NOVES TECNOLOGIES PER AL DIAGNÒSTIC MOLECULAR DE LA SÈPSIA

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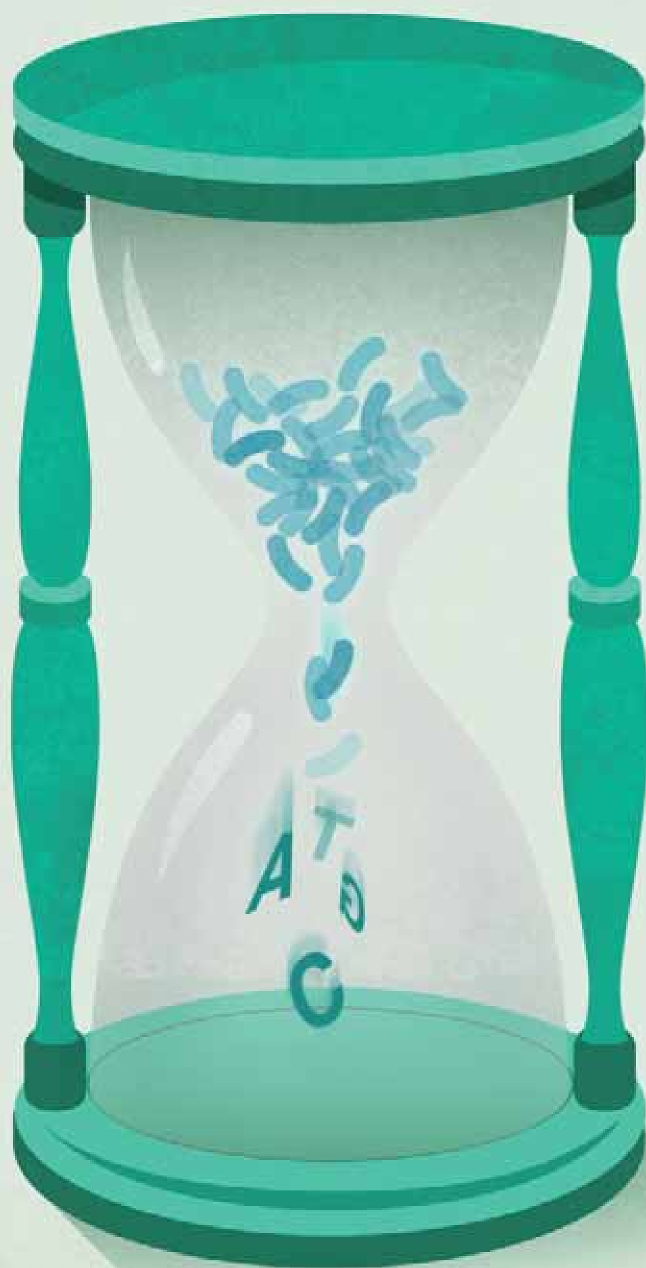

Objectius

Justificació de la unitat temàtica de la tesi

La sèpsia és un procés molt complex en el que es produeixen un conjunt de canvis humorals i cel·lulars com a resposta a la invasió de microorganismes, desencadenant-se una resposta inflamatòria sistèmica. En l'evolució clínica del pacient intervindran diferents factors com les característiques del microorganisme (inòcul, virulència), la immunocompetència de l'hostatger (patologia de base, factors genètics, etc.) i la rapidesa diagnòstica que ha de permetre establir de manera precoç el tractament antibiòtic i de suport apropiats. S'ha demostrat que la mortalitat relacionada amb la sèpsia augmenta fins a cinc vegades en els pacients que reben un tractament antibiòtic inadequat.

Per tant, en els casos de sèpsia i xoc sèptic, la supervivència del pacient depèn d'un diagnòstic etiològic precoç que permetrà instaurar el tractament correcte en les etapes inicials del procés. El baix rendiment de les tècniques microbiològiques convencionals, especialment en malalts sotmesos a tractament antibiòtic, fa que en moltes ocasions no s'arribi al diagnòstic etiològic i per tant es desconegui la sensibilitat del microorganisme. La incorporació progressiva de les tècniques de biologia molecular, basades en la detecció dels àcids nucleics microbians ha suposat un avenç a l'hora d'obtenir un diagnòstic ràpid, sensible i específic. De tota manera, tot i que s'han desenvolupat diferents mètodes per al diagnòstic molecular de la sèpsia, aquests no han arribat a implementar-se a la rutina assistencial del laboratori de microbiologia degut a diverses limitacions.

Per al desenvolupament de nous assaigs de diagnòstic in vitro és molt interessant l'establiment de col·laboracions entre les empreses que els produeixen i els centres de recerca associats als centres sanitaris. Els beneficis que obté l'empresa són diversos, ja que li permet aconseguir mostres clíniques reals per al desenvolupament i la validació analítica de l'assaig, així com poder realitzar una validació externa del mateix. A més, el fet de tenir una relació directa amb els destinataris finals de l'assaig es

tradueix en la generació d'un producte més competitiu comercialment. Per altra banda, per als centres de recerca també resulta beneficiós, ja que aquestes col·laboracions li permeten participar en l'avaluació i millora de nous assajos i l'oportunitat d'incorporar tecnologies pioneres.

Durant el desenvolupament d'aquesta tesi doctoral, s'ha col·laborat de forma molt estreta amb diverses empreses per al disseny i desenvolupament de noves eines diagnòstiques i també en la avaluació prèvia a la seva comercialització. Malauradament, alguns dels prototips no s'han acabat comercialitzant, per la qual cosa, degut a contractes de confidencialitat, aquest estudis no s'han pogut publicar. Per tant, aquesta tesi es basa en la tecnologia més prometedora que s'ha avaluat, la PCR/ESI-MS (consta d'una PCR d'ampli espectre seguida d'espectrometria de masses tipus electrospray), desenvolupada per Ibis Biosciences-Abbott Molecular. Aquesta tecnologia és molt versàtil, ja que permet la identificació de qualsevol tipus de patogen a partir de sang directa. La col·laboració amb aquesta empresa es va iniciar l'any 2008. Al 2009 es va realitzar una primera avaluació pilot de la tècnica, on es van obtenir resultats prometedors i on es van identificar punts crítics a millorar. Una segona avaluació de la tècnica utilitzant la versió PLEX-ID va donar lloc a la publicació del primer article utilitzant aquesta tecnologia per al diagnòstic de la sèpsia a partir de sang total (**Article I**). Cal destacar que la sensibilitat d'aquesta primera versió de la tecnologia va ser moderada. Per aquest motiu, l'empresa va voler redissenyar-la per tal d'augmentar la sensibilitat. Una tercera avaluació utilitzant la versió IRIDICA de la tecnologia (**Article II**) avala aquest augment de sensibilitat i explora la seva utilitat per al diagnòstic molecular de la sèpsia en diferents grups de pacients (provinents d'Urgències i ingressats a la Unitat de Cures Intensives). La nostra aportació s'ha basat en la validació externa d'aquesta tècnica abans i després de la seva comercialització, contribuint al seu desenvolupament.

Objectius

Objectiu general

- Avaluar la tecnologia PCR/ESI-MS per al diagnòstic molecular de la sèpsia i la seva aplicació en la rutina clínica (**Articles I i II**).

Objectius secundaris

1. Determinar la capacitat de la tècnica PCR/ESI-MS per a identificar un ampli ventall de patògens (**Articles I i II**).
2. Determinar la capacitat de la versió PLEX-ID per a detectar i identificar diferents patògens a partir d'hemocultius positius (**Article I**).
3. Determinar la capacitat de la tècnica PCR/ESI-MS per a detectar i identificar diferents patògens a partir de sang total (**Article I i II**).
4. Avaluar de la versió IRIDICA per al diagnòstic de la sèpsia en malalts crítics (**Article II**)
5. Implementació teòrica del sistema IRIDICA per al diagnòstic rutinari de la sèpsia.

OBJECTIVES

Justification of the thematic unit of the thesis

Sepsis is a complex process in which humoral and cellular immune responses lead to a systemic inflammatory response against invader microorganisms. The outcome of this situation depends on the specific microorganism (virulence factors, inoculum), the immune status of the patient (underlying pathology, genetic factors…), and the time needed to achieve a microbiological diagnosis, which will allow the establishment of a prompt antibiotic therapy as well as support measures.

Therefore, in cases of sepsis and septic shock, the patient survival depends on the early etiological diagnosis that will allow the establishment of the correct treatment in the initial stages of the process. However, conventional microbiologic methods have a low yield, especially in patients under antibiotic treatment; thus, in numerous cases the etiologic diagnosis is not achieved and the susceptibility to antibiotics is not known.

The progressive incorporation of molecular methods based on the detection of microbial nucleic acids has enhanced the chances of obtaining a rapid, sensitive and specific diagnostic. However, although several methods for the molecular diagnosis of sepsis have been developed, none of them have been implemented in the routine of a clinical microbiology laboratory due to several limitations.

For the development of new in vitro diagnostic tools, the co-operation between companies and research centers in hospitals is remarkably interesting. The company obtains several benefits, such as acquiring real clinical samples for the development and the analytical validation of the assay as well as performing an external validation of the assay. In addition, having a direct relationship with the final users of the assay may result in the generation of a more commercially competitive product. On the other hand, for the hospital research centers this relationship is also beneficial because it allows participating in the evaluation and im-

provement of new assays and having the opportunity to incorporate pioneer methods.

During the development of this doctoral thesis, our group has closely collaborated with several diagnostics companies in the design, development and premarket evaluation of new molecular assays for the detection of microorganisms directly from the patient's blood. Some of these diagnostic assays have not ended up being commercialized, and due to confidentiality agreements, those studies have not been published. Thus, this dissertation focuses on the most promising technology, the PCR/ESI-MS (broad range PCR coupled with electrospray ionization mass spectrometry), developed by Ibis Biosciences-Abbott Molecular. This technology allows the identification of any pathogen directly from blood.

The co-operation with this company started in 2008. In 2009, our group performed a pilot evaluation of the technology obtaining promising results and identifying some critical points to be improved. A second evaluation of the technique using the version PLEX-ID led to the publication of the first paper using this technology for the molecular diagnosis of bloodstream infections from whole blood (**Article I**). Given that the sensitivity of this first version of the technology was moderate, the company decided to refurbish it in order to increase the sensitivity. A third evaluation using the IRIDICA version of the technology (**Article II**) was performed in order to validate the new version. This paper endorses the increase in sensitivity and explores the usefulness if this technology for the molecular diagnosis of bloodstream infection in different patient groups (admitted to the Emergency room and the Intensive Care Unit). Our contribution has been based on an external validation of these techniques before and after their commercialization, contributing to their development.

Objectives

General objective

- To evaluate the PCR/ESI-MS technology for the molecular diagnosis of sepsis and its implementation on the clinical routine (**Articles I and II**).

Secondary objectives

1. To determine the system capability for the detection and identification of a wide range of pathogens (**Articles I and II**)
2. To determine the capability of the PLEX-ID version for the detection and identification of pathogens from positive blood cultures (**Article I**).
3. To determine the capability of the PCR/ESI-MS technology for the detection and identification of pathogens from whole blood (**Article I and II**).
4. To evaluate of the usefulness of the IRIDICA version for the diagnosis of sepsis in the critically ill patient.
5. Theoretical implementation of the IRIDICA system in the routine of a clinical laboratory for the molecular diagnosis of sepsis.

Article I

Rapid diagnosis of bloodstream infections with PCR followed by mass spectrometry

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Rapid Diagnosis of Bloodstream Infections with PCR Followed by Mass Spectrometry

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Abstract

Achieving a rapid microbiological diagnosis is crucial for decreasing morbidity and mortality of patients with a bloodstream infection, as it leads to the administration of an appropriate empiric antimicrobial therapy. Molecular methods may offer a rapid alternative to conventional microbiological diagnosis involving blood culture. In this study, the performance of a new technology that uses broad-spectrum PCR coupled with mass spectrometry (PCR/ESI-MS) was evaluated for the detection of microorganisms directly from whole blood. A total of 247 whole blood samples and paired blood cultures were prospectively obtained from 175 patients with a suspicion of sepsis. Both sample types were analyzed using the PCR/ESI-MS technology, and the results were compared with those obtained by conventional identification methods. The overall agreement between conventional methods and PCR/ESI-MS performed in blood culture aliquots was 94.2% with 96.8% sensitivity and 98.5% specificity for the molecular method. When comparing conventional methods with PCR/ESI-MS performed in whole blood specimens, the overall agreement was 77.1% with 50% sensitivity and 93.8% specificity for the molecular method. Interestingly, the PCR/ESI-MS technology led to the additional identification of 13 pathogens that were not found by conventional methods. Using the PCR/ESI-MS technology the microbiological diagnosis of bloodstream infections could be anticipated in about half of the patients in our setting, including a small but significant proportion of patients newly diagnosed. Thus, this promising technology could be very useful for the rapid diagnosis of sepsis in combination with traditional methods.

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Competing Interests: HE Carolan, R Sampath, DJ Ecker, LB Blyn, and LL Cummins are employees of Ibis Biosciences, an Abbott company. All the reagents and materials necessary for PCR/ESI-MS testing were supplied by Ibis Biosciences. There are no further patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

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Introduction

The administration of an empiric antibiotic therapy within the first hour of recognition of clinical symptoms of bloodstream infections is strongly recommended [1]. However, a rapid microbiological diagnosis is of paramount importance for the best outcome of the patient, since it allows for the administration of an appropriate empiric treatment on the basis of the clinician's knowledge of the antimicrobial susceptibility status of each bacterial species that is prevalent in that area. Once the identification is achieved, the initial therapy can be changed, if necessary, to assure an adequate antibiotic activity against the etiologic agent, or its spectrum reduced to prevent antimicrobial resistance development [2]. While susceptibility testing results are usually delayed 42–72 h, every hour gained to initiate proper antimicrobial therapy significantly increases the probability of patient survival [3].

The reference method used for the detection of pathogens in blood of septic patients is the blood culture (BC) followed by conventional identification methods. This methodology, while being necessary for the assessment of antimicrobial susceptibility, implies a delay of up to 48–72 h in the identification of the etiologic agent. Furthermore, despite being the reference diagnostic method, the BC has several limitations regarding sensitivity, especially in the case of previous antimicrobial therapy, and fastidious, slow-growing or uncultivable pathogens, often leading to a low diagnostic yield [4].

In order to speed the diagnostic process, it would be desirable to detect and identify the microorganisms directly from the patient's blood avoiding culture. Currently, molecular methods offer a rapid and reliable alternative to conventional culture, reducing the time to detection and increasing the sensitivity in the identification of certain microorganisms. As N. Mancini *et al.* recently reviewed [4], several assays are commercially available for the detection and

identification of microorganisms related to bloodstream infections directly from whole blood (WB). Among them, only SeptiFast (Roche, Mannheim, Germany) has been evaluated in several studies in the hospital setting leading to heterogeneous results [5–10]. Besides, this assay is limited to the 25 pathogens most commonly involved in sepsis; however, this syndrome can be caused by a broad range of pathogens and, thus, the diagnostic value is limited to the detection of the microorganisms included in the assay.

Recently, a new and promising technology has been described that uses a broad-spectrum PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS; Ibis Biosciences, an Abbott company, Carlsbad, CA, USA) to potentially identify any microorganism present in a clinical specimen [11–13]. Using mass spectrometry, the mass of each PCR amplicon is determined and the nucleotide base composition is unambiguously calculated and compared to a database, achieving the identification. This technology has shown promising results in the accurate detection of microorganisms directly from clinical specimens, including the detection of respiratory pathogens [14–16]. The goal of the present study was to test in a clinical setting the performance of this new technology for the identification of sepsis-related pathogens directly from WB. However, given the low concentration of microorganisms in this specimen type, a first evaluation was carried out on BC to confirm the ability of the PCR/ESI-MS technology to identify a variety of sepsis-related pathogens, and then its performance was assessed on WB.

Materials and Methods

Ethics Statement

Written informed consent was obtained from all patients or their guardians. This study was approved by the Clinical Research Ethics Committee at our institution ("Comité Ético de Investigación Clínica", CEIC).

Patients and Specimens

This was an observational study where a total of 175 patients with a suspicion of sepsis according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) criteria [17] were prospectively included between April 2010 and May 2011 (median age 60 years, range 1–94; 70 male and 102 female).

For each patient, a WB specimen was collected in an EDTA tube under aseptic conditions at the onset of fever or other clinical signs of sepsis, when the BC was inoculated for routine microbiological testing; for 42 patients, serial specimens were included, either from the same or different sepsis episodes, adding to a total of 247 WB specimens tested. For a subset of 206 WB specimens, an aliquot of the paired BC bottle/s was also obtained after incubation in order to compare the performance of the molecular method in the two specimen types. WB specimens were classified depending on their paired BC result as WB with a paired positive BC (n = 75) or WB with a paired negative BC (n = 172). WB specimens were frozen at −20°C for up to nine months until DNA extraction. Long term stability of WB samples under these storage conditions had previously been demonstrated by the manufacturer on spiked samples.

Conventional Microbiological Methods

For each adult patient, a set of two BC, including two aerobic and one anaerobic BC bottles, were inoculated with up to 10 mL of blood whereas the pediatric BC were filled with up to 2–5 mL depending on the child's age. The BC bottles were incubated in the Bactec 9240 BC system (Becton Dickinson, Franklin Lakes, NJ, USA) for up to 5 days before being called negative. The identification and susceptibility testing of the microorganism/s present in the positive BC bottles was achieved using the Vitek-2 Compact system (BioMérieux, Marseille-L'Étoile, France) after performing a Gram stain and a concentration protocol [18,19]. Conventional cultures were also performed following standard microbiological methods for identification and antibiotic susceptibility testing (disc diffusion and minimum inhibitory concentration methods as required).

DNA Extraction

For each patient DNA was extracted from 1.250 mL of WB and from 1.1 mL of the BC aliquot, according to the manufacturer's instructions. After a mechanical lysis step with the Precellys instrument (Bertin Technologies, Montigny-le-Bretonneux, France), the DNA was extracted using the magnetic-bead based extractor KingFisher Flex (ThermoScientific, Waltham, MA, USA) following the manufacturer's protocols. The eluted DNA (200 µL) was blind-coded regarding both the patient's identity and the BC result and frozen at −80°C for further analysis at Ibis Biosciences (Carlsbad, CA, USA).

DNA Amplification and Analysis

Two investigational assays were used to amplify DNA extracts from WB and BC specimens, respectively, according to the manufacturer's protocols. The assays were designed to detect a broad range of bacteria and *Candida* spp. as well as four antibiotic resistance genes: *mecA* (resistance to methicillin), *vanA* and *vanB* (resistance to vancomycin) and *kpc* (resistance to carbapenems) in a 96-well plate. After DNA amplification in a Mastercycler® ep S Thermal Cycler (Eppendorf, Eppendorf AG. Hamburg, Germany), according to the manufacturer's protocol, the plates were transferred to the ESI-MS instrument (Ibis Biosciences). The PCR products were analyzed using electrospray ionization time of flight mass spectrometry (ESI-TOF) to determine the mass of each amplicon strand. Using a built-in software analysis package, the base count of each amplicon was deduced from the measured masses and compared with the reference database. The combination of the results obtained from each primer pair was used to identify the microorganism/s present in the sample. Furthermore, the genetic material was quantified by using an internal calibrant added at a known concentration to each PCR well. Identification can be obtained from clinical specimens in 6–8 hours including DNA extraction, PCR amplification and ESI-TOF analysis.

Data Interpretation and Statistical Analysis

For each patient the results obtained with the PCR/ESI-MS technology on WB and its paired BC were compared with those obtained using conventional methods (BC was considered the gold standard). As discrepancies between both methods were found, to assess whether the microorganisms identified only by the PCR/ESI-MS corresponded to the true etiological agents of the sepsis episode and, thus, had clinical significance, the results were compared to a constructed "clinical infection criterion"; this new gold standard was based on clinical records review in order to identify the diagnosed focus of infection, as well as on the results of cultures from other specimens (i.e. microorganisms detected only by PCR/ESI-MS were considered true positives when the same microorganism had been isolated from a culture from another specimen type reflecting the focus of infection or supported by the nature of the underlying infection).

Since more than one microorganism could be detected per specimen, the methods were compared at two levels against the

two aforementioned gold standards: 1) the Cohen's Kappa coefficient of agreement was calculated (OpenEpi software) [20] between both methods considering all microorganisms identified (a direct comparison for each microorganism isolated by conventional methods vs. the same microorganism detected by the molecular method); and 2) positive and negative results by each method were computed in order to calculate the parameters of analytical performance of the molecular method (sensitivity, specificity, positive and negative predictive values), excluding specimens with polymicrobial detections, since they could not be properly classified (i.e. both methods agreed in some but not all microorganisms identified).

Results

1. Microbial Identification from Blood Culture Specimens

The performance of the PCR/ESI-MS at identifying a variety of sepsis-related microorganisms was firstly assessed on BC aliquots in comparison with conventional methods.

Agreement between isolated/detected microorganisms. When the PCR/ESI-MS was compared to the conventional methods using the BC as the gold standard, the same microorganism was identified in 78 out of a total of 96 identifications by either or both methods, while 128 specimens were negative by both methods. Thus, the overall agreement at the microorganism level was 92.0% ($\kappa = 0.830$) (**Table 1A**). Polymicrobial infections involving two or three microorganisms were detected by either or both methods in 14 (18.7%) cases among the 75 specimens with a positive BC (**Table 2**). Six of the seven microorganisms detected by BC that were missed by PCR/ESI-MS corresponded to polymicrobial specimens. However, the PCR/ESI-MS technology detected an additional five microorganisms not detected by BC that were clinically significant (**Table 3**). Thus, when a clinical infection criterion was used as the gold standard, the agreement rose to 94.2% ($\kappa = 0.879$) (**Table 1A**).

In one case of two closely related microorganisms, the PCR/ESI-MS software misidentified the microorganism (*Klebsiella pneumoniae* as *Citrobacter* spp.), and in a few cases, the identification was only achieved at the genus level (9.6% of the 83 pathogens detected with clinical significance: *Streptococcus* spp., *Citrobacter* spp., *Fusobacterium* spp., *Clostridium* spp. and *Salmonella* spp.). In the case of conventional methods, 3.5% of the 85 microorganisms were also identified at the genus level (*Serratia* spp., *Fusobacterium* spp. and *Bacteroides* spp.).

Parameters of analytical performance. The sensitivity, specificity, the PPV and the NPV of the PCR/ESI-MS were 96.7%, 97.7%, 95.2% and 98.5%, respectively, using the BC as the gold standard (**Table 4A**). Given that the molecular method detected a microorganism with clinical value in a negative BC (**Table 5**), these values were 96.8%, 98.5%, 96.8% and 98.5%, respectively when the clinical criterion gold standard was used (**Table 4A**).

2. Microbial Identification from Whole Blood Specimens

Given that the PCR/ESI-MS technology demonstrated a good performance on BC aliquots, we proceeded with the evaluation in the WB specimens obtained from the same patients.

Agreement between isolated/detected microorganisms. From a total of 110 microorganisms identified by either or both methods, 37 were identified both by BC and the PCR-ESI/MS technique, while no identification was achieved in 152 specimens by either method. Thus the overall agreement between methods was 72.1% ($\kappa = 0.316$) (**Table 1B**). The PCR/ESI-MS identified a total of 25 microorganisms that were not detected by BC (commented in **Table 6**), and the presence of 13 of them was supported by clinical facts. On the contrary, in four cases the presence of those microorganisms could not be supported by clinical evidence, and another eight microorganisms were considered contaminants from the skin flora that were found in the BC due to inadequate antisepsis before venipuncture (i.e. coagulase-negative staphylococci, *Propionibacterium acnes*, etc.). When the results were reanalyzed taking this clinical information into consideration, the agreement between the PCR/ESI-MS and the conventional methods increased to 77.1% ($\kappa = 0.472$) (**Table 1B**). A list of the microorganisms identified by either or both methods is depicted in **Table 3**.

Parameters of analytical performance. Polymicrobial infections were detected by either or both methods in 11 (14.7%) out of the 75 cases with a positive paired BC (**Table 2**). As described above for BC samples, only those specimens with a single pathogen were considered for analysis. When the BC was regarded as the gold standard, the sensitivity, specificity, the PPV and the NPV were 43.8%, 88.4%, 58.3% and 80.9%, respectively. However, taking into account the 10 cases with clinical

Table 1. Agreement between microorganisms isolated by conventional microbiological methods and detected by the PCR/ESI-MS method according to the gold standard used and the specimen type.

		Blood culture gold standard			Clinical infection criterion		
		Conventional methods			Conventional methods		
		Positive	Negative	Total	Positive	Negative	Total
A) PCR/ESI-MS in blood culture	Positive	78	11	**89**	83	6	**89**
	Negative	7	128	**135**	7	128	**135**
	Total	**85**	**139**	**224**	**90**	**134**	**224**
B) PCR/ESI-MS in whole blood	Positive	37	25	**62**	50*	12	**62**
	Negative	48	152	**200**	48	152	**200**
	Total	**85**	**177**	**262**	**98**	**164**	**262**

A) Overall agreement, blood culture gold standard: [(78+128)/224] = 92.0%, and clinical infection criterion: [(83+128)/224] = 94.2%.
B) Overall agreement, blood culture gold standard: [(37+152)/262] = 72.1%, and clinical infection criterion: [(50+152)/262] = 77.1%.
*Two detections correspond to different specimens from the same patient and sepsis episode.
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Table 2. Polymicrobial infections/detections by conventional methods or PCR/ESI-MS according to specimen type.

		PCR/ESI-MS	
Specimen	**Conventional methods**	**Blood culture**	**Whole blood**
1	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
	Enterococcus faecium	*Enterococcus faecium*	*Klebsiella oxytoca*
2	*Enterococcus faecalis*	*Enterococcus faecalis*	Not detected
	Pseudomonas aeruginosa	Not detected	*Pseudomonas aeruginosa*
	Klebsiella pneumoniae	Not detected	Not detected
3	*Citrobacter koseri*	*Citrobacter* spp.	*Citrobacter koseri*
	Hafnia alvei	Not detected	Not detected
4	*Enterobacter cloacae*	*Enterobacter cloacae* complex	*Enterobacter intermedius*
	Enterococcus faecalis	Not detected	Not detected
	CoNS[1]	CoNS	Not detected
5	*Streptococcus parasanguinis*	*Streptococcus* spp.	*Streptococcus thermophylus*
	Staphylococcus epidermidis	CoNS	Not detected
6	*Escherichia coli*	*Escherichia coli*	Not detected
	Staphylococcus aureus	*Staphylococcus aureus*	Not detected
7	*Serratia marcescens*	*Serratia marcescens*	Not detected
	Enterococcus faecium	Not detected	Not detected
8	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
	Staphylococcus epidermidis	CoNS	*Staphylococcus epidermidis*
9	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Not detected
	Not detected	*Citrobacter* spp.	Not detected
	Not detected	Not detected	*Staphylococcus warneri*
10	*Fusobacterium* spp.	*Fusobacterium* spp.	Not detected
	Not detected	*Clostridium* spp.	Not detected
11	*Staphylococcus epidermidis*	CoNS	Not detected
	Not detected	*Enterobacter cloacae* complex	Not detected
12	*Enterococcus faecalis*	*Enterococcus faecalis*	Not detected
	Not detected	*Enterobacter cloacae* complex	Not detected
13	*Bacteroides* spp.	*Bacteroides fragilis*	*Bacteroides fragilis*
	Not detected	*Clostridium* spp.	Not detected
14	*Serratia* spp.	*Serratia* spp.	Not detected
	Not detected	*Bacillus* spp.	Not detected
15	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium*
	Not detected	Not detected	*Candida albicans*
16	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
	Not detected	Not detected	*Corynebacterium* spp.
17	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
	Not detected	Not detected	*Enterobacter cloacae*

[1]*Staphylococcus epidermidis* and other coagulase-negative species (CoNS).
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significance detected by the PCR/ESI-MS only (**Table 6**), the values rose to 50%, 93.8%, 78.7% and 80.4% when the clinical infection criterion was used (**Table 4B**).

Variables such as the time to positivity of the BC, total DNA concentration and leukocyte count were compared between those specimens with a paired positive BC that had a positive detection and those with no detection by PCR/ESI-MS and no statistically significant differences were found (data not shown).

3. Detection of Antibiotic Resistances

Among all *Staphylococcus aureus* isolates (N = 11), two were reported to be methicillin-resistant *Staphylococcus aureus* (MRSA) by conventional methods. Both of them were correctly identified by the PCR/ESI-MS technology both in WB and paired BC specimens. Additionally, the presence of the *mecA* gene was reported by the molecular method in two other strains (one from WB and one from BC specimens) that were methicillin-susceptible *Staphylococcus aureus* (MSSA) according to the reference methods.

Table 3. Pathogens with clinical significance isolated by conventional microbiological methods and detected by the PCR/ESI-MS method.

		N° of microorganisms detected					
		Blood culture specimens			Whole blood specimens		
Group	Microorganism	BC only	PCR/ESI-MS and BC	PCR/ESI-MS only	BC only	PCR/ESI-MS and BC	PCR/ESI-MS only
Gramnegatives	*Bacteroides* spp.	0	1	0	0	1	0
	Citrobacter koseri	0	1	0	0	1	0
	Enterobacter cloacae	0	2	1	0	2	1
	Escherichia coli	0	16	0	8	8	3
	Fusobacterium spp.	0	1	0	1	0	0
	Hafnia alvei	1	0	0	1	0	0
	Klebsiella oxytoca	0	1	0	1	0	1
	Klebsiella pneumoniae	1	6	0	2	5	2
	Pseudomonas aeruginosa	1	4	0	1	4	0
	Salmonella enterica	0	1	0	1	0	0
	Serratia marcescens	0	2	0	2	0	0
Grampositives	*Clostridium* spp.	0	0	2	0	0	0
	Enterococcus faecalis	1	7	0	8	0	0
	Enterococcus faecium	3	5	0	5	3	0
	Staphylococcus aureus	0	9	0	3	6	2
	Methicillin-resistant *Staphylococcus aureus*	0	2	0	0	2	0
	Coagulase-negative staphylococci	0	11	0	10	1	0
	Streptococcus spp.[1]	0	2	0	0	2	0
	Streptococcus agalactiae	0	1	0	0	1	0
	Streptococcus pneumoniae	0	5	2	3	2	3
Yeasts	*Candida albicans*	0	1	0	1	0	1
Total		**7**	**78**	**5**	**47**	**38**	**13**

[1]*Streptococcus mitis* and *S. parasanguinis*.
BC, blood culture.
doi:10.1371/journal.pone.0062108.t003

Table 4. Agreement of unique isolation/detection specimens depending on the gold standard used and the specimen type.

		Blood culture gold standard			Clinical infection criterion		
		Conventional methods			Conventional methods		
		Positive	Negative	Total	Positive	Negative	Total
A) PCR/ESI-MS in blood culture	Positive	59	3	**64**	60	2	**64**
	Negative	2[a]	128	**128**	2[b]	128	**128**
	Total	**61**	**131**	**192**	**62**	**130**	**192**
B) PCR/ESI-MS in whole blood	Positive	28[c]	20	**48**	37	10	**47**
	Negative	36	152	**188**	37	152	**189**
	Total	**64**	**172**	**236**	**74**	**162**	**236**

A) Blood culture gold standard: 96.7% sensitivity, 97.71% specificity, 95.2% PPV, 98.5% NPV. Clinical infection criterion: 96.8% sensitivity, 98.5% specificity, 96.8% PPV, 98.5% NPV.
[a]In one specimen an *Enterococcus faecium* was isolated by blood culture whereas PCR/ESI-MS detected a coagulase-negative staphylococci.
[b]In another specimen a *Klebsiella pneumoniae* was isolated by blood culture and it was identified as *Citrobacter* spp. by PCR/ESI-MS.
B) Blood culture gold standard: 43.8% sensitivity, 88.4% specificity, 58.3% PPV, 80.9% NPV. Clinical infection criterion: 50.0% sensitivity, 93.8% specificity, 78.7% PPV, 80.4% NPV.
[c]The PCR/ESI-MS detected a coagulase-negative staphylococci while a *Klebsiella pneumoniae* was isolated by blood culture. In 10 specimens with a negative paired blood culture (two of them from the same patient and sepsis episode) the PCR/ESI-MS detected clinically significant microorganisms.
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Table 5. Discrepancies found between the PCR/ESI-MS in blood cultures and conventional methods.

	PCR/ESI-MS results	Conventional methods	Comments
Clinical evidence supporting PCR/ESI-MS results	CoNS[1], *Enterobacter cloacae*	*Staphylococcus epidermidis*	Bronchial aspirate culture positive for *E. cloacae* 3 days later. Respiratory superinfection.
	Streptococcus pneumoniae	Negative	Pneumonia antecedents. Previous splenectomy. PCR/ESI-MS detected *S. pneumoniae* in the paired whole blood specimen.
	Bacteroides fragilis, Clostridium spp.	*Bacteroides* spp.	Abdominal infection. Possible presence of different anaerobic microorganisms.
	Streptococcus spp., *Streptococcus pneumoniae*, CoNS	*Streptococcus mitis, Staphylococcus epidermidis*	Pneumonia.
	Fusobacterium spp., *Clostridium* spp.	*Fusobacterium* spp.	Soft tissue infection, possible presence of anaerobic microorganisms.
No clinical evidence supporting PCR/ESI-MS results	*Staphylococcus aureus*	Negative	
	Staphylococcus aureus	Negative	Pneumonia.
	Enterococcus faecalis, Enterobacter spp.	*Enterococcus faecalis*	Communitarian sepsis. Unknown focus.
	Klebsiella pneumoniae, Citrobacter spp.	*Klebsiella pneumoniae*	Communitarian sepsis. Unknown focus. Possible misidentification.
Misidentification	*Citrobacter* spp.	*Klebsiella pneumoniae*	
Skin or ambient contaminant	CoNS	*Enterococcus faecium*	
	Serratia marcescens, Bacillus spp.	*Serratia* spp.	

[1]*Staphylococcus epidermidis* and other coagulase-negative species.

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The other resistance-related genes (*vanA*, *vanB* or *kpc*) were not detected in any of the isolates included in this study by any method.

Discussion

Sepsis is a major cause of morbidity and mortality in hospitals around the world [21]. Since the rapid administration of an effective antimicrobial therapy is decisive for the best outcome of the patient, a prompt identification of the causal agent is highly desirable to readdress the initial empirical treatment if necessary [3]. This is the first study that clinically evaluated a new technology based on PCR and mass spectrometry for the detection of pathogens directly from WB.

Since sepsis may be caused by a wide diversity of pathogens, mainly bacteria and fungi, the identification of all of them may be challenging for diagnostic assays. Given the low concentration of bacteria/yeast in WB, the ability of the PCR/ESI-MS technology to detect a variety of sepsis-related pathogens was best assessed in BC. Being based on several broad-range PCR reactions, the investigational assay used for the identification of pathogens from BC showed a very good overall agreement (94.2%) with the conventional microbiological methods, with 21 different species identified. These results are in agreement with previous data [22]; 93 (90.3%) of the 103 microorganisms identified by conventional methods were also detected by PCR/ESI-MS (45 different species identified).

Recently, another mass spectrometry-based technology has been adapted to microbiological diagnosis; in this case, a Matrix-Assisted Laser Desorption/Ionization (MALDI-TOF) approach is used to obtain the protein spectrum of microbial pathogens from BC [23–25]. E. J. Kaleta *et al.* [26] compared the PCR/ESI-MS technology with the MALDI-TOF approach in BC specimens and a highly accurate identification at species level was achieved with both methodologies (95.2% and 94.3%, respectively). The main advantage of the MALDI-TOF approach is that the identification of pathogens can be achieved within minutes, but a previously grown culture is required, whereas the identification can be directly obtained from uncultured WB in 6–8 hours with the PCR/ESI-MS technology.

As opposed to working with BC aliquots, starting from the patient's blood would provide microbiologists with the possibility of anticipating the diagnosis up to 40 hours. However, the concentration of bacteria in WB may be as low as 1–10 CFU/mL [27]. Although the PCR/ESI-MS technology was able to detect very low levels of bacteria (1–5 genomes/well) in some cases, its performance was more limited in this specimen type than in BC, as expected. One inherent limitation in comparing molecular methods to culture is that current molecular methods use a smaller volume of blood than BC. This is because an excess of human DNA may hamper the detection of minor bacterial DNA or even inhibit the PCR reaction. Methods to overcome these problems and test larger blood volumes are being developed by the manufacturer as well as other groups [28–30]. Even so, our results show that using the PCR/ESI-MS technology, a microbiological diagnosis of sepsis could be achieved directly from the patient's blood with 50% sensitivity and 93.8% specificity when compared with conventional methods.

Other molecular methods for the diagnosis of sepsis directly from WB have been clinically evaluated. The SeptiFast test is based on a multiplex real-time PCR targeting the 25 most frequent pathogens involved in bloodstream infections. However, the results obtained with this assay are not very consistent across different studies, with sensitivity ranging between 61 and 90.9%, and specificity between 70 and 100% [5–10]. The overall agreement between microorganisms isolated by BC and identified with the PCR/ESI-MS in WB in our study was 77.5%, which is comparable to results published using the SeptiFast (69 to 77.8%) [5,31,32]. However, microorganisms not included in the mentioned assay were identified in seven cases in our study

Table 6. Discrepancies found between the PCR/ESI-MS in whole blood and conventional methods.

	PCR/ESI-MS results	Conventional methods	Comments
Clinical evidence supporting PCR/ESI-MS results	*Klebsiella pneumoniae, Klebsiella oxytoca*	*Klebsiella pneumonia, Enterococcus faecalis*	Hepatocellular carcinoma and biliar obstruction.
	Enterococcus faecium, Candida albicans	*Enterococcus faecium*	Intravascular catheter-related sepsis in patient with leukemia and neutropenia treated with caspofungin. *C. albicans* confirmed by sequencing.
	Escherichia coli, Enterobacter cloacae	*Escherichia coli*	Sepsis of abdominal origin.
	Escherichia coli	Negative	Urine culture positive for *E. coli.*
	Streptococcus pneumoniae	Negative	Pneumonia in a splectomized patient. Confirmed by sequencing.
	Staphylococcus aureus	Negative	Pneumonia due to *S. aureus.*
	Escherichia coli	Negative	Urine culture positive for *E. coli.*
	Streptococcus pneumoniae[1]	Negative	Bilateral pneumonia in an immunocompromised patient with a bronchial aspirate culture positive for *S. pneumoniae.*
	Klebsiella pneumoniae	Negative	Previous sepsis due to *K. pneumoniae.* Polycystic kidney disease.
	Staphylococcus aureus	Negative	Skin origin of sepsis. Culture positive for *S. aureus.*
	Klebsiella pneumoniae	Negative	Bilateral pneumonia. Previous urine culture positive for *K. pneumoniae.*
	Escherichia coli	Negative	Acute lymphoblastic leukemia. Primary origin of sepsis. Distended abdomen, possible translocation.
No clinical evidence supporting PCR/ESI-MS results	*Escherichia coli*	Negative	Pneumonia due to MRSA.[2]
	Staphylococcus aureus	Negative	Pneumonia due to *S. pneumoniae.*
	Escherichia coli	Negative	Chronic respiratory disease.
	Escherichia coli	Negative	
Skin contaminants	*Staphylococcus warneri*	*Klebsiella pneumoniae*	
	Pseudomonas aeruginosa, Corynebacterium spp.	*Pseudomonas aeruginosa*	
	Staphylococcus haemolyticus	Negative	
	Staphylococcus hominis	Negative	
	Staphylococcus epidermidis	Negative	*S. epidermidis* was detected and considered as contaminant in two specimens, one of them confirmed by sequencing.
	Propionibacterium acnes	Negative	*P. acnes* was detected and considered as contaminant in two specimens.

[1] *Streptococcus pneumoniae* was detected in two whole blood specimens with negative blood culture from the same patient during the same sepsis episode.
[2] Methicillin-resistant *Staphylococcus aureus.*
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(*Salmonella enterica*, *Bacteroides* spp., *Citrobacter koseri*, *Fusobacterium* spp., *Hafnia alvei*, and two cases of *Clostridium* spp.). The VYOO® test (SIRS-Lab, Jena, Germany) is based on a multiplex PCR followed by microarray detection that includes 34 bacterial pathogens, seven fungi and five antibiotic resistance genes, and showed a 46.2% concordance with conventional methods [29]. The SepsiTest (Molzym, Bremen, Germany) uses a universal 16S rRNA PCR followed by sequencing and a concordance of 86% with BC was reported for this assay [30].

While the PCR/ESI-MS offers a quantitative result and it detected from 1 to 399 genomes per PCR well, a lower detection limit is set for the final interpretation of results (3 genomes/well for most pathogens and at 5 genomes/well for the *Candida* spp.). However, this detection limit was risen up to 10 genomes/well by the manufacturer for the coagulase-negative staphylococci (CoNS) and other pathogens likely to be contaminants from the skin flora. While this strategy was designed to improve the specificity of the assay for the diagnosis of sepsis, it could also lead to a low percentage of false-negative results, given that the amount of genomes/well observed for microorganisms with clinical significance and those considered contaminants cannot be easily distinguished. In fact, in nine WB specimens with a positive paired BC the PCR/ESI-MS achieved the correct identifications but they were not reported as they were under the mentioned threshold, being three of them CoNS. On the other hand, eight cases corresponding to skin contaminants were above the limit of detection and, thus, reported as positive. In fact, the detection of such microorganisms is also an issue to be considered when interpreting the results of BC; in our center, in up to 5% of all positive blood cultures a skin contaminant is isolated due to poor aseptic practices at the time of inoculation. Those patients in which a skin contaminant had been identified by BC were excluded from this study.

Several factors may limit the interpretation of the results obtained in our study. Firstly, 42 patients were sampled at several time-points during the same sepsis episode; serial blood cultures are often obtained in the clinical practice for patients that are not evolving favorably. Given that the PCR/ESI-MS results are quantitative, future studies should explore the value of this technique for monitoring antibiotic efficacy and predict clinical

outcome of patients with sepsis. Secondly, in some cases the samples were drawn when patients were already under antibiotic treatment. The latter could lead to positive findings by PCR/ESI-MS in sepsis episodes with a negative BC. In the absence of a highly sensitive gold standard, reviewing clinical facts as well as other positive cultures is necessary in these cases. In this study, the clinical relevance of 12 out of 25 such cases was clinically supported by additional positive cultures or by the nature of the underlying infection, and 9 of those 12 patients were on antibiotic treatment. This data supports the fact that molecular technologies may be useful in those cases where the value of traditional culture is limited; the identification of the etiological pathogen in treated patients could have a clinical impact in patient outcome through the adjustment of the initially administered antimicrobial therapy. It also has to be taken into account that molecular methods are able to detect the DNA either from living or dead bacteria, as well as DNA released to the bloodstream by translocation [33], while blood culture and identification by the Vitek-2 system only detects viable microorganisms. Some of the PCR/ESI-MS findings could not be supported by clinical facts and, consequently, the results obtained should always be reviewed and interpreted by a clinical microbiologist considering all the available clinical data.

Despite molecular methods are more expensive than conventional ones, the overall benefits for the patient have to be considered. A rapid identification of the pathogen may lead to the optimization of the administered therapy and, thus, to a prompter recovery of the patient and a shorter stay at the ICU department. Cost/benefit studies regarding the use of molecular assays in combination with conventional methods have been performed using the SeptiFast assay and significant economic savings were reported due to the shortening of the ICU stay and a more rational use of antibiotics [34,35].

In conclusion, the PCR/ESI-MS technology could be a useful tool to achieve a rapid diagnosis in patients with clinical suspicion of sepsis. Our results show that a significant proportion of patients would beneficiate from an early diagnosis, and its use in combination with traditional methods could increase the number of microbiologically confirmed sepsis cases. Although more studies are necessary to asses the real clinical impact of this technology in the detection of pathogens in whole blood, this early identification of the pathogen could affect the antibiotic treatment and, therefore, the patient management and outcome. In addition, given its capability of detecting any pathogen, this technology offers a high versatility for the diagnosis of infectious diseases.

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Author Contributions

Critically reviewed the manuscript: MG MDQ HEC JMM FA LLC DJE RS LBB. Acquired the data: EJL HEC RS LLC LBB JMM FA. Conceived and designed the experiments: EM VA EJL DJE LLC. Performed the experiments: EJL EM LLC. Analyzed the data: EJL EM MG MDQ. Contributed reagents/materials/analysis tools: VA LLC DJE RS LBB. Wrote the paper: EJL EM VA.

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ARTICLE II

Evaluation of the broad-range PCR/ESI-MS technology in blood specimens for the molecular diagnosis of bloodstream infection

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RESEARCH ARTICLE

Evaluation of the Broad-Range PCR/ESI-MS Technology in Blood Specimens for the Molecular Diagnosis of Bloodstream Infections

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Abstract

Background

Rapid identification of the etiological agent in bloodstream infections is of vital importance for the early administration of the most appropriate antibiotic therapy. Molecular methods may offer an advantage to current culture-based microbiological diagnosis. The goal of this study was to evaluate the performance of IRIDICA, a platform based on universal genetic amplification followed by mass spectrometry (PCR/ESI-MS) for the molecular diagnosis of sepsis-related pathogens directly from the patient's blood.

Methods

A total of 410 whole blood specimens from patients admitted to Emergency Room (ER) and Intensive Care Unit (ICU) with clinical suspicion of sepsis were tested with the IRIDICA BAC BSI Assay (broad identification of bacteria and *Candida* spp.). Microorganisms grown in culture and detected by IRIDICA were compared considering blood culture as gold standard. When discrepancies were found, clinical records and results from other cultures were taken into consideration (clinical infection criterion).

Results

The overall positive and negative agreement of IRIDICA with blood culture in the analysis by specimen was 74.8% and 78.6%, respectively, rising to 76.9% and 87.2% respectively, when compared with the clinical infection criterion. Interestingly, IRIDICA detected 41 clinically significant microorganisms missed by culture, most of them from patients under antimicrobial treatment. Of special interest were the detections of one *Mycoplasma hominis* and two *Mycobacterium simiae* in immunocompromised patients. When ICU patients were

Clínica" (SEIMC) (EJL). All reagents and materials necessary for IRIDICA testing were supplied by Ibis Biosciences. This institution had no role in study design, decision to publish, or preparation of the manuscript.

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analyzed separately, sensitivity, specificity, positive and negative predictive values compared with blood culture were 83.3%, 78.6%, 33.9% and 97.3% respectively, and 90.5%, 87.2%, 64.4% and 97.3% respectively, in comparison with the clinical infection criterion.

Conclusions

IRIDICA is a promising technology that offers an early and reliable identification of a wide variety of pathogens directly from the patient's blood within 6h, which brings the opportunity to improve management of septic patients, especially for those critically ill admitted to the ICU.

Introduction

Bloodstream infection is a life-threatening illness due to the presence of microorganisms or their toxins in the blood [1]. The systemic deleterious host response to this infection can lead to severe sepsis and septic shock, which affect millions of people around the world with an increasing incidence [2]. Once the symptoms are recognized, the administration of antibiotic therapy during the first hour is strongly recommended [2], as every hour gained in initiating proper antimicrobial therapy significantly increases the probability of patient survival [3]. However, the current gold standard still relies on culture, which may take up to 3 days before obtaining the identification and performing the antimicrobial susceptibility testing. Thus, rapid identification of the causal agent directly from the patient's blood would be desirable, as it would allow clinicians to readdress the initial antibiotic therapy if necessary, before culture-based identification and susceptibility testing results are available.

A few years ago, an innovative technology based on universal PCR amplification coupled with mass spectrometry was described (PCR/ESI-MS) [4,5]. The first version of this technology, although promising, showed a moderate sensitivity ranging from 50% to 68% for the diagnosis of bloodstream infections (in comparison with blood culture results plus other microbiological findings) [6,7]. A newer version of this technology called IRIDICA (Ibis Biosciences, Carlsbad, CA) is in development. Its main improvement is an enhanced sensitivity, up to 83–91%, due to an increase in the volume of blood tested (5 mL instead of 1.25 mL in the former version), the optimization of PCR conditions and reagents to be tolerant of high loads of human DNA, and an improved downstream processing and analysis step to ensure high sensitivity [8]. The goal of this study was to analyze the clinical performance of this new platform for the diagnosis of bloodstream infections as well as its ability for identifying a wide range of pathogens.

Materials and Methods

Ethics statement

Written informed consent was obtained from all patients or their guardians. This study was approved by the Clinical Research Ethics Committee at Germans Trias i Pujol University Hospital ("Comité Ético de Investigación Clínica", CEIC).

Patients and specimens

This was an observational prospective study including a total of 405 patients admitted to the ICU or ER (median age 66 years, range 16–101; 246 male and 161 female) with a suspicion of

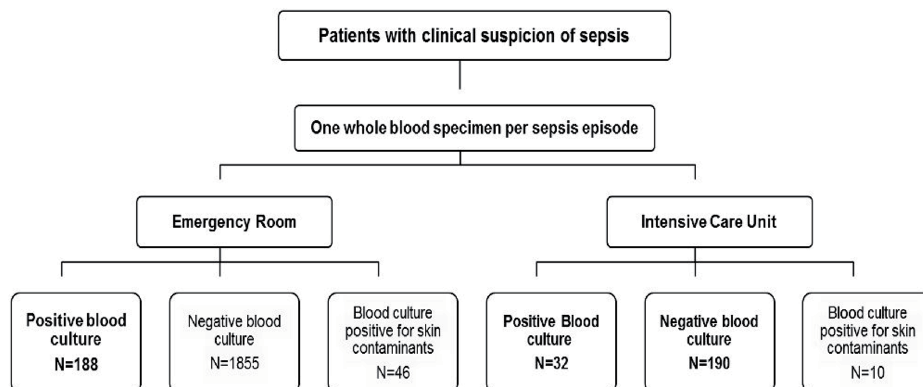

Fig 1. Flowchart depicting the study design.

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sepsis according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) criteria [9], and enrolled between September 2012 and March 2013 at a tertiary care center in Spain (Fig 1). For each patient, one extra whole blood specimen was collected in an EDTA tube under aseptic conditions at the same time as the inoculation of the blood culture for routine microbiological testing (at the onset of fever or other clinical signs of sepsis). For five patients, a blood specimen from two different sepsis episodes was included adding up to 410 specimens. The result of the paired blood culture for each specimen was recorded. Over the study period, a blood specimen was obtained for 222 ICU patients (median age 57.7 years, range 16–83; 138 male, 82 female), which were all tested by IRIDICA. These corresponded to 32 specimens with a paired positive blood culture and 190 with a paired negative blood culture. In order to further assess the ability of the technology for identifying a wider range of pathogens, we also included 188 specimens with paired positive blood culture from ER patients (median 71.5 years, range 20–101; 110 male, 80 female), as this clinical unit is the source of 60% of blood cultures sent to the Microbiology laboratory. Those patients in which skin contaminants were identified by blood culture were excluded from this study ($n = 56$ cases with 59 microorganisms isolated from a single positive culture bottle with coagulase-negative staphylococci ($n = 46$), *Streptococcus* spp. ($n = 5$), *Micrococcus* spp. ($n = 3$), *Corynebacterium* spp. ($n = 2$), *Bacillus* spp. ($n = 1$), *Propionibacterium* spp. ($n = 1$), and *Stenotrophomonas maltophilia* ($n = 1$)). Whole blood specimens were stored at -20°C until testing at Ibis Biosciences.

Conventional microbiological methods

For each adult patient, a set of two blood cultures, including two aerobic and one anaerobic blood culture bottles, were inoculated with up to 10 mL of blood each. The blood culture bottles were incubated in the Bactec 9240 blood culture system (Becton Dickinson, Franklin Lakes, NJ, USA) for up to 5 days. The identification and susceptibility testing of the microorganisms were achieved using the Vitek-2 Compact system (BioMérieux, Marseille-L'Étoile, France) directly from positive blood culture bottles after performing a Gram stain and a concentration procedure [10,11]. Conventional cultures were also performed, following standard

microbiological methods for identification and antibiotic susceptibility testing (disc diffusion and minimum inhibitory concentration methods) as required.

Specimen processing with IRIDICA

Specimen testing with IRIDICA (Ibis Biosciences) was performed according to the manufacturer's instructions using the IRIDICA BAC BSI Assay (Ibis Biosciences-Abbott Molecular (Des Plaines, IL). The work presented here was done using IRIDICA system under development. IRIDICA (CE-IVD) is now commercially available (http://iridica.abbott.com/). As previously described [8], this process includes automated DNA extraction, PCR set-up, PCR amplification, amplicon purification, and electrospray ionization time-of-flight mass spectrometry (PCR/ESI-MS), leading to microbial identification from whole blood in 6h. Computational matching of observed amplicon base compositions to a signature database provides broad-spectrum microbial identification. Briefly, 5 mL whole blood samples were chemically and mechanically lysed and an extraction control was added to each specimen for process monitoring purposes. DNA extraction and PCR set-up was automatically performed by a single instrument using pre-filled individual disposable sample preparation cartridges and pre-filled 16-well PCR reaction strips. The BAC BSI Assay utilizes several conserved-site primer pairs designed to amplify variable (and thereby discriminable) products from a broad range of bacteria and *Candida* spp., as well as primer pairs targeted to common antibiotic resistance loci conferring resistance to methicillin (*mecA*), vancomycin (*van*A and *van*B) and carbapenems (*KPC*). PCR products were then desalted and concentrated relative to human genomic DNA in an automated system and analyzed through ESI-MS. The base compositions of detected amplicon strands were deduced from the measured masses and compared with a reference database, leading to the identification of the microorganisms present in clinical samples. Internal calibrants present in each reaction allowed for relative (qualitative) approximation of target concentrations (expressed as levels), which in turn were used to limit noise- and contamination-derived background detections through thresholding of positive signals.

Data interpretation and statistical analysis

For each specimen, the results obtained with IRIDICA were compared with those obtained using conventional methods (blood culture was considered the gold standard). When discrepancies between these methods were found, the clinical significance of the discrepant results was determined by comparison with a constructed "clinical infection criterion"; for this purpose, a clinical microbiologist together with a clinician were asked to retrospectively evaluate the discrepant results obtained by IRIDICA and to interpret them in the same way as the blood culture results are evaluated: the clinical records of the patients were reviewed in order to identify the diagnosed focus of infection, as well as the results of cultures from other specimens (i.e. microorganisms detected only by IRIDICA were considered true positives when the same microorganism had been isolated from a culture from another specimen type reflecting the focus of infection or supported by the nature of the underlying infection).

Since polymicrobial detections are not uncommon in bloodstream infections, the results obtained by IRIDICA and blood culture were compared at two levels using the two aforementioned gold standards: 1) by microorganism: a direct comparison for each microorganism isolated by conventional methods *vs*. the same microorganism detected by the molecular method, taking into consideration all microorganisms identified; and 2) by specimen: for each specimen with a single detection, matched positive or negative results by each method were recorded. In the latter case, specimens with polymicrobial detections were excluded, as they could not be properly classified (i.e. both methods agreed in some but not all microorganisms identified). In

those terms, the Cohen's Kappa coefficient of agreement by microorganism, and the positive and negative agreement by microorganism and by specimen were calculated with OpenEpi software [12]. In the ICU setting positive and negative agreements were equivalent to sensitivity and specificity, as all blood specimens consecutively obtained during the study period were tested (including specimens with both positive and negative paired blood culture). The positive and negative predictive values were also calculated both by microorganism and by specimen in this subgroup of patients. IRIDICA performance was compared between ICU and ER subgroups using the Pearson's chi-squared test (χ^2). Clinical and molecular quantitative variables of interest were compared between groups using the Mann-Whitney U test (non-Normal distribution), and data was expressed as median and range. *P*-values <0.05 were considered significant. Statistical analyses were performed using the statistical software package SPSS v15.0.

Results

Data analysis was performed on 408 specimens (220 from ICU and 188 from ER), as an invalid IRIDICA result was obtained in two cases due to the lack of detection of the extraction control.

Overall agreement between both methods by microorganism

In comparison with blood culture, IRIDICA showed 73.3% positive concordance (detection of the same microorganism by the two methods) and 64.1% negative concordance (negative by the two methods) (Table 1). IRIDICA detected 80 microorganisms that did not grow in blood culture; 41 (51.2%) of these were supported by clinical facts (S1 Table). On the contrary, the presence of 7 (8.8%) microorganisms could not be supported by clinical evidence (S1 Table), and another 32 (40%) microorganisms were considered clinically irrelevant contaminants from the skin flora (i.e. coagulase-negative staphylococci, *Propionibacterium acnes*, etc.) or from the environment (i.e. *Methylobacterium* spp., *Pseudomonas putida*, etc.) (S2 Table). When the results were reanalyzed taking clinical information into consideration, the positive and negative agreement were, respectively, 77.2% and 78.6% (Table 1). All microorganisms with clinical significance were identified by IRIDICA at species level except for five (one *Acinetobacter baumannii* identified as *Acinetobacter* spp., one each *Streptococcus pneumoniae* and

Table 1. Agreement between methods according to the two gold standards used by microorganisms isolated by conventional microbiological methods and detected by IRIDICA.

	Global		Emergency Room		Intensive Care Unit	
	BC gold standard	Clinical infection criterion	BC gold standard	Clinical infection criterion	BC gold standard	Clinical infection criterion
Matched positives (*n*)	176	217[a]	147	152[a]	29	65 [a]
Matched negatives (*n*)	143	143	n.a.	n.a.	143	143
IRIDICA overcalls (*n*)	80	39	21	16	59	23
IRIDICA misses (*n*)	64	64	56	56	8	8
Overall agreement (%)	68.9	77.8	n.a.	n.a.	72.0	87.0
Positive agreement (%)	73.3	77.2	72.4	73.1	78.4 [b]	89.0 [b]
Negative agreement (%)	64.1	78.6	n.a.	n.a.	70.8 [b]	86.1 [b]

[a] IRIDICA overcalls with clinical significance were classified as matched positives according the clinical infection criterion.

[b] Positive and negative agreement correspond to sensitivity and specificity, as all blood specimens consecutively obtained during the study period were tested (including specimens with both positive and negative paired blood culture).

n.a., not applicable.

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Table 2. Microorganisms with clinical significance identified by either or both methods.

		Blood culture and IRIDICA	Blood culture only	IRIDICA only
Gram-positive bacteria	*Staphylococcus aureus*	8	3	3
	Coagulase-negative staphylococci	4	4	0
	Streptococcus pneumoniae	8	5	3
	Viridans streptococci	4	3	0
	Granulicatella spp.	1	0	0
	β-hemolytic *Streptococcus*	3	2	2
	Enterococcus spp.	15	6	1
	Streptococcus gallolyticus	1	3	1
	Listeria monocytogenes	1	2	0
	Clostridium spp.	0	1	0
	Bacillus spp.	0	1	0
	Lactobacillus spp.	0	1	0
	Mycobacterium simiae	0	0	2
	Subtotal	45	31	12
Gram-negative bacteria	*Escherichia coli*	85	18	7
	Klebsiella pneumoniae/oxytoca	13	0	5
	Enterobacter cloacae/aerogenes	10	3	3
	Proteus mirabilis	4	2	0
	Salmonella enterica sv. Enteritidis	1	0	0
	Serratia marcescens	1	1	0
	Pseudomonas aeruginosa	8	2	3
	Pseudomonas spp.	3	0	0
	Stenotrophomonas maltophilia	2	0	0
	Acinetobacter baumannii	1	0	0
	Elisabethkingia meningoseptica	1	0	0
	Haemophilus influenzae	0	1	1
	Mycoplasma hominis	0	0	1
	Subtotal	129	27	20
Anaerobic bacteria		0	1	4
Fungi	*Candida albicans*	0	1	2
	Candida tropicalis	1	0	1
	Candida parapsilosis	1	1	0
	Candida glabrata	0	1	0
	Candida lusitanie	0	1	0
	Candida famata	0	1	0
	Fungus detected, no ID provided	0	0	2
	Subtotal	2	5	5
TOTAL		**176**	**64**	**41**

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Streptococcus viridans group as *Streptococcus* spp., and two potential *Aspergillus* spp. as "Fungus detected, no identification can be provided"). The microorganisms with clinical significance isolated by culture or detected by IRIDICA are listed in Table 2.

Overall agreement between both methods by specimen

Polymicrobial infections with clinical significance were detected by either or both methods in 28 out of 245 specimens (11.4%) (S3 Table). Given that both methods agreed in some but not

Table 3. Agreement between methods according to the two gold standards used on specimens with a unique isolation/detection.

	Global		Emergency Room		Intensive Care Unit	
	BC gold standard	Clinical infection criterion	BC gold standard	Clinical infection criterion	BC gold standard	Clinical infection criterion
Matched positives (*n*)	148	166[a]	128	128	20	38 [a]
Matched negatives (*n*)	143	143	n.a.	n.a.	143	143
IRIDICA overcalls (*n*)	39	21	0	0	39	21
IRIDICA misses (*n*)	50	50	46	46	4	4
Overall agreement (%)	76.6	81.3	n.a.	n.a.	79.1	87.9
Positive agreement (%)	74.8	76.9	73.6	73.6	83.3[b]	90.5 [b]
Negative agreement (%)	78.6	87.2	n.a.	n.a.	78.6 [b]	87.2 [b]

[a] IRIDICA overcalls with clinical significance were classified as matched positives according the Clinical infection Criterion.

[b] Positive and negative agreement correspond to sensitivity and specificity, as all blood specimens consecutively obtained during the study period were tested (including specimens with both positive and negative paired blood culture).

n.a., not applicable.

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all the microorganisms identified, these apparent polymicrobial samples were excluded from this analysis. Among the rest of specimens ($n = 380$), when blood culture was taken as the gold standard the positive and negative agreement of IRIDICA were 74.8% and 78.6% respectively, and those values rose to 76.9% and 87.2% when re-analyzed with the clinical infection criterion (Table 3).

IRIDICA performance in ICU patients

In the analysis by microorganism, the overall agreement between methods was 72.0% ($\kappa = 0.315$) and the sensitivity, specificity and positive and negative predictive values of IRIDICA in comparison with blood culture were 78.4%, 70.8%, 33% and 95%, respectively (Table 1). When discrepancies found were evaluated using the clinical infection criterion (S1 Table), the overall agreement was 87% ($\kappa = 0.711$) and the values of analytical performance rose to 89%, 86.1%, 73.9% and 95%, respectively. Fourteen polymicrobial infections by either or both methods were excluded in order to perform the analysis by specimen (S3 Table). In those terms, the sensitivity, specificity and positive and negative predictive values in comparison with blood culture were 83.3%, 78.6%, 33.9% and 97.3% respectively, and rose to 90.5%, 87.2%, 64.4% and 97.3% respectively when considering the clinical infection criterion (Table 3).

IRIDICA performance in ER patients

For this subgroup, we only included patients with a positive blood culture. According to the blood culture positivity rate in our center (8.2–12.2%), about 1860 patients with a negative culture would have had to be included in order to reach the 188 positive blood cultures tested, which was not feasible. From the 203 microorganisms isolated by culture, 147 were correctly detected by IRIDICA. Thus, the positive agreement by microorganism in comparison with blood culture was 72.4% (Table 1). A total of five microorganisms with clinical significance were detected by IRIDICA only, giving a positive agreement of 73.1% when the clinical infection criterion was used. When analyzed by specimen, the positive agreement was 73.6% either comparing with blood culture or clinical infection criterion (128 matched detections out of 174 monomicrobial infections) (Table 3).

Table 4. Description of the antibiotic resistance markers detected in this study.

Antibiotic	Identification and AST by conventional methods	Identification and resistance markers by IRIDICA
Methicillin	Coagulase-negative staphylococci, resistant	*Staphylococcus haemolyticus*, *Stahphylococcus epidermidis*, *mec*A
	S. epidermidis, resistant	*S. epidermidis*, *mec*A
	Staphylococcus aureus resistant	*S. aureus*, *mec*A
	Negative	*S. aureus*, *mec*A (clinically significant)
	Negative	*S. epidermidis*, *mec*A (contaminant)
	Negative	*S. hominis*, *mec*A (contaminant)
	Negative	*S. aureus*, *mec*A (no clinical explanation was found for this detection)
Vancomycin/ Teicoplanin	*Enterococcus faecium*, susceptible	*E. faecium*, *van*A
	E. faecium, susceptible	*E. faecium*, *van*B
Carbapenems	-	-

AST, antibiotic susceptibility testing.

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Assessment of factors potentially influencing performance of the molecular method

In those sepsis cases with a positive blood culture, several variables regarding both the culture (time to positivity) and the paired whole blood specimen (leukocyte count, storage time at -20°C) were compared between IRIDICA-negative and -positive specimens in order to find out if they were related to positivity by the molecular method. No statistically significant associations were found in the median white cell count between IRIDICA-negative and -positive specimens (11.9×10^6 *vs*. 11.6×10^6 cells/mL). Whole blood specimens had been stored for variable periods of time (range, 5–23 months; median, 13 months), but the median storage time was comparable between the two groups of specimens (12 months in IRIDICA negative *vs*. 13 months in IRIDICA positive). The difference in the time to positivity of the blood culture was marginally significant; specimens with an IRIDICA-positive result, tended to have a paired blood culture that was called positive earlier (the time to positivity of the blood culture was shorter: 14.5 *vs*. 15.5 h, $p = 0.87$). When comparing ICU *vs*. ER patients, the median of genomes per well by IRIDICA was significantly higher ($p = 0.011$) in the ICU group (median, 97 genomes/well; range, 15–229) than in the ER group (median, 24.5 genomes/well; range, 3–370).

Detection of antibiotic resistance genes

The resistance markers detected by the molecular method in this study are depicted in Table 4. The resistance marker *mec*A was detected by IRIDICA in eight whole blood specimens, being four of them concordant with the result obtained with the conventional methods. From the other four detections, only one (*Staphylococcus aureus*, *mec*A) was supported by other microbiological findings, as the patient also had a bronchial aspirate positive for methicillin-resistant *S. aureus*. While none of the enterococci isolated and identified by conventional methods in this study were resistant to vancomycin or teicoplanin, IRIDICA detected the resistance markers *van*A and *van*B in two different specimens with an *Enterococcus faecium*.

Discussion

Sepsis is a severe syndrome where time is crucial to the optimal patient management. There is a clear need to administer the appropriate antimicrobial therapy as soon as possible, as it will have a positive impact on patient's survival [2,3]. However, the identification of the etiological agent from positive blood cultures may take 24–48h. Being able to detect and identify the

causal pathogen directly from blood would speed the diagnosis and, therefore, improve the management of septic patients. Although several molecular methods designed for this purpose have been commercially available for several years, reported sensitivities are moderate in most cases and are not consistent across studies [13–17]. Given that in bloodstream infections microorganisms are present at low levels, working directly from small volumes of whole blood is an inherent limitation of molecular methods [17,18]. In order to overcome this issue, the new version of the PCR/ESI-MS technology, named IRIDICA, features improvements in the methodology and instruments, such as an increase in the volume of blood analyzed (5 mL), and the enrichment of microbial DNA during the purification process [8].

The overall agreement between IRIDICA and conventional methods was 68.9% when analyzing by microorganism and 76.6% when analyzing by specimen. Overall, 64 microorganisms isolated by conventional methods were not detected by IRIDICA (8 from ICU patients and 56 from ER patients). It should be borne in mind that molecular diagnostic assays typically use smaller volumes of blood (5 mL in this case) than blood culture (up to 30 mL), as the high amounts of human DNA found in whole blood (mostly in white blood cells) may hamper the detection of pathogen DNA. This fact could lead to the suboptimal sensitivities commonly reported for molecular methods in comparison with blood culture [15]. In this sense, Bacconi *et al.* [8] demonstrated that IRIDICA performance was not hampered by the presence of up to 4.0×10^7 white blood cells/mL. In our study no significant difference in white blood cell count was found between IRIDICA-negative and -positive specimens. However, the two obtained invalid results corresponded to specimens from ICU patients with a high white blood cell count (4.5×10^7 and 7.2×10^7 cells/mL). It should also be considered that the IRIDICA software has specific thresholds for reporting different microorganisms and those detections below the threshold are not reported in order to increase the specificity (3–10 genomes/well for most pathogenic bacteria, and 10 genomes/well for those microorganisms that can also be found as skin contaminants). However, this could lead to false negative results in certain cases. In our study, four microorganisms that were considered the etiological agent of the sepsis episode were detected but not reported as they were below those levels (data not shown).

The use of blood culture as gold standard when evaluating molecular methods has limitations, given its low positivity rate (only about 10% of all blood cultures are positive) [19,20]. In this regard, IRIDICA was able to identify an extra 80 microorganisms that did not grow in blood culture. The detection of 41 (51.2%) of them was supported by the clinical condition of the patient or other positive cultures (five microorganisms from four ER patients and 36 microorganisms from 30 ICU patients), and 39 (40%) were easily identified as contaminants (skin or environmental). Finally, there were seven microorganisms (8.8%) that are not commonly found as contaminants and could not be explained by the review of clinical records. These results reinforce the idea that, like the results of the blood culture, the results obtained with molecular methods should be interpreted by the clinician in light of clinical signs and symptoms. Skin or ambient environmental contaminants can also be found in a small percentage of blood cultures due to insufficient aseptic practices during extraction (up to 5% of blood cultures in our setting, which is similar to the 5.4% contamination rate found by IRIDICA). When all the available clinical information was taken into account, IRIDICA showed a positive concordance of 77.2% with respect to clinically diagnosed sepsis. Furthermore, our results are comparable to those recently published by Bacconi *et al.*[8], who described a sensitivity ranging from 83 to 91% in comparison with conventional methods. These results evidence how using a higher volume of whole blood results in an increased detection rate in comparison with the previous version of the technology (50% positive agreement) [6].

The IRIDICA platform has been conceived for the rapid diagnosis of infections in critically ill patients. It should be noticed that this molecular method performed particularly well in the

subgroup of ICU patients when compared with the clinical infection criterion gold standard; the sensitivity was 89.0% in the analysis by microorganism *vs*. 73.1% in the ER ($p = 0.005$) and 90.5% in the analysis by specimen *vs*. 73.6% in the ER ($p = 0.02$). The different performance of IRIDICA in the ICU and ER settings could be explained, at least in part, by the inherent characteristics of the patients admitted to the ICU. These patients are severely ill and suffering from underlying pathologies that may increase the risk of developing sepsis. They also have a major risk of suffering from nosocomial infections due to the use of several intravascular devices, such as catheters. Besides, patients staying at the ICU setting for a long period of time may suffer from immunological impairment. All these factors may be related to the presence of higher bacterial loads (a significantly higher number of genomes per well was observed in ICU patients in comparison with those from the ER). Interestingly, the agreement between IRIDICA and the clinical infection criterion was higher than with the blood culture ($\kappa = 0.711$ *vs*. $\kappa = 0.315$), which points to the presence of clinically relevant microorganisms detected only by the molecular method (73.3% of these patients were under antimicrobial therapy). Finally, IRIDICA showed a negative predictive value of 95% in patients admitted to the ICU, indicating that this technology could be useful for ruling out infection in this setting when the clinical suspicion of sepsis is low.

The ability of detecting a broad range of pathogens was demonstrated in this study, as IRIDICA was able to detect 43 different species of bacteria and *Candida* spp. Interestingly, this technology also detected certain microorganisms that are not commonly recovered from blood culture, such as *Mycoplasma hominis* and *Mycobacterium simiae*. There were six cases reported by the software as bacteria or fungus "detected but not identified" (three of each, respectively). Those detections may indicate the presence of a microorganism that is not usually pathogenic (in the case of bacteria), or that the software does not have enough information to assign an identification (for instance, filamentous fungi are not specifically targeted by the assay but can be amplified by the primers directed to conserved ribosomal genes used for the detection of *Candida* spp.). While most of these cases (three bacteria and one fungus) were considered contaminants, in two cases the detection could have been due to the presence of *Aspergillus fumigatus* in blood, as both patients had respiratory cultures positive for this fungus and also had a positive galactomannan antigen detection. Given that aspergillosis can be a serious complication in immunocompromised patients, obtaining this information (fungus detected but not identified) may be useful in order to run additional confirmatory tests and guide treatment.

This study has several limitations. First, only the specimens from patients admitted to the ICU were consecutively included; while we obtained a dedicated blood specimen from most of the patients admitted to the ER, only those with a paired positive blood culture were included, in order to make the study feasible. Thus, the specimen set did not reflect the usual blood culture positivity rate in the clinical setting (around 10% in our hospital). Nevertheless, the ICU subanalysis does reflect the positivity rate in this department (7.5–13.7%), and no other studies have tested as many patients with a positive blood culture using this technology. Secondly, the specimens were analyzed retrospectively. Thus, we were not able to perform any further testing when discrepancies were found, especially in those involving resistance markers. Although it was surprising that the resistance markers *van*A and *van*B were detected in susceptible *Enterococcus faecium* isolates, this phenomenon has already been described when point mutations or deletions affect the regulatory genes *van*S and *van*R [21,22]. Thirdly, the specimens were stored for varying periods of time at -20°C until tested at IBIS Biosciences. However, long-term stability of whole blood samples under these storage conditions had previously been demonstrated by the manufacturer on spiked samples (unpublished data), and statistical analysis ruled out any significant association between the storage time and the IRIDICA positivity rate. Finally, in some cases blood samples were drawn when the patients were already under antibiotic

treatment, which could have led to the detection of clinically relevant microorganisms by the molecular method in patients with a negative BC.

When implementing molecular methods in the clinical microbiology laboratory, cost-effectiveness studies are necessary given that molecular methods are more expensive than conventional ones. However, a rapid identification of the pathogen may lead to the optimization of the administered therapy and, thus, to a prompter recovery of the patient and a shorter stay at the ICU department. Although prospective cost/benefit studies are needed to assess the real impact of this technology in the management of septic patients, significant economic savings have been reported for the molecular SeptiFast assay (Roche, Manheim, Germany) due to the shortening of the ICU stay and a more rational use of antibiotics [23,24].

In conclusion, the IRIDICA technology offers a rapid and reliable identification of pathogens for the diagnosis of sepsis directly from the patient's blood, with a better performance in ICU patients. When used in combination with conventional methods it could lead to an increase in the number of microbiologically confirmed sepsis cases. More importantly, a significant proportion of septic patients would benefit from an early identification of the pathogen leading to a prompter appropriate antibiotic treatment, which relates to patient survival rates.

Supporting Information

S1 Table. Clinical review of the discrepancies between IRIDICA and blood culture.
(DOC)

S2 Table. Microorganisms detected by IRIDICA considered as skin or ambient contaminants.
(DOCX)

S3 Table. Polymicrobial infections by either conventional or molecular methods (*n* = 28).
(DOCX)

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Author Contributions

Conceived and designed the experiments: EJ EM MG MDQ MJD FA VA. Performed the experiments: EJ BR CM. Analyzed the data: EJ EM MG MDQ BR CM. Contributed reagents/materials/analysis tools: VA. Wrote the paper: EJ EM MG. Collected specimens and informed consents: MJD FA. Interpretation of the clinical data of the manuscript: MJD FA MG MDQ BR CM VA. Final approval of the manuscript: EJ EM MG MDQ BR CM MJD FA VA.

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S1: Supplementary table 1. Clinical review of the discrepancies between IRIDICA and blood culture.

Clinical evidence supporting IRIDICA results					
Sample No.	**Department**	**Blood culture result**	**IRIDICA result**	**QC score/ level**	**Comments**
1	**ER**	*Escherichia coli* *Enterobacter aerogenes* *Enterococcus casseliflavus*	*Escherichia coli* *Klebsiella pneumoniae*	0.94/19 0.99/174	Sepsis of abdominal origin.
2	**ER**	*Escherichia coli*	*Escherichia coli* *Klebsiella pneumoniae*	0.92/3	Sepsis of abdominal origin.
3	**ER**	*Candida parapsilosis* *Enterobacter aerogenes*	*Candida parapsilosis* *Enterobacter aerogenes* *Klebsiella oxytoca*	0.99/177 0.97/19 0.88/5	Patient with a solid neoplasm and sepsis of abdominal origin.
4	**ER**	*Streptococcus constellatus*	*Escherichia coli* *Fusobacterium nucleatum*	0.96/3 0.97/37	Patient with peritonitis and sepsis of abdominal origin. Culture of ascetic liquid positive for *E. coli* three days after.
5	**ICU**	Negative	*Candida tropicalis*	0.96/123	Previous blood culture positive for *C. tropicalis*. Under antifungal treatment.
6	**ICU**	Negative	*Enterobacter cloacae complex* *Streptococcus pneumoniae*	0.93/3 0.96/12	Patient with a pneumonia caused by bronchoaspiration.
7	**ICU**	Negative	*Enterobacter cloacae complex* *Enterobacter cancerogenus*	0.96/86 0.94/40	Patient with several cultures positive for *E. cloacae* after surgery (bronchial aspirate, urine, wound suppuration). Under antibiotic treatment.
8	**ICU**	Negative	*Staphylococcus aureus*	0.99/27	Previous blood culture positive for *S. aureus*. Under antibiotic treatment.
9	**ICU**	Negative	*Haemophilus influenzae*	0.97/12	Patient with a pneumonia caused by bronchoaspiration.
10	**ICU**	Negative	*Mycobacterium simiae*	0.95/63	Leukaemia and neutropenia. Acute enterocolitis and severe respiratory insuffiency.
11	**ICU**	Negative	*Streptococcus pneumoniae*	0.98/209	Previous blood culture positive for *S. pneumoniae*. Under antibiotic treatment.
12	**ICU**	Negative	*Escherichia coli*	0.96/4	Intra-abdominal infection. Under antibiotic treatment.

13	**ICU**	Negative	*Klebsiella pneumoniae*	0.98/16	Acute pancreatitis. Abdominal fluid culture positive for *K. pneumoniae.* Under antibiotic treatment.
14	**ICU**	Negative	*Escherichia coli*	0.99/388	Patient with acute myeloid limfoma. Previous cultures positive for *E. coli* (pleural liquid and blood culture). Under antibiotic treatment.
15	**ICU**	Negative	*Pseudomonas aeruginosa*	0.99/38	Previous respiratory specimen positive for *P. aeruginosa.* Under antibiotic treatment.
16	**ICU**	Negative	*Staphylococcus aureus*	0.97/8	Endocarditis by *S. aureus.* Under antibiotic treatment.
17	**ICU**	Negative	*Pseudomonas aeruginosa*	0.98/190	Sepsis of respiratory origin. Mechanical ventilation. Under antibiotic treatment.
18	**ICU**	Negative	*Escherichia coli* *Escherichia coli/Shigella spp.*	0.96/68 0.95/79	Nephrolithiasis with a urethral obstruction.
19	**ICU**	Negative	*Mycobacterium simiae*	0.86/11	Alcoholic cirrhosis. Under antibiotic treatment.
20	**ICU**	Negative	*Escherichia coli/Shigella spp.*	0.96/26	Patient with a pneumonia caused by bronchoaspiration. Under antibiotic treatment.
21	**ICU**	Negative	*Escherichia coli*	0.97/4	Sepsis of abdominal origin. Purulent peritonitis. Under antibiotic treatment.
22	**ICU**	Negative	*Enterococcus faecium* *Candida albicans*	0.98/42 0.99/57	Patient with acute myeloid lymphoma. Neutropenic enterocolitis. Respiratory specimen positive for *C. albicans* 6 days after the specimen tested by IRIDICA. Under antibiotic (no antifungal) treatment.
23	**ICU**	Negative	*Streptococcus dysagalactiae* *Fungus detected. No ID provided*	0.99/157 0.95/15	Patient with multiple myeloma and aplasia. Sepsis of respiratory origin. Previous blood culture positive for *S. dysagalactiae.* Respiratory specimen positive for *Aspergilllus* spp. and galactomannan antigen positive a few days later. Under antibiotic treatment.
24	**ICU**	Negative	*Fusobacterium nucleatum*	0.98/24	Patient with acute myeloid lymphoma and neutropenia presenting severe mucositis. Under antibiotic treatment.
25	**ICU**	Negative	*Fusobacterium nucleatum*	0.97/9	Patient presenting acute peritonitis.
26	**ICU**	Negative	*Prevotella denticola*	0.94/13	Sepsis of abdominal origin. Purulent peritonitis. Under antibiotic treatment.
27	**ICU**	Negative	*Streptococcus oralis/pneumoniae*	0.97/24	Meningitis by *S. pneumoniae.* Under antibiotic treatment.
28	**ICU**	Negative	*Pseudomonas aeruginosa*	0.99/480	Sepsis of abdominal origin.

29	**ICU**	Negative	*Streptococcus pyogenes*	0.96/29	Sepsis due to cellulitis. Under antibiotic treatment.
30	**ICU**	*Elisabethkingia meningoseptica*	*Elisabethkingia meningoseptica* *Candida albicans*	0.97/39 0.97/3	Patient with neutropenia. Sepsis of primary origin. Under antibiotic (no antifungal) treatment. Two specimens included from the same episode.
31		Negative	*Mycoplasma hominis*	0.99/105	Patient with neutropenia. Sepsis of primary origin. Vulvar abcess. Under antibiotic treatment. Same patient that case 30.
32	**ICU**	*Streptococcus mitis*	*Streptococcus mitis* *Streptoccus spp.*	0.97/152 0.97/31	Blood culture positive for *S. mitis.*
33	**ICU**	*Enterococcus faecium*	*Enterococcus faecium* *Fungus detected. No ID provided*	0.97/58 0.97/58	Sepsis of primary origin. Respiratory specimen positive for *Aspergillus* spp. and galactomannan antigen positive. Under antibiotic treatment.
34	**ICU**	*Candida albicans*	*Staphylococcus aureus mecA*	0.96/7	Respiratory culture positive by methicillin-resistant *S. aureus* (MRSA). Under antibiotic treatment.
35	**ICU**	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* *Klebsiella oxytoca*	 0.98/81	Sepsis of abdominal origin.
No clinical evidence supporting IRIDICA results					
1	**ICU**	Negative	*Escherichia coli*	0.97/17	Patient with a cranioencephalic traumatism. Respiratory culture positive by *S. constellatus.* Under antibiotic treatment.
2	**ICU**	*Staphylococcus aureus*	*Fusobacterium nucleatum*	0.96/80	Sepsis due to *S. aureus.*
3	**ER**	Negative	Methicillin-resistant *S. aureus* (MRSA)	0.91/1	Patient with multiple myeloma and aplasia. Sepsis of respiratory origin (same specimen than case 23)
4	**ER**	*Escherichia coli*	*Escherichia coli* *Enterobacter cloacae complex*	0.97/15 0.90/7	Sepsis of urologic origin.
5	**ER**	*Enterococcus gallinarum*	*Clostridium perfingens*	0.97/53	Sepsis of urologic origin.
6	**ER**	Negative	*Citrobacter freundii*		Sepsis of urologic origin.
7	**ER**	*Escherichia coli*	*Escherichia coli* *Shigella boydii*	0.96/42 0.95/5	Sepsis of urologic origin. Possible misidentification due to high genetic similarity.

ER: Emergency Room, **ICU:** Intensive care Unit

S2: Supplementary table 2. Microorganisms detected by IRIDICA considered as skin or ambient contaminants.

Microorganism	N
Propionibacterium acnes	10
Coagulase-negative Staphylococci	4
Bacteria detected. No ID provided	3
Fungus detected. No ID provided	3
Corynebacterium spp.	2
Micrococcus luteus	2
Acinetobacter johnsonii	1
Acinetobacter junii	1
Methylobacterium zatmanii	1
Pectobacterium carotovum	1
Pseudomonas entomophila/putida	1
Pseudomonas mendocina	1
Streptococcus spp.	1
Sphinobacterium multivorum	1
TOTAL	**32**

S3: Supplementary table 3. Polymicrobial infections by either conventional or molecular methods (N=28).

	Department	Conventional methods	IRIDICA
Blood culture and IRIDICA (concordant ID)			
1	**ER**	*Proteus mirabilis*	*Proteus mirabilis*
		Enterococcus faecalis	*Enterococcus faecalis*
2	**ER**	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
		Streptococcus viridans	*Streptococus* spp.
3	**ER**	*Escherichia coli*	*Escherichia coli*
		Not isolated	*Escherichia coli/Shigella* spp.
4	**ER**	*Enterobacter cloacae*	*Enterobacter cloacae* complex
		Enterococcus faecium	*Enterococcus faecium*
5	**ER**	*Escherichia coli*	*Escherichia coli*
		Not isolated	*Escherichia coli/Shigella* spp.
6	**ICU**	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
		Candida tropicalis	*Candida tropicalis*
7	**ICU**	*Streptococcus mitis*	Viridans/mitis group
		Not isolated	*Streptoccus* spp.
8	**ICU**	*Enterococcus faecium*	*Enterococcus faecium*
		Coagulase-negative Staphylococci	*Staphylococcus epidermidis*
			Staphylococcus haemolyticus
Blood culture detected more microorganisms than IRIDICA			
1	**ER**	*Escherichia coli*	*Escherichia coli*
		Proteus mirabilis	Not detected
2	**ER**	*Escherichia coli*	*Escherichia coli*
		Enterococcus faecalis	Not detected
		Enterobacter cloacae	Not detected
3	**ER**	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
		Escherichia coli	Escherichia coli
		Enterococcus gallinarum	Not detected
4	**ER**	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
		Enterococcus gallinarum	Not detected
5	**ER**	*Escherichia coli*	*Escherichia coli*
		Enterobacter aerogenes	Not detected
		Enterococcus casselifavus	Not detected
		Not isolated	*Klebsiella pneumoniae*
IRIDICA detected more microorganisms than blood culture			
1	**ER**	*Candida parapsilosis*	*Candida parapsilosis*
		Enterobacter aerogenes	*Enterobacter aerogenes*
		Not isolated	*Klebsiella oxytoca*
2	**ER**	*Escherichia coli*	*Escherichia coli*
		Not isolated	*Klebsiella pneumoniae*
3	**ICU**	*Enterococcus faecium*	*Enterococcus faecium*
		Not isolated	Fungus detected. No ID provided
4	**ICU**	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
		Not isolated	*Klebsiella oxytoca*
5	**ICU**	*Elisabethkingia meningoseptica*	*Elisabethkingia meningoseptica*
		Not isolated	*Candida albicans*
Blood culture and IRIDICA detected different microorganisms			
1	**ER**	*Streptococcus constellatus*	Not detected
		Not isolated	*Escherichia coli*
		Not isolated	*Fusobacterium nucleatum*
2	**ICU**	*Candida albicans*	Not detected
		Not isolated	*Staphylococcus aureus mec*A
3	**ICU**	*Candida parapsilosis*	Not detected

		Not isolated	*Staphylococcus aureus*
Only by blood culture			
1	**ER**	*Escherichia coli*	Not detected
		Streptococcus mitis	Not detected
2	**ICU**	*Lactobacillus* spp.	Not detected
		Candida glabrata	Not detected
Only by IRIDICA			
1	**ICU**	Not isolated	*Enterobacter cloacae complex*
		Not isolated	*Enterbacter cancerogenus*
2	**ICU**	Not isolated	*Escherichia coli*
		Not isolated	Escherichia coli/Shigella spp.
3	**ICU**	Not isolated	*Enterococcus faecium*
		Not isolated	*Candida albicans*
4	**ICU**	Not isolated	*Streptococcus dysagalactiae*
		Not isolated	Fungus detected. No ID provided
5	**ICU**	Not isolated	*Enterobacter cloacae complex*
		Not isolated	*Streptococcus pneumoniae*

ER: Emergency Room, **ICU:** Intensive care Unit