 (46%)	27 (53%)	29 (40%)	0.165	12 (50%)	15 (56%)	0.692	0.173
FQ-R *Enterobacteriaceae*[c]	20/56 (36%)	12/27 (44%)	8/29 (28%)	0.266	3/12 (25%)	9/15 (33%)	0.069	0.053
ESBL-P *Enterobacteriacae*[c]	6/56 (11%)	3/27 (11%)	3/29 (10%)	1.000	1/12 (8%)	2/15 (13%)	1.000	1.000
Infection by Gram-negative bacilli	74 (60%)	37 (73%)	37 (51%)	0.018	16 (67%)	21 (78%)	0.375	0.018
FQ-R Gram-negative bacilli[c]	21/74 (28%)	13/37 (35%)	8/37 (22%)	0.197	3/16 (19%)	10/21 (48%)	0.068	0.040
Infection by *Enterococcus*	13 (11%)	4 (8%)	9 (13%)	0.408	1 (4%)	3 (11%)	0.612	1.000
Days of antimicrobial therapy[d]	58 (51–63)	56 (44–60)	60 (54–68)	0.020	56 (41–60)	55 (48–62)	1.000	0.104
Need for 2 debridements or more	24 (20%)	7 (14%)	17 (24%)	0.173	2 (8%)	5 (19%)	0.425	0.587
Exchange of removable components	77 (63%)	47 (92%)	30 (42%)	<0.001	24 (100%)	23 (85%)	0.113	<0.001
Time to debridement (days)[e]	5.0 (3.0–10.0)	5.0 (3.0–8.0)	6.5 (4.0–12.8)	0.083	4 (3–6)	6 (3–11)	0.200	0.684
Overall failure[f]	44 (37%)	15 (31%)	29 (41%)	0.261	6 (26%)	9 (36%)	0.459	0.634
Failure while on therapy[f,g]	24/44 (55%)	11/15 (73%)	13/29 (45%)	0.072	4/6 (67%)	7/9 (78%)	1.000	0.130
Failure after therapy[f,g]	20/44 (46%)	4/15 (27%)	16/29 (55%)		2/6 (33%)	2/9 (22%)		
Overall mortality[f]	26 (22%)	17 (35%)	9 (13%)	0.004	4 (17%)	13 (52%)	0.012	<0.001
Mortality related to infection[f]	13 (11%)	10 (21%)	3 (4%)	0.005	2 (9%)	8 (32%)	0.075	0.001

Categorical variables expressed in absolute number (and percentage); continuous variables expressed in median (and interquartile range).
Abbreviations: HHA: hip hemiarthroplasty; THA: total hip arthroplasty; NC-HHA: non-cemented HHA; C-HHA: cemented HHA; MSSA: methicillin-susceptible S. aureus; MRSA: methicillin-resistant S. aureus; FQ-R: fluoroquinolone-resistant; ESBL-P: extended spectrum beta-lactamase producing.

[a] Type of infection according to Tsukayama.
[b] Time to infection: time from prosthesis placement to beginning of symptoms (8 hematogenous cases excluded).
[c] Percentages and comparisons referred to resistant strains in each etiologic group.
[d] For patients finishing the scheduled treatment without failing (n = 91).
[e] Time to debridement: time from beginning of symptoms to surgery of debridement.
[f] 5 patients excluded, with unknown outcome.
[g] percentages given in rapport to total of failures.
[h] Comparison between THA and C-HHA.

The number of infections by Gram-negative bacilli is less than the simple sum of episodes by *P. aeruginosa* and episodes by Enterobacteriaceae, since there are polymicrobial infections caused by several Gram-negative microorganisms.

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it is also placed on young active patients with hip fracture. By contrast, the placement of a HHA is a standardized emergent procedure for the treatment of hip fracture in the elderly. HHA may be cemented (C-HHA) or non-cemented (NC-HHA). The former device implies a more sophisticated surgical technique but better functional results, and is usually reserved for patients with acceptable previous mobility.

Clinical and surgical management

Diagnosis of PJI was made from surgical, joint-aspirated or blood cultures, alongside the presence of typical clinical signs and symptoms such as joint pain or other inflammatory signs, the presence of a sinus tract communicating with the prosthesis, and/or the presence of purulence surrounding the implant.[8] Microorganisms were identified following standard criteria[9] after being seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate and MacConkey agar) and incubated for at least 10 days. One or more positive culture for virulent pathogenic bacteria and $\geq$2 positive cultures showing the same antibiotic susceptibility profile for potential contaminant bacteria, such as coagulase-negative *Staphylococcus* or *Propionibacterium*, were needed in the presence of a compatible clinical picture.[8] PJI was classified according to Tsukayama et al.[6]

Patients were treated following current recommendations.[1,2] Those with early post-surgical and haematogenous infections, stable device and non-badly damaged surrounding periprosthetic tissue underwent DAIR (debridement of purulent and necrotic tissue and the exchange of removable prosthesis components, antimicrobial therapy and implant retention). This approach has been described elsewhere.[7] Wide antimicrobial therapy (i.e. vancomycin plus ceftazidime) is initially administered and the antimicrobial spectrum is then narrowed once aetiology has been identified and the antibiotic susceptibility profile is available. The use of an intravenous route is maintained for most beta-lactam antibiotics. For the other antimicrobials, a switch to the oral route is made according to the patient's oral tolerance. Therapy usually lasts 8 weeks.

At the time of debridement, NC-HHA are not usually osteo-integrated, since they are non-cemented and have usually been placed a short time before. The removal of exchangeable components of the prosthesis is highly recommended in DAIR-management,[7,10,11] since it allows the removal of the biofilm attached to these pieces, and also a better debridement of some bone and device surfaces that would have not been reached without moving apart these components. Thus, in our institution treatment of an early post-surgical infection of NC-HHA does not follow a standard DAIR: the device is easily removed and exchanged for another non-cemented prosthesis in the same procedure, once thorough debridement has been performed. For the purposes of this study, these patients are compared along with THA and C-HHA patients who underwent DAIR.

Patients with late-chronic infection usually undergo prosthesis removal, 6 weeks of antibiotic therapy and, when possible, prosthesis replacement in a second step. Patients with positive intraoperative cultures (PIOC), who have actually undergone a 1-step exchange procedure, receive a prolonged course of antibiotics.

Study design, analysis and outcome

A comparative analysis of baseline features and type of PJI among patients with HHA and THA was made. Rheumatoid arthritis was defined by diagnostic criteria.[12] Renal chronic impairment was defined as a stable level of creatinine >150 μmol/l. Liver cirrhosis was considered after compatible clinical, analytical and/or pathological findings. Chronic heart and lung diseases were also considered.

Since clinical signs and symptoms and microorganisms responsible for the infection differ widely according to the type of PJI, a further comparative analysis of aetiologies, clinical presentation and outcome was performed for cases undergoing DAIR. Here, failure was defined as: 1) death related with the infection; and/or 2) removal of the prosthesis for any cause within the first 2 years after debridement, or due to relapse or persistence of the infection at any time; and/or 3) need for any kind of salvage therapy after DAIR, including extra debridements 6 weeks after the first one, and/or additional antimicrobial therapy upon completion of the original course and/or long-term suppressive antimicrobial therapy. Apart from failure, an exploratory analysis of mortality due to infection among groups was also performed.

Table 3 Microorganisms responsible for the episode of infection among cases managed with DAIR.[a]

	HHA	THA	Total
Gram-positive bacteria			
Staphylococcus aureus	13	32	45
MRSA	4	5	9
Coagulase-negative *Staphylococcus*	10	9	19
Enterococcus sp[b]	4	10	14
Streptococcus group G	0	1	1
Corynebacterium sp	4	1	5
Propionibacterium sp	0	1	1
Gram-negative bacteria			
Escherichia coli	16	12	28
Proteus sp	9	13	22
Klebsiella sp	5	7	12
Enterobacter sp	3	2	5
Salmonella enteritidis	0	1	1
Citrobacter koseri	0	1	1
Morganella sp	2	3	5
Serratia sp	1	0	1
Providencia sturartii	0	1	1
Pseudomonas aeruginosa	17	16	33
Acinetobacter baumani	2	0	2
Anaerobic bacteria[c]	1	8	9

HHA: hip hemiarthroplasty. THA: total hip arthroplasty. MRSA: methicillin-resistant *S. aureus*.

[a] 205 isolates in 123 episodes; three episodes with negative cultures.

[b] 8 *E. faecalis*, 1 *E. faecium*, 1 *E. durans*.

[c] Anaerobic bacteria: 3 *Peptostreptococcus* sp, 2 *Bacteroides* sp, 1 *Actinomyces* sp, 1 *Porphyromonas* sp, 1 *Prevotella* sp, 1 *Veillonella* sp.

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Statistical analysis

Categorical variables were compared with X^2 or Fisher's exact test. Continuous variables were compared with the Mann–Whitney *U* test. Since the time when the failure happens is important in the setting of bone and joint infections, including PJI, parameters predicting failure among patients undergoing DAIR were examined by univariate analysis with Kaplan–Meier curves (log-rank test) and univariate Cox regression. Failure was considered as the main event, while loss of follow-up, death unrelated with the infection or a new unrelated episode of PJI were considered as censored times. Follow-up was considered from the first debridement to the time of failure or the censored time. A multivariate analysis with Cox regression was conducted to identify independent parameters predicting failure. Variables showing a p value < 0.30 in the univariate analysis were included in a stepwise backward selection process to build a multivariate model. All analyses were 2-tailed. A p value < 0.05 was considered statistically significant. Data were analysed using SPSS (version 15.0).

Results

Of a total of 348 PJI episodes during the study period, 210 (60%) involved hip prosthesis, occurring in 197 patients with a median age of 74 years [interquartile range (IQR): 64–80] and of whom 124 (63%) were women. Sixty-two (39%) patients carrying a HHA [29 (48%) NC-HHA and 33 (53%) C-HHA] and 148 (61%) a THA had similar baseline characteristics, except the former were older and had more age-related comorbidities (Table 1). Rheumatoid arthritis and revision prosthesis were more frequent among THA. The majority of infections among HHA were early post-surgical, whereas the type of PJI was more varied among THA.

A total of 123 (59%) patients underwent DAIR [120 (88%) of the 136 patients with acute onset, plus 3 patients with late-chronic infection]: 51 (41%) HHA and 72 (59%) THA. Differences in basal features were similar as those observed in the whole series (data not shown). Table 2 summarizes their clinical presentation, aetiology, treatment and outcome. While clinical presentation was very similar among patients with different hip devices, the aetiology was different: infection by methicillin-susceptible *Staphylococcus aureus* (MSSA) was more frequent in THA, while Gram-negative bacilli (GNB) were more frequent in HHA, with a higher prevalence of fluoroquinolone resistance in the C-HHA group. A more detailed description of aetiologies among these cases is shown in Table 3.

Table 2 also shows that medical and surgical DAIR management was very similar among different groups, except for a higher rate of exchange of removable components among HHA, also observed when considering only C-HHA vs. THA.

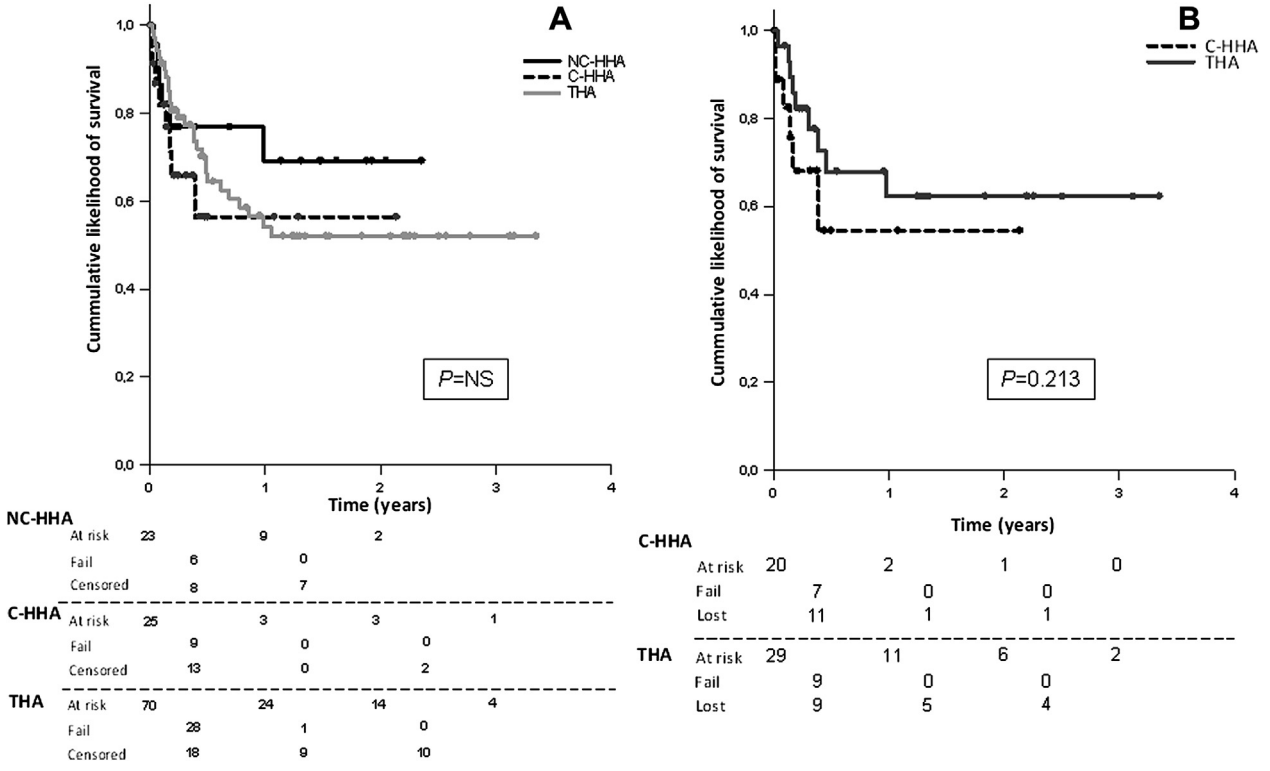

Figure 1 Cumulative likelihood of survival of THA, C-HHA and NC-HHA after DAIR THA: total hip arthroplasty (grey continuous line); C-HHA: cemented hip hemiarthroplasty (black discontinuous line); NC-HHA: non-cemented hip hemiarthroplasty (black continuous line); DAIR: debridement, antibiotics and implant retention. Labels: at risk denotes de number of patients at risk of failing at the beginning of the period (year); fail denotes the patients actually failing during the period; lost denotes the number of patients lost for follow-up during the period (censored times). (A). All cases submitted to DAIR with known outcome: $n = 118$; Log-rank test, $p = 0.333$. (B). Subanalysis of post-surgical cemented hip device infection in which removable components were exchanged during debridement: THA, $n = 29$; C-HHA, $n = 20$; Log rank test, $p = 0.213$.

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Table 4 Univariate analysis of parameters predicting failure ($n = 118$)

	Categories (failure/*n*)	Survival time (days)[b]	HR (95%CI)	*p*
Sex	Women (27/78)	743 ± 72	0.78 (0.43–1.44)	0.437
	Men[a] (17/40)	621 ± 92		
Age (years)	–	–	0.99 (0.97–1.01)	0.461
Rheumatoid arthritis	Yes (4/9)	444 ± 118	1.25 (0.45–3.49)	0.683
	No[a] (40/109)	721 ± 61		
Diabetes mellitus	Yes (13/30)	417 ± 83	1.75 (0.91–3.37)	0.105
	No[a] (31/88)	757 ± 65		
Immunosuppressant therapy	Yes (9/16)	188 ± 43	2.31 (1.10–4.84)	0.041
	No[a] (35/102)	760 ± 61		
Any comorbidity	Yes (22/70)	741 ± 69	0.72 (0.40–1.30)	0.271
	No[a] (22/48)	625 ± 88		
Prosthesis number	Revision (12/23)	583 ± 109	1.35 (0.69–2.64)	0.386
	Primary[a] (31/93)	752 ± 67		
Hip prosthesis type	THA (29/70)	697 ± 72	1.52 (0.63–3.68)	0.345
	C-HHA (9/25)	449 ± 87	2.17 (0.77–6.14)	0.145
	NC-HHA[a] (6/23)	631 ± 80	–	–
Time to infection[c]	In days	–	1.00 (1.00–1.00)	0.319
	>30days (4/9)	505 ± 159	1.46 (0.52–4.12)	0.496
	≤30 days[a] (34/101)	760 ± 62		
Type of infection	Hematogenous (6/8)	185 ± 74	2.72 (1.14–6.46)	0.045
	Post-surgical[a] (38/110)	751 ± 60		
Sinus tract	Yes (6/22)	765 ± 130	0.77 (0.33–1.83)	0.547
	No[a] (38/95)	690 ± 64		
Temperature>37 °C	Yes (22/52)	660 ± 88	1.31 (0.72–2.39)	0.373
	No[a] (21/64)	645 ± 64		
Prosthesis Rx	Sings of infection (6/8)	330 ± 119	2.23 (0.94–5.31)	0.099
	Normal[a] (35/106)	771 ± 61		
CRP (mg/l)	–	–	1.00 (1.00–1.01)	0.398
ESG (mm/h)	–	–	0.99 (0.96–1.02)	0.550
Leukocytes ($x10^9$/l)	–	–	1.08 (1.01–1.14)	0.035
Bacteremia	Yes (4/7)	215 ± 83	1.96 (0.70–5.49)	0.241
	No[a] (40/111)	729 ± 60		
Polymicrobial infection	Yes (20/56)	694 ± 92	1.24 (0.68–2.25)	0.480
	No[a] (24/62)	694 ± 69		
Infection by *S. aureus*	Yes (20/44)	668 ± 90	1.23 (0.68–2.24)	0.491
	No[a] (24/74)	711 ± 72		
Infection by MRSA	Yes (8/9)	133 ± 58	3.93 (1.81–8.52)	0.003
	No[a] (36/109)	767 ± 60		
Infection by *P. aeruginosa*	Yes (6/31)	779 ± 85	0.45 (0.19–1.06)	0.043
	No[a] (38/87)	654 ± 67		
Infection by *Enterobacteriaceae*	Yes (21/55)	666 ± 90	1.20 (0.66–2.16)	0.555
	No[a] (23/63)	702 ± 71		
Infection by FQ-R Gram-negative bacilli	Yes (8/21)	609 ± 143	1.59 (0.73–3.44)	0.263
	No[a] (36/97)	737 ± 62		
Infection by *Enterococci*	Yes (7/12)	459 ± 165	3.22 (1.42–7.34)	0.014
	No[a] (37/106)	748 ± 60		
Removable components exchange	Yes (22/73)	801 ± 74	0.68 (0.38–1.23)	0.204
	No[a] (22/45)	587 ± 83		
Need for 2 debridements or more	Yes (15/23)	317 ± 70	2.18 (1.17–4.08)	0.020
	No[a] (29/95)	804 ± 64		
Time to debridement[d]	In days	–	0.99 (0.95–1.04)	0.753
	>2days (32/95)	765 ± 64	0.51 (0.26–0.99)	0.061
	≤2 days[a] (12/23)	484 ± 120		
	>7days (17/44)	719 ± 92	0.97 (0.53–1.78)	0.916
	≤7 days[a] (27/74)	678 ± 70		

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Table 4 (*continued*)

	Categories (failure/*n*)	Survival time (days)[b]	HR (95%CI)	*p*
Length of antimicrobial therapy[e]	In days	–	1.01 (0.97–1.04)	0.696
	>60days (11/33)	758 ± 104	2.11 (0.88–5.10)	0.096
	≤60 days[a] (9/60)	938 ± 64		

Patients with unknown outcome ($n = 5$) excluded from this analysis.
[a] Reference category for each univariate analysis.
[b] Survival time expressed in mean ± standard deviation.
[c] Time to infection: time from prosthesis placement to beginning of symptoms (hematogenous cases excluded).
[d] Time to debridement: time from beginning of symptoms to surgery of debridement.
[e] Length of antimicrobial therapy considered only in patients who finished the scheduled treatment without failing.
HR (95%CI): hazard ratio (95% confidence interval). THA: total hip arthroplasty. HHA: hip hemiarthroplasty. C-HHA: cemented HHA. NC-HHA: non-cemented HHA. Prosthesis Rx: prosthesis radiography at diagnosis. CRP: C-reactive protein. ESR: erythrocyte sedimentation rate. MSSA: methicillin-susceptible *S. aureus*. MRSA: methicillin-resistant *S. aureus*. FQ-R: fluoroquinolone-resistant.

Overall failure of DAIR management was 37%, there being no significant differences between the groups (Fig. 1A). HHA infections were more likely to be associated with early failure while still on therapy. Notably, significantly higher infection-related mortality was observed among HHA, especially among C-HHA. Median follow-up among patients not failing was 347 days (IQR: 92–692), being shorter in patients with C-HHA as compared with NC-HAA or THA [105 days (IQR 50–180) vs 454 days (IQR 122–775); $p = 0.002$]. A sub-analysis of failure comparing post-surgical C-HHA and THA cases in which removable components were exchanged is shown in Fig. 1B: mean survival time was 1.19 years (95% CI 0.63–1.75) and 2.20 years (95% CI 1.62–2.82), respectively ($p = 0.213$).

Tables 4 and 5 summarize, respectively, the univariate and multivariate analysis of parameters predicting failure. The latter shows that haematogenous cases with a high inflammatory pattern, frequently needing more than one debridement, had a higher likelihood of failure, as did those caused by MRSA or *Enterococcus*.

Discussion

This study specifically addresses the differences observed in hip devices in the setting of PJI. We have found that patients carrying infected HHA or THA have different baseline features and present with different PJI, both clinically and microbiology.

This is consistent with the indications for HHA or THA, which respond to different kind of patients. The placement of a HHA is a standardised emergent procedure for a rapid recovery after hip fracture in elderly patients without high physical demands.[5] By contrast, surgery for placing a THA is usually elective in the context of younger patients with degenerative joint disease (such as rheumatoid arthritis or arthrosis). THA may also be the treatment for a hip fracture, but this is commonly reserved for young and highly active patients.

Indeed, we have observed that HHA patients are usually older and thus present with more comorbidities than do those with THA. Further differences may also be found among patients with C-HHA or NC-HHA, especially regarding age, and this is consistent with the indication of either device. The main goal after a hip fracture is to restore the patient's previous functional status.[4] C-HHA involves slightly more complex surgery but functional results seem to be better than with NC-HHA.[13,14] Hence, the former are normally reserved for younger patients with previously better mobility.

We also observed differences between HHA and THA regarding the type of PJI. HHA infections were fundamentally early post-surgical, while among THA the percentage of early post-surgical and late-chronic infections was lower and higher, respectively, with a significant number of haematogenous infections also being observed.

Among patients undergoing DAIR, important aetiological differences were observed. HHA infections were frequently caused by GNB, whereas THA infections were more often caused by *S. aureus*, something that may be explained by the younger age[15] and the higher rate of rheumatoid arthritis and haematogenous cases in this group.[6,16,17] Resistant microorganisms were more frequently found in HHA infections. The proportion of MRSA among staphylococcal PJI was 31% for HHA and 16% in THA. The proportion of fluoroquinolone-resistant GNB infections was 48% among C-HHA vs. 22% for THA infections. Again, this is probably due to age differences and the fact that many HHA patients have a close relationship with long-term care facilities and the health-care system.[18,19] The rate of polymicrobial infection was high in the present series as compared with data previously reported, although some variability may

Table 5 Multivariate analysis of parameters predicting failure.

	HR (CI95%)	*p*
Hematogenous infection	3.87 (1.52–9.83)	0.005
Leukocytes ($\times 10^9$/l)	1.10 (1.03–1.18)	0.006
Need for 2 debridements or more	2.47 (1.24–4.94)	0.010
Infection by MRSA	3.75 (1.66–8.50)	0.002
Infection by *Enterococcus*	4.83 (1.98–11.9)	0.001

The following parameters were included in an initial model of multivariate analysis: diabetes, immunosuppressant therapy, cemented hip hemiarthroplasties vs other hip devices, hematogenous infection, radiographic signs of infection, leukocyte count, bacteremia, infection by MRSA, infection by fluoroquinolone-resistant Gram-negative bacilli, infection by *Enterococcus*, exchange of removable components during debridement and need for 2 debridements or more.

be observed in different studies.[10,20,21] Interestingly, we did not found statistical differences of polymicrobial PJI among THA and HHA groups.

It is likely that the patients' baseline characteristics and the emergency of the procedure have had influence on these differences found in the microbiology and clinical presentation of PJIs. The fact that HHA are usually placed during an emergency procedure on older and fragile patients, who frequently present no control of their rectal and urinary sphincters, undoubtedly increases the likelihood of early post-surgical infections due to Gram-negative flora and *Enterococcus* sp.

Overall, HHA patients had similar odds of being cured and retaining their prosthesis as did those with THA. This was somewhat surprising, since the former were older and presented more comorbidities and a higher rate of resistant microorganisms. These factors would, *a priori*, imply a worse prognosis,[22] but this is likely to have been balanced by some other parameters that also influence outcome and which are more frequently observed in the THA group. One such parameter would be the higher proportion of haematogenous infections among THA. Our multivariate model indicated worse outcomes for haematogenous infections, as reported previously.[10,23,24] In addition, and as mentioned above, most cases of THA were caused by *S. aureus*, which classically has been related with worse outcomes as compared with other aetiologies.[7,11,23,25]

There were a further two notable differences between THA and HHA patients regarding DAIR management. First, as previously mentioned, patients carrying a NC-HHA did not undergo standard DAIR because the implant was not retained — its removal does not imply complex surgery or bleeding, and it can be easily replaced by a new NC-HHA, thereby enabling better debridement and removal of bacterial biofilm. This could account for a not-so-bad prognosis for NC-HHA. Most patients with infected NC-HHA underwent this one-step exchange strategy, so it is uncertain if alternative approaches would have been followed by different results.

Second, exchanging removable prosthetic components significantly improves the outcome of patients with PJI.[7,10,11] This could account for the worse prognosis among THA patients, among whom only 42% underwent removal of exchangeable components. Improving this surgical approach would potentially ameliorate the outcome of these patients. Among the various reasons for this difference between C-HHA and THA surgical debridement, hip dislocation and exchange of the prosthetic head is technically easier in C-HHA, and sometimes the polyethylene component of THA is cemented, thus making it more difficult to remove. A sub-analysis of a more homogeneous cohort of patients sought to illustrate this balance: when excluding haematogenous cases and those with debridement in which no removable pieces were exchanged, a trend towards a better outcome was observed among THA infections (Fig. 1B).

In line with previous studies[7,10,22,26] our multivariate analysis identified certain parameters, mainly related with the clinical presentation and aetiology of the infection, that had an independent influence on the likelihood of failure. It is acknowledged, however, that other parameters may also have influenced in this probability of failure, for example, immunosuppressant therapy, the presence of fistula, bacteraemia, polymicrobial infection, a significant delay in debridement or, as previously discussed, the exchange of removable pieces.[10,25] Our sample may not have been large enough to detect these other parameters with less weight but which remain clinically significant.

Another aspect to bear in mind when interpreting the present results is our wide definition of failure. This included dramatic outcomes such as death, which was analysed at the same level as other less definitive outcomes, such as the need for salvage therapy, which may eventually lead to good functional status in the patient. This definition of outcome was chosen on the basis of previous studies by our group and for comparison purposes.[10,23,27]

Regarding the specific association with mortality, the present analysis showed that death related with the infection was higher among patients carrying a HHA. While failure in THA patients normally implied the need for salvage therapy and loss of the prosthesis, among HHA cases it frequently meant a fatal outcome. A recent communication also reported a higher raw mortality rate among patients carrying an infected HHA [Del Toro et al., 51st ICAAC 2011, abstract K-1568]. These differences are consistent with the recipient of the prosthesis: HHA patients were older and had more comorbidities. In this population a hip-fracture implies *per se* an important challenge that may sometimes jeopardize the life of the patient,[28,29] and infection of the prosthetic device could easily promote progression to death.

In our series, patients with NC-HHA presented less infection-related mortality than did subjects with C-HHA. This is probably because the former, despite being older, had fewer comorbid conditions. Furthermore, and as discussed above, the debridement of NC-HHA implied a 1-step exchange of the prosthesis, probably leading to better control of the infection and, therefore, reducing the rate of infection-related deaths. We do not believe that patients with C-HHA would necessarily have had a better prognosis if removal of the prosthesis had been attempted. Indeed, in contrast to NC-HHA, C-HHA are soundly fixed after implantation, and a longer surgery with more bleeding, implying transfemoral osteotomy of the bone, would have been necessary.

Our work has some limitations. First it is an observational study. However, information has been prospectively gathered, thus increasing its quality. Furthermore, observational studies are probably the best quality of information we may ever have in this subject, since the indication for a specific hip-device is made according to the patients' baseline features and hip condition, and cannot be randomized. Second, although the sample is wide enough for this kind of studies, statistical power may have not been enough to identify some parameters also associated with failure. Finally, follow-up in patients carrying a C-HHA was shorter than the rest. This is likely to be related with their old and fragile condition, and many of them may have died for causes not related with the infection not long after the hip fracture. This shorter expectancy of life might have prevented some of these patients from presenting a relapse of their infection, thus biasing our results and explaining in part the similar likelihood of failure observed between HHA and THA patients.

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In summary, our analysis shows that carriers of infected THA or HHA have different characteristics. Although the specific type of hip device does not independently influence the likelihood of failure after DAIR, different parameters in the two groups produce ultimately similar outcomes: while HHA patients were older, had more comorbidities and a higher rate of resistant microorganisms, they were more thoroughly debrided than were patients carrying a THA, who had a higher frequency of haematogenous infections and *S. aureus* as the aetiology. Importantly, PJI among HHA patients led to a higher probability of death, especially among C-HHA. This highlights the need for early diagnosis and treatment, and a proactive attitude in response to an infected HHA.

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References

1. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004;**351**:1645—54.
2. Cobo J, Del Pozo JL. Prosthetic joint infection: diagnosis and management. *Expert Rev Anti Infect Ther* 2011;**9**: 787—802.
3. Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. *N Engl J Med* 2009;**361**:787—94.
4. Zuckerman JD. Hip fracture. *N Engl J Med* 1996;**334**:1519—25.
5. Hopley C, Stengel D, Ekkernkamp A, Wich M. Primary total hip arthroplasty versus hemiarthroplasty for displaced intracapsular hip fractures in older patients: systematic review. *BMJ* 2010;**340**:c2332.
6. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. *J Bone Jt Surg Am* 1996;**78**:512—23.
7. Byren I, Bejon P, Atkins BL, Angus B, Masters S, McLardy-Smith P, et al. One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. *J Antimicrob Chemother* 2009;**63**:1264—71.
8. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2013;**56**:1—10.
9. Murray PR, Baron EJ, Jorgesen JH, Landry ML, Pfaller MA, editors. *Manual of clinical microbiology*. 9th ed. Washington, D.C: American Society for Microbiology; 2007.
10. Lora-Tamayo J, Murillo O, Iribarren JA, Soriano A, Sánchez-Somolinos M, Baraia-Etxaburu JM, et al. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. *Clin Infect Dis* 2013;**56**:182—94.
11. Choi HR, von Knoch F, Zurakowski D, Nelson SB, Malchau H. Can implant retention be recommended for treatment of infected TKA? *Clin Orthop Relat Res* 2011;**469**:961—9.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315—24.
13. Parker MI, Pryor G, Gurusamy K. Cemented versus uncemented hemiarthroplasty for intracapsular hip fractures: a randomized controlled trial in 400 patients. *J Bone Jt Surg Br* 2010;**92**: 116—22.
14. Luo X, He S, Li Z, Huang D. Systematic review of cemented versus uncemented hemiarthroplasty for displaced femoral neck fractures in older patients. *Arch Orthop Trauma Surg* 2012;**132**:455—63.
15. Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. *Clin Infect Dis* 2009;**49**:1036—43.
16. Jacobsson G, Dashti S, Wahlberg T, Andersson R. The epidemiology of and risk factors for invasive *Staphylococcus aureus* infections in western Sweden. *Scand J Infect Dis* 2007;**39**: 6—13.
17. Dubost JJ, Soubrier M, Sauvezie B. Arthrite septique à pyogène de l'adulte. *Rev Rhum [Éd Fr]* 2000;**67**:17—27.
18. Manzur A, Gudiol F. Methicillin-resistant *Staphylococcus aureus* in long-term-care facilities. *Clin Microbiol Infect* 2009;**15**: 26—30.
19. Lautenbach E, Fishman NO, Bilker WB, Castiglioni A, Metlay JP, Edelstein PH, et al. Risk factors for fluoroquinolone resistance in nosocomial *Escherichia coli* and *Klebsiella pneumoniae* infections. *Arch Int Med* 2002;**162**:2469—77.
20. Peel TN, Cheng AC, Bising KL, Choonq PF. Microbiological aetiology, epidemiology, and clinical profile of prosthetic joint infections: are current antibiotic prophylaxis guidelines effective? *Antimicrob Agents Chemother* 2012;**56**:2386—91.
21. Marculescu CE, Cantey JR. Polymicrobial prosthetic joint infections: risk factors and outcome. *Clin Orthop Relat Res* 2008; **466**:1397—404.
22. Martínez-Pastor JC, Muñoz-Mahamud E, Vilchez F, García-Ramiro S, Bori G, Sierra J, et al. Outcome of acute prosthetic joint infections due to Gram-negative bacilli treated with open debridement and retention of the prosthesis. *Antimicrob Agents Chemother* 2009;**53**:4772—7.
23. Rodríguez D, Pigrau C, Euba G, Cobo J, García-Lechuz J, Palomino J, et al. Acute hematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. *Clin Microbiol Infect* 2010;**16**:1789—95.
24. Sendi P, Banderet F, Graber P, Zimmerli W. Clinical comparison between exogenous and hematogenous periprosthetic joint infections caused by *Staphylococcus aureus*. *Clin Microbiol Infect* 2011;**17**:1098—100.
25. Marculescu CE, Berbari EF, Hanssen AD, Steckelberg JM, Harmsen SW, Mandrekar JN, et al. Outcome of prosthetic joint infections treated with debridement and retention of components. *Clin Infect Dis* 2006;**42**:471—8.
26. Vilchez F, Martínez-Pastor JC, García-Ramiro S, Bori G, Maculé F, Sierra J, et al. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to *Staphylococcus aureus* treated with debridement. *Clin Microbiol Infect* 2011;**17**:439—44.
27. Cobo J, García San Miguel L, Euba G, Rodríguez D, García-Lechuz JM, Riera M, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. *Clin Microbiol Infect* 2011;**17**: 1632—7.
28. Kesmezacar H, Ayhan E, Unlu MC, Seker A, Karaca S. Predictors of mortality in elderly patients with an intertrochanteric or a femoral neck fracture. *J Trauma* 2010;**68**:153—8.
29. Petersen MB, Jørgensen HL, Hansen K, Duus BR. Factors affecting postoperative mortality of patients with displaced femoral neck fracture. *Injury* 2006;**37**:705—11.

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Linezolid in late-chronic prosthetic joint infection caused by gram-positive bacteria

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ABSTRACT

Linezolid may be an interesting alternative for prosthetic joint infection (PJI) due to its bioavailability and its antimicrobial spectrum. However, experience in this setting is scarce. The aim of the study was to assess linezolid's clinical and microbiological efficacy, and also its tolerance. This was a prospective, multicenter, open-label, non-comparative study of 25 patients with late-chronic PJI caused by Gram-positive bacteria managed with a two-step exchange procedure plus 6 weeks of linezolid. Twenty-two (88%) patients tolerated linezolid without major adverse effects, although a global decrease in the platelet count was observed. Three patients were withdrawn because of major toxicity, which reversed after linezolid stoppage. Among patients who completed treatment, 19 (86%) demonstrated clinical and microbiological cure. Two patients presented with clinical and microbiological failure, and one showed clinical cure and microbiological failure. In conclusion, linezolid showed good results in chronic PJI managed with a two-step exchange procedure. Tolerance seems acceptable, though close surveillance is required.

1. Introduction

Prosthetic joint infection (PJI) is a major health problem of increasing incidence. In chronic and in some acute PJI, removal of hardware is often necessary (Del Pozo and Patel, 2009; Zimmerli et al., 2004), being a 2-step exchange the most common procedure, with success rates around 90% (Jämsen et al., 2009; Zimmerli et al., 2004). Briefly, this technique involves the removal of the infected prosthesis in the first stage, replacement by an antibiotic-loaded cement spacer, administration of systemic antibiotics, and finally the placement of a new prosthesis. The aim is to provide a sterile surgical site for the new arthroplasty.

However, cultures systematically performed at prosthesis reimplantation have demonstrated that sterility is not always guaranteed, as positive results have been found in 6–20% of cases (Bejon et al., 2010; Della Valle et al., 1999; Mont et al., 2000; Murillo et al., 2008). In most of these cases, the isolates are coagulase-negative Staphylococci (CNS) resistant to the antimicrobials used during the previous weeks (Mont et al., 2000; Murillo et al., 2008).

Six weeks of intravenous antibiotic treatment is usually recommended (Del Pozo and Patel, 2009; Hanssen and Spangehl, 2004; Jämsen et al., 2009; Murillo et al., 2008) but the emergence of alternative antimicrobials with good bioavailability may mean that the intravenous route is no longer necessary. It has also been suggested that antibiotics with extended anti-staphylococcal spectrum may be of use in avoiding persistence or superinfection by resistant CNS (Cabo et al., 2011; Murillo et al., 2008).

Linezolid possesses a wide anti-Gram-positive bacteria (GPB) spectrum, including all CNS species, and has 100% bioavailability and good diffusion in bone tissue (Clemmet and Markham, 2000; Rana et al., 2002). These properties may make it a suitable alternative for the treatment of chronic PJI. However, clinical experience with linezolid in this setting is scarce (Rao and Hamilton, 2007; Senneville et al., 2006) and toxicity is a matter of concern (Legout et al., 2010; Rayner et al., 2004; Senneville et al., 2006; Vihn and Rubinstein, 2009).

We undertook a prospective multicentre study of patients with PJI caused by GPB treated with a two-step exchange procedure and therapy with linezolid for 6 weeks. The aims of the present study

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were: 1) to analyze the clinical rate of success and microbiological eradication in patients treated with linezolid; and 2) to assess the safety of this antimicrobial during a 6-week therapy schedule, especially in the elderly population.

2. Patients and methods

2.1. Setting

This prospective, open-label, non-randomized, non-comparative, multicentre study was performed at seven teaching hospitals in Spain between 2007 and 2009. The study was approved by the local ethic committees.

2.2. Study population

Patients undergoing two-steps exchange procedure as treatment of chronic PJI caused by GPB were eligible for this study. These were mainly patients with late-chronic infection according to Tsukayama criteria (Tsukayama et al., 1996), but also patients who underwent salvage therapy for acute infections or relapses.

Clinical diagnosis was based on the presence of typical symptoms and signs, such as joint pain, inflammatory signs, and fistula. Microbiological diagnosis was established from surgical or arthrocentesis samples showing 2 or more positive cultures for the same bacteria with identical antibiogram profile (Atkins et al., 1998). The following baseline characteristics and data were recorded: age, sex, type of prosthesis, Charlson co-morbidity index (Charlson et al., 1987), concomitant treatment, haemogram and biochemistry profile.

The samples obtained during the operation (synovial fluid, periprosthetiic tissue and bone) were seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate and MacConkey agar). They were incubated for at least 7 days. Microorganisms and antibiotic susceptibility were identified according to standard criteria (Kloos and Lambe, 1991).

The following exclusion criteria were applied: impossibility of removing all the components of the prosthetic or one-step exchange procedure; use of cement spacers loaded with vancomycin; need for an antibiotic with anti-GPB activity other than linezolid for more than 7 days; breastfeeding; pregnancy; age less than 18 years; non-controlled hypertension or diseases that could lead to severe hypertension; liver cirrhosis; thrombocytopenia less than 60,000 platelets/μL or anemia of central origin; creatinine clearance less than 20 mL/min; peripheral neuropathy.

2.3. Treatment protocol

Patients underwent a two-step exchange procedure. During the first step surgery, all prosthetic components were removed and a thorough debridement was performed. A cement spacer could be placed in the surgical site; loading with antibiotics other than vancomycin was allowed. Empirical antibiotic therapy was then started. Patients signed written informed consent and linezolid 600 mg every 12 hours, either intravenous or oral, was started within 7 days of the first step surgery. Daily dose was not modified depending on patient's weight or renal function. The co-administration of other antibiotics with no activity against GPB was permitted in patients with polymicrobial infection. Haemogram and biochemistry profiles were performed each week during antimicrobial therapy, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). After a total antimicrobial therapy of 42 days, the reimplantation surgery could be performed. Before placing the new prosthesis and administering prophylactic antibiotics, 4–6 samples were taken from the surgical site and cultured, following a similar protocol as in the first step. Patients were followed up at the outpatient clinic for at least 12 months with prosthesis X-ray, haemogram, and biochemistry profile.

2.4. Outcome definitions

Patients were considered to be clinically cured if there was progressive disappearance of inflammatory signs, significant decrease in CRP, no signs of infection during reimplantation surgery, and no signs of infection due to the same bacteria during follow-up. The need for a new debridement after the first step surgery and before reimplantation was not considered a failure per se. After reimplantation, patients who developed an early post-surgical infection due to different bacteria were not considered to have failed, but to have had a new episode of PJI.

Patients were considered to be microbiologically cured if cultures taken at surgical site during reimplantation were negative. One or more cultures with the same bacteria causing the original infection were required to consider persistence. If 2 or more cultures yielded the same bacteria, but were different from that causing the original episode, it was considered superinfection.

While on therapy with linezolid, patients were interviewed every week for the presence of new symptoms or signs which could be considered an adverse event. Specifically, patients were questioned on whether they had experienced nausea, vomiting, dizziness, abdominal pain, diarrhea, headache, somnolence, paresthesias or other symptoms suggesting neuropathy, blurred vision, hypoacusis, tinnitus, disturbances in the taste or dysgeusia, insomnia, anxiety, behavior disturbances, mood alterations, cough, dyspnea, chest pain, palpitations, arthalgias, myalgias, rashes, pruritus or muco-cutaneous candidiasis. Toxicity was defined as mild if it was transitory or could be managed without stopping linezolid. It was considered to be severe if the life of the patient was exposed to serious risk, if hospitalization needed to be prolonged, or if linezolid had to be withdrawn. Thrombocytopenia was defined as a platelet count less than 100,000 platelets/mm^3 or less than 75% of the baseline count. Anemia was defined as haemoglobin less than 9.0 g/dL or less than 75% of baseline haemoglobin. In order to avoid the interference of blood transfusions during or immediately after surgery, baseline haemoglobin was measured one week after surgery.

Since platelets may behave as acute-phase reactants and may therefore present a progressive decline after surgery, a matched study was performed comparing patients treated with linezolid (cases) and 25 historical controls. These historical controls were patients with chronic-PJI managed at two of the participating hospitals, treated with a 2-step exchange procedure and six weeks of antimicrobial therapy other than linezolid. Cases and historical controls were age and sex-matched.

2.5. Statistical analysis

A per protocol analysis was performed, evaluating the outcome among patients who tolerated linezolid for the programmed schedule. A potential association for the development of AE was evaluated for serveral variables (age, sex, BMI, Charlson score, creatinine and concomitant treatment with pyridoxine or serotoninegic antidepressants) by means of a univariate analysis, using χ^2 or Fisher exact test for categorical variables, and the t test or Mann-Whitney's U test for continuous variables. All analyses were performed using SPSS software (version 15.0).

3. Results

Twenty-five patients were recruited: 20 were women (80%), and the median age was 73 years (range 59–89). Median Charlson-score was 1 (range 0–3), being 0 in 11 (44%) patients. Median body mass index (BMI) was 28.7 kg/m^2 (range 21.3–36.8). Median creatinine

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was 0.54 mg/dl (maximum 1.13 mg/dl). Sixteen patients had knee-prostheses and nine had hip-prostheses. Five cases had revision prostheses (2 of them were tertiary implants). Apart from joint pain, inflammatory signs were observed in 22 patients (88%). A fistula was present in 10 cases (40%). Radiographic prosthetic loosening was seen in 14 patients (56%). Infection was considered late-chronic in 17 cases (beginning of infection >3 months after placement of the device), while it had presented acutely in 8: in 6, an unsuccessful attempt of debridement, antibiotics and implant retention had been made, leading to a chronic scenario, and the two-step exchange procedure was a salvage therapy; in the remaining 2, the retention of the implant was not feasible and the prosthesis was directly removed.

Table 1 shows the microbiologic etiology. Infection was polymicrobial in 2 patients. All microorganisms were susceptible to linezolid.

Signs of infection during first step surgery were found in 23 patients (92%). A cement spacer was placed in 23 patients (92%), in 16 cases (64%) loaded with gentamycin, and in 5 (20%) with gentamycin plus clindamycin. Four patients (16%) needed at least one extra debridement: 2 due to leakage of synovial fluid, 1 due to hemarthrosis, and 1 because of persistent suppuration. Cultures taken during this extra debridement were negative. However, one of these patients developed superinfection after the debridement, with positive cultures for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Adverse effects (AE) of linezolid are shown in Table 2. Nineteen patients (76%) presented with some kind of adverse effect during therapy, mostly mild. The majority of symptoms developed after 2–3 weeks of therapy. We found no association between the likelihood of the development of any AE and age, sex, BMI, Charlson score, creatinine or concomitant treatment with pyridoxine. Of note, 5 (20%) patients were also being administered serotoninergic antidepressants, but we did not observe a higher likelihood of AE either.

Linezolid was withdrawn in three patients (12%) because of toxicity after a median of 23 days (range 17–24). One patient developed pancytopenia [haemoglobin 7.0 g/dL, leucocytes 3,700/mm^3 (granulocytes 85%) and platelets 113,000/mm^3]. Another patient presented with rectorrhagia in the setting of thrombocytopenia (56,000/mm^3). The third case developed severe mucocutaneous candidiasis. All these severe AE reversed after withdrawal of linezolid. The other 22 patients (88%) tolerated linezolid for a median of 42 days (range 30–88).

Median platelet count at baseline was 326,000 platelets/mm^3 (range 176,000–379,000). Only one patient demonstrated thrombocytopenia under 100,000/mm^3 and bleeding. However, 19 patients (76%) showed platelet counts less than 75% of the baseline level at some point during therapy. Fig. 1 compares the decrease in platelet counts in cases and historical controls. In cases, the platelet count decreased by a mean of 82,417 ± 117,901 platelets/mm^3, while in historic controls it decreased by a mean of 2,920 ± 94,387 platelets/mm^3 ($P = 0.007$).

Median haemoglobin 1 week after surgery was 10.0 g/dL (range 9.0–12.7 g/dL). Two patients (8%) needed blood transfusion while on treatment with linezolid, and overall 9 patients (36%) met the definition of anemia after a median of 4 weeks of linezolid (range 2–6 weeks). However, the overall haemoglobin decrease among non-transfused patients was non-significant (−0.51 g/dL, 95% confidence interval: −1.28 g/dL to +0.25 g/dL; $P = 0.178$).

Outcomes are summarized in Fig. 2. Among the 22 patients who completed the scheduled treatment with linezolid, 20 (91%) were clinically cured and a new prosthesis could be reimplanted a median of 56 days (interquartile range 15–112) after the end of antimicrobial therapy. Two patients (10%) developed an early post-surgical infection of their new prosthesis. Median follow-up was 423 days (interquartile range 214 days).

Clinical failure was observed in 2 patients (9%). One had a PJI caused by *Staphylococcus capitis* which had been unsuccessfully treated with 2 previous 2-step exchanges. Again, *S. capitis* was isolated in the samples taken at the surgical site during reimplantation (still susceptible to linezolid). The other case was a chronic infection of a primary knee-arthroplasty by *S. aureus*. After the prosthesis was removed the patient needed two more debridements and finally an arthrodesis was performed. During these operations, superinfection by Gram-negative bacteria and persistence of *S. aureus* were observed.

Cultures at reimplantation were positive in both cases of clinical failure. Cultures were taken in 19 of the 20 patients with clinical cure (95%), and were positive in only one case (5%) originally caused by CNS. This case had shown a very slow clinical improvement after the removal of the prosthesis: cultures taken during replacement isolated

Table 2
Clinical adverse effects (AE) of Linezolid during therapy.

Adverse effect	Mild toxicity	Antibiotic withdrawal	Median time to develop AE (weeks)
Nausea	10 (40%)	None	3
Vomiting	7 (28%)	None	2
Abdominal pain	8 (32%)	None	3
Diarrhea	4 (16%)	None	2.5
Headache	3 (12%)	None	5
Dizziness	6 (24%)	None	3
Neuropathy	-	-	-
Drowsiness	2 (8%)	None	5
Paresthesia	1 (4%)	None	2
Blurred vision	1 (4%)	None	6
Hearing loss	-	-	-
Tinnitus	-	-	-
Unspecific taste distortion	6 (24%)	None	3
Metallic taste	3 (12%)	None	4
Insomnia	5 (20%)	None	1
Anxiety	5 (20%)	None	2
Behavior disorders	2 (8%)	None	4
Mood disorders	6 (24%)	None	2.5
Cough	1 (4%)	None	3
Dyspnea	1 (4%)	None	1
Chest pain	-	-	-
Palpitations	1 (4%)	None	6
Arthralgias	2 (8%)	None	5
Myalgias	2 (8%)	None	5
Exanthema	1 (4%)	1 (4%)	3.5
Pruritus	5 (20%)	None	3
Candidiasis	5 (16%)	1 (4%)	3
Thrombocytopenia*	19 (76%)	1 (4%)	4
Anemia**	9 (36%)	None	4

* Platelet count below 100,000/mm^3 or below75% of the baseline count.
** Haemoglobin below 90 g/L or below 75% of haemoglobin 1 week after surgery.

Table 1
Microbiological etiology of 25 cases* of PJI by Gram-positive bacteria.

Microorganism		n (%)
Staphylococcus aureus†		4 (15)
Coagulase-negative Staphylococci (CNS)‡		18 (66)
S. epidermidis	13	
S. lugdunensis	1	
S. capitis	1	
S. hominis	1	
CNS sp.	2	
Streptococci		3 (11)
S. intermedius	1	
S. viridans	1	
S. agalactiae	1	
Propionibacterium acnes		1 (4)
Corynebacterium striatum		1 (4)

* Two cases of polymicrobial infection.
† No strains methicillin-resistant.
‡ 6 strains (33%) methicillin-resistant.

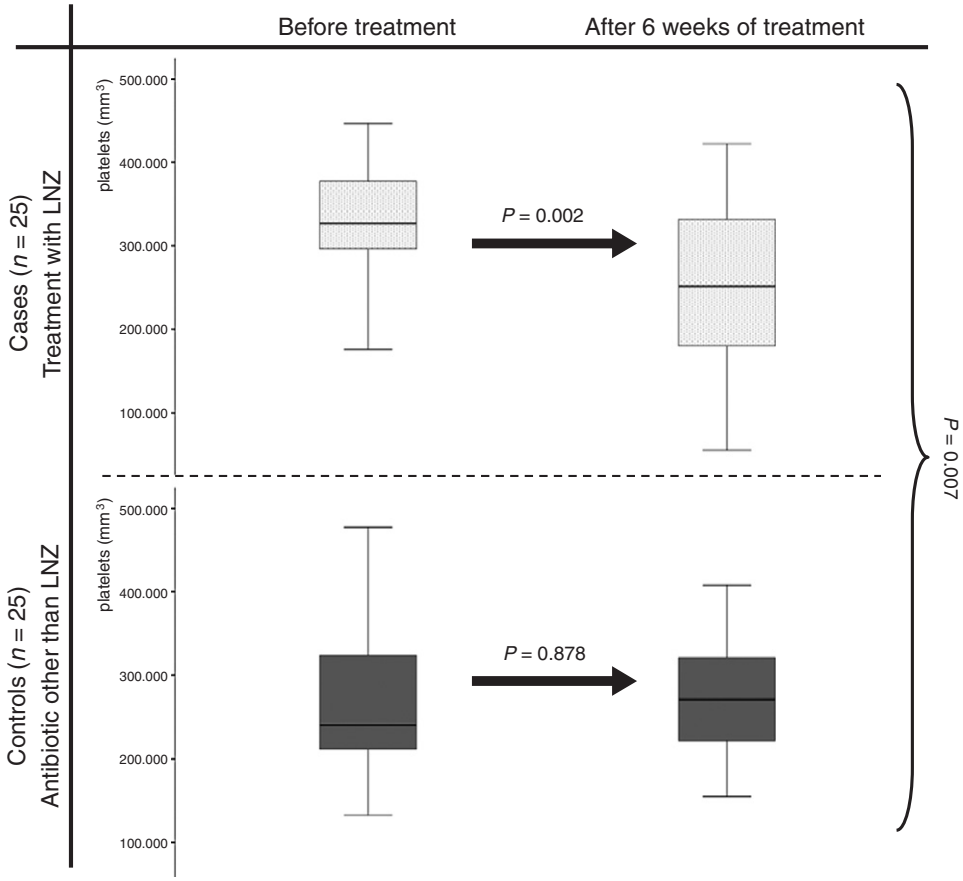

Fig. 1. Comparative platelet count evolution after 6 weeks of treatment with linezolid vs. 6 weeks of treatment with an alternative therapy.

S. mitis, *S. parasanguis* and *P. acnes*, which required a prolonged course of clindamicin.

Overall, among the 22 patients who received the scheduled treatment with linezolid, 19 (86%) patients presented with clinical improvement and sterile surgical site at second-stage surgery. These results were similar when only the cases of staphylococcal etiology were considered.

4. Discussion

This study assesses the effectiveness of linezolid for the management of PJI caused by GPB treated with a two-step exchange procedure. Most of our patients had chronic PJI, according to clinical data and the frequent finding of radiological prosthetic loosening. Among patients treated with linezolid for six weeks, the success rate

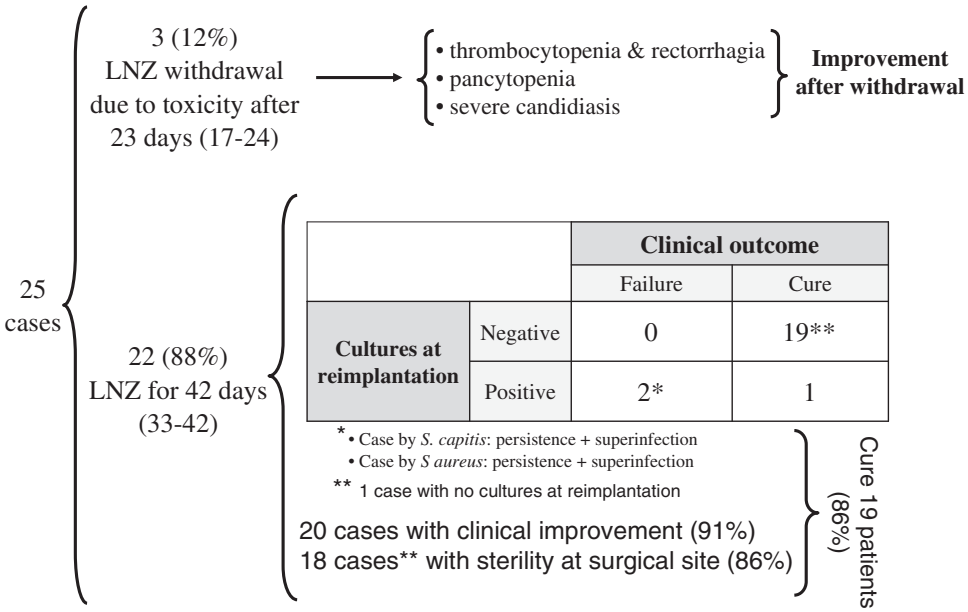

Fig. 2. Outcome chartflow.

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(assessed on the basis of both clinical improvement and sterility at the surgical site) was 86%. This percentage is similar to the rates reported in other series with a similar surgical approach (Bejon et al., 2010; Jämsen et al., 2009; Mittal et al., 2007; Zimmerli et al., 2004). However, these studies are often retrospective and heterogeneous, and include different microorganisms, different systemic and locally-eluted antibiotics (generally gentamycin) or different outcome definitions. Most do not include microbiological eradication as a complementary goal for treatment.

The aim of exchanging an infected prosthesis in a two-step surgical procedure is to guarantee, as far as possible, that the new implant will not be contaminated with the bacteria responsible for the original infection (Bejon et al., 2010; Cabo et al., 2011; Della Valle et al., 1999; Hanssen and Spangehl, 2004; Mont et al., 2000). In our series, surgical site at reimplantation was found not to be sterile in the two patients with clinical failure and in one case among the 20 patients with clinical improvement (5%). This 5% rate of positive cultures among patients with no clinical or surgical signs of infection at the time of reimplantation, is in the lower range as compared with other series, which show a positive culture rate of 5 to 30% of the patients (Bejon et al., 2010; Cabo et al., 2011; Della Valle et al., 1999; Mont et al., 2000; Murillo et al., 2008). Of interest, in these other series wide anti-GPB antibiotics were not systematically used.

Although interpretation of cultures taken at second step surgery is controversial and non-standardized, monitoring the sterility of surgical site seems to be important. Relapse is more likely in the case of positive cultures (Mont et al., 2000) and an additional prolonged course of antibiotics may be needed (Bejon et al., 2010).

As a foreign body, the cement-spacer may perpetuate the infection (Jämsen et al., 2009; Zimmerli et al., 2004). It is not known if systemic antibiotics can prevent the attachment of bacteria to the spacer. Although most of our patients carried spacers impregnated with anti-staphylococcal agents, such as gentamicin and clindamycin, none of them were loaded with a broad anti-Gram-positive antimicrobial such as vancomycin, in order to assess the influence of linezolid in the sterility of surgical site.

Microorganisms cultured at the time of reimplantation tend to be Gram-positive, are usually CNS (Bejon et al., 2010; Mont et al., 2000; Murillo et al., 2008), and are generally resistant to the antibiotics used after the removal of the prosthesis (Cabo et al., 2011; Murillo et al., 2008). The presence of these microorganisms could either imply the selection of several CNS strains from an original polyclonal infection, or superinfection by new CNS strains at some point during the healing process (Bejon et al., 2010; Cabo et al., 2011; Della Valle et al., 1999; Hanssen and Spangehl, 2004; Murillo et al., 2008). Both hypotheses raise the question of whether the use of universal anti-GPB antimicrobial therapy would avoid the finding of CNS at reimplantation.

A recent randomized trial has compared the clinical and microbiological efficacy of two different regimes of daptomycin with that of standard treatment (mostly vancomycin), the former showing better results (Byren et al., 2012). This could suggest that not all treatments are equally effective regarding the sterility of surgical site in spite of having a wide anti-Gram-positive spectrum. Our rate of sterility among clinical successes was 95%, but our study was non-comparative.

Most groups administer intravenous antibiotics for six weeks after the removal of the prosthesis (Bejon et al., 2010; Byren et al., 2012; Jämsen et al., 2009) but this strategy is not supported by comparative studies. Thanks to the drug's excellent bioavailability, treatment with linezolid may be an effective systemic approach which can be administered on an outpatient basis (Clemmet and Markham, 2000; Rana et al., 2002).

However, concerns have been raised in the literature regarding AE when prolonged courses of linezolid are required (Senneville et al., 2006). We found AE of varying severity in 76% of our patients, and in 12% the antibiotic had to be stopped. These results corroborate those of different case series of patients treated with linezolid for several weeks, which report AE rates of 24–64%, leading to linezolid withdrawal in 6–35% (Birmingham et al., 2003; Bishop et al., 2006; Rao and Hamilton, 2007; Rayner et al., 2004; Senneville et al., 2006). Although our patients were notably older than the ones included in these reports, they did not show a higher incidence of AE, nor was there a statistical association between age and AE.

Bone marrow suppression and neurological toxicity are matters of particular concern. In our series, no cases of polyneuropathy or optic neuritis were seen, but these conditions have been reported in the case of longer courses (Birmingham et al., 2003; Bishop et al., 2006; Legout et al., 2010; Rao and Hamilton, 2007; Rubinstein et al., 2003; Vihn and Rubinstein, 2009). Anemia occurred in 36% of our patients, a figure similar to that found in previous reports, and seems to be more frequent in elderly diabetic patients with renal impairment (Bishop et al., 2006; Legout et al., 2010; Rao and Hamilton, 2007; Rayner et al., 2004; Senneville et al., 2006).

Relevant thrombocytopenia occurred in only one of our cases, but in general all our patients showed a decrease in the platelet count compared with historical controls not treated with linezolid. Thrombocytopenia has been reported in 9–30% of patients exposed to prolonged courses of linezolid, usually after 2–3 weeks of therapy (Birmingham et al., 2003; Bishop et al., 2006; Rao and Hamilton, 2007) and more often in patients with renal impairment (Bishop et al., 2006; Soriano et al., 2007) or a low baseline platelet count (Vihn and Rubinstein, 2009).

Our observation that both anemia and thrombocytopenia are reversible after withdrawal of linezolid corroborates previous reports (Birmingham et al., 2003; Bishop et al., 2006; Rao and Hamilton, 2007; Vihn and Rubinstein, 2009). These data and the fact that hematological toxicity appeared after 3–4 weeks of therapy suggest that this accumulative and dose-related effect may be acceptable in therapeutic schedules during less than 6 weeks. It has been reported that linezolid levels may be lower in overweight patients (Di Paolo et al., 2010), but this seems unlikely to explain the low rate of serious toxicity in our patients, since the median BMI was below 30 mg/m2. The toxicity of linezolid may even be reduced if it is combined with rifampin (Soriano et al., 2007), which is known to decrease serum levels of linezolid (Egle et al., 2005) or if shorter courses of antibiotics are considered (Hsieh et al., 2009; McKenna et al., 2009).

Our study has several limitations: the number of patients is small and it is non-comparative. It also has some strengths: it is multicentre and prospective, and cases included make up a very homogenous cohort of patients. In this setting, linezolid has shown good results in terms of cure rate and sterility at the surgical site. Tolerance seems acceptable, though close surveillance is required.

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Appendix A

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References

Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, et al. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS collaborative study group. J Clin Microbiol 1998;36:2932–9.

Bejon PA, Berendt A, Atkins BL, Green N, Parry H, Masters S, et al. Two-stage revision for prosthetic joint infection: predictors of outcome and the role of reimplantation microbiology. J Antimicrob Chemother 2010;65:569–75.

Birmingham MC, Rayner CR, Meagher AK, Flavin SM, Batts DH, Schentag JJ. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate-use program. Clin Infect Dis 2003;36:159–68.

Bishop E, Melvani S, Howden BP, Charles PGP, Grayson ML. Good clinical outcomes but high rates of adverse reactions during linezolid therapy for serious infections: a proposed protocol for monitoring therapy in complex patients. Antimicrob Agents Chemother 2006;50:1599–602.

Byren I, Reqe S, Campanaro E, Yankelev S, Anastasiou D, Kuropatkin G, et al. Randomized controlled trial of the safety and efficacy of Daptomycin versus standard-of-care therapy for management of patients with osteomyelitis associated with prosthetic devices undergoing two-stage revision arthroplasty. Antimicrob Agents Chemother 2012;56:5626–32.

Cabo J, Euba G, Saborido A, González-Panisello M, Domínguez MA, Agulló JL, et al. Clinical outcome and microbiological findings using antibiotic-loaded spacers in two-stage revision of prosthetic joint infections. J Infect 2011;63:23–31.

Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83.

Clemmet D, Markham A. Linezolid. Drugs 2000;59:815–27.

Del Pozo JL, Patel R. Infection associated with prosthetic joints. N Engl J Med 2009;361:787–94.

Della Valle CJ, Bogner E, Desai P, Lonner JH, Adle E, Zuckerman JD. Analysis of frozen sections of intraoperative specimens obtained at the time of reoperation after hip or knee resection arthroplasty for the treatment of infection. J Bone Joint Surg Am 1999;81:684–90.

Egle H, Trittler R, Kümmerer K, Lemmen SW. Linezolid and rifampin: drug interaction contrary to expectations? Clin Pharmacol Ther 2005;77:451–3.

Hanssen AD, Spangehl MJ. Treatment of the infected hip replacement. Clin Orthop 2004;420:63–71.

Hsieh PH, Huang KC, Lee PC, Lee MS. Two-stage revision of infected hip arthroplasty using an antibiotic-loaded spacer: retrospective comparison between short-term and prolonged antibiotic therapy. J Antimicrob Chemother 2009;64:392–7.

Jämsen E, Stogiannidis I, Malmivaara A, Pajamäki J, Puolakka T, Konttinen Y. Outcome of prosthesis exchange for infected knee arthroplasty: the effect of treatment approach. A systematic review of the literature. Acta Orthop 2009;80:67–77.

Kloos WE, Lambe Jr DW. Staphylococcus. In: Balows A, Hausler WJ, Hermann KL, Isenberg HD, Shadomy HJ, editors. Manual of Clinical Microbiology. 5th ed. Washington, DC.: ASM; 1991. p. 222–37.

Legout L, Valette M, Dezeque H, Nguyen S, Lemaire X, Loïez C, et al. Tolerability of prolonged linezolid therapy in bone and joint infection: protective effect of rifampicine on the occurrence of anaemia? J Antimicrob Chemother 2010;65:2224–30.

McKenna PB, O'Shea K, Masterson EL. Two-stage revision of infected hip arthroplasty using a shortened post-operative course of antibiotics. Arch Orthop Trauma Surg 2009;129:489–94.

Mittal Y, Fehring TK, Hanssen A, Marculescu C, Odum SM, Osmon D. Two-stage reimplantation for periprosthetic knee infection involving resistant organisms. J Bone Joint Surg Am 2007;89:1227–31.

Mont MA, Walkman BJ, Hungerford DS. Evaluation of preoperative cultures before second-stage reimplantation of a total knee prosthesis complicated by infection. J Bone Joint Surg Am 2000;82:1552–7.

Murillo O, Euba G, Calatayud L, Domínguez MA, Verdaguer R, Pérez A, et al. The role of intraoperative cultures at the time of reimplantation in the management of infected total joint arthroplasty. Eur J Clin Microbiol Infect Dis 2008;27:805–11.

Di Paolo A, Malacarne P, Guidotti E, Danesi R, Del Tacca M. Pharmacological issues of linezolid: an updated critical review. Clin Pharmacokinet 2010;49:439–47.

Rana B, Butcher I, Grigoris P, Murnaghan C, Seaton RA, Tobin CM. Linezolid penetration into osteo-articular tissues. J Antimicrob Chemother 2002;50:747–50.

Rao N, Hamilton CW. Efficacy and safety of linezolid for Gram-positive orthopedic infections: a prospective case series. Diagn Microbiol Infect Dis 2007;59:173–9.

Rayner CR, Baddour LM, Birmingham MC, Norden C, Meagher AK, Schentag JJ. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. Infection 2004;32:8-14.

Rubinstein E, Isturiz R, Standiford HC, Smith LG, Oliphant TH, Cammarata S, et al. Worldide assessment of linezolid's clinical safety and tolerability: comparator-controlled phase III studies. Antimicrob Agents Chemother 2003;47:1824–31.

Senneville E, Legout L, Valette M, Yazdanpanah Y, Beltrand E, Caillaux M, et al. Effectiveness and tolerability of prolonged linezolid treatment in chronic osteomyelitis: a retrospective study. Clin Ther 2006;28:1155–63.

Soriano A, Ortega M, García S, Peñarroja G, Bové A, Marcos M, et al. Comparative study of the effects of pyridoxine, rifampin, and renal function on hematological adverse events induced by linezolid. Antimicrob Agents Chemother 2007;51:2559–63.

Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg Am 1996;78:512–23.

Vihn DC, Rubinstein E. Linezolid: a review of safety and tolerability. J Infect 2009;59:S59–74.

Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645–54.

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Activity of Colistin Combined with Doripenem at Clinically Relevant Concentrations against Multidrug-resistant Pseudomonas aeruginosa in an In Vitro Dynamic Biofilm Model

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Keywords:	Colistin, Doripenem, Polymyxins, combination, biofilm, Pseudomonas aeruginosa, carbapenem-resistant, multiresistant

Activity of Colistin Combined with Doripenem at Clinically Relevant Concentrations against Multidrug-resistant *Pseudomonas aeruginosa* in an *In Vitro* Dynamic Biofilm Model

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Running title: Colistin and doripenem against MDR *Pseudomonas aeruginosa* biofilm

Key words: Colistin, doripenem, combination, biofilm, *Pseudomonas aeruginosa*, carbapenem-resistant

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Synopsis

Objectives: Colistin combination therapy may be required to treat biofilm-associated infections. We evaluated bacterial killing and emergence of colistin resistance with colistin and doripenem combinations against biofilm-embedded and planktonic multidrug-resistant (MDR) *Pseudomonas aeruginosa*.

Methods: One colistin-susceptible reference strain (PAO1) and two colistin-susceptible MDR clinical isolates (HUB1 and HUB2; both carbapenem-resistant) were investigated over 72 h in the CDC biofilm reactor, a dynamic biofilm model. Two colistin regimens (constant concentrations of 1.25 mg/L and 3.50 mg/L), one doripenem regimen (C_{max} 25 mg/L 8-hourly), and their combination were employed. Microbiological response was examined by log changes and absolute bacterial counts.

Results: For biofilm-embedded bacteria, bactericidal activity was only observed with monotherapy with colistin at 3.50 mg/L. The emergence of colistin resistance occurred with colistin monotherapy against two strains (both colistin regimens for PAO1 and the 3.50 mg/L regimen for HUB1). Colistin 3.50 mg/L plus doripenem resulted in ~2 - 3 $\log_{10}$ CFU/cm^2 initial killing against both clinical isolates and remained synergistic at 72 h. The emergence of colistin resistance was not observed in biofilm-embedded bacteria with either combination. For planktonic bacteria, bactericidal activity was not observed with any monotherapy regimen, although enhanced bacterial killing was observed with doripenem plus colistin 3.50 mg/L against all isolates. Colistin-resistance was observed with colistin monotherapy against all isolates, but did not emerge with combination regimens

Conclusions: Doripenem enhanced the killing of colistin against biofilm-embedded cells in both carbapenem-susceptible and -resistant strains, and the combination minimised the emergence of colistin resistance.

Introduction

Rapidly increasing antibiotic resistance and a dearth of new antibiotics in the drug development pipeline represent a major global medical challenge.[1, 2] Infections by multidrug-resistant (MDR) Gram-negative bacilli such as *Pseudomonas aeruginosa* are particularly problematic, with no new antibiotics to treat these infections to become available in the foreseeable future.[2, 3] MDR *P. aeruginosa* has been identified by the Infectious Diseases Society of America (IDSA) as one of the top six pathogens threatening healthcare systems.[1, 4] As a versatile pathogen with the ability to cause diverse types of infections, *P. aeruginosa* is of central importance in a broad range of nosocomial and community-acquired infections, including biofilm-associated infections.[5-9] 'Old' polymyxins, particularly colistin, are often the only therapeutic option.[4, 10-13]

Colistin is administered parenterally in the form of sodium colistin methanesulfonate (CMS), an inactive prodrug.[14] However, the emerging pharmacokinetic (PK) and pharmacodynamic (PD) data suggest that caution is required with the use of colistin monotherapy due to suboptimal exposure and emergence of resistance.[15-17] Both *in vitro*[18-25] and *in vivo*[26-28] studies have shown the potential for the rapid emergence of colistin resistance with monotherapy, including against *P. aeruginosa*, very likely due to amplification of pre-existing colistin-resistant subpopulations.[19, 21, 22, 29] Such observations highlight the importance of investigating rational combinations of colistin with other antibiotics to minimize the emergence of colistin resistance.[19, 21]

Treatment of biofilm-related infections caused by MDR *P. aeruginosa,* including those associated with a foreign body is particularly problematic and the clinical prognosis is poor.[7, 30, 31] Biofilms are complex bacterial communities embedded in a self-produced polymeric matrix which protects the cells from environmental, immune system and antimicrobial threats.[32, 33] Bacterial cells growing in a biofilm may become substantially more resistant to antibiotic treatment than the planktonic cells,[34, 35] a phenomenon contributing to the

chronicity of MDR bacterial infections.[33, 36] Although significant synergy has been reported for the combination of polymyxin and a carbapenem against non-biofilm infections caused by *P. aeruginosa,*[19, 21, 37] for biofilm-related infections there is a paucity of PK/PD information regarding such combinations.[38-43] Thus, the aim of the present study was to investigate the activity of colistin alone, and in combination with doripenem, on bacterial killing and emergence of resistance of biofilm-embedded carbapenem-resistant *P. aeruginosa* by simulating the PK of both antibiotics in humans using an *in vitro* dynamic biofilm model.

Materials and Methods

Bacterial isolates

Three colistin-susceptible but heteroresistant strains of *P. aeruginosa* were employed in this study: a reference strain PAO1 (American Type Culture Collection, Rockville, MD, USA), and two clonally unrelated carbapenem-resistant clinical isolates: HUB1 (extensively drug-resistant, XDR) and HUB2 (MDR). Heteroresistance to colistin was defined as an isolate with a minimum inhibitory concentration (MIC) ≤2 mg/L in which subpopulations were able to grow in the presence of ≥4 mg/L colistin in population analysis profiles (PAPs; determined as previously described).[19, 21] XDR was defined as resistance to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), and MDR was defined as resistance to at least one agent in three or more antimicrobial categories.[44] Both clinical isolates caused outbreaks in the Hospital Universitario de Bellvitge in Barcelona, Spain, and contained a VIM-2 metallo-β-lactamase (HUB1)[45] or a PSE-1 β-lactamase plus a MexXY-OprM efflux-pump (HUB2).[46] MICs to colistin (sulfate) and doripenem for each isolate are shown in the Table and were determined using broth microdilution in cation-adjusted Mueller-Hinton broth (CAMHB) and cation-adjusted 1%-Tryptisone soy broth (CA-1%TSB) (for both media, Ca^{2+} at 23.0 mg/L, Mg^{2+} at 12.2 mg/L; Oxoid, Hampshire, England).[47] Determination of MIC in CAMHB were performed for comparison with those observed in CA-1%TSB, which was the growth medium used in our model. Resistance to colistin[47] and doripenem[48] in *P. aeruginosa* was defined as MIC ≥4 mg/L. Isolates were stored in tryptone soy broth (Oxoid) with 20% glycerol (Ajax Finechem, Seven Hills, New South Wales, Australia) in cryovials (Simport Plastics, Quebec, Canada) at -80°C.

Antibiotics and reagents

For MIC determinations and *in vitro* PK/PD studies, colistin sulfate (C4461, lot number SLBD8306V; ≥15,000 U/mg) and doripenem (lot 0137Y01) were used (Sigma-Aldrich, Castle Hill, Australia). Colistin sulfate was employed in the current study as colistin is the active antibacterial entity formed *in vivo* after administration of its inactive prodrug CMS.[14] Stock solutions of colistin and doripenem were prepared immediately prior to each experiment using Milli-Q water (Millipore Australia, Australia) and 0.9% saline, respectively, and sterilised by filtration with a 0.20-μm cellulose acetate syringe filter (Millipore, Bedford, MA).

Binding of colistin and doripenem in CA-1%TSB

The binding of colistin and doripenem in CA-1%TSB was determined by ultracentrifugation (Optimal MAX-TL, Beckman Coulter, Inc). Colistin and doripenem were spiked in CA-1%TSB at 3.5 mg/L and 25 mg/L, respectively. An aliquot (200 μL) of drug-containing CA-1%TSB was transferred to centrifuge tubes (polycarbonate, 7 × 20 mm, Beckman Coulter, Inc) and incubated for 30 min at 37°C (n = 3). Tubes were then subjected to ultracentrifugation using a TLA-100 fixed-angle rotor (Beckman Coulter, Inc) at 279,000 *g* for 4 h at 37°C, and 50 μL was removed from the upper part of the supernatant of two replicate tubes. The contents of the third tube were resuspended and two 50-μL samples were removed from the central part of the tube. All samples were stored at -80˚C until analysis. Concentrations of colistin and doripenem were determined in two replicates by HPLC.[21, 49] The percentage of colistin and doripenem bound in CA-1%TSB was calculated as follows:

$$Binding\ percentage = \left(1 - \frac{\text{Supernatant concentration}}{\text{Resuspended concentration}}\right) \times 100\%$$

The final binding percentage was calculated as the average of two values.

In vitro PK/PD biofilm model

Experiments to examine the bacterial killing and emergence of resistance to various dosage regimens of colistin and doripenem alone, and in combination, were conducted over 72 h using a CDC biofilm reactor (CBR) (BioSurface Technologies, Bozeman, MT). This dynamic model consisted of a 1-L glass reactor connected to a 10-L carboy containing sterile drug-free CA-1%TSB. The broth was pumped through the model with mixing and shear generated by a magnetic stir bar operating at 130 rpm. The volume of broth in the glass reactor was maintained at 350 mL and a waste vessel was connected. Eight polypropylene coupon holders were suspended from the lid, each containing three removable Teflon coupons (diameter 12.7 mm) on which biofilm formed. The biofilm-growing surface across both faces of each coupon was 2.53 cm^2.Our protocol for biofilm growth was based upon previously published methods.[50-52] Briefly, prior to each experiment isolates were subcultured onto nutrient agar plates and incubated at 35˚C for 24 h. One colony was then selected and grown overnight in 10 mL of TSB, from which early log-phase growth was obtained. A 1-mL aliquot of this early log-phase bacterial suspension was inoculated into the model at 37˚C and a 28-h conditioning phase commenced. This consisted of 24-h incubation in drug-free CA-1%TSB, after which the model was emptied and fresh sterile drug-free CA-1%TSB was pumped into the model at a flow rate of 11.67 mL/min for 4 h prior to the commencement of antibiotic treatment (i.e. 0 h).For viable counting and examination of emergence of colistin resistance in the biofilm-embedded cells, three coupons at each of 0, 4, 8, 24, 32, 48, 56 and 72 h were aseptically removed, rinsed twice in 5 mL of phosphate-buffered saline (PBS, pH 7.4) to remove planktonic cells, and placed in sterile tubes containing 10 mL of PBS. Biofilm-embedded cells were recovered by three alternating 1-min cycles of vortexing and sonication at 43 kHz (Soniclean, Therbaton, Australia) followed by a final 1-min vortexing.[52] CA-1%TSB (1 mL) was also removed from the model at each time point for viable counting and examination of emergence of colistin resistance in planktonic cells (below).

Two colistin regimens and one doripenem regimen were examined as monotherapy and as their respective combinations. Colistin was simulated as a continuous infusion at 1.25 mg/L or 3.50 mg/L. This was achieved by bolus administration of colistin at 0 h to the model to achieve the desired concentration of 1.25 mg/L or 3.50 mg/L, as well as by spiking the media in the carboy with colistin at the appropriate concentration. Our approach mimicked the 'flat' plasma concentration-time profiles of formed colistin at steady state observed in critically-ill patients receiving CMS.[15-17, 53] For doripenem regimens, administration occurred as a bolus dose every 8 h into the model to achieve the desired steady-state peak concentration (C_{max}) of 25 mg/L. The flow rate to the glass reactor vessel (4 mL/min) was chosen to simulate a doripenem elimination half-life ($t_{1/2}$) of 1 h in patients.[54] All control and drug-containing regimens except those containing colistin at 3.50 mg/L (as monotherapy or in combination) were performed in two replicates with three coupons examined at each time point per run (i.e. 6 coupons in total); additionally, two broth samples from each model were collected at each time point for enumeration of planktonic cells (below). For regimens involving colistin 3.50 mg/L, one experiment was conducted with three coupons per time point for measurements of biofilm-embedded cells and one sample for assessing planktonic cells. Flow rates were calibrated prior to each experiment and monitored through the experiment to ensure the system was performing optimally.

Microbiological response and emergence of resistance to colistin

For enumeration of biofilm-embedded and planktonic viable cells, the respective samples were serially diluted with sterile saline and 50 µL was spirally plated onto drug-free nutrient agar (Media Preparation Unit) using an automatic spiral plater (WASP, Don Whitley Scientific, West Yorkshire, UK). Serial 10-fold dilutions and spiral plating, which further diluted the samples, minimized antibiotic carryover. Colonies were counted using a ProtoCOL automated colony counter (Synbiosis, Cambridge, UK) after 24 h of incubation at

35°C and 48 h for the plates with small colonies. In order to evaluate the emergence of colistin resistance, both biofilm-embedded and planktonic (broth) samples were additionally plated in a similar manner onto nutrient agar containing 4 mg/L colistin (Media Preparation Unit).

Pharmacokinetic validation

Samples (100 µL) collected in duplicate from the model were placed in 1.5-mL microcentrifuge tubes (Greiner Bio-One, Frickenhausen, Germany) and immediately stored at -80ºC; all samples were assayed within 4 weeks to avoid any potential degradation. Concentrations of colistin and doripenem were measured using HPLC as previously described[21, 49] with assay ranges of 0.10 to 6.00 mg/L for colistin and 0.5 to 32 mg/L for doripenem. For both colistin and doripenem assays, analysis of quality control samples revealed that measured and nominal concentrations differed by <10% and coefficients of variation were <10.2%.

Pharmacodynamic analysis

The antibacterial activity of efficacy of mono- or combination regimens was examined using the log change method, i.e. comparing the change in $\log_{10}$ CFU/cm^2 (biofilm-embedded cells) or $\log_{10}$ CFU/mL (planktonic cells) from 0 h (CFU_0) to time *t* (4, 8, 24, 32, 48, 56 or 72 h; CFU_t) as shown: log change = $\log_{10}(CFU_t) - \log_{10}(CFU_0)$.[19, 21] Treatments were considered to be bactericidal (99.9% kill) when they led to a ≥3 $\log_{10}$ CFU/cm^2 or CFU/mL reduction, compared to the corresponding counts at zero time. Monotherapy or combination regimens causing a reduction of ≥1-$\log_{10}$ CFU/cm^2 or CFU/mL at a specified time were considered active. Synergy was defined as ≥2 $\log_{10}$ CFU/cm^2 or CFU/mL killing for the combination relative to the most active corresponding monotherapy at a specified time;[55]

212 additivity was defined as 1 to <2 $\log_{10}$ CFU/cm^2 or CFU/mL greater killing for the
213 combination. The emergence of colistin resistance (i.e. ability to grow on plates with a
214 colistin concentration of 4 mg/L) was examined using absolute bacterial counts ($\log_{10}$
215 CFU/cm^2 or $\log_{10}$ CFU/mL) for biofilm-embedded and planktonic cells, respectively.

216

217 **Results**

218 ***Pharmacokinetic validation and binding of colistin and doripenem in CA-1%TSB***

219 The achieved colistin concentrations (mean ± SD) were 1.20 ± 0.18 (n = 55) and 3.80
220 ± 1.03 (n = 30) mg/L for the targeted concentrations of 1.25 and 3.50 mg/L, respectively.
221 Measured doripenem C_{max} concentrations were 20.3 ± 3.08 (n = 90) for the targeted value of
222 25.0 mg/L. The observed mean $t_{1/2}$ for the simulated intermittent doripenem dosage
223 regimens was 1.02 ± 0.11 h (n = 15) for the targeted value of 1.0 h. The percentages of
224 colistin and doripenem bound in CA-1%TSB (2.00% and 0%, respectively) were negligible,
225 indicating practical equivalence of total and unbound concentrations. The percentage of time
226 that unbound concentrations of doripenem exceeded the MIC (fT$_{>MIC}$) was 100%, 0% and
227 21% for PAO1, HUB1 and HUB2, respectively. The area under the unbound colistin
228 concentration-versus-time curves over 24 h divided by the MIC (fAUC/MIC) for the three
229 strains was 14.4 and 45.6 for the regimes of colistin 1.25 and 3.50 mg/L, respectively.

230

231 ***Microbiological response***

232 The presence of colistin-resistant subpopulations in all isolates is evident in the PAPs
233 obtained prior to colistin treatment (Figure 1). The time-course profiles of bacterial numbers
234 of biofilm-embedded and planktonic bacteria for control (drug-free) experiments are shown in
235 Figure 2. Log changes of viable cell counts in the presence of colistin, doripenem or the

combination, and the emergence of colistin-resistant bacteria are shown in Figure 3 (biofilm-embedded cells) and Figure 4 (planktonic bacteria).

The colistin monotherapy regimen at 1.25 mg/L was ineffective against biofilm-embedded bacteria of PAO1 and produced only modest, non-bactericidal killing of HUB1 and HUB2 (Figure 3). The high-concentration colistin monotherapy regimen (3.50 mg/L) produced greater and more rapid initial killing against biofilm-embedded bacteria of all strains, but with subsequent regrowth by 72 h such that bactericidal activity was only observed at this time against HUB1. The colistin monotherapy 3.50 mg/L regimen against PAO1 and HUB1 resulted in the emergence of colistin resistance within the biofilm; however, against HUB2, no colistin-resistant colonies were detected with either colistin regimen. For all isolates the emergence of colistin resistance was more pronounced against planktonic bacteria (Figure 4, lower panels). Doripenem monotherapy achieved rapid and sustained killing (although not bactericidal) against biofilm-embedded PAO1 across 72 h (Figure 3, top panels), but against planktonic bacteria regrowth occurred rapidly following initial killing (Figure 4, top panels). Not surprisingly, doripenem monotherapy was ineffective against the carbapenem-resistant isolates (HUB1 and HUB2).

The combination of colistin 1.25 mg/L plus doripenem showed some additive effects against biofilm-embedded bacteria during the first 24 – 32 h of treatment, especially against PAO1 (Figure 3, top panel). However, this combination was not bactericidal in biofilm against any strain and against both clinical isolates it was generally no better than the most active colistin monotherapy. Against planktonic PAO1 additivity was observed across 72 h with this combination; however, against the clinical isolates the only benefit over colistin monotherapy was additivity within the first 24 h against HUB2 (Figure 4, top panels). The emergence of colistin-resistant subpopulations was greater in the planktonic bacteria compared with the biofilm-embedded bacteria (Figures 3 and 4, lower panels).

The combination of colistin 3.50 mg/L plus doripenem resulted in greater and more sustained killing than either corresponding monotherapy across 72 h. Against biofilm-embedded PAO1 this combination was synergistic up to 48 h and additive at 56 and 72 h, while against both clinical isolates greater initial killing (of ~2 - 3 $\log_{10}$ CFU/cm^2 compared to equivalent monotherapy) was followed by slow regrowth but nevertheless remained synergistic at 72 h (Figure 3, top panels). Against planktonic bacteria this combination produced ~1.5 $\log_{10}$ CFU/mL greater bacterial killing than with the corresponding monotherapy for all strains, with primarily synergistic (PAO1 and HUB2) or additive (HUB1) effects observed across the 72 h (Figure 4, top panels). No emergence of colistin resistance was observed with this combination in either biofilm-embedded or planktonic bacteria (Figures 3 and 4, lower panels).

Discussion

Foreign-body infections by MDR *P. aeruginosa* are of great clinical concern, with a limited number of therapeutic options currently available. Increasingly, colistin is being used as a last-line therapy for treatment of such infections.[4, 7, 11, 45, 46, 56] Indeed, the two carbapenem-resistant clinical isolates employed in the present study were responsible for outbreaks in a teaching hospital in Barcelona (Spain), including cases of prosthetic joint infections, vascular graft infections, and vascular and urinary catheter infections; fortunately both isolates remained susceptible to colistin.[45, 46]

The emergence of colistin resistance has been reported in *P. aeruginosa in vitro* with colistin monotherapy;[19-21, 57-59] regrowth is due, at least in part, to amplification of pre-existing colistin-resistant subpopulations present in colistin-heteroresistant isolates.[19, 20] Additionally, recent studies indicate that the plasma colistin concentrations achieved in critically-ill patients with the currently recommended CMS dosage regimens are sub-optimal in many cases;[15, 17] unfortunately toxicity, especially nephrotoxicity,[60] precludes dose escalation.[61] Plasma colistin concentrations of ~2 - 3 mg/L are typically achieved at steady state following intravenous administration of CMS, with some patients achieving concentrations of up to ~10 mg/L.[15-17, 53] We administered colistin as a continuous infusion to simulate the 'flat' profiles of formed colistin observed in critically-ill patients at steady state across a CMS dosage interval.[15, 17] Similarly, doripenem is typically administered 8-hourly with peak concentrations of ~25 mg/L achieved after a standard 500 mg dose.[48] While binding of doripenem to plasma proteins is very low (~8%),[48] the unbound fraction of colistin in human plasma is yet to be fully defined. However, assuming the unbound fraction of colistin in humans is similar to that observed in rats (i.e. ~50% unbound), and given the minimal binding of both colistin and doripenem in CA-1%TSB, the dosage regimens of colistin and doripenem employed in the present study reflect clinically achievable unbound (free) plasma concentration-time profiles in patients.

The difficulties of achieving adequate colistin concentrations, as outlined above, are exacerbated with biofilm infections where MICs and minimum bactericidal concentrations (MBCs) are substantially increased. Indeed, Hengzhuang *et al*.[39, 40] recently reported that the minimal biofilm inhibitory concentration (MBIC) and minimal biofilm eradication concentration (MBEC) of colistin and imipenem against *P. aeruginosa* are substantially higher than the MICs and MBCs of planktonic bacteria. For colistin against *P. aeruginosa*, Hengzhuang *et al*. reported that the concentrations inhibiting biofilm cell growth were ~4 times higher than the concentrations required to inhibit planktonic growth.[39] Against PAO1 mature biofilms, the MBIC and MBEC for colistin was 16 mg/L and 128 mg/L, respectively, with the corresponding values for imipenem being 32 mg/L and 1,024 mg/L.[40] Such MBICs for colistin are unattainable by intravenous administration of CMS at clinically tolerable doses, and alternative strategies must therefore be applied in order to adequately treat biofilm infections caused by MDR *P. aeruginosa*.

In light of the PK/PD difficulties of colistin, combination therapy has been suggested as a promising approach to increase bacterial killing and minimize the emergence of colistin resistance.[15, 19, 21, 38, 62] Previous studies have investigated the pharmacodynamics of colistin/carbapenem combinations on planktonically grown isolates of colistin-susceptible and -resistant *P. aeruginosa* (including carbapenem-resistant strains) using static and dynamic time-kill methods.[19, 21, 37] These studies demonstrated increased bacterial killing and a reduction or prevention of the emergence of colistin resistance with this combination, the activity being greater with imipenem and doripenem than with meropenem against *P. aeruginosa*.[37] In combination against planktonic cells, the activity of colistin and a second antibiotic may be complementary by targeting distinct bacterial subpopulations with different antimicrobial susceptibilities. In addition to this so-called subpopulation synergy (where different drugs target cells with different susceptibilities), mechanistic synergy has also been proposed for combinations involving colistin whereby each drug acts on different metabolic pathways or otherwise enhances killing by the second drug.[63] For the latter situation, it has

been suggested that the ability of colistin to disrupt the Gram-negative outer membrane, increasing its permeability, may allow greater access of doripenem to the critical penicillin-binding proteins located on the cytoplasmic membrane where the carbapenems act.[21]

While bacterial cells growing in a biofilm differ from planktonic cells in a number of important ways,[41, 64-67] the concept of cellular heterogeneity and differential susceptibility of subpopulations to antibiotics may be similar. Metabolically distinct subpopulations within the biofilm structure have been identified with varying susceptibilities to specific antimicrobials. For example, less metabolically active cells located in deeper layers of the biofilm may retain susceptibility to colistin but not other antibiotics.[41-43] Indeed, the combination of colistin with tobramycin has shown benefits in an *in vitro* biofilm model.[38] To the best of our knowledge, our study is the first to investigate the activity of colistin/carbapenem combinations against biofilm-embedded *P. aeruginosa*. using an *in vitro* dynamic biofilm model. The CDC biofilm reactor is a well-accepted validated tool for performing *in vitro* PK/PD experiments involving biofilm, allowing simulation of clinically relevant phamacokinetics. In our model we utilized 1%TSB, a nutrient-restricted media with which the growing of P. aeruginosa biofilm has been standardized in the CDC biofilm reactor.[51] Furthermore, the cation concentrations in 1%TSB were adjusted as concentrations of Ca^{2+} and Mg^{2+} can affect colistin antibacterial activity.[47]

In agreement with previous non-biofilm studies involving colistin regimens,[19-21, 57, 58] regrowth was generally observed with colistin monotherapy against biofilm-embedded cells (Figure 3, upper panels). The presence of colistin heteroresistance in all isolates (Figure 1) may explain, at least in part, these observations as amplification of pre-existing colistin-resistant subpopulations likely contributed to regrowth. The diminished activity of β-lactams in the setting of foreign-body and biofilm infections has previously been reported,[36, 68, 69] and was similarly observed here against the doripenem-susceptible reference isolate. As expected, no bacterial killing was observed for the two clinical isolates with doripenem monotherapy. However, against biofilm-embedded cells the addition of doripenem to colistin

resulted in synergy in bacterial killing over corresponding monotherapy against all three isolates across the 72 h, primarily with the 3.50 mg/L colistin combination regimen. Similar to the results achieved against biofilm-embedded bacteria, improvements in bacterial killing of planktonic bacteria were primarily observed with the combination regimen containing 3.50 mg/L colistin. As per our previous investigation with colistin/doripenem combinations against planktonic *P. aeruginosa*,[21] this combination similarly eliminated or reduced the emergence of colistin resistance. Against planktonic cells, minor colistin resistance with combination therapy was only observed with the lower colistin concentration (1.25 mg/L) combination regimen.

Previous *in vitro*[38, 40, 41] and *in vivo*[39] studies have demonstrated the need for very high concentrations of colistin when used as monotherapy to achieve any substantial killing of biofilm-embedded bacterial cells. In a recently published study utilising a mouse lung infection biofilm model, a colistin serum concentration of 64× MIC (i.e. 128 mg/L) was required to achieve a 1 $\log_{10}$ decrease in CFU/lung.[39] This concentration far exceeds the upper limits of clinically achievable colistin concentrations (~10 mg/L) following intravenous administration of CMS.[15, 17, 70] Indeed, our results show poor killing with colistin monotherapy at achievable free concentrations and further support the view that colistin should not be used as monotherapy against *P. aeruginosa*, especially in the setting of foreign-body infections. However, our results have also shown that a colistin concentration of 3.50 mg/L in combination with doripenem is able to produce greater and more sustained bacterial killing than either antibiotic alone. Interestingly, this also applied to the two carbapenem-resistant isolates.

There are a number of possible reasons for the strain-to-strain differences in both biofilm-embedded and planktonic bacterial killing observed in this investigation (Figures 3 and 4). Firstly, biofilm-forming ability and particular biofilm characteristics are strain-dependent;[33, 51] the three strains used in this investigation established biofilms with varying

initial cell densities following the conditioning phase (Figure 2). Second, the different PAPs observed for the three strains prior to antibiotic treatment (Figure 1) indicate slightly different frequencies of pre-existing colistin-resistant subpopulations at the commencement of therapy. Finally, the two clinical isolates had a different mechanism(s) of carbapenem resistance (a carbapenemase in HUB1[45] and an efflux-pump plus a β-lactamase in HUB2).[46] The interplay of these factors likely contributed to the strain-to-strain differences in the bacterial killing observed.

The use of our *in vitro* PK/PD biofilm model deserves some additional comments. First, the killing of planktonic cells by the mono- and combination therapy in the present study was reduced in comparison to that observed in our previous PK/PD study with comparable colistin and doripenem exposures.[21] It is very likely that the reduced efficacy was due to continuous release of bacterial cells from the biofilm in the CDC model. In addition, the antimicrobial treatment in our experiments commenced after 28 hours of biofilm growth. This must be taken into account when interpreting the results, since the maturity of the biofilm has important influence in the activity of antimicrobials.[40, 68]

In summary, we have shown for the first time that clinically relevant dosage regimens of colistin and doripenem in combination increase bacterial killing of biofilm-embedded *P. aeruginosa*, including carbapenem-resistant isolates, with negligible emergence of colistin resistance. These findings provide important PK/PD information for foreign-body infections caused by MDR *P. aeruginosa*. Further investigations using validated animal biofilm models are warranted and are currently underway in our laboratory.

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Transparency declaration

We do not have any financial, commercial or proprietary interest in any drug, device or equipment mentioned in this paper.

References

1. Talbot GH, Bradley J, Edwards JE, Jr. et al. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis* 2006; **42**: 657-68 (Erratum in Clin Infect Dis 2006; 42:1065).
2. Boucher HW, Talbot GH, Benjamin DK, Jr. et al. 10 x '20 Progress--development of new drugs active against gram-negative bacilli: an update from the Infectious Diseases Society of America. *Clin Infect Dis* 2013; **56**: 1685-94.
3. Payne DJ, Gwynn MN, Holmes DJ et al. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov* 2007; **6**: 29-40.
4. Boucher HW, Talbot GH, Bradley JS et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 1-12.
5. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs* 2007; **67**: 351-68.
6. Hauser AR, Jain M, Bar-Meir M et al. Clinical significance of microbial infection and adaptation in cystic fibrosis. *Clin Microbiol Rev* 2011; **24**: 29-70.
7. Papagelopoulos PJ, Mavrogenis AF, Giannitsioti E et al. Management of a multidrug-resistant Pseudomonas aeruginosa infected total knee arthroplasty using colistin. A case report and review of the literature. *The Journal of arthroplasty* 2007; **22**: 457-63.
8. Neut D, Hendriks JG, van Horn JR et al. Pseudomonas aeruginosa biofilm formation and slime excretion on antibiotic-loaded bone cement. *Acta orthopaedica* 2005; **76**: 109-14.
9. Hoiby N. Recent advances in the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. *BMC medicine* 2011; **9**: 32.
10. Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009; **22**: 535-43.
11. Li J, Nation RL, Turnidge JD et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 2006; **6**: 589-601.
12. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005; **40**: 1333-41.
13. Landman D, Georgescu C, Martin DA et al. Polymyxins revisited. *Clin Microbiol Rev* 2008; **21**: 449-65.
14. Bergen PJ, Li J, Rayner CR et al. Colistin methanesulfonate is an inactive prodrug of colistin against Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2006; **50**: 1953-8.
15. Garonzik SM, Li J, Thamlikitkul V et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011; **55**: 3284-94.
16. Mohamed AF, Karaiskos I, Plachouras D et al. Application of a loading dose of colistin methanesulfonate in critically ill patients: population pharmacokinetics, protein binding, and prediction of bacterial kill. *Antimicrob Agents Chemother* 2012; **56**: 4241-9.
17. Plachouras D, Karvanen M, Friberg LE et al. Population pharmacokinetic analysis of colistin methanesulphonate and colistin after intravenous administration in critically ill patients with gram-negative bacterial infections. *Antimicrob Agents Chemother* 2009; **53**: 3430-6.
18. Bergen PJ, Bulitta JB, Forrest A et al. Pharmacokinetic/pharmacodynamic investigation of colistin against Pseudomonas aeruginosa using an in vitro model. *Antimicrob Agents Chemother* 2010; **54**: 3783-9.
19. Bergen PJ, Forrest A, Bulitta JB et al. Clinically Relevant Plasma Concentrations of Colistin in Combination with Imipenem Enhance Pharmacodynamic Activity against Multidrug-Resistant Pseudomonas aeruginosa at Multiple Inocula. *Antimicrob Agents Chemother* 2011; **55**: 5134-42.

20. Bergen PJ, Li J, Nation RL et al. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with Pseudomonas aeruginosa in an in vitro pharmacodynamic model. *J Antimicrob Chemother* 2008; **61**: 636-42.
21. Bergen PJ, Tsuji BT, Bulitta JB et al. Synergistic Killing of Multidrug-Resistant Pseudomonas aeruginosa at Multiple Inocula by Colistin Combined with Doripenem in an In Vitro Pharmacokinetic/Pharmacodynamic Model. *Antimicrob Agents Chemother* 2011; **55**: 5685-95.
22. Poudyal A, Howden BP, Bell JM et al. In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. *J Antimicrob Chemother* 2008; **62**: 1311-8.
23. Tan CH, Li J, Nation RL. Activity of colistin against heteroresistant Acinetobacter baumannii and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2007; **51**: 3413-5.
24. Cai Y, Li R, Liang B et al. In vitro Antimicrobial Activity and Mutant Prevention Concentration of Colistin against Acinetobacter baumannii. *Antimicrob Agents Chemother* 2010.
25. Rodriguez CH, De Ambrosio A, Bajuk M et al. In vitro antimicrobials activity against endemic Acinetobacter baumannii multiresistant clones. *Journal of infection in developing countries* 2010; **4**: 164-7.
26. Ketthireddy S, Lee DG, Murakami Y et al. In vivo pharmacodynamics of colistin against Pseudomonas aeruginosa in thighs of neutropenic mice (abstract A-4, p1). *In: Abstracts of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, Illinois, September 17-20*: American Society for Microbiology, 2007.
27. Matthaiou DK, Michalopoulos A, Rafailidis PI et al. Risk factors associated with the isolation of colistin-resistant gram-negative bacteria: a matched case-control study. *Crit Care Med* 2008; **36**: 807-11.
28. Dudhani RV, Turnidge JD, Nation RL et al. fAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against Acinetobacter baumannii in murine thigh and lung infection models. *J Antimicrob Chemother* 2010; **65**: 1984-90.
29. Owen RJ, Li J, Nation RL et al. In vitro pharmacodynamics of colistin against Acinetobacter baumannii clinical isolates. *J Antimicrob Chemother* 2007; **59**: 473-7.
30. Gump WC, Walsh JW. Intrathecal colistin for treatment of highly resistant Pseudomonas ventriculitis. Case report and review of the literature. *J Neurosurg* 2005; **102**: 915-7.
31. Falagas ME, Kapaskelis AM, Kouranos VD et al. Outcome of antimicrobial therapy in documented biofilm-associated infections: a review of the available clinical evidence. *Drugs* 2009; **69**: 1351-61.
32. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis* 2002; **8**: 881-90.
33. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318-22.
34. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001; **9**: 34-9.
35. Moskowitz SM, Foster JM, Emerson J et al. Clinically feasible biofilm susceptibility assay for isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. *J Clin Microbiol* 2004; **42**: 1915-22.
36. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001; **358**: 135-8.
37. Zusman O, Avni T, Leibovici L et al. Systematic review and meta-analysis of in vitro synergy of polymyxins and carbapenems. *Antimicrob Agents Chemother* 2013; **57**: 5104-11.
38. Herrmann G, Yang L, Wu H et al. Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm Pseudomonas aeruginosa. *J Infect Dis* 2010; **202**: 1585-92.

512 39. Hengzhuang W, Wu H, Ciofu O et al. In vivo pharmacokinetics/pharmacodynamics of colistin
513 and imipenem in Pseudomonas aeruginosa biofilm infection. *Antimicrob Agents Chemother* 2012;
514 **56**: 2683-90.
515 40. Hengzhuang W, Wu H, Ciofu O et al. Pharmacokinetics/pharmacodynamics of colistin and
516 imipenem on mucoid and nonmucoid Pseudomonas aeruginosa biofilms. *Antimicrob Agents*
517 *Chemother* 2011; **55**: 4469-74.
518 41. Pamp SJ, Gjermansen M, Johansen HK et al. Tolerance to the antimicrobial peptide colistin in
519 Pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr
520 and mexAB-oprM genes. *Mol Microbiol* 2008; **68**: 223-40.
521 42. Haagensen JA, Klausen M, Ernst RK et al. Differentiation and distribution of colistin- and
522 sodium dodecyl sulfate-tolerant cells in Pseudomonas aeruginosa biofilms. *J Bacteriol* 2007; **189**: 28-
523 37.
524 43. Cai Y, Wang R, Liang BB et al. In-vitro bactericidal activity of colistin against biofilm-
525 associated Pseudomonas aeruginosa and Acinetobacter baumannii. *J Hosp Infect* 2009; **72**: 368-70.
526 44. Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant
527 and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for
528 acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268-81.
529 45. Pena C, Suarez C, Tubau F et al. Nosocomial spread of Pseudomonas aeruginosa producing
530 the metallo-beta-lactamase VIM-2 in a Spanish hospital: clinical and epidemiological implications.
531 *Clin Microbiol Infect* 2007; **13**: 1026-9.
532 46. Pena C, Suarez C, Tubau F et al. Nosocomial outbreak of a non-cefepime-susceptible
533 ceftazidime-susceptible Pseudomonas aeruginosa strain overexpressing MexXY-OprM and producing
534 an integron-borne PSE-1 betta-lactamase. *J Clin Microbiol* 2009; **47**: 2381-7.
535 47. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial
536 Susceptibility Testing: Twenty-Third Informational Supplement (M100-S23). CLSI. Wayne, PA, USA.,
537 2013.
538 48. Ortho-McNeil-Janssen Pharmaceuticals. Doribax (doripenem for injection) package insert.
539 Ortho-McNeil-Janssen Pharmaceuticals, Inc., Raritan, NJ, 2007.
540 49. Li J, Milne RW, Nation RL et al. Stability of colistin and colistin methanesulfonate in aqueous
541 media and plasma as determined by high-performance liquid chromatography. *Antimicrob Agents*
542 *Chemother* 2003; **47**: 1364-70.
543 50. Donlan RM, Piede JA, Heyes CD et al. Model system for growing and quantifying
544 Streptococcus pneumoniae biofilms in situ and in real time. *Appl Environ Microbiol* 2004; **70**: 4980-8.
545 51. Goeres DM, Loetterle LR, Hamilton MA et al. Statistical assessment of a laboratory method
546 for growing biofilms. *Microbiology* 2005; **151**: 757-62.
547 52. Parra-Ruiz J, Vidaillac C, Rose WE et al. Activities of high-dose daptomycin, vancomycin, and
548 moxifloxacin alone or in combination with clarithromycin or rifampin in a novel in vitro model of
549 Staphylococcus aureus biofilm. *Antimicrob Agents Chemother* 2010; **54**: 4329-34.
550 53. Markou N, Markantonis SL, Dimitrakis E et al. Colistin serum concentrations after
551 intravenous administration in critically ill patients with serious multidrug-resistant, gram-negative
552 bacilli infections: a prospective, open-label, uncontrolled study. *Clin Ther* 2008; **30**: 143-51.
553 54. Matthews SJ, Lancaster JW. Doripenem monohydrate, a broad-spectrum carbapenem
554 antibiotic. *Clin Ther* 2009; **31**: 42-63.
555 55. Pillai SK, Moellering RC, Eliopoulos GM. Antimicrobial Combinations. In: Lorian V, ed.
556 *Antibiotics in Laboratory Medicine*: Philadelphia, PA : Lippincott Williams & Wilkins 2005.
557 56. Montero M, Horcajada JP, Sorli L et al. Effectiveness and safety of colistin for the treatment
558 of multidrug-resistant Pseudomonas aeruginosa infections. *Infection* 2009; **37**: 461-5.
559 57. Bulitta JB, Yang JC, Yohonn L et al. Attenuation of colistin bactericidal activity by high
560 inoculum of Pseudomonas aeruginosa characterized by a new mechanism-based population
561 pharmacodynamic model. *Antimicrob Agents Chemother* 2010; **54**: 2051-62.

562 58. Gunderson BW, Ibrahim KH, Hovde LB et al. Synergistic activity of colistin and ceftazidime
563 against multiantibiotic-resistant Pseudomonas aeruginosa in an in vitro pharmacodynamic model.
564 *Antimicrob Agents Chemother* 2003; **47**: 905-9.
565 59. Tam VH, Schilling AN, Vo G et al. Pharmacodynamics of polymyxin B against Pseudomonas
566 aeruginosa. *Antimicrob Agents Chemother* 2005; **49**: 3624-30.
567 60. Hartzell JD, Neff R, Ake J et al. Nephrotoxicity associated with intravenous colistin
568 (colistimethate sodium) treatment at a tertiary care medical center. *Clin Infect Dis* 2009; **48**: 1724-8.
569 61. Bergen PJ, Landersdorfer CB, Zhang J et al. Pharmacokinetics and pharmacodynamics of 'old'
570 polymyxins: what is new? *Diagn Microbiol Infect Dis* 2012; **74**: 213-23.
571 62. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical
572 impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol*
573 *Rev* 2009; **22**: 582-610.
574 63. Bulitta JB, Li J, Poudyal A et al. Quantifying synergy of colistin combinations against MDR
575 Gram-negatives by mechanism-based models (abstract A1-573, p41). *In: Abstracts of the 49th*
576 *Annual Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco,*
577 *CA, September 12-15*: American Society for Microbiology, 2009.
578 64. Ciofu O, Mandsberg LF, Bjarnsholt T et al. Genetic adaptation of Pseudomonas aeruginosa
579 during chronic lung infection of patients with cystic fibrosis: strong and weak mutators with
580 heterogeneous genetic backgrounds emerge in mucA and/or lasR mutants. *Microbiology* 2010; **156**:
581 1108-19.
582 65. Hoiby N, Krogh Johansen H, Moser C et al. Pseudomonas aeruginosa and the in vitro and in
583 vivo biofilm mode of growth. *Microbes Infect* 2001; **3**: 23-35.
584 66. Tyson GW, Chapman J, Hugenholtz P et al. Community structure and metabolism through
585 reconstruction of microbial genomes from the environment. *Nature* 2004; **428**: 37-43.
586 67. Jensen PO, Bjarnsholt T, Phipps R et al. Rapid necrotic killing of polymorphonuclear
587 leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by Pseudomonas
588 aeruginosa. *Microbiology* 2007; **153**: 1329-38.
589 68. Tanaka G, Shigeta M, Komatsuzawa H et al. Effect of the growth rate of Pseudomonas
590 aeruginosa biofilms on the susceptibility to antimicrobial agents: beta-lactams and fluoroquinolones.
591 *Chemotherapy* 1999; **45**: 28-36.
592 69. Hengzhuang W, Ciofu O, Yang L et al. High beta-lactamase levels change the
593 pharmacodynamics of beta-lactam antibiotics in Pseudomonas aeruginosa biofilms. *Antimicrob*
594 *Agents Chemother* 2013; **57**: 196-204.
595 70. Mizuyachi K, Hara K, Wakamatsu A et al. Safety and pharmacokinetic evaluation of
596 intravenous colistin methanesulfonate sodium in Japanese healthy male subjects. *Curr Med Res Opin*
597 2011; **27**: 2261-70.

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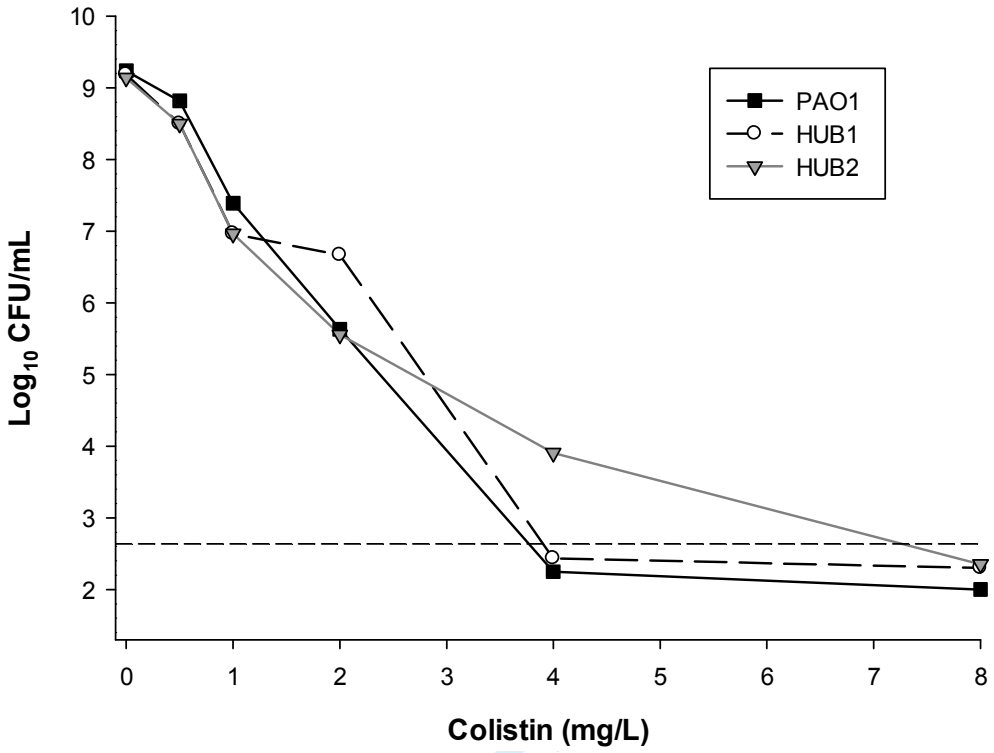
Table. MICs (mg/L)* for the *P. aeruginosa* isolates examined in this study

	Colistin		Doripenem	
	CAMHB	CA-1%TSB	CAMHB	CA-1%TSB
PAO1	1	2	1	<0.125
HUB1	2	2	>128	128
HUB2	1	2	16	8

* CLSI breakpoints for colistin were ≤2 mg/L for susceptibility, 4 mg/L for intermediate, and ≥8 mg/L for resistance. For doripenem, the breakpoints were ≤2 mg/L for susceptibility and >2 mg/L for resistance.[48]



606

607 **Figure 1.** Baseline PAPs of the reference strain PAO1 and clinical isolates HUB1 and HUB2

608 at an initial inoculum of ~10^9 CFU/mL. The *y* axis starts from the limit of detection, and the

609 limit of quantification is indicated by the horizontal broken line.

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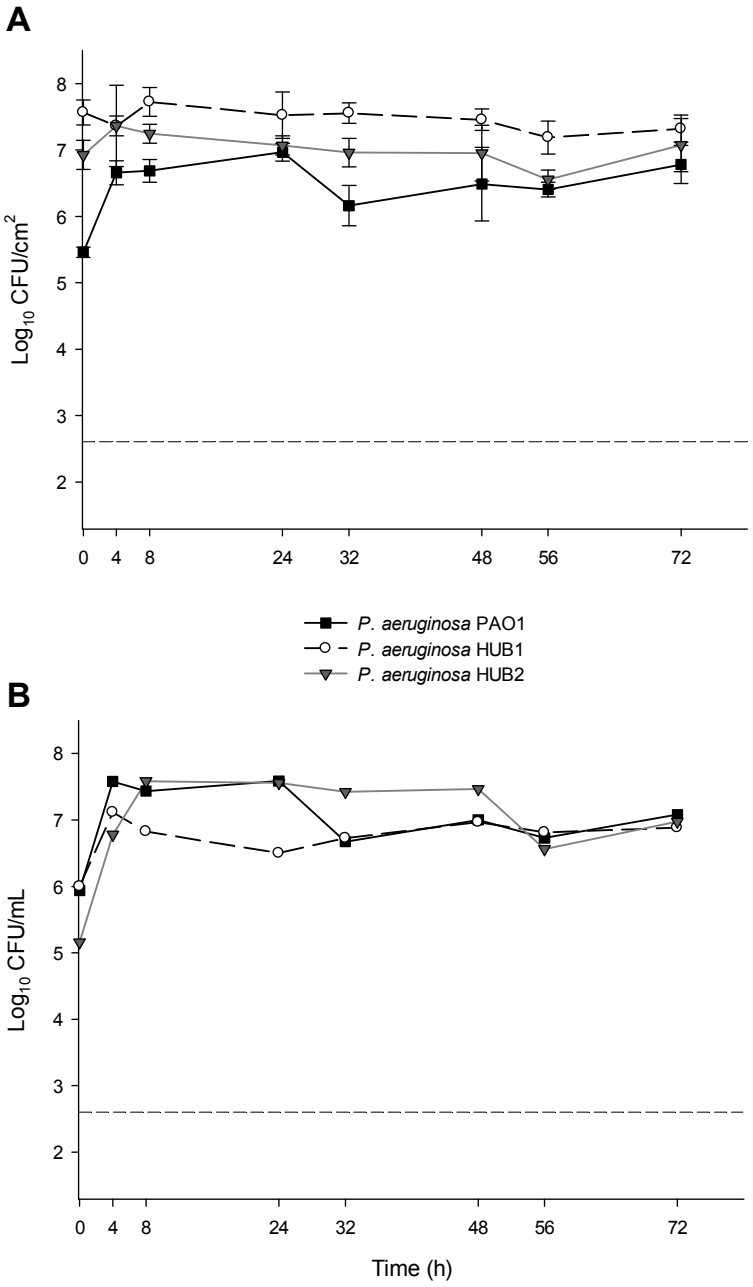
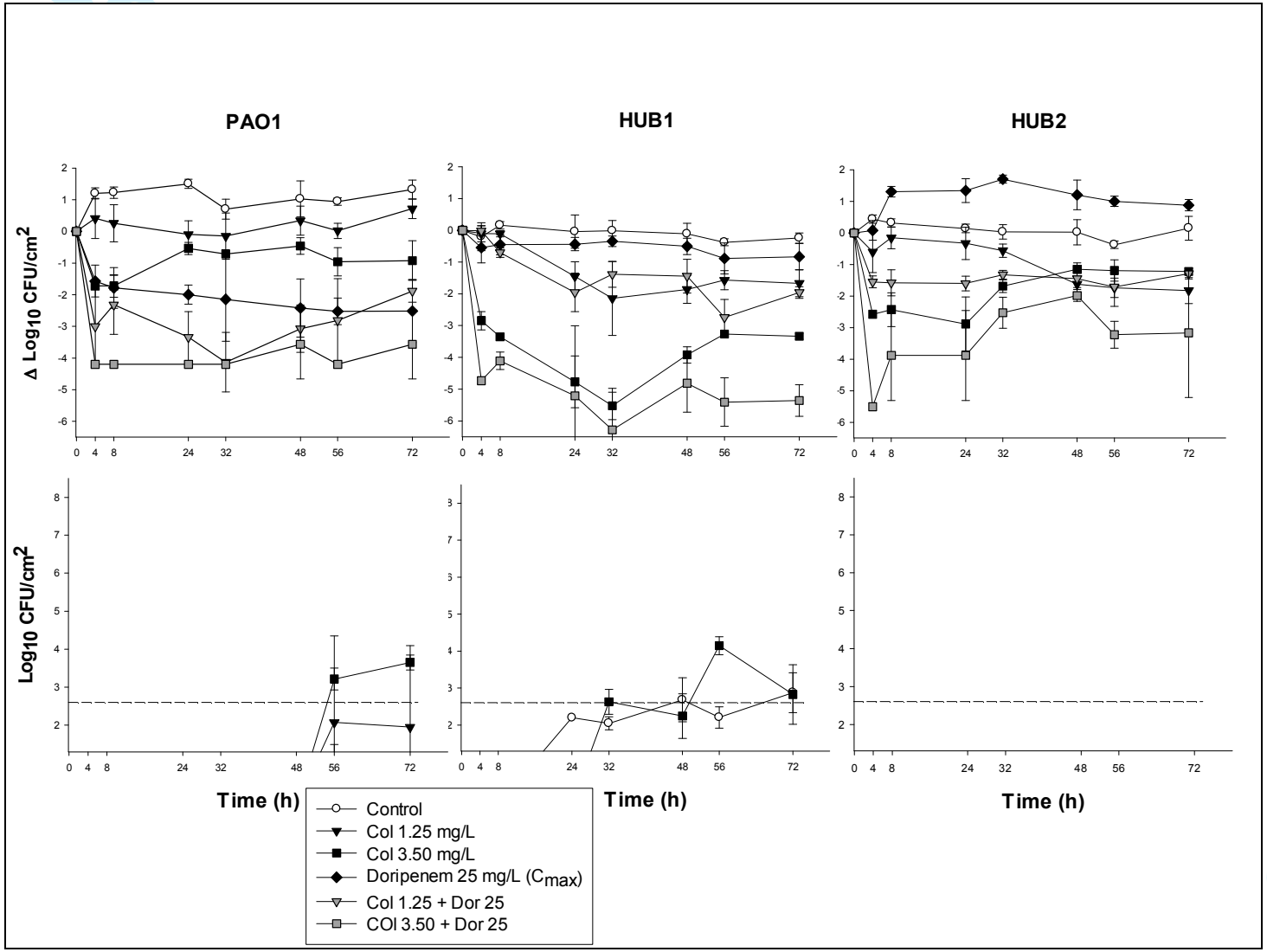


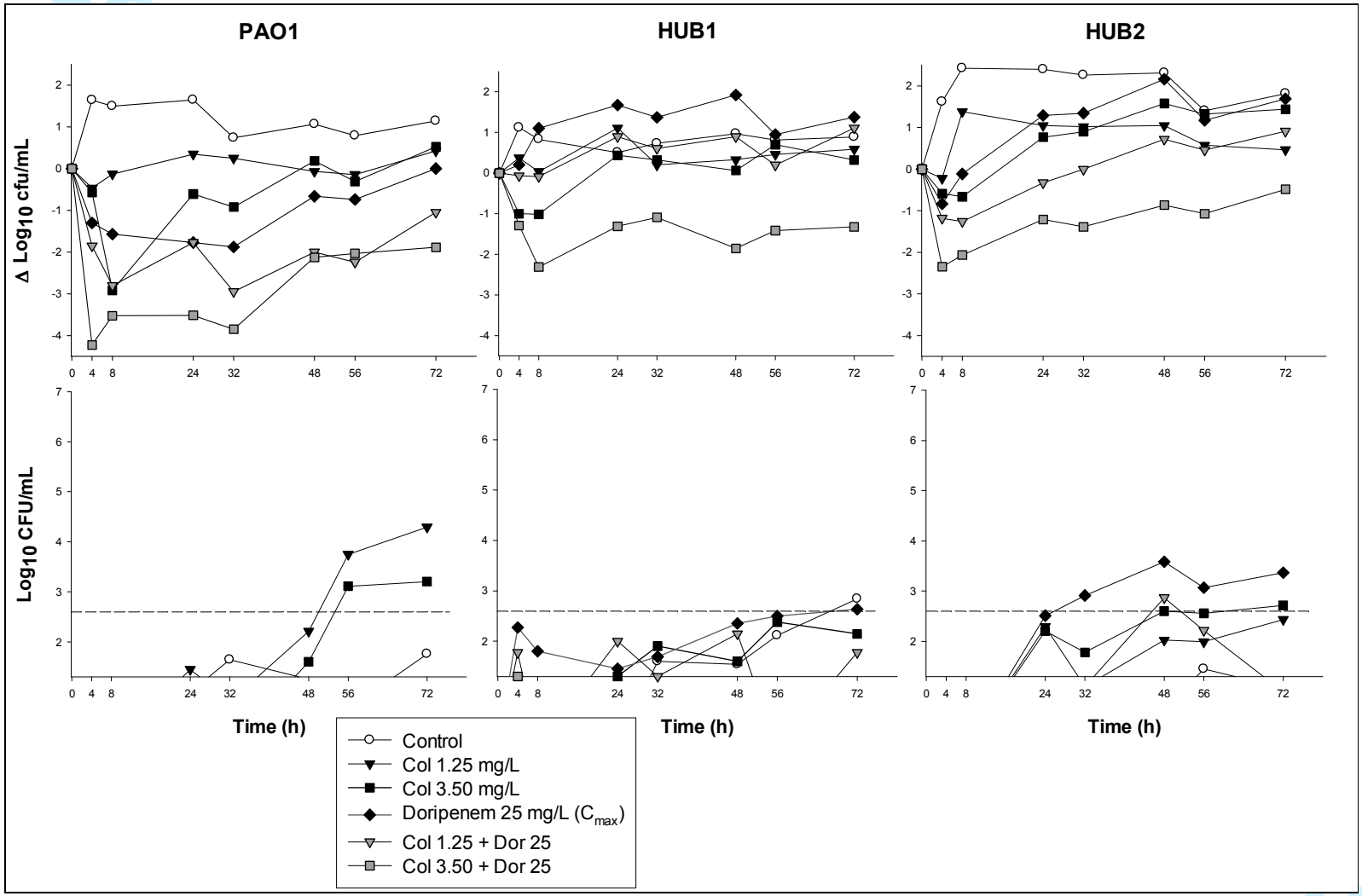
613

614 **Figure 2.** Bacterial growth in the absence of colistin and doripenem (i.e. growth controls) for
615 biofilm-embedded (Panel A) and planktonic (Panel B) bacteria for the three strains of
616 *P. aeruginosa*. Time on the *x* axis begins immediately after the 28 h-conditioning phase and
617 commencement of drug regimens. The *y* axis starts from the limit of detection, and the limit
618 of quantification is indicated by the horizontal broken line. Data are presented as means ±
619 standard deviation of the mean (panel A) or as mean (panel B).


PAO1
HUB1
HUB2
Δ Log10 CFU/cm2
Log10 CFU/cm2
Time (h)
Time (h)
Time (h)
Control
Col 1.25 mg/L
Col 3.50 mg/L
Doripenem 25 mg/L (Cmax)
Col 1.25 + Dor 25
COl 3.50 + Dor 25

620

Figure 3. Upper panels: Bacterial killing by colistin (Col) alone at two different clinically relevant concentrations, doripenem (Dor) alone, and in combination against biofilm-embedded cells of three different *P. aeruginosa* strains; results expressed using the log change method. Lower panels: Emergence of colistin resistance (i.e. colonies able to grow in the presence of ≥4 mg/L colistin) among biofilm-embedded *P. aeruginosa* across the treatment period with the same treatment regimens; results expressed as the absolute number of recovered bacteria. For the lower panels, the limit of quantification is indicated by the horizontal broken line. Data are presented as means ± standard deviation of the mean.


PAO1
HUB1
HUB2
Δ Log10 cfu/mL
Log10 CFU/mL
Time (h)
Time (h)
Time (h)
Control
Col 1.25 mg/L
Col 3.50 mg/L
Doripenem 25 mg/L (Cmax)
Col 1.25 + Dor 25
Col 3.50 + Dor 25

627

628

629 **Figure 4.** Upper panels: Bacterial killing by colistin (Col) alone at two different clinically relevant concentrations, doripenem (Dor) alone, and in
630 combination against three different strains of *P. aeruginosa* recovered from the media within the reactor (i.e. planktonic cells); results expressed
631 using the log change method. Lower panels: Emergence of colistin resistance (i.e. colonies able to grow in the presence of ≥4 mg/L colistin)
632 among planktonic *P. aeruginosa* across the treatment period with the same treatment regimens; results expressed as the absolute number of
633 recovered bacteria. For the lower panels, the limit of quantification is indicated by the horizontal broken line.

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Current Opinion in Microbiology

PK/PD models in antibacterial development

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There is an urgent need for novel antibiotics to treat life-threatening infections caused by bacterial 'superbugs'. Validated *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) and animal infection models have been employed to identify the most predictive PK/PD indices and serve as key tools in the antibiotic development process. The results obtained can be utilized for optimizing study designs in order to minimize the cost and duration of clinical trials. This review outlines the key *in vitro* PK/PD and animal infection models which have been extensively used in antibiotic discovery and development. These models have shown great potential in accelerating drug development programs and will continue to make significant contributions to antibiotic development.

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Introduction

Rapidly increasing antibiotic resistance and the lack of new antibiotics in the drug discovery pipeline are presenting a significant unmet global medical need [1]. Antimicrobial resistance has been identified as one of the three greatest threats to human health. An urgent global call for the discovery of new antibiotics, *The 10 × '20 Initiative*, has been made recently [1]. In antibiotic discovery and development, pharmacokinetic/pharmacodynamic (PK/PD) and animal infection models play essential roles and bridge the gap between *in vitro* susceptibility and clinical evaluations of new antibiotics. Identification of PK/PD relationships in an early discovery stage provides a quantitative tool to enable rational go or no-go decision making and predictions of clinical pharmacological profiles of superior leads. This review outlines the key PK/PD models that have been extensively used in antibiotic discovery and development.

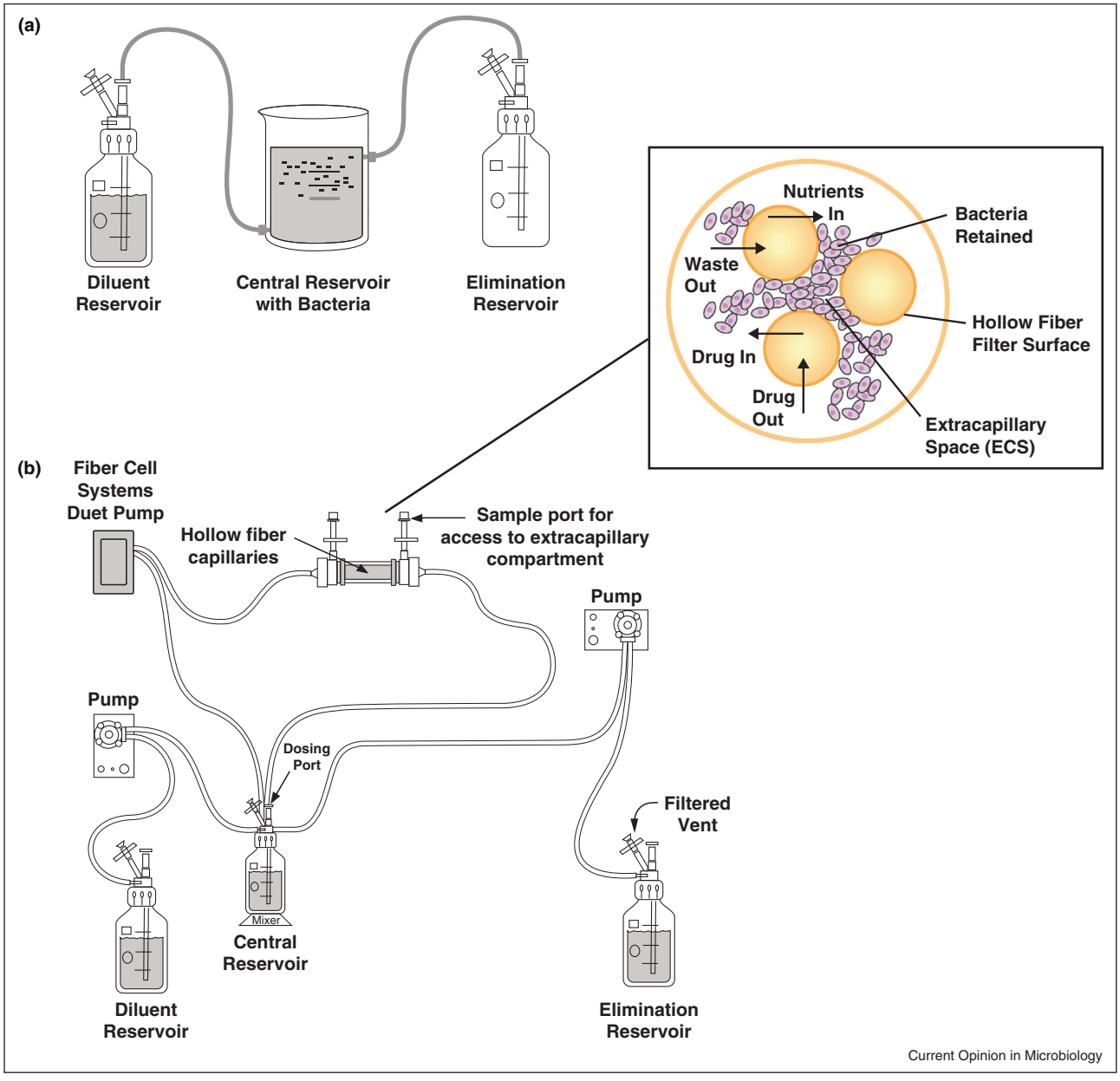
In vitro PK/PD models

In vitro PK/PD models essentially fall into one of two categories: one-compartment or two-compartment models (Figure 1) [2••,3]. One-compartment models typically consist of a central reservoir containing the organism, a diluent reservoir and a waste reservoir. Drug is administered to the central reservoir with drug elimination achieved by pumping drug-free media into the central reservoir; this setup, while necessary for mimicking the PK of antibiotics in patients (i.e. simulation of the desired antibiotic half-life), simultaneously eliminates bacteria. This unintended consequence can be problematic for antibiotics with short elimination half-lives and is the primary disadvantage of one-compartment PK/PD models. To overcome this problem filters have been utilized to prevent bacterial loss, but are prone to blockage [4].

Two-compartment PK/PD models are similar to one-compartment models, but prevent bacterial elimination by physically separating bacteria from the central reservoir within a small peripheral compartment (typically 10–20 mL). The most common example is the hollow fiber infection model (HFIM) containing thousands of small tubular fibers (filters) in a cartridge through which medium is pumped [5•]. Pores on the fibers retain the microorganisms while allowing the free diffusion of drugs and other molecules (e.g. glucose). Drug is administered into, and eliminated from, the central reservoir with antibiotic concentrations equilibrating rapidly with the peripheral (bacterial containing) compartment. Importantly, both absorption and elimination kinetics of the antibiotic under investigation can be precisely and independently controlled. The versatility of both one-compartment and two-compartment models allows for the simulation of virtually any desired elimination half-life observed in patients.

These PK/PD models have played an important role in the determination of the key PK/PD indices driving antibacterial activity (i.e. C_{max}/MIC [the peak concentration divided by the MIC], AUC/MIC [the area under the concentration-time curve over 24 hours at steady-state divided by the MIC] or $T_{>MIC}$ [the cumulative percentage of a 24-hour period that the drug concentration exceeds the MIC at steady-state pharmacokinetic

Figure 1



In vitro PK/PD models. **(a)** The one-compartment model. The volume remains constant but the test organism is not constrained. **(b)** Hollow fiber two-compartment model. Bacterial cells reside in the hollow fiber cartridge. The nutrient broth continually re-circulates through the central reservoir and cartridge. Drug is administered to the central reservoir and the elimination kinetics is controlled by the addition of fresh drug-free medium to the central reservoir. Figures adapted from Ref. [5•] with permission.

conditions]) [2••]. Identification of the most predictive PK/PD index and the associated values required for different magnitudes of killing is essential for the rational design of optimal dosing strategies in animal and clinical studies. Dose-fractionation studies in *in vitro* PK/PD models are more easily performed than in animal models. A recent example is the work by Bergen *et al.* that identified AUC/MIC as the main driver of antibacterial activity for colistin [6]. This information subsequently contributed to the first scientifically based dosing guidelines for colistin in critically ill patients [7]. Such *in vitro* dose-fractionation is increasingly applied to dosage regimen optimization of other antibiotics [8]. The PK/PD information obtained is crucial for designing optimal dosing strategies for further evaluations in animal models and clinical trials.

In vitro PK/PD models are also increasingly being used in the assessment of the emergence of resistance with antibiotic monotherapy and combination therapy [9–12], and demonstrate that emergence of resistance is a complex interplay of the PK and PD of antibiotics [13,14]. Thus the PK profiles simulated in PK/PD models provide more clinically relevant information than static models. The utility of PK/PD models in this regard is exemplified in the study by Tam *et al.* [13]. Using a hollow fiber infection model, it was demonstrated that, in a heterogeneous bacterial population with multiple subpopulations of varying drug susceptibility, low to medium exposures (based on AUC/MIC) of quinolones selectively amplified resistant subpopulation(s) whereas high drug exposures suppressed this. Bergen *et al.* demonstrated that of three dosage regimens each providing a similar exposure to colistin, emergence of resistance was substantially greater and occurred earlier with the two colistin regimens employing the longer dosage intervals [15]. Additionally, *in vitro* PK/PD models have been employed to identify antibiotic breakpoints deemed crucial for the suppression of resistance development [16].

In addition to being less costly and resource-intensive, *in vitro* PK/PD models permit investigations of considerable duration (e.g. weeks) that may not be feasible in animals. Furthermore, PK/PD models allow for the use of high inocula without the ethical concerns associated with excessive early mortality of the animals; the latter is particularly important for the investigation of resistance development as a high bacterial load (e.g. 10^8 colony forming units [CFU] per mL) is usually required to increase the probability of detection of resistant mutants [14]. In addition, these models can be used to examine microorganisms for which animal models are not well established. Results obtained from *in vitro* PK/PD models have shown good correlations with human and animal data [17,18]. The lack of immune components in *in vitro* models is both a limitation and an advantage. While this presents difficulties in extrapolating results to immunocompetent hosts, *in vitro* models permit the direct evaluation of the activity of antibiotics themselves in the absence of host defenses, mimicking the situation in the immunocompromised. It is for this reason that PK/PD models have been particularly useful in the study of anti-tubercular drugs [18].

In summary, *in vitro* PK/PD studies provide important insights into the therapeutic potential of lead compounds in early antibiotic development, and assist in the design of optimal dosage strategies for animal studies and clinical trials.

In vitro biofilm models

Microorganisms are frequently biofilm-embedded in nature and also in the clinic such as in catheter or prosthetic joint infections, chronic sinusitis and infective endocarditis [19]. Biofilm can result in increased antimicrobial tolerance by altering bacterial metabolism, retarding the diffusion of antibiotics, increasing the enzymatic-inactivation of antibiotics in the extracellular matrix, and impairing bacterial clearance by the immune system [19]. In *in vitro* biofilm models, factors including restriction of nutrients and oxygen, surface material, shearing force and the age of the biofilm may significantly influence the maturity of the biofilm and its response to antimicrobials [20••,21,22]. The classic concepts of MIC and minimal bactericidal concentration for planktonic cells have a poor clinical correlation in a biofilm scenario. Minimal biofilm inhibitory (MBIC) and eradicative (MBEC) concentrations more accurately reflect the activity of antimicrobials in biofilm [23]. Measurements of MBIC and MBEC can be achieved by microtiter plate-based models using automatized technology. The Calgary device [23] has been widely used, and numerous variations (e.g. addition of magnetic beads to the media used in the Biofilm Ring Test [24] or microcalorimetric assays [25]) have been recently incorporated into this static biofilm model. However, for examining the anti-biofilm PK/PD of antibacterials, dynamic models are required to mimic antibacterial PK *in vivo*. In the plug flow reactors, microbiological broth flows in one direction and solutes diffuse in a radial direction [21]. Another recent development is the drip flow biofilm reactor that is able to grow biofilm under low shearing forces [21]. Similarly, microfluidic devices (e.g. BioFlux) allow multiple parallel experiments for growing biofilm under low flow rates and shearing forces [22]. In continuous flow stirred tank reactors, homogenous mixing and diffusion of solutes occurs throughout the reactor [20••]. Two representative examples are the Rotating Disk Reactor [26] and the CDC Biofilm Reactor [27]. In addition, in these models imaging techniques (e.g. advanced fluorescence microscopy and integrated nuclear magnetic resonance and confocal laser scanning microscopy) are commonly used for evaluating antimicrobial diffusion, and changes in the biofilm ultra-structure and on viable but non-culturable bacteria after antibiotic treatment [28,29•,30].

Animal infection models

Animal infection models serve an important role in simulating the pathophysiology of infections in patients and as a platform for preclinical assessments of new antibiotics, as well as optimizing antibiotic use [31]. Pertaining to this review, animal models have been instrumental for evaluating antimicrobial PK/PD, notably the relationships between *in vitro* activity, bacterial growth, size of the inoculum, the timing of treatment, PK and *in vivo* efficacy [32]. Disadvantages of animal models include the variations in the PK of antibiotics compared to that in humans. In attempts to simulate human PK and usually prolong the half-life of the drug in animals, multiple doses or inducing transient renal impairment in animals by administration of uranyl nitrite can be employed [33].

In addition, allometric scaling should be considered when designing dosage regimens in animals. This section provides a practical overview of the most commonly used animal models in antibiotic drug discovery.

Thigh infection models. The mouse thigh infection model is the most common animal model to examine antibiotic PK/PD relationships [33,34]. The model is reproducible and relatively inexpensive. Mice are rendered neutropenic by treatment with cyclophosphamide on days −4 and −1, producing neutropenia by day 0 [33,34]. Log-phase bacterial cells (normally 10^5 to 10^6 CFU, depending on bacterial strains) are injected into each thigh under light anesthesia. An important consideration is the time difference between inoculation and the commencement of therapy. The tested compound is administered over 24 hours with multiple dosing regimens depending on the half-life and the PK/PD indices under investigation. The efficacy of the antibacterial agent is commonly determined by subtracting the $\log_{10}$ CFU/thigh at 24 hours of the treated mice from that of the control mice at 0 hours. PK/PD indices of $T_{>MIC}$, AUC/MIC or C_{max}/MIC can be related to the *in vivo* efficacy, most commonly by a sigmoid model [34]. Notable examples of the application of the mouse thigh infection model to study PK/PD relationships for antibiotic development include cephalosporin PPI-0903 [35] and linezolid [36]. In the linezolid study, it was revealed that a dosage regimen of 600 mg twice daily (AUC/MIC of 50–100) would be effective against pathogens with MICs as high as 2–4 mg/L [36].

Septicemia models. This model has been instrumental for evaluating the *in vivo* efficacy of numerous antibiotics [37,38•]. The model has been implemented across a number of animal species; however, for reasons of economy mice and rats are most commonly used. The simplicity of the endpoint analysis lends the mouse septicemia model to the routine use for preclinical *in vivo* efficacy assessment of novel antimicrobials [38•]. For mice, in most instances, the model involves rendering the animal neutropenic through the administration of 100–150 mg/kg of cyclophosphamide once a day for three days. The unanesthetized animal is then infected by an intraperitoneal injection of 0.1–0.5 mL of a log-phase bacterial suspension. Antibiotic(s) is administered by subcutaneous injection one hour postinoculation over multiple dosage regimens for a period of up to 72 hours. Other drug administration routes can be used depending on the prospective formulation of the compound. Endpoints for this model can be morbidity (% survival) and bacterial load (CFU) in the blood. Compared to the thigh infection model, the mouse septicemia model is significantly less time consuming and labor intensive as tissue homogenization and filtration are not required for viable counting.

Endocarditis models. Bacterial endocarditis can be a very difficult infection to treat due to inaccessibility of organisms within the core of the vegetations to the immune system and poor penetration of antibiotics into the infected endocardial vegetations [39]. The latter can also set ideal conditions for bacteria to develop resistance. Moreover, bacteria within the core of the vegetations display low metabolic activity rendering them less susceptible to antibiotics [40]. Endocarditis animal models have been developed in several species including mouse, rat, rabbit, pig, dog and opossum [41]. Endpoints used in this model include CFU/g vegetation and morbidity; blood samples are also collected to test for sepsis and relapse of infection following treatment. Endocarditis models have been extensively used for antibacterial PK/PD studies [41]. For fluoroquinolones, it was reported that an AUC/MIC $\geq$ 100 is required for bacterial clearance over three to six days of therapy [41].

Urinary tract infection (UTI) models. UTI is a significant urologic disease in women, predominantly caused by uropathogenic *Escherichia coli* from the intestinal flora that colonize the urethra and bladder [42]. UTI may even ascend from the bladder to the kidneys causing permanent damage and scarring [43]. Several animal models of ascending unobstructed UTI have been developed for antibacterial pharmacology and discovery [44]. Female mice are routinely used to simulate ascending UTI in women; however, male mice can also be employed [45]. After the animal is anesthetized, a catheter is inserted into the urethra and a needle is inserted into the catheter opening through which ~50 μL of bacterial suspension is delivered (usually 10^7 to 10^9 CFU/mouse). The mouse should not be given liquids for one hour before and after bacterial challenge to reduce urine output. Careful attention should be paid to the growth media used for the preparation of the inoculum as certain medium conditions provide for the expression of virulence factors required for uropathogenesis. The infection usually peaks one day post challenge and resolves over two to three weeks, depending upon the bacterial strain, the genetic background of inbred mice, and the absence of inoculation-associated vesicoureteral reflux. The endpoints are usually bacterial cultures of bladder and kidney homogenates. Additional parameters monitored may include morbidity and blood cultures, while homogenates of liver and spleen can also be taken to monitor dissemination of the infection outside the urinary tract. The mouse UTI model was recently employed to demonstrate the *in vivo* efficacy of ACHN-490, a new aminoglycoside with good *in vitro* activity against MDR Gram-negative and select Gram-positive pathogens [38•]. ACHN-490 treatment (0.125–8 mg/kg/12 hours for three days) effectively reduced $\log_{10}$CFU counts in the kidneys, bladder and urine of treated animals [38•].

Wound infection models. Infection remains the major cause of morbidity in wound patients worldwide [46•]. Numerous external traumatic wound infection models

have been developed to simulate various forms of traumatic injury and evaluate antibacterial treatment [46•]. Examples of animal wound infection models include skin abrasions, burns and excision wounds. Albino Hartley guinea pigs are typically used for wound models; their dorsal hair is clipped and a black grid is drawn on the back of the animal where the lesions are created. The main factors which determine the severity of the traumatic wound infection model include bacterial inoculum, size of the wound and immune-competence of the animals. The end-point for these models usually includes histopathological examination of sections of lesions and counting of viable bacteria recovered from the inoculation sites to determine the inoculation producing 50% probability of infection (ID_{50}). ID_{50} values are determined by logistic regression from a plot of the infection rate *versus* the bacterial inoculum size, and can be employed to access the efficacy of antibacterial agents. The assessment of antibacterial agents in wound models has generally yielded good correlation between their *in vitro* activity and *in vivo* efficacy in humans [47].

Animal biofilm models. Several biofilm-related animal models have been developed with or without the addition of foreign material, including central venous catheter models, subcutaneous foreign body infection models and osteomyelitis infection models [20••]. The infection may be established by direct inoculation into a specific organ or space (e.g. the otitis media model), manipulation of the infection site (e.g. cortical bone drilling before inoculation in osteomyelitis models), or implantation of a foreign body (e.g. device-related osteomyelitis) [20••]. The microorganisms inoculated are usually planktonic but capable of attaching to surfaces and developing biofilm. Sessile biofilm-embedded microorganisms have also been used for inoculation to mimic specific clinical scenarios [48]. Recently, an *in vivo* polymicrobial biofilm wound infection model was developed to study interspecies interactions in biofilm and their relation to wound chronicity [49].

In addition, a number of recent animal infection models have been adapted for the real-time monitoring of infections using luminescent bacteria [50,51]. This allows for the monitoring of infections in live animals in a non-invasive manner. Nevertheless, the sensitivity of bioluminescence is generally lower compared to viable counting methods; hence, such models may not be able to differentiate a marked bactericidal action from mild antibacterial effect.

Antimicrobial PK/PD modeling

State-of-the-art data analysis to optimize the knowledge gained from the *in vitro* and animal model data is critical for antibiotic development. Traditional PK/PD target approaches aim to maximize $T_{>MIC}$, AUC/MIC or C_{max}/MIC with the targets for stasis and different magnitudes of bacterial killing derived from pre-clinical models. Combined with population PK modeling, the PK/PD target approach allows the prediction of the likelihood of target attainment in a patient population (including for dosage regimens not previously studied in clinical trials) [52]. More recently, mechanism-based mathematical (MBM) models [53•] have been developed to incorporate firstly, multiple biologically relevant mechanisms (e.g. antibacterial action and resistance), secondly, concentration-time courses of single or multiple antibiotics, thirdly, effects of antibiotic exposure on bacterial killing and emergence of resistance in heterogeneous bacterial subpopulations with different antibiotic susceptibilities, and fourthly, effects of the immune system. On the basis of *in vitro* PK/PD data (e.g. from the hollow fiber infection model), MBM models can establish a quantitative relationship between PK profiles in patients and the time course of bacterial killing and resistance for further pre-clinical and clinical evaluations.

Conclusion

One of the significant challenges in antibiotic development is to establish the correlation between *in vitro* susceptibility and clinical efficacy. Hence, validated *in vitro* PK/PD and animal infection models serve as key tools in the antibiotic development process and have been widely employed for identifying the most predictive PK/PD indices. After analysis using comprehensive mathematical modeling, the results obtained set a quantitative basis for optimizing study designs in order to minimize the cost and duration of expensive clinical trials. In summary, *in vitro* PK/PD and animal infection models have shown great potential in increasing success rates and accelerating the drug development process, and will continue to make a significant contribution to the search for new antibiotics.

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References and recommended reading

- • of special interest
- •• of outstanding interest

1. Infectious Diseases Society of America: **The 10 × '20 Initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020**. *Clin Infect Dis* 2010, **50**:1081-1083.

2. •• Gloede J, Scheerans C, Derendorf H, Kloft C: ***In vitro* pharmacodynamic models to determine the effect of antibacterial drugs**. *J Antimicrob Chemother* 2010, **65**:186-201.

This excellent review provides a detailed overview of *in vitro* PK/PD models including their classification, working principles, development and implementation.

3. Lister PD: **The role of pharmacodynamic research in the assessment and development of new antibacterial drugs**. *Biochem Pharmacol* 2006, **71**:1057-1065.

4. Budha NR, Lee RB, Hurdle JG, Lee RE, Meibohm B: **A simple *in vitro* PK/PD model system to determine time-kill curves of drugs against Mycobacteria**. *Tuberculosis (Edinb)* 2009, **89**:378-385.

5. • Cadwell JJS: **The hollow fibre infection model for antimicrobial pharmacodynamics and pharmacokinetics**. *Adv Pharmacoepiderm Drug Safety* 2012, **S1**:007.
An excellent review on the history of PK/PD models. The uses, advantages and limitations of HFIM are discussed in detail.

6. Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL: **Pharmacokinetic/pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an *in vitro* model**. *Antimicrob Agents Chemother* 2010, **54**:3783-3789.

7. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira FP, Forrest A, Nation RL: **Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients**. *Antimicrob Agents Chemother* 2011, **55**:3284-3294.

8. Nicasio AM, Bulitta JB, Lodise TP, D'Hondt RE, Kulawy R, Louie A, Drusano GL: **Evaluation of once-daily vancomycin against methicillin-resistant *Staphylococcus aureus* in a hollow-fiber infection model**. *Antimicrob Agents Chemother* 2012, **56**:682-686.

9. Singh R, Tam VH: **Optimizing dosage to prevent emergence of resistance — lessons from *in vitro* models**. *Curr Opin Pharmacol* 2011, **11**:453-456.

10. Drusano GL, Sgambati N, Eichas A, Brown DL, Kulawy R, Louie A: **The combination of rifampin plus moxifloxacin is synergistic for suppression of resistance but antagonistic for cell kill of *Mycobacterium tuberculosis* as determined in a hollow-fiber infection model**. *MBio* 2010, **1**:e00139-210.

11. Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, Paterson DL, Nation RL, Li J: **Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an *in vitro* pharmacokinetic/pharmacodynamic model**. *Antimicrob Agents Chemother* 2011, **55**:5685-5695.

12. Deris ZZ, Yu HH, Davis K, Soon RL, Jacob J, Ku CK, Poudyal A, Bergen PJ, Tsuji BT, Bulitta JB *et al.*: **The combination of colistin and doripenem is synergistic against *Klebsiella pneumoniae* at multiple inocula and suppresses colistin resistance in an *in vitro* pharmacokinetic/pharmacodynamic model**. *Antimicrob Agents Chemother* 2012, **56**:5103-5112.

13. Tam VH, Louie A, Deziel MR, Liu W, Drusano GL: **The relationship between quinolone exposures and resistance amplification is characterized by an inverted U: a new paradigm for optimizing pharmacodynamics to counterselect resistance**. *Antimicrob Agents Chemother* 2007, **51**:744-747.

14. Drusano GL, Louie A, Deziel M, Gumbo T: **The crisis of resistance: identifying drug exposures to suppress amplification of resistant mutant subpopulations**. *Clin Infect Dis* 2006, **42**:525-532.

15. Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW: **Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic model**. *J Antimicrob Chemother* 2008, **61**:636-642.

16. Mouton JW, Brown DF, Apfalter P, Canton R, Giske CG, Ivanova M, MacGowan AP, Rodloff A, Soussy CJ, Steinbakk M *et al.*: **The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach**. *Clin Microbiol Infect* 2012, **18**:E37-E45.

17. Bulitta JB, Landersdorfer CB, Forrest A, Brown SV, Neely MN, Tsuji BT, Louie A: **Relevance of pharmacokinetic and pharmacodynamic modeling to clinical care of critically ill patients**. *Curr Pharm Biotechnol* 2011, **12**:2044-2061.

18. Vaddady PK, Lee RE, Meibohm B: ***In vitro* pharmacokinetic/pharmacodynamic models in anti-infective drug development: focus on TB**. *Future Med Chem* 2010, **2**:1355-1369.

19. Stewart PS, Costerton JW: **Antibiotic resistance of bacteria in biofilms**. *Lancet* 2001, **358**:135-138.

20. •• Coenye T, Nelis HJ: ***In vitro* and *in vivo* model systems to study microbial biofilm formation**. *J Microbiol Methods* 2010, **83**:89-105.
An excellent review on *in vitro* and *in vivo* experimental biofilm models.

21. Goeres DM, Hamilton MA, Beck NA, Buckingham-Meyer K, Hilyard JD, Loetterle LR, Lorenz LA, Walker DK, Stewart PS: **A method for growing a biofilm under low shear at the air-liquid interface using the drip flow biofilm reactor**. *Nat Protoc* 2009, **4**:783-788.

22. Kim J, Park HD, Chung S: **Microfluidic approaches to bacterial biofilm formation**. *Molecules* 2012, **17**:9818-9834.

23. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A: **The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms**. *J Clin Microbiol* 1999, **37**:1771-1776.

24. Chavant P, Gaillard-Martinie B, Talon R, Hebraud M, Bernardi T: **A new device for rapid evaluation of biofilm formation potential by bacteria**. *J Microbiol Methods* 2007, **68**:605-612.

25. Furustrand Tafin U, Corvec S, Betrisey B, Zimmerli W, Trampuz A: **Role of rifampin against *Propionibacterium acnes* biofilm *in vitro* and in an experimental foreign-body infection model**. *Antimicrob Agents Chemother* 2012, **56**:1885-1891.

26. Schwartz K, Stephenson R, Hernandez M, Jambang N, Boles BR: **The use of drip flow and rotating disk reactors for *Staphylococcus aureus* biofilm analysis**. *J Vis Exp* 2010, **46**:e2470.

27. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM: **Statistical assessment of a laboratory method for growing biofilms**. *Microbiology* 2005, **151**:757-762.

28. Hannig C, Follo M, Hellwig E, Al-Ahmad A: **Visualization of adherent micro-organisms using different techniques**. *J Med Microbiol* 2010, **59**:1-7.

29. • Daddi Oubekka S, Briandet R, Fontaine-Aupart MP, Steenkeste K: **Correlative time-resolved fluorescence microscopy to assess antibiotic diffusion-reaction in biofilms**. *Antimicrob Agents Chemother* 2012, **56**:3349-3358.
This study demonstarted that a combination of advanced fluorescence microscopic tools can be employed to characterize the dynamics of fluorescently labeled antibiotics in a biofilm model.

30. McLean JS, Ona ON, Majors PD: **Correlated biofilm imaging, transport and metabolism measurements via combined nuclear magnetic resonance and confocal microscopy**. *ISME J* 2008, **2**:121-131.

31. Andes D, Craig WA: **Animal model pharmacokinetics and pharmacodynamics: a critical review**. *Int J Antimicrob Agents* 2002, **19**:261-268.

32. Cars O, Odenholt-Tornqvist I: **The post-antibiotic sub-MIC effect *in vitro* and *in vivo***. *J Antimicrob Chemother* 1993, **31(Suppl D)**:159-166.

33. Craig WA, Redington J, Ebert SC: **Pharmacodynamics of amikacin *in vitro* and in mouse thigh and lung infections**. *J Antimicrob Chemother* 1991, **27(Suppl C)**:29-40.

34. Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA: **Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model**. *J Infect Dis* 1988, **158**:831-847.

35. Andes D, Craig WA: **Pharmacodynamics of a new cephalosporin, PPI-0903 (TAK-599), active against methicillin-resistant *Staphylococcus aureus* in murine thigh and lung infection models: identification of an *in vivo* pharmacokinetic–pharmacodynamic target**. *Antimicrob Agents Chemother* 2006, **50**:1376-1383.

36. Andes D, van Ogtrop ML, Peng J, Craig WA: ***In vivo* pharmacodynamics of a new oxazolidinone (linezolid)**. *Antimicrob Agents Chemother* 2002, **46**:3484-3489.

37. Drusano GL, Johnson DE, Rosen M, Standiford HC: **Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas* sepsis**. *Antimicrob Agents Chemother* 1993, **37**:483-490.

38. • Reyes N, Aggen JB, Kostrub CF: ***In vivo* efficacy of the novel aminoglycoside ACHN-490 in murine infection models**. *Antimicrob Agents Chemother* 2011, **55**:1728-1733.
This study demonstrated that murine septicemia and neutropenic thigh models play a key role in antibacterial discovery.

39. Cremieux AC, Maziere B, Vallois JM, Ottaviani M, Azancot A, Raffoul H, Bouvet A, Pocidalo JJ, Carbon C: **Evaluation of antibiotic diffusion into cardiac vegetations by quantitative autoradiography**. *J Infect Dis* 1989, **159**:938-944.

40. Durack DT, Beeson PB: **Experimental bacterial endocarditis. II. Survival of a bacteria in endocardial vegetations**. *Br J Exp Pathol* 1972, **53**:50-53.

41. Andes DR, Craig WA: **Pharmacodynamics of fluoroquinolones in experimental models of endocarditis**. *Clin Infect Dis* 1998, **27**:47-50.

42. Sobel JD: **Bacterial etiologic agents in the pathogenesis of urinary tract infection**. *Med Clin North Am* 1991, **75**:253-273.

43. Andriole VT: **Urinary tract infections in the 90s: pathogenesis and management**. *Infection* 1992, **20(Suppl 4)**:S251-S256.

44. Rubin RH, Shapiro ED, Andriole VT, Davis RJ, Stamm WE: **Evaluation of new anti-infective drugs for the treatment of urinary tract infection. Infectious Diseases Society of America and the Food and Drug Administration**. *Clin Infect Dis* 1992, **15(Suppl 1)**:S216-S227.

45. Hopkins WJ, Hall JA, Conway BP, Uehling DT: **Induction of urinary tract infection by intraurethral inoculation with *Escherichia coli*: refining the murine model**. *J Infect Dis* 1995, **171**:462-465.

46. • Dai T, Kharkwal GB, Tanaka M, Huang YY, Bil de Arce VJ, Hamblin MR: **Animal models of external traumatic wound infections**. *Virulence* 2011, **2**:296-315.
A comprehensive review on *in vivo* animal wound infection models.

47. Fallon MT, Shafer W, Jacob E: **Use of cefazolin microspheres to treat localized methicillin-resistant *Staphylococcus aureus* infections in rats**. *J Surg Res* 1999, **86**:97-102.

48. Williams DL, Costerton JW: **Using biofilms as initial inocula in animal models of biofilm-related infections**. *J Biomed Mater Res B Appl Biomater* 2012, **100**:1163-1169.

49. Dalton T, Dowd SE, Wolcott RD, Sun Y, Watters C, Griswold JA, Rumbaugh KP: **An *in vivo* polymicrobial biofilm wound infection model to study interspecies interactions**. *PLoS ONE* 2011, **6**:e27317.

50. Zhang H, Kalkeri G, Mani N, Grossman TH: **Development and validation of a multi-dose neutropenic rat thigh infection model using real-time monitoring of *Staphylococcus aureus* growth *in vivo***. *In Vivo* 2008, **22**:667-672.

51. Xiong YQ, Willard J, Kadurugamuwa JL, Yu J, Francis KP, Bayer AS: **Real-time *in vivo* bioluminescent imaging for evaluating the efficacy of antibiotics in a rat *Staphylococcus aureus* endocarditis model**. *Antimicrob Agents Chemother* 2005, **49**:380-387.

52. Ambrose PG, Bhavnani SM, Rubino CM, Louie A, Gumbo T, Forrest A, Drusano GL: **Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore**. *Clin Infect Dis* 2007, **44**:79-86.

53. • Landersdorfer CB, Ly NS, Xu H, Tsuji BT, Bulitta JB: **Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design**. *Antimicrob Agents Chemother* 2013, **57**:2343-2351.
This paper describes the first mechanism-based model for antibiotic combinations.