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Exploring antimicrobial resistance and bacterial dynamics: A comprehensive One Health investigation at the livestock–wildlife–human interface in rural western Uganda

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Informen:

Que la memòria titulada "Exploring antimicrobial resistance and bacterial dynamics: A comprehensive One Health investigation at the livestock-wildlife-human interface in rural western Uganda", presentada per Andrea Dias Alves per a la obtenció del títol de Doctora en Veterinària per la Universitat Autònoma de Barcelona, s'ha realitzat sota la nostra direcció i, un cop considerada satisfactòriament finalitzada, autoritzem la seva presentació per tal que sigui avaluada per la comissió corresponent.

I, perquè així consti als efectes que siguin oportuns, firmem el present informe a Bellaterra, 1 de setembre del 2024.

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Acknowledgments / Agraïments / Agradecimientos

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1. Abstract

Although antimicrobial drugs have played a crucial role in combating infections, their effectiveness is significantly limited in developing countries due to factors like poverty, inadequate sanitation, limited access to medications, and weak healthcare systems. These challenges are further compounded by the emergence of antimicrobial resistance (AMR) and infectious diseases. The lack of resources to study and manage susceptibility patterns, along with the scarcity of reliable data, hampers the development of effective treatment and the optimal use of antimicrobial agents, thereby exacerbating the impact of infectious diseases and AMR. In Uganda, extensive agricultural and livestock practices, combined with high levels of human-animal interaction, create conditions that facilitate both the spread of AMR but also the transmission of pathogenic bacteria across species and environments. Although research has been conducted, it has predominantly focused on human medicine and food-producing animals, leaving a gap in understanding both the prevalence of AMR and the dynamics of pathogen transmission in wildlife and the broader environmental context. This doctoral thesis explores the detection and transmission of AMR and bacterial pathogens in western Uganda, focusing on the interaction between humans, animals and the environment. The first study reviews published and unpublished literature to assess AMR in *E. coli* strains from humans, animals, and environmental sources, revealing a lack of quantification of the resistance burden in Uganda, particularly in domestic animals, wildlife and the environment. Additionally, through a questionnaire survey, this study highlights a significant lack of awareness and a widespread misuse of antimicrobials in both human and animal health contexts. Building on these findings, the third study demonstrates the widespread presence of resistance genes, particularly CTX-M-15, in both cephalosporin-resistant *E. coli* and *Klebsiella pneumoniae* isolated from different species in regions similar to those previous surveyed. This highlights the extensive spread of AMR in both human settlements and natural protected areas in western Uganda. In addition, the second study identifies *Salmonella* and *Campylobacter* spp. in livestock and wildlife across regions closely aligned with those where AMR was reported. Lower pathogen rates were observed in wildlife

from remote areas, suggesting a potential spillover from livestock or humans. Finally, the presence of potentially pathogenic enteric bacteria in chimpanzees, goats and humans from Budongo Central Forest Reserve (BCFR) and nearby regions is investigated in the fourth study of this thesis. The study finds that bacteria in chimpanzees are similar to those in goats and humans, supporting previous research that agricultural expansion near wildlife habitats enhances pathogen circulation at the livestock-human-chimpanzee interface. Overall, this thesis provides insights into the dynamics of AMR and pathogen detection in regions with significant human, animal, and environmental interactions. The findings underscore the importance of adopting a broader One Health approach to fully understand and manage pathogen and AMR transmission dynamics in rural areas of western Uganda.

1. Resum

Tot i que els antimicrobians han jugat un paper crucial en la lluita contra les infeccions, la seva efectivitat és limitada en països en desenvolupament degut a factors com la pobresa, la manca d'higiene, l'accés limitat als medicaments i la feblesa dels sistemes de salut. Aquests reptes es veuen agreujats per l'aparició de la resistència als antimicrobians (RAM) i de les malalties infeccioses. La manca de recursos per estudiar i gestionar els patrons de susceptibilitat, juntament amb manca de dades fiables, dificulta el desenvolupament de tractaments eficaços i l'ús òptim d'agents antimicrobians, agreujant així l'impacte de les malalties infeccioses i la RAM. A Uganda, les pràctiques agrícoles i ramaderes extensives, juntament amb els alts nivells d'interacció entre humans i animals, creen condicions idònies que faciliten tant l'expansió de la RAM com la transmissió de bacteris patògens entre espècies i el medi ambient. Tot i els estudis realitzats, aquests s'han centrat predominantment en medicina humana i en producció alimentària, deixant un buit en la comprensió de la dinàmica de la RAM i dels agents patògens en la fauna salvatge i el medi ambient. Aquesta tesi doctoral explora la detecció i transmissió de la RAM i de bacteris patògens a l'oest d'Uganda, centrant-se en la interacció entre humans, animals i el seu entorn. El primer estudi revisa la literatura publicada i no publicada per avaluar la RAM en soques d'*E. coli* provinents d'humans, animals i fonts ambientals, revelant una manca d'estudis sobre RAM a Uganda, particularment en animals domèstics, fauna salvatge i el medi ambient. A més, a través d'una enquesta realitzada, es destaca una mancança significativa de conscienciació sobre aquesta problemàtica, així com un ús inadequat generalitzat dels antimicrobians tant en el context de salut humana com animal. Basant-se en aquests resultats, el tercer estudi demostra la presència extensa de gens de resistència, particularment el CTX-M-15, tant en aïllats d'*E. coli* com de *Klebsiella pneumoniae* resistents a cefalosporines, aïllats en diferents especies en regions similars a les prèviament estudiades. Això ressalta l'extensa propagació de la RAM tant en assentaments humans com en àrees naturals protegides de l'oest d'Uganda. A més, el segon estudi identifica *Salmonella* i *Campylobacter* spp. en bestiar i fauna salvatge en regions que coincideixen en gran mesura amb aquelles on es van

detectar gens de resistència. S'ha observat una menor prevalença de patògens en fauna salvatge d'àrees remotes, suggerint una possible transmissió des del bestiar o els humans. Finalment, el quart estudi investiga la presència de bacteris entèrics potencialment patògens en ximpanzés, cabres i humans del Bosc de Budongo (BCFR) i regions properes. L'estudi evidencia que els bacteris trobats en ximpanzés són similars als trobats en cabres i humans, recolzant resultats d'estudis anteriors on s'evidencia que l'expansió agrícola prop dels hàbitats de la fauna salvatge millora la circulació de patògens a la interfície bestiar-humà-ximpanzé. En conclusió, aquesta tesi proporciona una visió general sobre la dinàmica de la RAM i la presència de patògens en regions amb diferents nivells d'interacció entre humans, animals i el medi ambient. Els resultats subratllen la importància d'adoptar un enfocament "Una Sola Salut" per comprendre i gestionar la dinàmica de transmissió de patògens i la RAM en zones rurals de l'oest d'Uganda.

1. Resumen

A pesar del papel fundamental de los agentes antimicrobianos en la lucha contra las infecciones, su efectividad en países en desarrollo se ha visto limitada por factores como la pobreza, la falta de higiene, el acceso restringido a los medicamentos y la debilidad de los sistemas de salud. Estos problemas se han visto agravados con la aparición de la resistencia a los antimicrobianos (RAM) y la emergencia de las enfermedades infecciosas. La insuficiencia de recursos para estudiar y gestionar los patrones de susceptibilidad, junto con falta de datos fiables, dificulta el desarrollo de tratamientos eficaces y el uso óptimo de los agentes antimicrobianos, intensificando así el impacto de las enfermedades infecciosas y la RAM. En Uganda las prácticas agrícolas y ganaderas, junto con los elevados niveles de interacción entre humanos y animales, crean condiciones idóneas que favorecen tanto la expansión de RAM como la transmisión de bacterias patógenos entre especies y el medio ambiente. A pesar de los estudios realizados, la investigación se ha enfocado principalmente en medicina humana y en animales de producción, dejando un vacío en la comprensión de la dinámica de la RAM en la fauna salvaje y el medio ambiente. Esta tesis doctoral aborda la detección y transmisión de la RAM y de bacterias patógenas en el oeste de Uganda, enfocándose en la interacción entre humanos, animales y el entorno. El primer estudio revisa la literatura publicada y no publicada para evaluar la RAM en cepas de *E. coli* aisladas de humanos, animales y fuentes ambientales, revelando una falta de estudios sobre la RAM en Uganda, especialmente en animales domésticos, fauna salvaje y el medio ambiente. Además, una encuesta realizada en el mismo estudio revela una falta de concienciación sobre la problemática de la RAM y un uso inadecuado de los antimicrobianos de forma generalizada tanto en salud humana como animal. Basándose en estos hallazgos, el tercer estudio demuestra la extensa presencia de genes de resistencia, especialmente el CTX-M-15, en cepas de *E. coli* y *Klebsiella pneumoniae* resistentes a cefalosporinas aisladas en diferentes especies en regiones similares a las estudiadas anteriormente. Esto pone de relieve la amplia propagación de la RAM tanto en asentamientos humanos como en áreas naturales protegidas del oeste de Uganda. Asimismo, el segundo estudio identifica *Salmonella* y *Campylobacter* spp. en ganado y fauna salvaje en regiones

que coinciden en gran medida con aquellas donde se detectaron genes de resistencia. Se observó una menor prevalencia de patógenos en fauna salvaje de áreas remotas, sugiriendo una posible transmisión desde el ganado o los humanos. Finalmente, el cuarto estudio investiga la presencia de bacterias entéricas potencialmente patógenas en chimpancés, cabras y humanos del Bosque de Budongo (BCFR) y regiones cercanas. El estudio revela que las bacterias en chimpancés son similares a las encontradas en cabras y humanos, respaldando investigaciones anteriores que indican que la expansión agrícola cerca de hábitats de fauna salvaje incrementa la circulación de patógenos en la interfaz ganado-humano-chimpancé. En conclusión, esta tesis ofrece una visión integral de la dinámica de la RAM y la presencia de patógenos en regiones con diferentes niveles de interacción entre humanos, animales y el medio ambiente. Los resultados resaltan la importancia de adoptar un enfoque de "Una Sola Salud" para comprender y gestionar adecuadamente la dinámica de transmisión de patógenos y RAM en las áreas rurales del oeste de Uganda.

2. General introduction

2.1. Uganda

"*For magnificence, for variety of form and colour, for profusion of brilliant life – bird, insect, reptile, beast – for vast scale – Uganda is truly 'the Pearl of Africa.'*"

Winston Churchill (1874 - 1965)

General information

Uganda, officially known as the Republic of Uganda, is an East African country bordered by South Sudan to the north, Rwanda and Tanzania to the south, Kenya to the east and the Democratic Republic of Congo (DRC) to the west (Fig. 1.2.1). It spans approximately 241,139 km^2 and is one of the 13 countries worldwide crossed by the equator.

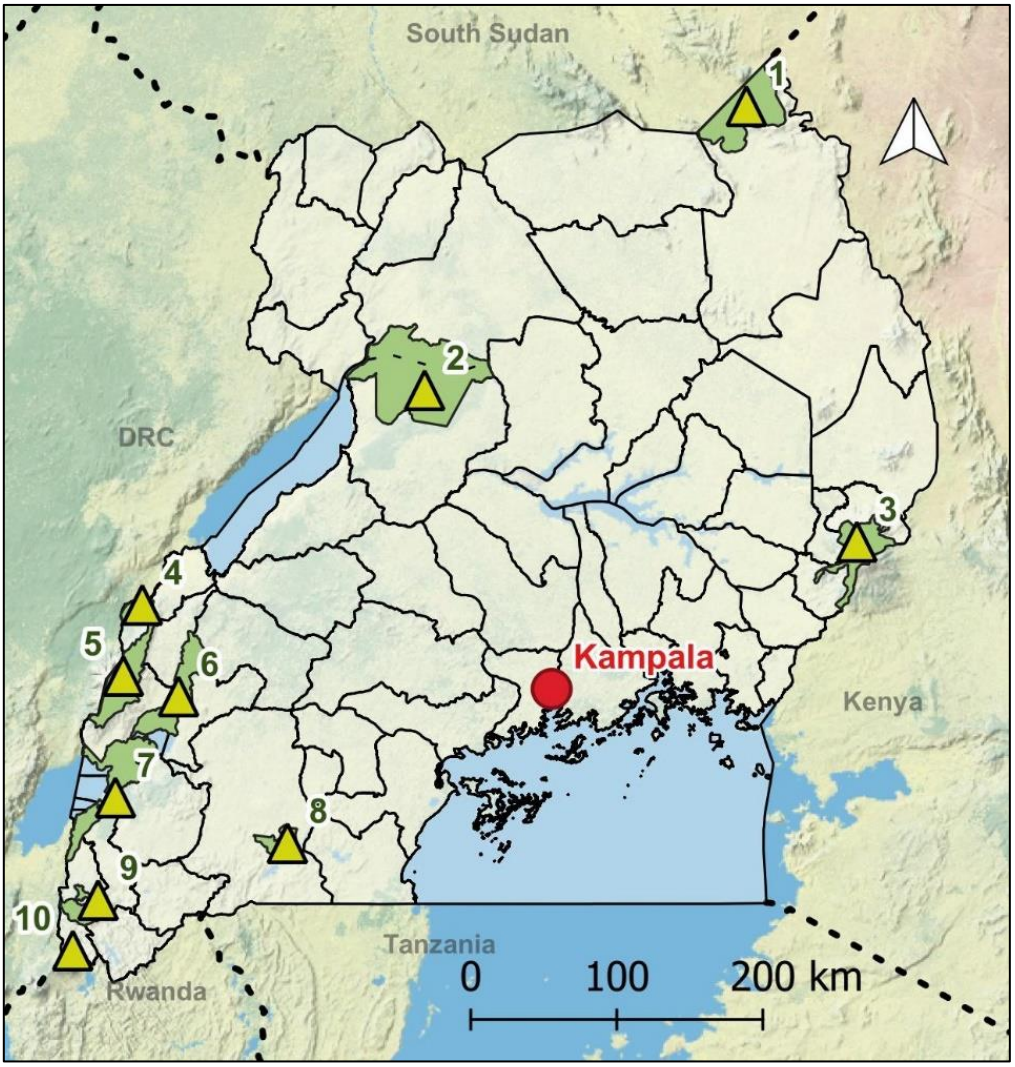


Figure 1.2.1. Map of Uganda and its National Parks (NP): Kidepo Valley NP (1), Murchison Falls NP (2), Mount Elgon NP (3), Semuliki NP (4), Rwenzori Mountains NP (5), Kibale NP (6), Queen Elizabeth NP (7), Lake Mburo NP (8), Bwindi Impenetrable NP (9) and Mgahinga Gorilla NP (10).

Currently, Uganda is administratively divided into Central, Eastern, Northern and Western regions, which are further subdivided into 121 districts, in addition to the city of Kampala. Kampala serves as the capital and the largest city in the country,

situated around seven hills and hosting more than one-fourth of the country's urban population (Fig. 2.2.1). Other notable urban centres in Uganda include Entebbe, Jinja, Mbale, Masaka and Gulu. In 2014, Uganda's population was recorded at 35 million inhabitants (UBOS 2016), which stands today at around 45 million (UBOS 2023). The predominant ethnic group comprises Bantu-language speakers, concentrated in southern and western Uganda, while eastern and northern Uganda are inhabited by the Nilotic- or Cushitic-speaking groups.

Despite Luganda being Uganda's most widely spoken language, English has been the official language since 1962. Nevertheless, the country is home to over 30 local languages spoken in various regions. In addition to Luganda and English, Swahili is also commonly spoken by many Ugandans, particularly in areas bordering Kenya and Tanzania (Briggs 2020).

Religion plays a significant role in Ugandans' lives, with fewer than 1% of the population identifying as atheist or agnostic. Most of the Ugandan population adheres to Christianity, with a division between Roman Catholics and Protestants. Additionally, a substantial Muslim population lives in eastern Uganda. There are also communities that practice minority religions or traditional beliefs, such as the Karimojong in the northeast, who abstain from adopting external faiths (Byrnes 1992).

Figure 2.2.1. Kampala, the capital city of the Republic of Uganda.

History

It is broadly recognised that human evolution unfolded in the Rift Valley and the plains of East Africa, supported by DNA evidence and archaeological findings (Wood 2010). However, the origins of the modern inhabitants of East Africa are still subjected to debate due to the absence of written history before the arrival of Europeans in 1862. Since 1,000 BCE, East Africa experienced two major human migrations by West African populations. In Uganda, during the early centuries of the current era, Bantu speakers expanded into the well-watered areas of the Great Lakes region, particularly around Lake Victoria. This population included the Ankole, Baganda (people of Buganda who speak Luganda), Banyoro, Basoga and Batoro. They were skilled iron workers, and their landscape modifications facilitated subsequent pastoralist migrations. In the mid-15th century, the Nilotic-speaking Luo left south-eastern Sudan and south-west Ethiopia across Uganda from the northeast to the southwest, following the course of the Nile River (Wrigley 1981).

Traditions affirm that Bunyoro-Kitara was the first kingdom established, with the Batembuzi being the first dynasty to rule over it, succeeded by the Bachwezi, who significantly influenced modern Uganda (Dunbar 1965; Briggs 2020). However, the Bachwezi dynasty collapsed, and by the end of the 15th century, several other kingdoms sharing Bachwezi inheritance emerged: Buganda and Ankole in modern-day Uganda, Rwanda and Burundi, and the Karagwe kingdom in what is now northwestern Tanzania (Reid 2017). Between the 17th and 19th centuries, the Bunyoro kingdom experienced a decline and lost its dominance, yielding to Buganda, which expanded its territory westward from the Victoria Nile (Briggs 2020). During this period, Arab slave traders and Europeans arrived in Uganda (Reid 2017; Briggs 2020).

In 1894, Uganda became a protectorate of the British Empire by joining the kingdoms of Buganda, Bunyoro, Ankole and Toro. Sir Harry H. Johnston was ordered as the first governor of Uganda. Despite Uganda being inhabited by different ethnic groups, its borders enclosed two different divisions previously mentioned: the relatively centralized Bantu kingdoms of the south, which represent the most

significant proportion of Uganda's population, and the more decentralized Nilotic and Sudanic people to the north (Reid 2017). Under British colonial rule, economic growth and education were concentrated in the south, with the Bantu population occupying most of the important roles in academia, politics, and religion. In contrast, the British primarily recruited the northern population into the armed and paramilitary forces, concentrating military power in northern Uganda and creating an imbalance that would shape the political landscape of postcolonial Uganda (Baker 2001; Reid 2017).

In the late 1950s, the Ugandan population focused on achieving self-government. Despite political, ethnic and geographical fragmentation, Uganda gained independence in 1962 (Fig. 3.2.1) (Carter 1962). After gaining formal independence in 1962, political instability, conflicts, and the devastating impact of environmental problems and the AIDS epidemic hindered economic development and modernization for many years (Reid 2017). Milton Obote (1924-2005) served as the prime minister (1962-1966) and president of Uganda (1966-1971). However, he lost the support of Ugandans and was overthrown by Idi Amin in 1971, who established a dictatorial regime. During the 19th and 20th centuries, a significant number of South Asians immigrated to Uganda. However, Idi Amin ordered the expulsion of the entire Asian community and the appropriation of their businesses and goods, which brought significant adverse consequences to the Ugandan economy. Moreover, Amin resorted to arbitrary violence to maintain his position, leading to the death of more than 300,000 people and the torture of countless others for ethnic, political, and financial reasons during his presidency (Reid 2017; Briggs 2020). In 1979,

Figure 3.2.1. Uganda Independence monument (Kampala).

Tanzanian troops, alongside armed Ugandan exiles, invaded Uganda and forced Amin into exile (Roberts 2014). Although Obote returned to the presidency in 1980, Yoweri Museveni formed the National Resistance Movement (NRM) to prevent a recurrence of the past and, in 1985, Obote was forced into exile (Ingham 1994). Since 1986, Yoweri Museveni has served as the president of Uganda and continues to hold the position to this day.

Nowadays, Bantu speakers comprise over two-thirds of the population, including Eastern Lacustrine (Baganda, Basoga and smaller groups in Uganda, Kenya and Tanzania), as well as Western Lacustrine Bantu (Banyankole, Banyoro, Batoro and smaller groups). Nilotic speakers, the second largest ethnic group in Uganda, include communities that speak Eastern (Iteso and Karamojong regions) and the Western Nilotic languages (Acholi, Alur and Langi regions). A minor part of the population speaks Sudanic languages in the north, including the Kakwa, Lugbara, Ma'di, Nubians, and other small groups in northwestern Uganda (Fig. 4.2.1) (Baker 2001; Ricart-Huguet and Green 2018). In addition to these groups, Uganda is home to a large refugee population including individuals from Burundi, Rwanda, Somalia and South Sudan (Baker 2001; Murahashi 2021). Since 1986, the Yoweri Museveni government has brought relative stability and economic growth and invited the previously deported Asian community to return. To address the damage caused by previous governments, foreign investment in agriculture and industry, mainly from Western countries and Asian residents, has been pursued. During the 1990s and early 2000s, Uganda's economy achieved stability and high growth rates, enabling the government to focus on poverty eradication, industry expansion, and tourism. However, rebel activity has increased, mainly due to the Lord's Resistance Army (LRA) (Dunn 2007), which challenges Museveni's government. In addition to security, inflation, unemployment, and corruption continue to be important problems in the country (Reid 2017; Briggs 2020).

Figure 4.2.1. Map showing the main ethnic groups in Uganda. Source: Baker (2001).

Economy

Due to President Museveni's economic reforms and relative stability, Uganda has experienced consistent economic growth over the last three decades. The main contributors to the national Gross Domestic Product (GDP) are the industry and service sectors, encompassing the exportation of agricultural products and tourism, with the farming industry closely following (Fig. 5.2.1) (Ssewanyana et al. 2011). The production of livestock and crop cultivation, particularly in the southern districts, which benefit from Uganda's fertile soils and high rainfall, has ensured Uganda's self-sufficiency in food since independence. The major export cash crops are coffee and cotton, followed by tea, cut flowers and tobacco. The extensive coverage of lakes and rivers provides a promising outlook for the fishing sector. Although mining has not play a significant role in the Ugandan economy, the reopening of the Kilembe copper mine and the discovery of oil in the vicinity of Lake Albert may reshape the significance of this sector (Briggs 2020).

Figure 5.2.1. Land dedicated to agriculture (Kisoro district).

Ecosystems and biodiversity

Geographically, Uganda is situated at the heart of the great African plateau, demarcated by various mountains and valleys. The country is located in an elevated basin, which separates the eastern branch of the Great Rift Valley (extending into Kenya and Tanzania) and the western branch (which forms the border between

Uganda and DRC), known as the Albertine Rift (Howard 1991). The convergence of the borders of Uganda, the DRC and Rwanda occurs on the volcanic Virunga Mountains, including part of Mount Muhavura (4,125 metres), Mount Sabinio (3,645 metres) and Mount Gahinga (3,474 metres) on the Ugandan side. Alongside this volcanic chain, the western Ugandan border features the Rwenzori Mountains and the Western Rift Valley, which contains Lake Albert and the Albert Nile River. Between the Virunga and Rwenzori Mountains, home to Uganda's highest massif, Margherita Peak (5,109 metres), lie Lakes Edward and George. To the north, the South Sudanese border is marked by the Imatong Mountains, with an elevation of around 1,800 metres, while the north-eastern border is determined by Mounts Morungole (2,750 metres), Moroto (3,084 metres), and Kadam (3,068 metres). Along the Kenyan frontier, Mount Elgon rises at 4,321 metres which, despite not being the highest Ugandan massif, has the world's largest base among volcanically formed mountain ranges. Lastly, to the south of these mountains, Lake Victoria and an eastern extension of the Rift Valley can be found (White 1983; Briggs 2020).

Figure 6.2.1. Savannah and tropical rainforest from Uganda.

Due to Uganda's equatorial location, the country is characterized by an equable tropical climate throughout the year. The annual rainfall varies from 500 to 2,800 mm depending on the region (Nsubuga et al. 2014). However, increased climate variability attributed to climate change has been observed recently, leading to a higher frequency of extreme weather events, including erratic rainfall patterns (Nsubuga and Rautenbach 2018).

Despite its relatively small size, Uganda stands out as one of Africa's most biodiverse countries. It encompasses a diverse range of landscapes, including volcanic mountains, extensive savannah plains, small hills, woodlands, and tropical rainforests (Winterbottom and Eilu 2006) (Fig. 6.2.1). Notably, western Uganda is part of the Albertine Rift, recognized as one of Africa's most biodiverse regions (Plumptre et al. 2007). This area features extensive waterbodies, vast savannahs, grasslands, and rainforests, hosting more vertebrate species than any other region on the continent, despite significant species loss during decades of internal conflicts (Brooks et al. 2001; Plumptre et al. 2007). Uganda is home to 10.2% and 7.5% of the global recorded species of birds and mammals, respectively (Fig. 7.2.1). Additionally, it boasts 19% and 14% of Africa's amphibian and reptile species richness, along with approximately 1,249 butterfly species (Winterbottom and Eilu 2006). Moreover, Uganda has more primate species than any other country of similar size. It is a critical habitat for over half of the world's mountain gorilla population (*Gorilla gorilla beringei*), several communities of Eastern chimpanzees (*Pan troglodytes schweinfurthii*), and the Golden monkey (*Cercopithecus mitis kandti*), which is endemic to the Virunga Mountains, among other species (UWA 2023a).

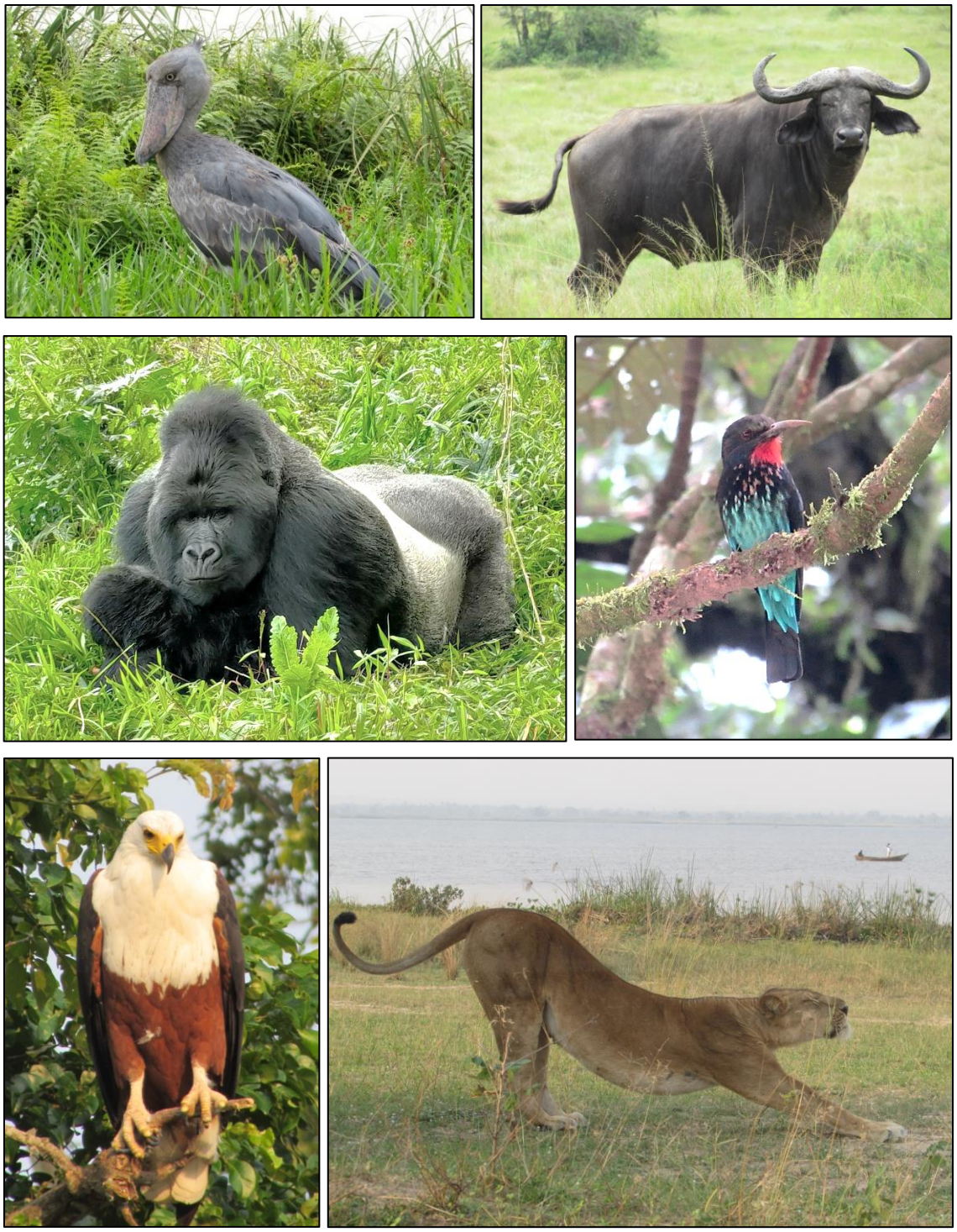


Figure 7.2.1. Wildlife from Uganda: shoebill (*Balaeniceps rex*), African buffalo (*Syncerus caffer*), mountain gorilla (*Gorilla gorilla beringei*), black bee-eater (*Merops gularis*), African fish eagle (*Haliaeetus vocifer*) and lioness (*Panthera leo*).

The high level of biodiversity observed in Uganda can be attributed to its strategic location, which encompasses terrestrial and aquatic habitats. The aquatic habitats, consisting of numerous freshwater lakes and swamps, significantly contribute to this rich biodiversity (Winterbottom and Eilu 2006). Notably, Lake Victoria, situated in the

southeastern corner of the country, ranks as the world's second-largest inland freshwater lake and serves as one of the sources of the Nile River. Beyond Lake Victoria, Uganda is home to five other major lakes: Edward and George in the southwest, Albert to the west, Kyoga in central Uganda and Bisina in the east. Complementing these lakes are eight major rivers, with the Victoria Nile in central Uganda and the Albert Nile in the northwest emerging as the primary rivers of Uganda (Winterbottom and Eilu 2006; Briggs 2020) (Fig. 8.2.1).

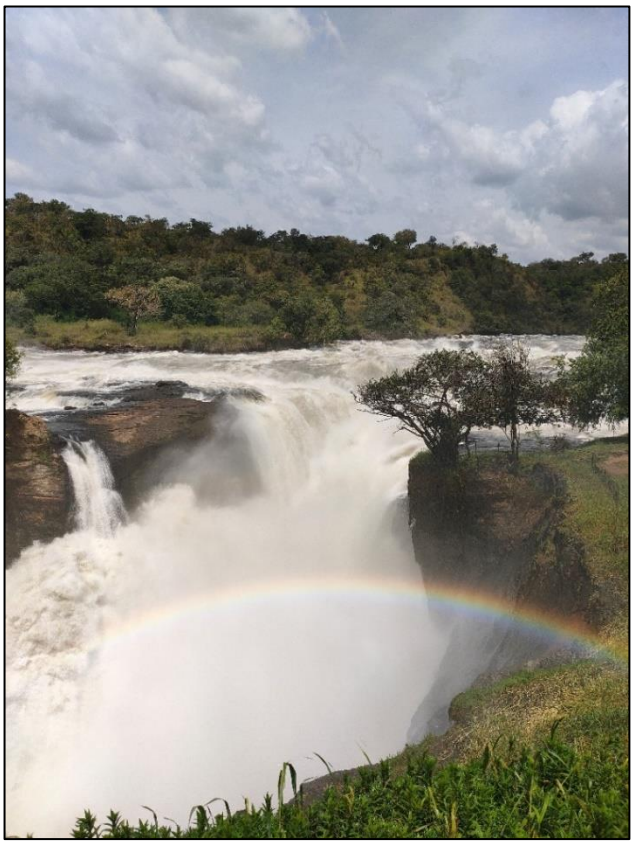


Figure 8.2.1. The confluence of the Albert Nile River at Murchison Falls in Uganda.

Several protected areas were created to conserve Ugandan biodiversity, several protected areas were created, including 10 National Parks (Fig. 1.2.1), 13 wildlife reserves and 10 wildlife sanctuaries (UWA 2023b). Most of the wildlife and forest reserves are adjuncts to the savannah national parks, with Murchison Falls being Uganda's largest National Park, covering approximately 3,800 km^2. These protected areas are managed by the Uganda Wildlife Authority (UWA) and the National Forest Authority (NFA). However, several activities threaten Ugandan biodiversity. The discovery of oil reserves is causing local impacts on wildlife due to activities related to oil exploration. Additionally, the increasing population density has resulted in a higher demand for land for cultivation and water. These factors, combined with natural resource extraction, contamination, infectious diseases, and introduction of invasive species are leading to land degradation and habitat loss for wildlife species (Winterbottom and Eilu 2006; Shackleton et al. 2017; Kusiima et al. 2022).

2.2. The One Health Approach and Antimicrobial resistance

"*Between animal and human medicine there are no dividing lines - nor should be there. The object is different, but the experience obtained constitutes the basis of all medicine.*"

Rudolf Virchow (1821-1902)

One Health definition and history

The term One Health is defined as "a collaborative, multisectoral, and transdisciplinary approach – working at the local, regional, national, and global levels– with the goal of achieving optimal health outcomes by recognising the interconnection between people, animals, plants, and their shared environment" (CDC 2022). Although this term was first used in the early 2000s, the concept and its principles date back to the cultures and beliefs of ancient civilisations.

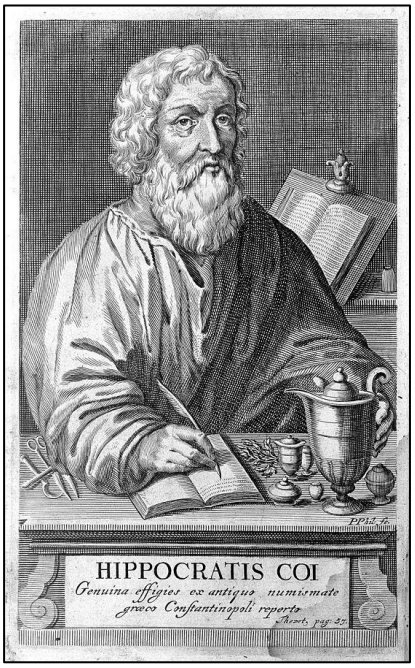


Figure 1.2.2. Hippocrates' portrait from an 18th century edition of *Hippocratis Aphorismi*. Strasbourg, König,

During Greece's Classical period, the idea of One Health emerged in the "Airs, Waters, Places" text by the physician Hippocrates (460 BCE – 367 BCE) (Fig. 1.2.2), where an interdependence between public health and a clean environment was recognised (Wear 2008). Shortly afterwards, Aristotle (384 BCE – 322 BCE), a Greek philosopher and scientist, introduced the practice of comparative medicine, studying common characteristics and diseases across humans and other mammals (Dunlop and Williams 1996).

About 2,000 years later, the role of the environment in the spread of diseases to humans and animals was demonstrated by the Italian physician, veterinarian and epidemiologist Giovanni Maria Lancisi (1654-1720) while addressing rinderpest in cattle (Mantovani and Zanetti 1993). The progress made in the 17th and 18th centuries regarding the interconnectedness of human, animal and environmental health validated the correctness of ancient Greek theories.

In 1762, formal education in animal health and its association with human health was established with the foundation of the first veterinary faculty in Lyon, France, by Claude Bourgelat (1712-1779) (La Berge 1992). In the 19th century, the German physician and pathologist Rudolf Virchow became interested in the link between

human and animal health by studying the parasite *Trichinella spiralis* in swine. Virchow used the term "zoonosis" to indicate an infectious disease transmitted between humans and animals, emphasizing that there should be no dividing lines between human and animal medicine (Schultz 2008; Evans and Leighton 2014). Furthermore, vaccine development and the study of the aetiology of infectious diseases by Louis Pasteur and Robert Koch, respectively, reinforced the understanding that human and animal health are interrelated (Mackenzie et al. 2013). The term "One Medicine" was first coined by William Osler (Cardiff et al. 2008) and later consolidated by the epidemiologist and parasitologist Calvin Schwabe who, through his work with Dinka pastoralists (Majok and Schwabe 1996), evidenced the close interaction of humans and animals for health, livelihood and nutrition (Schwabe 1984).

In the early 21st century, the One Health concept emerged as a re-conceptualisation of the "One Medicine" concept, including not only human and animal health but also environmental and wildlife health (Zinsstag et al. 2011). Accelerated environmental changes associated with human population growth and activities, such as land use change, urbanisation, and international trade, are linked to the emergence of infectious diseases, as illustrated by several viral epidemics (Evans and Leighton 2014; Destoumieux-Garzón et al. 2018). On the other hand, it is well known that animal domestication facilitated the transfer of pathogens between wild animals and humans (Day 2011). Nowadays, 60% of significant emerging infectious diseases (EIDs) arise from domestic and wild animals, and 75% of emerging infectious human diseases have an animal origin (WOAH 2023). The Wildlife Conservation Society meeting in 2004 resulted in the publication of the "Manhattan principles", which promoted the inclusion of wildlife health as an essential component of the strategic framework to mitigate the risk of infectious disease transmission through the human-animal-ecosystem interface (Wildlife Conservation Society 2004). Four years later, the World Health Organisation (WHO), the World Organisation for Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO) developed a tripartite agreement to collaborate across multiple sectors to tackle health risks at the human-animal-ecosystem interfaces (FAO/OIE/WHO 2010) (Fig.

2.2.2). Since then, multiple activities, including research programs, publications, international meetings, and health management measures, have created a solid scientific cooperation that has expanded the One Health community and its networks (Evans and Leighton 2014).

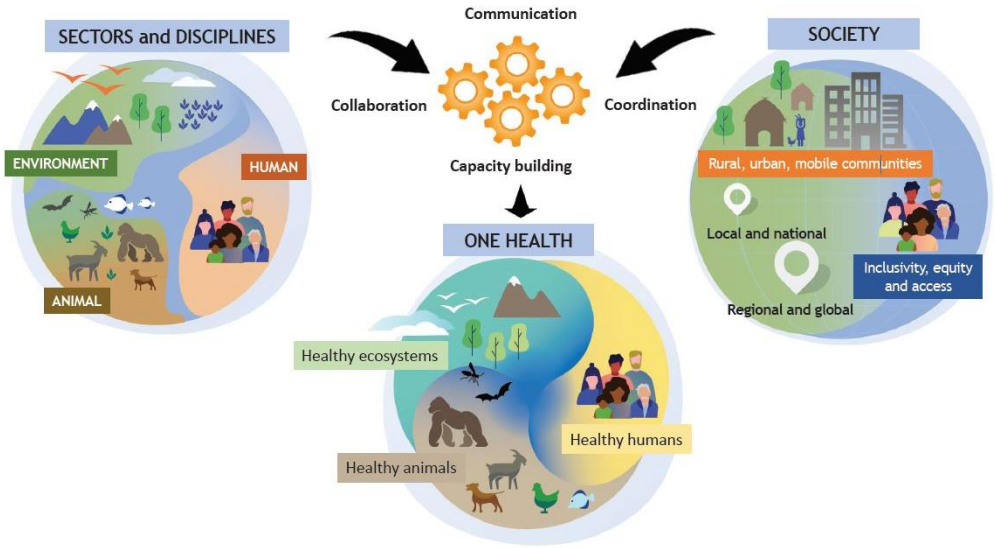


Figure 2.2.2. One Health definition visual (One Health High-Level Expert Panel (OHHLEP) et al. 2022).

In addition to the emerging and endemic zoonoses, other global health concerns such as antimicrobial resistance (AMR) in human and animal pathogens, pollution and environmental contaminants, neglected tropical diseases, and food insecurity are included in the One Health approach (Evans and Leighton 2014). All these factors contribute to a global health crisis, especially in low-income countries. In these regions, close contact with domestic and wild animals, traditional food consumption practices, exposure to zoonotic diseases in poor livestock-dependent communities, inadequate human and animal health services, and various social, political, and economic issues limit the capacity to manage health risks (Cleaveland et al. 2017). Strengthening integrated and cross-sectoral collaboration involving all stakeholders, innovation, investment, and governance is essential to support this approach worldwide.

Emerging infectious diseases

Emerging and re-emerging infectious diseases (EIDs) are defined as newly identified diseases, previously known infections that acquire new virulence factors, and infections that spread to previously unaffected regions, reflecting instances of newly introduced, evolved, or rapidly changing diseases (Kobayashi 2018). Emerging infectious diseases represent a major threat to public health worldwide. Three significant changes are associated with emerging diseases: environmental changes, changes in human and animal demography and behaviour, and pathogen changes over time (Morens et al. 2004; Kobayashi 2018).

To become established, infectious agents must first be introduced into vulnerable populations, and then they must have the ability to spread from human to human and cause disease. However, unlike other diseases, infectious diseases are unpredictable and have the potential to cause global outbreaks. Over the past century, the interaction between human populations and the environment has undergone significant changes (Jones et al. 2013). Population growth and expansion (including migration to urban areas, international travel, and human conflicts), along with changes in land use (such as agricultural expansion and deforestation), have resulted in a higher incidence of EIDs (Daszak et al. 2000; Morens et al. 2004; Jones et al. 2013). Due to the frequent interaction between human-animal-environment interfaces, most EIDs are zoonoses -those naturally transmitted from animals to humans- and wildlife reservoirs constitute an important public health problem (Jones et al. 2008; WHO 2020a). Zoonotic agents can be transmitted through direct contact, by inhalation (airbone), by inoculation (vector-borne), or by ingestion through contaminated food and water (foodborne) (WHO 2020a).

The first "Global Burden of Foodborne Disease Report" by the WHO in 2015 revealed that approximately 600 million illnesses and 420,000 deaths were attributed to foodborne diseases (FBD) in 2010 (WHO 2015). However, there are significant differences in the burden of these diseases among countries. Bacterial agents are the primary cause of FBD, with *Campylobacter* and nontyphoidal

Salmonella (NTS) being the leading causes in high-income countries. While NTS also poses a significant threat in low- and middle-income countries, diseases such as typhoid fever, foodborne cholera, and enterotoxigenic *Escherichia coli* (ETEC) contribute more prominently to diarrhoeal diseases than *Campylobacter* (WHO 2015). The health burden of FBD is comparable to that of malaria, HIV/AIDS, and tuberculosis (the "Big Three"), particularly affecting children under five years old in low- and middle-income countries (Havelaar et al. 2015). The heightened burden is related to several interconnected factors, including limited access to safe water, poor sanitation and food hygiene practices, limited resources for food safety regulation and enforcement, a high prevalence of foodborne pathogens, and limited access to healthcare. Furthermore, monitoring trends in FBD in these countries is challenging due to inaccurate reporting. Addressing the burden of FBDs in low- and middle-income countries (LMICs) requires comprehensive strategies which should focus on improving water and sanitation infrastructure, promoting food safety education and training, strengthening regulatory frameworks, enhancing healthcare systems, and implementing interventions to reduce the prevalence of foodborne pathogens in both the environment and the food supply chain (WHO 2015; Grace 2023).

Since ancient times, zoonoses have affected human health, and wildlife has always played a role (Roeder and Taylor 2002). The ecological changes influencing the epidemiology of zoonoses can be of natural or anthropogenic origin, including human population and agricultural expansion, habitat degradation, and climatic changes (Harper and Armelagos 2010; Jones et al. 2013). Furthermore, the movement of pathogens, vectors, and animal hosts can also influence zoonoses expansion. Today, infectious agents harboured by an animal, human or vector can be transported worldwide in less than 24 hours (Kruse et al. 2004). One example is the case of West Nile virus (WNV) infection, which has spread throughout Europe and America. The virus was first isolated in 1937 in Uganda (Smithburn et al. 1940); however, at the end of the 1990s, it rapidly spread in North America and neighbouring countries (Gubler 2007). The dominant vectors of the virus are *Culex pipiens* mosquitoes, and the American robin (*Turdus migratorius*) is considered the

most important host for WNV maintenance and transmission in the United States (Kilpatrick et al. 2006; Hamer et al. 2009). Bovine tuberculosis (*Mycobacterium bovis*) epidemiology was also influenced by the anthropogenic movement of animals through cattle importation into Africa during the colonial era and thereafter spread to wildlife (Cosivi et al. 1995), with the African buffalo (S*yncerus caffer*) serving as one of the maintenance hosts (De Vos et al. 2001). Ebola virus disease (EVD) was first reported in 1976 in the Democratic Republic of the Congo (DRC) and Sudan, with high case fatality rates in humans and non-human primates (International Commission 1978; WHO/International Study Team 1978). Ebola outbreaks occur intermittently in tropical regions of sub-Saharan Africa, and its transmission is attributed to contact with infected human or non-human primates' carcasses (Leroy et al. 2004). The re-emergence of EVD outbreaks in Gabon and DRC was concomitant with increased mortality among gorillas and chimpanzees, both endangered species (Rouquet et al. 2005). Even though many wildlife species serve as reservoirs of pathogens with public health significance, EIDs originating from wildlife also pose a significant threat to global biodiversity conservation.

EIDs represent a significant and evolving challenge, particularly in light of the ongoing crisis of AMR (WHO 2020b). Pathogens continuously undergo adaptive processes in response to environmental pressures, leading to the emergence of novel virulence factors and the acquisition of AMR mechanisms (Davies and Davies 2010). Recent data underscore the alarming pace of AMR, which has spread globally (WHO 2020b), highlighting the urgent need for coordinated and international efforts to mitigate the impact of AMR and protect public health.

A brief history of antibiotics

The introduction of antimicrobial agents into clinical use was one of the most significant medical advances of the 20th century (Katz and Baltz 2016). Antibiotics have extended the average human lifespan by treating infectious diseases and have played a crucial role in cancer treatment, organ transplantation, and open-heart surgery (Elzinga 2009).

Antibiotic-producing microbes to prevent diseases date back to ancient times, with traditional remedies used in Serbia, China, Greece and Egypt more than 2,000 years ago (Pećanac et al. 2013). The oldest preserved medical document is Eber's papyrus from ancient Egypt, dating back to about 1,500 B.C. (Haas 1999). In the 19th century, scientists such as Joseph Lister, Louis Pasteur, Jules François Joubert, and Ernest Duchesne observed inhibitory effects between microorganisms, although antimicrobial molecules were not purified. It was not until 1909 when Paul Ehrlich developed an arsenic derivative active against *Treponema pallidum*, the causative agent of syphilis (Gelpi et al. 2015). These chemical compounds with antimicrobial properties were marketed as Salvarsan® in 1911, and their discovery was crucial for drug development in the modern chemotherapeutic era (Kaufmann 2008). In 1930, due to the rapid expansion of the German chemical industry, Gerhard Domagk and colleagues discovered the antibiotic effects of sulphanilamide (Domagk 1935), a molecule previously synthesised by Paul Gelmo (Gelmo 1908). Sulphonamides followed Salvarsan's route becoming the first class of mass-produced antimicrobials commercialized as Protosil® in 1935. Despite being the first broad-spectrum antimicrobials in clinical use, sulpha drugs were displaced by the accidental discovery of Alexander Fleming.

In 1928, Fleming found a forgotten Petri dish contaminated by a fungus inhibiting the growth of *Staphylococcus aureus* colonies (Fleming 1929). However, it was not until 1940 that Howard Florey, Ernst Chain and colleagues at Oxford developed and produced this antibiotic (Abraham et al. 1941). Initially, penicillin was a rare and expensive drug. However, from 1946 onwards, improvements in chemical and pharmaceutical techniques allowed its production in larger quantities and global exportation (Bryskier 2005), becoming a commonly used antibiotic for a broad range of infectious diseases and showing fewer side effects than sulphonamides (Durand et al. 2019). Due to bacteria's ability to produce their antibiotics, in the late 1930s Selman Waksman performed the first systematic research on the antimicrobial activity of soil bacteria using a culture-based approach (Lewis 2012). Through this method, he discovered numerous antibiotics, including streptomycin (Jones et al. 1944), which revolutionised the treatment of tuberculosis, actinomycin (Waksman

et al. 1946) and neomycin (Waksman and Lechesralier 1949). The introduction of penicillin and streptomycin reduced mortality and prolonged life expectancy from 42 years in 1960 to more than 70 years in 1967 (Bryskier 2005). Waksman's method inspired the pharmaceutical industry, leading to the golden age of antibiotic discovery and development between the 1940s and the 1970s when most antibiotics were discovered (Fig. 3.2.2). However, despite most of these antibiotics still being in clinical use, their effectiveness has been affected by the rise of AMR (Katz and Baltz 2016).

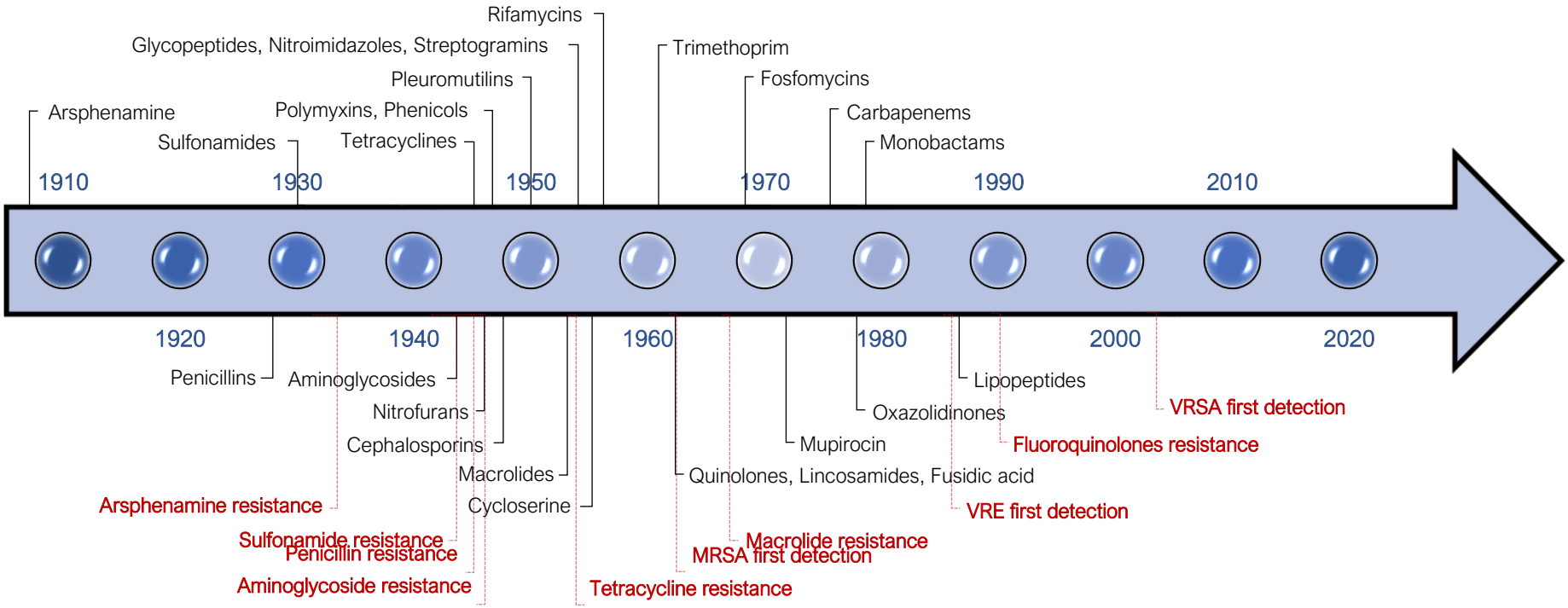


Figure 3.2.2. Antibiotic discovery timeline and first AMR detection to the main antibiotic classes (adapted from Hutchings et al. 2019).

Emergence of antimicrobial resistance (AMR)

In the hundreds of millions of years during which microorganisms have naturally produced antibiotics to act on bacteria sharing the same ecological niche, evolutionary pressure has compelled the vulnerable bacteria to devise survival resistance mechanisms (D'Costa et al. 2011). From an evolutionary perspective, bacteria employ two major genetic strategies to counteract harmful antibiotic molecules: mutations in genes commonly associated with the mechanism of action of the compound and the acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer (HGT) (Munita and Arias 2016).

In the 20th century, the rise of antibiotic production and their use to treat infectious diseases led to the progressive development of bacterial strains' resistance to one class of antibiotics after another, generating the risk of new health and socioeconomic imbalances (Walsh 2003). The widespread use of broad-spectrum antibiotics in humans, animals, and agriculture is considered the main driver of AMR emergence (D'Costa et al. 2011; Prescott 2014). Over the last few decades, this antibiotic abuse has exerted selective pressure on susceptible bacteria and favoured the survival of resistant strains, which represent a predominant threat to global health and the economy (Kumar et al. 2022).

Four years before the discovery of penicillin by Fleming, AMR was first reported in Salvarsan (Beckh and Kulchar 1939), and it did not stop there. Since the introduction of the first modern antibiotics, significant antibiotic resistance to different classes of drugs has emerged over the following decades. Despite their relatively recent discovery, the first cases of therapy failure in sulphonamides (Petro 1943), penicillins (Barber 1947) and streptomycins (Finland et al. 1946) due to acquired resistance were observed in the 1940s. In the following years, resistance to many more antibiotics was declared (Dixo 1967; Falkow 1975). In 1959, methicillin was introduced, the first designed antibiotic to avoid penicillinases, enzymes that inactivate penicillins (Knox 1960). Only two years later, methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported (Jevons 1961). Since the emergence of multidrug-resistant infections, vancomycin became the last resort

antibiotic to treat Gram-positive bacteria, such as enterococci and staphylococci, but resistance to this antibiotic was first observed in 1986 (Uttley et al. 1988) and 2002 (Sievert et al. 2008), respectively. Furthermore, in the 1990s, resistance to carbapenems was reported in Gram-negative bacteria (Lee et al. 1991; Bradford et al. 1997). This led to a profuse search for different antibiotic alternatives. Nowadays, only a small number of antibiotics are being developed (WHO 2019a), and few antibiotic groups can combat AMR (Uddin et al. 2021). In the 1960s, the earliest evidence of plasmid-mediated antibiotic resistance was first reported, demonstrating the ability of plasmids to carry and transfer resistance genes.

Multi-drug resistant bacteria have increased alarmingly over the last few decades, and solutions are urgently needed to tackle resistant bacteria (Khameneh et al. 2016). The WHO Global AMR Surveillance System (GLASS) Report confirmed that AMR has reached worrying levels around the world (WHO 2020b), with resistant bacterial infections associated with higher mortality, prolonged hospital stays, and increased costs (De Kraker et al. 2011). At present, AMR causes 700,000 deaths annually, but it is estimated that this number will increase to 10 million by 2050 (O'Neill 2016). For that reason, in 2019, WHO included AMR as one of the top ten threats to global health (WHO 2021a). However, AMR is emerging in humans, livestock, wildlife, and the environment (Huijbers et al. 2015; Arnold et al. 2016; Hoelzer et al. 2017).

Human population growth and habitat loss have created exceptional opportunities for wildlife to come into contact with humans and livestock, facilitating pathogen and AMR transmission between species (Jones et al. 2008). However, most AMR research has been focused on clinical and hospital settings. Despite the rising interest in AMR research on wildlife worldwide, information about the role of wildlife and the natural environment in AMR dissemination is still lacking in some countries (Greig et al. 2015; Huijbers et al. 2015; Tinoco Torres et al. 2021). As resistant bacteria and antimicrobials can be excreted by humans and domesticated animals, proximity to human settlements influences antibiotic resistance profiles of wildlife microbiota (Sjölund et al. 2008; Schaefer et al. 2009; Literak et al. 2010; Oravcova

et al. 2012). In the Galapagos, molecular studies showed that reptiles living closer to humans had more common AMR markers than those from natural areas (Wheeler et al. 2012). Similarly, Ugandan mountain gorillas from areas overlapping with human settlements carried resistant *E. coli* genetically similar to people and livestock compared to those from remote areas (Rwego et al. 2008). However, not only wildlife is exposed to these resistant organisms. Despite antibiotic-producing bacteria occurring naturally in the environment (Anukool et al. 2004), their mixing with resistant bacteria from anthropogenic sources provides exceptional conditions for the increase in the emergence of new resistant strains (Van Elsas and Bailey 2002). The natural environment, especially water, is a significant source of AMR bacteria to humans (Taylor et al. 2011).

Antimicrobial resistance in low- and middle-income countries

The first WHO Global report on the surveillance of AMR, published in 2014, showed the extent of this threat worldwide and the weaknesses of existing surveillance programs. However, the majority of AMR studies have focused on high-income countries, compared to low- and middle-income countries (LMICs) (WHO 2014; Prestinaci et al. 2015), where the decline of antimicrobial effectiveness has contributed to increased morbidity and mortality of common infections such as malaria, tuberculosis, respiratory and enteric diseases (Leopold et al. 2014; WHO 2014; Prestinaci et al. 2015).

Several interconnected factors drive the emergence of AMR in developing countries, most associated with the unnecessary and excessive use of antimicrobials in humans and food-producing animals (Davies and Davies 2010; Kimera, Mshana, et al. 2020). Inadequate healthcare systems, poor sanitation and malnutrition, which are related to poverty and lack of resources, lead to poor immune status, greater exposure to infectious diseases and inadequate access to drugs, especially in rural areas (Byarugaba 2004; Ayukekbong et al. 2017). Poverty, which is linked to limited education due to the impossibility of attending school (Alividza et al. 2018), contributes to inappropriate antibiotic use by unrestricted access to antibiotics without prescription and self-medication (Ocan et al. 2015; Belachew et al. 2021).

Additionally, the HIV/AIDS epidemic has expanded the immunocompromised population in those regions, further increasing the risk of the acquisition of resistant organisms (Byarugaba 2004). These factors, along with the presence of civil conflicts and the lack of accurate supervision from regulatory agencies in some developing countries, have aggravated AMR (Ayukekbong et al. 2017; Maltezou et al. 2017).

Past, present and future of AMR diagnostics

For over 50 years, antimicrobial susceptibility tests (AST), including disk diffusion, broth microdilution, and agar dilution assays, have been the gold-standard laboratory methods used to determine the antimicrobial sensitivity of AMR organisms (H. Wang et al. 2022). In the disk diffusion method, antibiotic-impregnated disks are placed onto an agar plate pre-inoculated with bacteria. After culturing overnight, the diameter of the zone of inhibition is measured. In agar dilution and broth microdilution, bacterial cells are suspended in serial dilutions with testing antibiotics in agar plates and micro-wells, respectively. The lowest concentration of antibiotics at which bacteria cannot grow is recorded as the minimum inhibitory concentration (MIC) value. Breakpoints for each bacteria-antibiotic pair are determined by the Clinical Laboratory Standards Institute (CLSI) in the USA and the European Committee on Antibiotic Susceptibility Testing (EUCAST) in Europe (Burnham et al. 2017; H. Wang et al. 2022). However, these traditional phenotypic diagnostics are expensive, labour-intensive, and time-consuming, as they rely on previous bacteria culturing and identification, delaying the choice of appropriate antimicrobial therapy (Burnham et al. 2017). To address the drawbacks of traditional AMR diagnostics, microbiologists have developed various phenotypic detection technologies. These include the luciferase assay-aided AST, which measures intracellular ATP of bacteria (Thore et al. 1977), the luciferase reporter phage that detects antibiotic resistance in *Mycobacterium tuberculosis* (Riska et al. 1999), forward laser scattering technology to discriminate different susceptibility phenotypes of Gram-positive bacteria (Idelevich et al. 2017), and a direct-on-target microdroplet growth assay capable of screening several antibiotics simultaneously

and assessing its susceptibility patterns in 15-30 minutes (Kang et al. 2019), among others (H. Wang et al. 2022).

Nowadays, molecular analysis is commonly used to detect genetic mechanisms of resistance when traditional antimicrobial sensitivity methods are too time-consuming or nonconclusive. These techniques are also employed in epidemiological investigations following an outbreak or in AMR surveillance for public health interventions (Anjum et al. 2017). The advent of molecular diagnostics has revolutionized the field by enabling rapid, sensitive, and specific detection of resistance mechanisms in bacterial and other microbial populations. Molecular techniques such as polymerase chain reaction (PCR), whole-genome sequencing (WGS), and metagenomics have provided powerful tools for identifying resistance genes and mutations and illuminating the complex dynamics of AMR dissemination in diverse ecosystems.

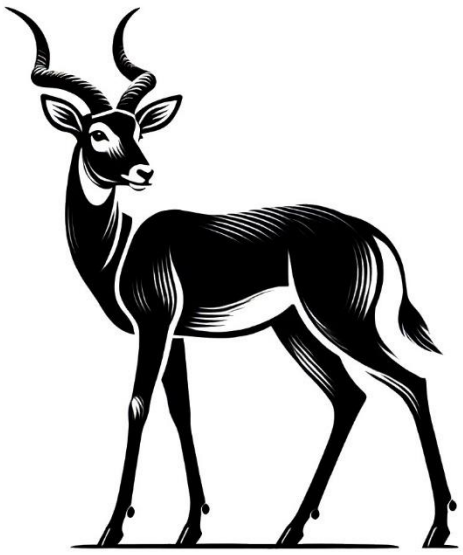
PCR was developed in the 1980s by Kary Mullis (Saiki et al. 1988), enabling rapid and exponential amplification of DNA sequences. Conventional PCR evolved to real-time PCR (rt-PCR), which was faster and safer and did not require agarose gel electrophoresis and ethidium bromide. However, both methods were optimized to a multiplex PCR, where several target DNA fragments could be amplified simultaneously by adding several primers. This assay offered a more rapid and precise identification of AMR, as it was described by Strommenger et al. (2003), which detected nine clinically antibiotic resistance genes of *Staphylococcus aureus* in a single run using a multiplex PCR (Strommenger et al. 2003).

The 1990s saw the rise of Whole Genome Sequencing (WGS), a breakthrough that enabled the sequencing of complete genomes. It represented a significant advancement in AMR studies, surpassing conventional techniques by providing a holistic view of an organism's genetic makeup (Palmer and Kishony 2013). Through WGS, diverse resistance genes can be simultaneously detected, offering nuanced insights into the intricate resistance landscape. This technology's ability to identify single nucleotide polymorphisms (SNPs) and mutations associated with resistance

provides essential information on the genetic underpinnings of AMR development (Anjum et al. 2017). Moreover, WGS facilitates high-resolution strain typing, enabling the tracking of transmission dynamics and the spread of resistant strains within and between populations, a vital aspect in epidemiological studies (Pornsukarom et al. 2018). Beyond gene detection, WGS sheds light on mobile genetic elements such as plasmids, crucial in transferring resistance genes between bacteria (Smoglica et al. 2023). The potential of WGS extends to precision medicine, allowing tailored antimicrobial treatments based on specific pathogen characteristics, potentially improving therapeutic outcomes. In essence, WGS emerges as a powerful and versatile tool, advancing our understanding of AMR mechanisms and transmission dynamics to address the global challenge of AMR.

At the end of the 90s, advances in DNA sequencing and biotechnology allowed scientists to explore the genetic diversity of microbial communities in natural environments through metagenomics (Handelsman et al. 1998). Metagenomics involves the extraction and sequencing of DNA directly from environmental samples or clinical specimens to analyse their genetic material instead of focusing on cultivable organisms in the laboratory. This technique has now emerged as a tool for AMR monitoring as it can quantify thousands of transmissible resistance genes in a single sample using short-read or long-read next-generation sequencing data. Compared to conventional methods, superior results have been observed at the herd/community level (Munk et al. 2018) in epidemiological investigations and global AMR surveillance (Petersen et al. 2015; Van Gompel et al. 2019). In conclusion, metagenomics and WGS are exceptional tools to address AMR within a One Health surveillance framework, enabling precise comparisons among different reservoirs.

3. Hypotheses and objectives



This thesis aimed to ascertain the presence of selected pathogens of significance to human and animal health, and to investigate the presence of resistance genes and their transmission among humans, livestock, wildlife, and the environment in different national parks and neighbouring areas in western Uganda. The urgency of this research is underscored by the increasing threat of AMR and its potential impact on public health.

Antimicrobial resistance is one of the biggest challenges facing global public health. Unfortunately, surveillance and control measures for drug resistance in African countries are limited, especially in low-resource rural settlements, where the choice of antibiotic is often empirical, and there is no regulation on drugs acquisition (Mbonye et al. 2016; Sulis et al. 2021). The first hypothesis of this thesis is that there are a limited number of comprehensive studies on AMR in Uganda, combined with inadequate knowledge among rural farmers about antimicrobial use, which may exacerbate the spread of resistance within these communities. By conducting a systematic review of published and unpublished literature focused on detecting AMR in *E. coli* strains isolated from humans, animals, and the environment, the first study of this thesis aims to provide insights into the extent of research conducted in different regions of Uganda. Additionally, we incorporated survey results from farmers residing in rural areas to assess their knowledge regarding antimicrobial use for both their animals and them.

The increasing rise of infectious pathogens is fuelled by the expanding anthropogenic influence on nature, including factors such as climate change, loss of biodiversity, degradation of habitats, and a rising frequency of interactions between domestic and wild animals and humans (Schmeller et al. 2020). The second study of this thesis hypothesizes that individuals residing in areas characterized by increased interaction between humans and animals, including both domestic and wild species, exhibit a higher prevalence of foodborne pathogens.

The spread of AMR represents a global public health crisis, undermining our capacity to treat bacterial infections effectively. It poses a significant challenge in

developing countries, where limited healthcare infrastructure and inadequate sanitation systems accelerate the spread of resistant microbes. The coexistence of humans, domestic animals and wildlife further facilitates the transmission of resistant strains across species. Additionally, the discharge of antimicrobial residues into the environment through waste exacerbates the problem, contributing to the development of resistance in environmental sources. In the third study of this thesis, we hypothesize that in Uganda, regions characterized by heightened interaction among humans, wildlife, and livestock may exhibit elevated levels of AMR compared to areas with limited contact.

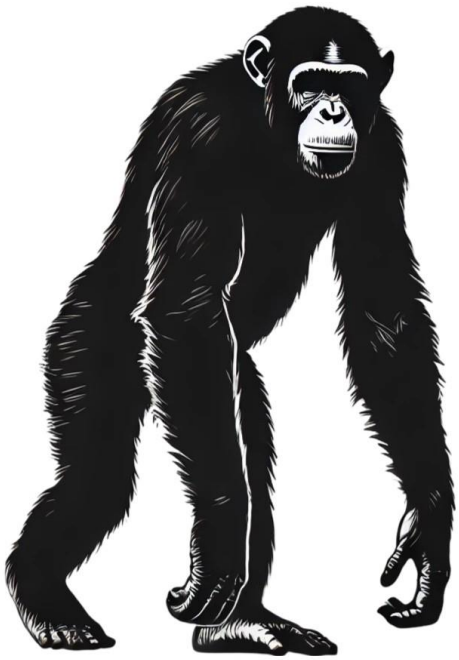
Budongo Central Forest Reserve (BCFR), the largest forest reserve in Uganda, is cohabited by different species of primates, including Eastern chimpanzees. Over the past four decades, land use and cover changes around this reserve have significantly altered the landscape, driven mainly by sugarcane plantations (Mwavu and Witkowski 2008), facilitating increased contact between chimpanzees, and humans and livestock from surrounding villages. In the fourth study of this thesis, we hypothesize that the increased interplay among these species contributes to a higher prevalence of shared pathogens, as habitat loss creates exceptional opportunities for pathogen exchange between species.

The objectives of the present thesis are:

1. To conduct a comprehensive review of available data on the prevalence of AMR in *Escherichia coli* isolates from humans, livestock, wildlife, and the environment across different regions of Uganda. Additionally, a questionnaire-based survey was also conducted to assess antibiotic use among herd owners in various areas of western Uganda.
2. To investigate the presence of selected foodborne pathogens (*Salmonella*, *Campylobacter* and *Arcobacter* spp.) in different rural areas from western Uganda characterized by varying levels of interaction between domestic animals, wildlife, and humans.

3. To evaluate and characterize antimicrobial-resistant *Escherichia coli* and *Klebsiella pneumoniae* in samples obtained from humans, livestock, wildlife, and the environment in western Uganda.
4. To investigate the influence of habitat overlap on the transmission of pathogenic bacteria between chimpanzees, humans and goats from western Uganda.

4. Studies



4.1. Study 1:

A Systematic Literature Review of Multidrug Resistant *Escherichia coli* in Uganda and a Questionnaire-Based Survey on Antibiotic Use

Abstract

Antimicrobial Resistance (AMR) has emerged as a global threat to human and animal health. However, surveillance and control measures in low-resource settings are limited. This study aims to evaluate the occurrence of AMR *Escherichia (E.) coli* among humans, livestock, wildlife, and the environment in different regions of Uganda. Data available from 2001 to 2022 were retrieved from relevant databases as well as grey literature dissertations archived in the College of Veterinary Medicine, Animal Resources and Bio-Security (Makerere University, Kampala, Uganda). A total of 130 studies were included in the final analysis. Additionally, a questionnaire-based survey involving 200 herd owners was conducted from November 2021 to February 2022 to gather information on antibiotic use in livestock and farmers in western Uganda. High levels of resistance to commonly used drugs, including amoxicillin, tetracycline and trimethoprim/sulfamethoxazole, were reported in all species and the environment across different periods and regions, especially in humans. Resistance to third and fourth-generation cephalosporins was also observed in all studied subjects. Furthermore, 40% of the studies reported multi-drug resistance in *E. coli* isolates. According to our interviews, 17 and 14.4% of the respondents admitted to never consulting a veterinarian or a physician before administering antibiotics to their animals or taking antibiotics themselves. Additionally, 66.7% recognized acquiring antibiotics without a prescription. Finally, 45.9% admitted not waiting the withdrawal period before consuming meat or milk. It is urgent to extend the One Health approach to minimize antimicrobial resistance emergence and spread, especially in resource-constrained areas. Appropriate strategies using collective actions are needed to combat AMR in Uganda.

4.1.1. Introduction

Antimicrobial Resistance (AMR) is an emerging and leading global threat to both human and animal health (O'Neill 2016). The WHO Global AMR Surveillance System (GLASS) Report indicates that AMR has reached concerning levels worldwide, rendering conventional treatments for common infections ineffective (WHO 2020b). AMR causes 700,000 deaths annually, and it is estimated that it could lead to 10 million deaths per year by 2050 (O'Neill 2016). However, resistant bacteria do not only emerge in humans but also in livestock, wildlife species, and the environment (Huijbers et al. 2015; Arnold et al. 2016; Hoelzer et al. 2017). Human population growth and increasing habitat fragmentation favour human, livestock and wildlife interactions, creating exceptional opportunities for AMR transmission (Weiss et al. 2018). Although the interconnectedness of human, animal and environmental health is especially relevant in the context of AMR, most research is focused on humans and food-producing animals, and little is known about AMR dynamics in wildlife and the natural environment (Wellington et al. 2013; Vittecoq et al. 2016).

Antibiotic misuse is one of the leading causes of the current AMR pandemic. Unfortunately, surveillance and control measures for drug resistance in African countries are limited (Sulis et al. 2021). In low-resource rural settlements, with limited access to medical or veterinarian assistance, the choice of antibiotics is often empirical, and there are no regulations on antibiotic acquisition. Additionally, the high burden of life-threatening infections and poor hygiene and sanitation contribute to the increased use and misuse of antibiotics, aggravating the AMR threat in these areas (Yeika et al. 2021). In 2019, the highest rates of AMR burden were reported in sub-Saharan Africa (Antimicrobial Resistance Collaborators 2022). Various reviews studying AMR in Africa, particularly in East Africa, have found a high level of resistance to commonly used antibiotics (such as amoxicillin, ampicillin and trimethoprim/sulfamethoxazole) and emerging resistance to gentamicin and third-generation cephalosporins (Ampaire et al. 2016; Tadesse et al. 2017). Uganda, a low-income country located in East Africa, heavily relies on agriculture and subsistence farming as its main economic activities (FAO 2023). Antibiotics are readily available in local drug shops, and there is poor regulation on prescription,

selling practices and antibiotic use (Mbonye et al. 2016), which could facilitate the expansion of AMR.

The present study aims to review available information on the occurrence of AMR in *E. coli* isolates obtained from humans, livestock, wildlife, and environmental across various regions of Uganda, and to conduct a questionnaire-based survey to evaluate antibiotic use by herd owners from different regions of western Uganda.

4.1.2. Materials and Methods

4.1.2.1. Systematic review

Search Strategy and Data Sources

A comprehensive search was conducted in multiple electronic databases, including PubMed, Scopus, Science Direct and Academic Google databases, to identify relevant scientific papers published in English using the PRISMA flow diagram (Fig. 1.4.1). The search was conducted without any date restrictions. The complete search syntax is available in the Supplementary material S1.4.1.

Additionally, grey literature (Bachelors and MSc dissertations) from 2001 to 2022 available in the College of Veterinary Medicine, Animal Resources and Bio-Security's library from Makerere University (Kampala, Uganda) was included in the review.

Inclusion and Exclusion Criteria

Based on the information provided in the abstract and the full-text articles, studies reporting phenotypic AMR prevalence in *E. coli* isolates obtained from different samples, including livestock, wildlife, environment and humans from Uganda, were included in the review. To ensure consistency, only studies with the number of resistant and total isolates clearly described were considered.

Studies in which *E. coli* was not identified, exhibited genetic resistance patterns instead of phenotypic patterns, or focused on antibiotic residues were excluded.

Quality assessment and bias

The quality of each article was evaluated using a Newcastle-Ottawa Quality Assessment Scale adapted for cross-sectional studies. The following quality criteria were assessed: (1) study population; (2) representativeness of the sample; and (3) ascertainment of the antimicrobial susceptibility testing method. The mean score of two authors were taken for final decision, including studies with score greater than or equal to three. Furthermore, a funnel plot and Egger's regression test were used to assess publication bias. Asymmetry of the funnel plot and/or statistical significance of Egger's regression test ($p < 0.05$) was suggestive of publication bias.

Data extraction

Relevant information was extracted for each included study, encompassing the first author's name, publication year, and duration of data collection. Additionally, the study's region of origin, sampling details (species of study, sample type, sample size and clinical syndrome), and the methodology employed for antibiotic susceptibility testing (disc diffusion or minimum inhibitory concentration [MIC]) were recorded. The number of the *E. coli* isolates analysed, and the count of resistant isolates were also extracted.

Moreover, the cut-off value reference for susceptibility testing (CLSI, EUCAST or others) was noted from each study. Data regarding ESBL-producing isolates and Multi-Locus Sequence Typing (MLST) were not included due to its limited availability.

4.1.2.2. Questionnaire-based survey

Additionally, a questionnaire-based survey was conducted between November 2021 and February 2022, involving direct interviews with herd owners in three regions of rural western Uganda. The questionnaires were developed by a multidisciplinary team comprising veterinarians, epidemiologists, and sociologists. The main objective was to collect information on antibiotic usage in both livestock and humans (Supplementary material S2.4.1). The questionnaire was originally designed in English and the contents were translated during the interviews by an Animal Health Assistant (Department of Animal Health) when required.

The questionnaire encompassed four categories: herd owner's personal information, herd's information, antibiotic use in animals, and antibiotic consumption by herd owners. To assess the correlation between antibiotic use, geographic area or level of education, Pearson's Chi-squared test ($\chi 2$) was implemented (with Yates' correction when appropriate). *p*-values less than 0.05 were considered statistically significant. Statistical analyses were performed using the R version 4.3.0 (R Core Team 2022).

4.1.3. Results

4.1.3.1. Systematic review

Data and study characteristics. In total, 485 studies were identified. Of those, 130 studies met the inclusion criteria and were included in the final analysis (Fig. 1.4.1).

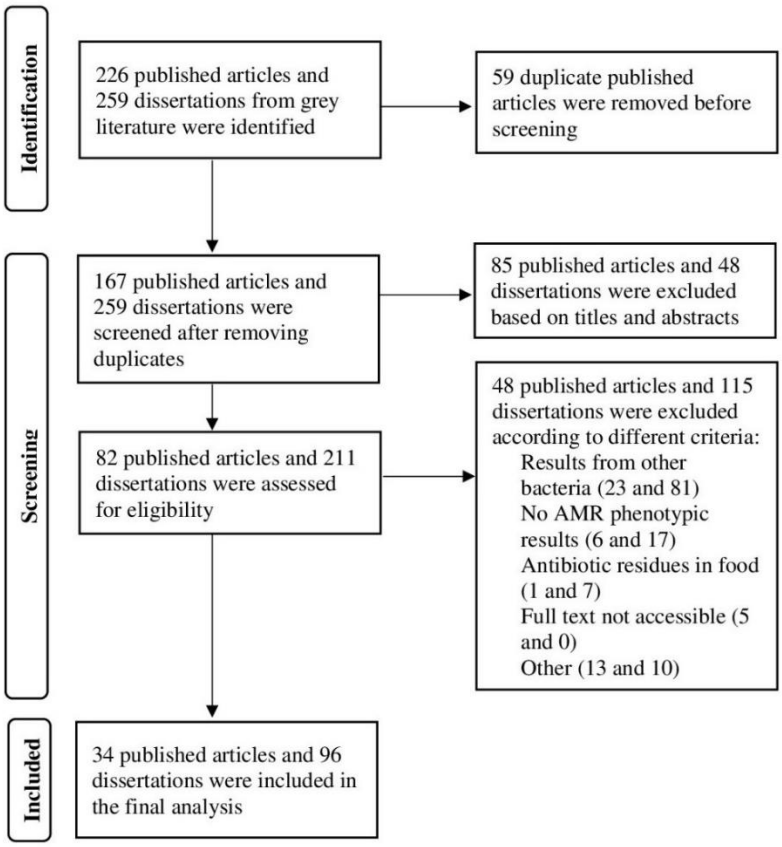


Figure 1.4.1. PRISMA flow diagram illustrating the screening process for studies included in the *Escherichia coli* antimicrobial resistance meta-analysis.

The different characteristics of the published and unpublished studies included in this review are summarized in Table 1.4.1.

Table 1.4.1. Characteristics of the articles included in the systematic review, number and frequency of studies with those characteristics and their references. Only published studies are included in the "References" column.

Characteristic	N studies (%)	References
Publication year		
2001-2005	16 (12.3)	
2006-2010	28 (21.5)	(Anguzu and Olila 2007; Goldberg et al. 2007; Kyabaggu et al. 2007)
2011-2015	31 (22.8)	(Odongo et al. 2013; Seni et al. 2013; Agwu et al. 2015; Kateregga et al. 2015; Okoche et al. 2015)
2016-2020	48 (36.9)	(Agaba et al. 2017; Bebell et al. 2017; Ssajjakambwe et al. 2017; Kajumbula et al. 2018; Okubo et al. 2018; Stanley et al. 2018; Weiss et al. 2018; Ball et al. 2019; Hope et al. 2019; Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Katongole et al. 2020; Odoki et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020)
2021-2022	17 (13.1)	(Abongomera et al. 2021; Ikwap et al. 2021; Johnson et al. 2021; Kakooza et al. 2021; Obakiro et al. 2021; Tumuhamye et al. 2021; Zamarano et al. 2021; Bager et al. 2022; Carrasco Calzada et al. 2022; Nakawuki et al. 2022)
Study subject		
Humans	87 (66.9)	(Anguzu and Olila 2007; Kyabaggu et al. 2007; Odongo et al. 2013; Seni et al. 2013; Agwu et al. 2015; Kateregga et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Bebell et al. 2017; Kajumbula et al. 2018; Stanley et al. 2018; Hope et al. 2019; Katongole et al. 2020; Odoki et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020; Abongomera et al. 2021; Johnson et al. 2021; Obakiro et al. 2021; Tumuhamye et al. 2021; Zamarano et al. 2021; Carrasco Calzada et al. 2022; Nakawuki et al. 2022)
Livestock	22 (16.9)	(Ssajjakambwe et al. 2017; Okubo et al. 2018; Ball et al. 2019; Ikwap et al. 2021; Kakooza et al. 2021)
Wildlife	4 (3.1)	(Bager et al. 2022)
Environment	7 (5.4)	
Humans and Livestock	5 (3.8)	(Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020)

Humans and Wildlife	1 (0.8)	(Goldberg et al. 2007)
Livestock and Wildlife	1 (0.8)	
Livestock and Environment	1 (0.8)	
Humans, Livestock and Wildlife	2 (1.5)	(Weiss et al. 2018)
Study area		
Central	89 (68.5)	(Kyabaggu et al. 2007; Seni et al. 2013; Kateregga et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Kajumbula et al. 2018; Okubo et al. 2018; Ball et al. 2019; Katongole et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020; Abongomera et al. 2021; Kakooza et al. 2021; Tumuhamye et al. 2021)
West	15 (11.5)	(Goldberg et al. 2007; Agwu et al. 2015; Bebell et al. 2017; Stanley et al. 2018; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Odoki et al. 2020; Bager et al. 2022)
East	14 (10.8)	(Anguzu and Olila 2007; Obakiro et al. 2021; Nakawuki et al. 2022)
North	4 (3.1)	(Odongo et al. 2013; Ikwap et al. 2021; Carrasco Calzada et al. 2022)
Southwest	8 (6.2)	(Ssajjakambwe et al. 2017; Weiss et al. 2018; Hope et al. 2019; Johnson et al. 2021; Zamarano et al. 2021)
Sample type		
Fecal samples	39 (30)	(Goldberg et al. 2007; Okubo et al. 2018; Stanley et al. 2018; Weiss et al. 2018; Ball et al. 2019; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Bager et al. 2022)
Urine samples	39 (30)	(Kyabaggu et al. 2007; Odongo et al. 2013; Bebell et al. 2017; Odoki et al. 2020; Odongo et al. 2020; Abongomera et al. 2021; Johnson et al. 2021; Carrasco Calzada et al. 2022; Nakawuki et al. 2022)
Wound swabs	13 (10)	(Anguzu and Olila 2007; Seni et al. 2013; Hope et al. 2019)
Water samples	9 (6.9)	
Blood samples	5 (3.8)	(Kajumbula et al. 2018; Tumuhamye et al. 2020; Zamarano et al. 2021)
Other	25 (19.2)	(Agwu et al. 2015; Kateregga et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Ssajjakambwe et al. 2017; Kajumbula et al. 2018; Katongole et al. 2020; Turyasiima et al. 2020; Ikwap et al. 2021; Kakooza et al. 2021; Obakiro et al. 2021; Tumuhamye et al. 2021)
Study design		

Cross-sectional study	110 (84.6)	(Odongo et al. 2013; Seni et al. 2013; Kateregga et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Ssajjakambwe et al. 2017; Stanley et al. 2018; Ball et al. 2019; Hope et al. 2019; Katongole et al. 2020; Odoki et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020; Abongomera et al. 2021; Johnson et al. 2021; Tumuhamye et al. 2021; Zamarano et al. 2021; Carrasco Calzada et al. 2022; Nakawuki et al. 2022)
Prospective study	3 (2.3)	(Bebell et al. 2017)
Retrospective study	4 (3.1)	(Kakooza et al. 2021; Obakiro et al. 2021)
Not specified	13 (10)	(Anguzu and Olila 2007; Goldberg et al. 2007; Kyabaggu et al. 2007; Agwu et al. 2015; Kajumbula et al. 2018; Okubo et al. 2018; Weiss et al. 2018; Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Ikwap et al. 2021; Bager et al. 2022)
Drug susceptibility method		
Disk Diffusion	119 (91.5)	(Anguzu and Olila 2007; Goldberg et al. 2007; Kyabaggu et al. 2007; Odongo et al. 2013; Seni et al. 2013; Agwu et al. 2015; Kateregga et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Bebell et al. 2017; Ssajjakambwe et al. 2017; Weiss et al. 2018; Hope et al. 2019; Katongole et al. 2020; Odoki et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020; Abongomera et al. 2021; Johnson et al. 2021; Kakooza et al. 2021; Tumuhamye et al. 2021; Zamarano et al. 2021; Bager et al. 2022; Carrasco Calzada et al. 2022; Nakawuki et al. 2022)
MIC	7 (5.4)	(Okubo et al. 2018; Ball et al. 2019; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Iramiot, Kajumbula, Bazira, Kansiime, et al. 2020; Ikwap et al. 2021)
Not specified	4 (3.1)	(Kajumbula et al. 2018; Stanley et al. 2018; Obakiro et al. 2021)
Laboratory Standard		
CLSI	119 (91.5)	(Goldberg et al. 2007; Odongo et al. 2013; Seni et al. 2013; Agwu et al. 2015; Okoche et al. 2015; Agaba et al. 2017; Okubo et al. 2018; Stanley et al. 2018; Weiss et al. 2018; Ball et al. 2019; Hope et al. 2019; Iramiot,

		Kajumbula, Bazira, Kansiime, et al. 2020; Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Katongole et al. 2020; Odoki et al. 2020; Odongo et al. 2020; Tumuhamye et al. 2020; Turyasiima et al. 2020; Abongomera et al. 2021; Johnson et al. 2021; Kakooza et al. 2021; Tumuhamye et al. 2021; Zamarano et al. 2021; Bager et al. 2022; Nakawuki et al. 2022)
EUCAST	3 (2.3)	(Bebell et al. 2017; Ikwap et al. 2021; Carrasco Calzada et al. 2022)
Not specified	8 (6.2)	(Anguzu and Olila 2007; Kyabaggu et al. 2007; Kateregga et al. 2015; Ssajjakambwe et al. 2017; Kajumbula et al. 2018; Obakiro et al. 2021)

Antimicrobial resistance patterns according to the study subject. *E. coli* isolates from four study subjects (humans, livestock, wildlife, and the environment) were tested against different antibiotics. Since 2011, studies on AMR increased considerably, especially in humans and livestock (Fig. 2.4.1). Furthermore, this same year, the environment started to be included in AMR investigations. Although the last period (2021-2022) showed fewer studies, it only comprised two years of research.

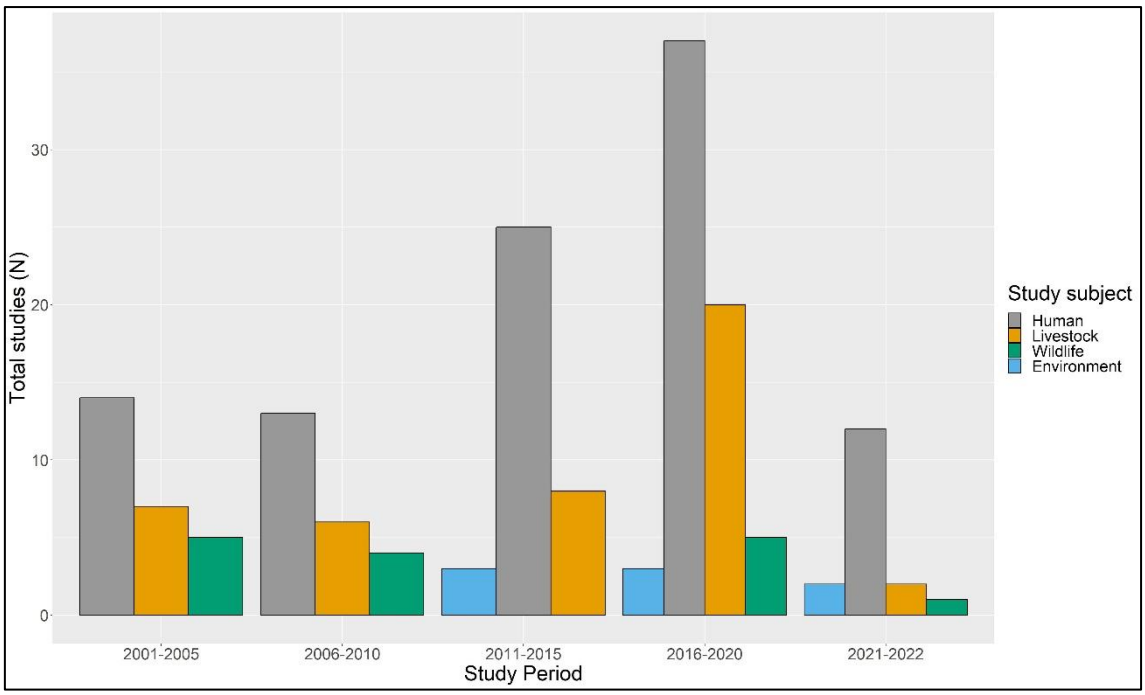


Figure 2.4.1. Total studies performed in humans, livestock, wildlife and environment according to the study periods in Uganda.

In total, human isolates were tested against 62 antibiotics, followed by livestock (48), wildlife (30) and the environment (21). The prevalence and weighted pooled prevalence of *E. coli* isolates are reported in the Supplementary material (Supplementary Table S3.4.1). AMR to commonly used drugs, like amoxicillin and trimethoprim/sulfamethoxazole, was higher in human isolates compared to environmental, livestock and wildlife isolates. In the case of sulfamethoxazole, livestock showed higher levels of resistance compared to humans. Resistance to third and four generation cephalosporins was found in all study subjects, but specially in human isolates, where prevalence ranged from 20.8 to 61.1%. Imipenem and meropenem showed low levels of resistance in livestock (1.2 and 0% respectively) and humans (5.8 and 7.3% respectively) compared to commonly used antibiotics, and no resistance was observed in wildlife. No study tested environmental isolates against carbapenems. Overall, the highest weighted pooled prevalence was observed in penicillin (79.1%), erythromycin (69.7%) and metronidazole (67.2%).

Antimicrobial resistance patterns over time**.** Reports of antibiotic resistance in *E. coli* isolates according to the selected studies began in 2001; therefore, the first period analysed was from 2001 to 2005, followed by 2006 to 2010, 2011 to 2015, 2016 to 2020, and 2021 to 2022. Except for erythromycin and tetracycline, the highest prevalence of resistance reported to commonly available antibiotics was in 2011-2015 (Table 2.4.1). Despite resistance prevalence of seven of the commonly used antibiotics decreased from 2011-2015 to 2016-2020, it increased again in 2021-2022. In the last three periods of study, an increase in ceftazidime resistance was observed. Generally, amoxicillin and erythromycin showed the highest weighted pooled prevalence of resistance across periods (67.4 and 78.8% respectively).

Antimicrobial resistance patterns across study areas**.** Central and eastern Uganda showed the highest levels of resistance to the nine commonly used antibiotics (Table 3.4.1). Resistance to ceftazidime was higher in western (72.5%), eastern (70.3%) and central (46.1%) Uganda compared to other regions. Overall, gentamicin,

ciprofloxacin and chloramphenicol showed the lowest levels of resistance compared to erythromycin and amoxicillin.

Table 2.4.1. Prevalence, weighted pooled prevalence and CI 95% of *E. coli* isolates from the different study periods to eight different antibiotics: amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN) and trimethoprim/sulfamethoxazole (SXT).

	Prevalence (CI 95%) 2001-2005	Prevalence (CI 95%) 2006-2010	Prevalence (CI 95%) 2011-2015	Prevalence (CI 95%) 2016-2020	Prevalence (CI 95%) 2021-2022	Weighted pooled prevalence (CI 95%)
AMX	43.3 (38.3-47.8)	69.1 (65.1-72.6)	96.1 (92.6-97.9)	69.8 (65.3-74)	77.4 (69.3-83.9)	67.4 (65.2-69.5)
AMC	44.3 (37.6-51.2)	NA	69 (63.6-73.8)	41.2 (38.5-43.9)	67.9 (65.1-70.4)	55.8 (54-57.6)
CAZ	5.6 (2.6-11.7)	19.4 (11.4-30.8)	72.5 (66.4-77.8)	49.4 (44.5-54.1)	59.5 (55.8-63.1)	53.2 (50.8-55.8)
CHL	31.2 (27.8-34.8)	38.6 (35.4-41.9)	59.1 (54.3-63.7)	9.8 (8.7-10.9)	21.4 (18.4-24.6)	22.3 (21.2-23.4)
CIP	6.7 (4.5-9.9)	17.2 (14.8-19.7)	48.3 (44.5-51.9)	13.3 (12.2-14.4)	28.8 (26.4-31.1)	20.2 (19.3-21.2)
ERY	77.4 (72.6-81.5)	89.5 (86.7-91.8)	80.9 (73.5-86.4)	58.2 (51.1-64.8)	0	78.8 (76.5-80.9)
GEN	8.3 (6.5-10.6)	14 (11.8-16.6)	46.8 (42.9-50.7)	14.2 (13-15.3)	0	30.5 (29.4-31.6)
TET	48.6 (44.8-52.4)	51.8 (48.4-55)	76.7 (73.8-79.4)	25.4 (23.9-27)	81 (77.8-83.7)	45 (43.7-46.1)
SXT	41.9 (37.6-46.1)	55 (51.2-58.7)	85.7 (82.4-88.5)	36.6 (34.9-38.2)	67.8 (65-70.2)	49.2 (48-50.4)

Table 3.4.1. Prevalence, weighted pooled prevalence and CI 95% of *E. coli* isolates from the different study areas to eight different antibiotics: amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN) and trimethoprim/sulfamethoxazole (SXT).

	Prevalence (CI 95%) Central	Prevalence (CI 95%) East	Prevalence (CI 95%) North	Prevalence (CI 95%) Southwest	Prevalence (CI 95%) West	Weighted pooled prevalence (CI 95%)
AMX	68.7 (66.7-71.2)	95.7 (78.9-99.2)	44.2 (31.6-57.6)	76.6 (64.5-85.5)	44.3 (39.5-49.2)	62.9 (60.5-65)
AMC	49.2 (47-51.4)	74.5 (70.7-78)	47.4 (34.9-60)	58.3 (44.4-71.2)	59.2 (54.1-63.9)	55.2 (53.4-57)
CAZ	46.1 (42.7-49.5)	70.3 (66.4-74)	7.7 (3-18.1)	13.2 (6.5-24.8)	72.5 (59-82.8)	52.5 (50-55)
CHL	32.4 (30.7-34.2)	20.1 (35.4-41.9)	NA	6.3 (5.3-7.5)	22.8 (19.3-26.7)	22.3 (21.2-23.4)
CIP	29.1 (27.5-30.6)	39.2 (35.8-42.5)	20.2 (14.1-27.8)	1.4 (0.9-2.1)	7.8 (6.3-9.6)	19.9 (19-20.8)
ERY	84.9 (82.5-87)	61 (50-70.7)	NA	26.3 (11.8-48.8)	77.5 (70.6-83.1)	81.9 (79.5-83.9)
GEN	26.6 (25-28.1)	53.2 (49.7-56.7)	11.6 (7.2-18.3)	1.4 (0.9-2.1)	6.2 (4.9-7.8)	19.7 (18.7-20.5)
TET	62.4 (60.6-64.2)	92.7 (90.2-94.6)	25 (11.2-46.8)	12.8 (11.3-14.5)	17.5 (13.4-22.5)	46.8 (45.4-48)
SXT	59.5 (57.6-61.2)	75 (71.7-78)	59.7 (48.5-69.9)	17.9 (16.2-19.8)	54.9 (51.5-58.2)	49.5 (48.2-50.6)

Multidrug resistance (MDR). Fifty-two out of 130 studies reported MDR in *E. coli* isolates, defined as resistance to at least three antibiotic classes, in *E. coli* isolates. Only 63.5% of these studies defined the resistance pattern observed, where the most common pattern observed (24.2%) was resistance to penicillins (amoxicillin or ampicillin), tetracyclines and trimethoprim/sulfamethoxazole. MDR was reported in all study periods.

Publication bias. Funnel plots of standard error supplemented by statistical tests confirmed that there is strong evidence of publication bias on studies reporting the prevalence of *E. coli* resistant isolates in Uganda (Egger's test, $p = 0.000$).

4.1.3.2. Questionnaire survey

In total, 200 herd owners consented to participate in the survey. Table 4.4.1 summarizes the sociodemographic characteristics of the participants, including data about sex, age, education level and district.

Table 4.4.1. Demographic characteristics of herd owners (N = 200). *Don't' know/Don't answer.

Category	Number of participants (%)
Sex	
Male	147 (73.5)
Female	53 (26.5)
Age range	
18 to 24	7 (3.5)
25 to 44	83 (41.5)
45 to 64	87 (43.5)
>65	15 (7.5)
DK/DA*	8 (4)
Education	
None	35 (17.5)
Primary school	99 (49.5)
Secondary school	50 (25)
University	14 (7)
DK/DA*	2 (1)
District	
Buliisa	50 (25)
Kasese	67 (33.5)
Kiruhura	1 (0.5)
Kisoro	66 (33)
Mbarara	1 (0.5)
Rubirizi	15 (7.5)

The most common livestock owned by farmers were goats, followed by cattle, poultry and sheep (Table 5.4.1). According to herd owner's responses, 86.5% (n = 173) and 69% (n = 27) of the herds shared grazing areas or water sources with neighbours' herds and with wildlife, respectively. A total of 63% of herd owners (n = 126) knew what antibiotics were, while 33.5% (n = 67) did not and 3.5% (n = 7) didn't know/didn't answer. Regarding antimicrobial prescription to treat the animals, 24% (n = 48) of the farmers always consulted a veterinarian or animal health assistant before starting it, 57.5% (n = 115) sometimes, 17% (n = 34) never and 1.5% (n = 3) didn't know/didn't answer. This practice was significantly more common in herd owners from north-western Uganda districts than those from south-western districts ($\chi^2 = 22.2$, df = 6, $p = 0.001$). Among those who consulted a veterinarian, 67.5% (n = 110) always followed the treatment period prescribed, while 24.5% (n = 40) and 3.7% (n = 6) followed the period sometimes or never, respectively. Seven of them (4.3%) didn't know/didn't answer.

Table 5.4.1. Number and percentage of cattle, sheep, goat, poultry, dog, pig, duck and cat owners, according to the number of animals owned by each of the 200 farmers surveyed.

Number of animals	Cattle owners (%)	Sheep owners (%)	Goat owners (%)	Poultry owners (%)	Dog owners (%)	Pig owners (%)	Duck owners (%)	Cat owners (%)
2 to 5	31 (25.2)	42 (60)	53 (34.9)	23 (21.3)	57 (96.6)	8 (50)	10 (41.7)	8 (100)
6 to 10	23 (18.7)	14 (20)	43 (28.3)	34 (31.5)	1 (1.7)	5 (31.3)	9 (37.5)	-
11 to 20	13 (10.6)	7 (10)	30 (19.7)	28 (26)	1 (1.7)	2 (12.5)	3 (12.5)	-
21 to 50	24 (19.5)	6 (8.6)	17 (11.2)	17 (15.7)	-	1 (6.3)	2 (8.3)	-
51 to 100	17 (13.8)	1 (1.4)	6 (3.9)	4 (3.7)	-	-	-	-
101 to 150	9 (7.3)	-	3 (1.9)	-	-	-	-	-
151 to 200	1 (0.8)	-	-	-	-	-	-	-
> 200	5 (4.1)	-	-	2 (1.9)	-	-	-	-

Regarding the use of antibiotics in animals, 148 herd owners (74%) recognised that they had treated their animals with antibiotics at least once during the last twelve months, while 37 (18.5%) had never used antibiotics with their animals. Fifteen (7.5%) herd owners didn't know/didn't answer. Furthermore, 41.2% of the herd owners admitted having administered antibiotics to their animals for disease prevention, especially in cattle. A significant association between region of study and antibiotic administration in animals was observed, with north-western and western

districts showing higher levels of antibiotic use than south-western districts (χ^2=48.6, df=4, p<0.001).

The most common condition treated in domestic animals during the twelve months previous to the questionnaire were infectious diseases (69/260; 26.5%), followed by injuries and skin disorders (63/260; 24.2%), reproductive disorders (31/260; 11.9%), gastroenteric diseases (31/260; 11.9%), respiratory diseases (30/260; 11.5%), articular disorders (16/260; 6.2%), non-specific clinical symptoms such as pain and anorexia (10/260; 3.8%), prevention (7/260; 2.7%), and ocular and neurological disorders (3/260; 1.2%). Penicillins, tetracyclines and sulphonamides were the most frequent antibiotics used in the different clinical conditions (Fig. 3.4.1). However, 76.4% of the herd owners did not have written records of the antibiotic treatments.

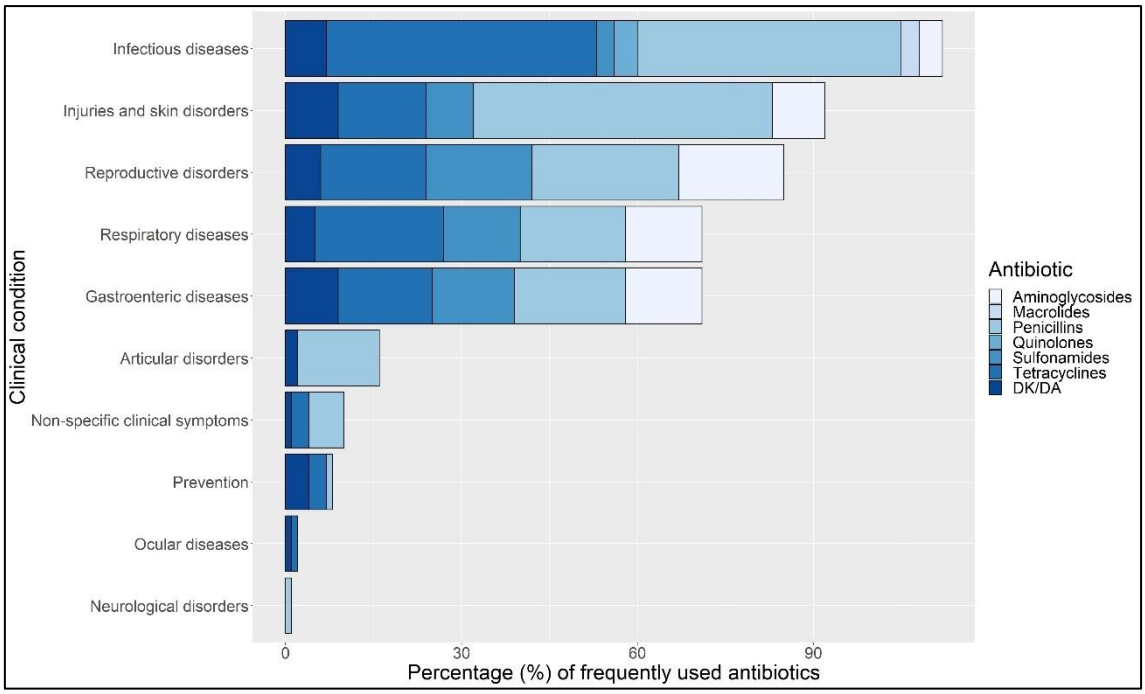


Figure 3.4.1. Clinical reason to administer antibiotics to the animals and frequency of antibiotics used.

Regarding the antimicrobial withdrawal period before slaughter or milk consumption, 50.7% of the herd owners recognized waiting the established period, while 45.9% did not and 3.4% didn't know/didn't answer. Among the 75 farmers who respected this period, fifty-one (68%) waited 1 to 14 days, six (8%) 15 to 29 days, eight

(10.7%) 30 to 59 days, seven (9.3%) 60 to 90 days and three (4%) more than 90 days. Concerning antibiotic disposal, 39.9% of the herd owners had agreed on disposing their antibiotic into the environment, while 32.4% kept them in their homes, 17.6% throw them to the toilet/latrines, 8.1% burned it, 6.8% tossed out in the trash and 2% didn't know/didn't answer.

Herd owners were also asked about their own antibiotic consumption. Out of 200 farmers, 55.5% (n = 111) had taken antibiotics during the last twelve months, while 39% (n = 78) did not and 5.5% (n = 11) didn't know/didn't answer. Antibiotic use by farmers was significantly associated with the area of study, being their use higher in the north-western region compared to the western and south-western regions (χ^2 = 35.5, df = 4, p < 0.001). Herd owners' surveyed reported respiratory symptoms (nasal discharge, sinusitis and cough) as the most common reason of antibiotic intake (59/111; 53.2%), followed by pain (chest pain, back pain, joint pain and headache) (32/111; 28.8%), diagnosed infectious diseases (malaria, gonorrhoea, brucellosis and tuberculosis) (15/111; 13.5%), fever (15/111; 13.5%), injuries (8/111; 7.2%), general malaise (6/111; 5.4%) and gastrointestinal symptoms (vomiting and diarrhoea) (3/111; 2.7%). Penicillins (63.1%) and trimethoprim/sulfamethoxazole (12.6%) were the most frequent antibiotics used by farmers, followed by tetracyclines (1.8%), aminoglycosides (0.9%) and macrolides (0.9%). A total of 22.5% of them didn't know/didn't answer.

Among the herd owners who had consumed antibiotics, 16 (14.4%) recognized they did not consult a doctor before taking them. A statistical association was observed between doctor consultation and the level of education, with unschooled people showing the lowest level of doctor visits (χ^2=19.8, df=8, p=0.01). However, 66.7% of the farmers that have taken antibiotics recognized they could acquire antibiotics in drug stores without medical prescription. Although 84.7% of the farmers did not buy antibiotics unprescribed and a 1.8% didn't know/didn't answer, 13.5% of the herd owners admitted they had taken antibiotics prescribed by a veterinarian or animal health assistant intended for use in their herd, which was significantly higher in farmers with low level of educational attainment (χ^2 = 16.5, df = 8, p = 0.03).

4.1.4. Discussion

The control of antimicrobial resistance pandemics is an urgent global public health priority. Our study analysed and compared published and unpublished literature from Uganda, reporting AMR prevalence in *E. coli* isolates, using a One Health approach. Through interviews with a significant number of farmers, we aimed to scrutinize the patterns of antibiotic consumption by herd owners and their use in farmed animals in western rural areas.

Humans and domestic animals, which were the most studied populations in Uganda according to the literature reviewed, showed a high median resistance to penicillins and trimethoprim/sulfamethoxazole, which are the cheapest antibiotics available in Uganda (Joloba et al. 2001). Significant levels of resistance to erythromycin and tetracyclines were also observed. Our results are similar to studies in both, people and cattle from other African countries, where amoxicillin, tetracycline and trimethoprim/sulfamethoxazole were the most common resistant phenotypes reported (Mainda et al. 2015; Defrancesco et al. 2017; Azabo et al. 2022). Despite the low number of studies encountered, this review revealed the presence of AMR *E. coli* in wildlife and the environment, including not only commonly used antibiotics in Uganda, but also 3rd and 4th generation cephalosporins. The presence of extended spectrum beta-lactamases in wildlife were first reported in 2006 (Costa et al. 2006), and first detected in African wildlife in 2009 (Literak et al. 2009). Although antibiotic-producing bacteria occur naturally in the environment, antibiotics and their metabolites can also be introduced into the environment through anthropogenic sources, converting soil, water and other habitats in hotspots for antibiotic resistance gene transfer (Van Elsas and Bailey 2002). Inadequate treated wastes from humans and domestic animals are generally assumed to be the main sources of AMR in wildlife (Vittecoq et al. 2016). Current surveillance programs predominantly focus on AMR in humans and livestock, while the environmental and wildlife perspective is usually ignored. Despite an increase of AMR studies on the natural environment has been observed worldwide, including East-African countries, our review highlights the need to tackle AMR from a One Health perspective, as colonisation of wildlife and environmental sources by bacteria resistant to last-

generation antibacterial agents could have important implications for public, ecosystem and animal health (Guenther et al. 2011). Furthermore, mitigation strategies on the transmission of AMR from livestock or human populations to wildlife and the environment are needed, particularly in countries with low control measures on antibiotic use.

The present study has revealed the presence of publication bias, which can be attributed to several factors, such as the limited research infrastructure and resources in Uganda, leading to a scarcity of studies AMR in *E. coli*. Moreover, the challenging conditions and limited access to healthcare facilities in low-resource settings have imposed constraints on sample size and diversity, resulting in smaller studies that are more likely to remain unpublished (Laxminarayan et al. 2016; Sulis et al. 2021). Although the inclusion of grey literature was intended to address some of these biases, the lack of standardized reporting and indexing these sources could have further contributed to its underrepresentation, as relevant studies might not have been easily discoverable through conventional search methods. Despite these challenges, this study sheds light on the critical issue of AMR in Uganda and provides valuable insights for policymakers and stakeholders to devise targeted interventions and strategies to combat the rising threat of AMR in low-resource settings.

Regarding the survey performed in rural areas from western Uganda, the frequency of antibiotic use amongst livestock and farmers was high. Penicillin, sulphonamides, and tetracyclines were the most common antibiotics used by both herd owners and farmed animals, comparable to previous reports from rural settings in Uganda and Tanzania (Katakweba et al. 2012; Nayiga et al. 2020). Furthermore, our survey indicates that farmers did not use newer generation antimicrobials, probably due to unaffordability of the costs of second and third-line antibiotics or their unavailability in health care facilities or drug stores in these remote areas. This could contribute to greater morbidity in both, humans and animals (Laxminarayan and Heymann 2012).

Despite their common use, a high proportion of the herd owners, as revealed through our survey, demonstrated irrational antibiotic usage in both animals and humans. Some of the observed bad practices included self-medication, using antibiotics prescribed for their animals, employing antibiotics as prophylaxis and non-specific clinical symptoms such as pain, anorexia and weakness, disregarding withdrawal periods in farmed animals, and inappropriate antibiotic disposal. Some of these habits were significantly higher in farmers with low educational attainment. In developing countries, there is a lack of resources and infrastructure (WHO 2016). Unfortunately, the negative effects of AMR are amplified due to poor sanitation, contaminated food and water, overpopulation, and increased susceptibility to infections (Laxminarayan et al. 2013). People from LMICs, especially those in rural areas, are more exposed to infectious diseases and may be more susceptible due to malnutrition or immunodeficiency, thereby increasing the need of antimicrobial therapy (Laxminarayan et al. 2013). However, factors such as misinformation, lack of education, and inaccessibility to health care and diagnostic facilities expose individuals with low economic means to self-medication, lack of access to effective antibiotics, sub-inhibitory doses of antimicrobials due to inability to afford complete treatment courses, drug sharing with friends and neighbours, or use of low-quality or expired drugs (Okeke et al. 1999). Hence, antibiotic misuse and poverty are directly associated with antibiotic resistance's spread in LMICs (Okeke et al. 2005; Alividza et al. 2018; WHO 2021a). Furthermore, in Sub-Saharan African countries, non-prescribed dispensing of antibiotics in drug stores has been identified as one of the main contributing factors to the widespread misuse of antibiotics in communities (Belachew et al. 2021). Given the global threat of AMR, a similar study including the community, physicians and pharmacists is recommended to provide a broader picture of the status of antibiotic use and awareness of antimicrobial consumption in Uganda. There is an urgent need for guidelines and awareness about AMR to reduce self-medication and inappropriate antibiotic use in the country.

4.1.5. Conclusions

Despite AMR is one of the greatest global public health threats, little has been done to quantify the burden of resistance in Uganda, especially in domestic animals,

wildlife, and the environment. It is urgent to extend the One Health approach to minimize antimicrobial resistance emergence and spread, particularly in resource-constrained areas. Furthermore, our survey highlights the lack of knowledge and awareness of appropriate antibiotic stewardship in both human and veterinary medicine in rural areas. Implementing appropriate strategies that involve collective actions and educational training to encourage best practices among practitioners, veterinarians, farmers, policy makers and the community is needed to combat the increased morbidity and mortality related to AMR in Uganda.

Supplementary material Study 1

S1.4.1. Search syntax.

("Antimicrobial" OR "antibiotic") AND ("resistance" OR "resistant" OR "susceptibility" OR "susceptible") AND ("Escherichia coli" OR "E. coli") AND "Uganda"

S2.4.1. Questionnaire model about drug use by herd owners in Uganda. This questionnaire included a survey on antiparasitic and anti-inflammatory use for other study purposes.

OWNER'S PERSONAL INFORMATION

a) Name: _____

b) Phone number: _____

c) Sex:
□ Male □ Female

d) Year of birth: _____

e) Education:
□ None □ Secondary school
□ Primary school □ University
□ DK/DA

f) District / Village / Community / National Park: _____
_____

HERD INFORMATION

1) Herd size and animal species. C: Cattle; S: Sheep; G: Goat; P: Poultry; D: Dog; Ot: Others.
□ 2-5 animals: _____ □ 50-100 animals: _____
□ 6-10 animals: _____ □ 100-150 animals: _____
□ 11-20 animals: _____ □ 150-200 animals: _____
□ 21-50 animals: _____ □ >200 animals: _____

2) Does your herd share grazing areas with other herds?
□ Yes □ No □ DK/DA

3) Does your herd share grazing areas or water sources with wildlife?
□ Yes □ No □ DK/DA

DRUG KNOWLEDGE

4) Do you know what antibiotics are?
□ Yes □ No □ DK/DA

5) Do you know what antiparasitics are?
□ Yes □ No □ DK/DA

6) Do you know what anti-inflammatories are?
□ Yes □ No □ DK/DA

VETERINARY RECOMMENDATIONS

7) Do you consult a veterinarian/Animal Health Assistant before start giving a medicine to your herd?
□ Never □ Always
□ Sometimes □ DK/DA
Veterinarian's name and phone number: _____

(If question 7 answer is Never, go to question 9)

8) Do you follow the treatment period prescribed by your veterinarian/Animal Health Assistant?
□ Never □ Always
□ Sometimes □ DK/DA

9) Other than the veterinarian/Animal Health Assistant, who is allowed to administer medicines to your herd?
□ Owner □ Others: _____
□ Employees □ DK/DA
□ Family member

ANTIBIOTIC USE (HERD)

10) Do you administer antibiotics to some animal of your herd?
□ Yes □ No □ DK/DA

(If question 10 answer is No, go to question 18)

11) How many times have you administered antibiotics to some animal of your herd during the past 12 months?
_____ times

(If question 7 answer is 0, go to question 18)

12) Which was the reason to administer antibiotics and which ones have you administered (brand name)?
□ Pneumonia: _____
□ Mastitis: _____
□ Metritis: _____
□ Foot rot: _____
□ Injuries: _____
□ Enteritis: _____
□ Others (why and which antibiotics): _____
: _____
□ DK/DA

13) When you administer an antibiotic to your herd, do you wait the established period before slaughter?
□ Yes (specify period): _____ days □ No □ DK/DA

14) Do you maintain written records for antibiotic treatments?
□ Yes □ No □ DK/DA

15) Where do you release antibiotic residues?
□ Toilet □ Water/soil (environment) □ Others (specify): _____
□ Garbage □ Special container □ DK/DA

16) Do you give antibiotics to your herd to prevent diseases?
□ Never □ Always

□ Sometimes □ DK/DA

(If question 16 answer is Never or DK/DA, go to question 18)

17) Specify in which animals' species do you use antibiotics to prevent diseases:
□ Cattle □ Poultry
□ Sheep □ Dog
□ Goat □ Others: _____

ANTIPARASITIC USE (HERD)

18) Do you administer antiparasitics to some animal of your herd?
□ Yes □ No □ DK/DA

(If question 18 answer is No, go to question 22)

19) How many times have you administered antiparasitics to some animal of your herd during the past 12 months?
_____ times

20) Which was the reason to administer antiparasitics and which ones have you administered (brand name)?
1._____
2._____
3._____

21) When you administer an antiparasitic to an animal, do you treat the whole herd at the same time?
□ Yes □ No □ DK/DA

22) Do you use medicated feed with antiparasitics to treat your herd?
□ Yes □ No □ DK/DA

(If question 22 answer is No, go to question 24)

23) When you administer an antiparasitic to your herd, do you wait the established period before slaughter?
□ Yes (specify period): _____ days □ No □ DK/DA

24) Do you maintain written records for antiparasitic treatments?
□ Yes □ No □ DK/DA

25) Where do you release antiparasitic residues?
□ Toilet □ Water/soil (environment) □ Others (specify): _____
□ Garbage □ Special container □ DK/DA

ANTI-INFLAMMATORIES USE (HERD)

26) Do you administer anti-inflammatories to some animal of your herd?
□ Yes □ No □ DK/DA

(If question 26 answer is No, go to question 32).

27) How many times have you administered anti-inflammatories to some animal of your herd during the past 12 months?
_____ times

28) Which was the reason to administer anti-inflammatories and which ones have you administered (brand name)?
1._____
2._____
3._____

29) When you administer anti-inflammatories to your herd, do you wait the established period before slaughter?
□ Yes (specify period): _____ days □ No □ DK/DA

30) Do you maintain written records for anti-inflammatory treatments?
□ Yes □ No □ DK/DA

31) Where do you release anti-inflammatories residues?
□ Toilet □ Water/soil (environment) □ Others (specify): _____
□ Garbage □ Special container □ DK/DA

ANTIBIOTIC USE (HERD OWNER)

32) Have you ever taken antibiotics?
□ Yes □ No □ DK/DA

(If question 32 answer is No, go to question 39)

33) How many times have you consumed antibiotics during the past 12 months?
_____ times

34) Which was the reason to take antibiotics and which ones have you consumed (brand name)?
1._____
2._____
3._____

35) Do you consult a doctor before starting an antibiotic?
□ Yes □ No □ DK/DA

36) When you take an antibiotic, do you follow the treatment period prescribed by your doctor?
□ Never □ Always
□ Sometimes □ DK/DA

37) Can you acquire antibiotics in pharmacies without medical prescription?
□ Yes □ No □ DK/DA

38) Have you ever taken antibiotics prescribed by a veterinarian/Animal Health Assistant to your herd?
□ Yes □ No □ DK/DA

ANTIPARASITIC USE (HERD OWNER)

39) Have you ever taken antiparasitics?
□ Yes □ DK/DA
□ No

(If question 39 answer is No, go to question 46)

40) How many times have you consumed antiparasitics during the past 12 months?
_____ times

41) Which was the reason to take antiparasitics and which ones have you consumed (brand name)?
1._____
2._____
3._____

42) Do you consult a doctor before starting an antiparasitic?
□ Yes □ No □ DK/DA

43) When you take an antiparasitic, do you follow the treatment period prescribed by your doctor?
□ Never □ Always
□ Sometimes □ DK/DA

44) Can you acquire antiparasitics in pharmacies without medical prescription?
□ Yes □ No □ DK/DA

45) Have you ever taken antiparasitics prescribed by a veterinarian/Animal Health Assistant to your herd?
□ Yes □ No □ DK/DA

ANTI-INFLAMMATORIES USE (HERD OWNER)

46) Have you ever taken anti-inflammatories?
□ Yes □ No □ DK/DA

(If question 46 answer is No, end the survey)

47) How many times have you consumed anti-inflammatories during the past 12 months?
_____ times

48) Which was the reason to take anti-inflammatories and which ones have you consumed (brand name)?
1._____
2._____
3._____

49) Do you consult a doctor before starting anti-inflammatories?
□ Yes □ No □ DK/DA

50) When you take anti-inflammatories, do you follow the treatment period prescribed by your doctor?
□ Never □ Always
□ Sometimes □ DK/DA

51) Can you acquire anti-inflammatories in pharmacies without medical prescription?
□ Yes □ No □ DK/DA

52) Have you ever taken anti-inflammatories prescribed by a veterinarian/Animal Health Assistant to your herd?
□ Yes □ No □ DK/DA

Observations: _____

Table S3.4.1. Median resistance and interquartile range of *E. coli* isolates from the different study subjects to all tested antibiotics.

Antibiotics	Humans (Number of isolates) Median (IQR)	Livestock (Number of isolates) Median (IQR)	Wildlife (Number of isolates) Median (IQR)	Environment (Number of isolates) Median (IQR)
Amikacin	(845) 5.8 (0-21.2)	(713) 12.5 (6.3-18.8)	(39) 0 (0-4.5)	NA
Amoxicillin	(1125) 86.7 (57.4-100)	(703) 40 (9-75.8)	(105) 0 (0-4)	(59) 43.5 (31.8-65.5)
Amoxicillin/clavulanate	(2521) 61.1 (40-83.5)	(490) 26.2 (0.6-62.8)	NA	(33) 15.2 (15.2-15.2)
Ampicillin	(3161) 93.3 (66.7-100)	(2321) 47.5 (26.5-80)	(943) 22.9 (2.5-40)	(282) 63 (60-90.9)
Ampicillin/sulbactam	(55) 56 (56-56)	NA	(72) 11 (11-11)	NA
Azithromycin	(182) 68.7 (34.4-73.4)	(537) 0 (0-0)	NA	NA
Aztreonam	(387) 28.4 (16.5-41.5)	(344) 13.5 (4.8-23)	(27) 57.8 (36.6-68.3)	NA
Cefalexin	(203) 82 (45.4-83.2)	(687) 0 (0-0)	NA	NA
Cefazolin	(404) 69.7 (31.1-98.5)	(903) 8.7 (4.1-89.9)	(48) 28.6 (28.6-28.6)	NA
Cefepime	(1575) 41.5 (17.1-67)	(949) 3.5 (0-28.8)	(9) 100 (100-100)	(149) 0 (0-0)
Cefixime	(1407) 50 (37.2-90.5)	(736) 7 (3.5-28)	NA	NA
Cefotaxime	(246) 51.6 (11-61)	(863) 0 (0-0)	NA	(168) 0 (0-0)
Cefotaxime/clavulanate	(87) 4.8 (0-34.6)	(14) 7.1 (7.1-7.1)	NA	(119) 0 (0-0)
Cefoxitin	(479) 27.6 (10.3-81.8)	(863) 0 (0-0)	NA	(149) 14.5 (14.5-14.5)
Cefpirome	(590) 67 (35-72)	(680) 43 (39.5-46.5)	NA	NA
Ceftazidime	(1227) 56.7 (5.3-73.4)	(822) 0 (0-14.3)	(9) 100 (100-100)	(100) 100 (100-100)
Ceftazidime/avibactam	(25) 64 (64-64)	NA	NA	NA
Ceftazidime/clavulanate	(40) 45 (45-45)	NA	NA	NA
Ceftiofur	(1434) 47.4 (13.8-77.5)	(706) 1.9 (0-36)	(48) 0 (0-25)	NA
Ceftriaxone	(1104) 36.4 (0-64.5)	(1263) 0 (0-0)	(741) 0 (0-0)	(81) 23.2 (11.6-34.7)
Cefuroxime	(1288) 49.8 (15.8-89.6)	(916) 12.5 (1-19.8)	(66) 6.3 (3.1-7.8)	(176) 61.2 (41.7-80.6)
Cephalothin	(820) 76.8 (37.4-91.2)	(1341) 27 (18.4-41.7)	(778) 12.7 (10.3-18.8)	NA
Chloramphenicol	(2323) 48.3 (29.5-74.5)	(2252) 8 (2.7-32.2)	(846) 0.4 (0-2.7)	(109) 34.9 (20.5-49.2)
Ciprofloxacin	(3851) 30 (14.6-50)	(2345) 0.5 (0-11.4)	(904) 0 (0-0)	(189) 17 (10.5-37.5)
Clindamycin	(91) 100 (100-100)	(563) 94 (91-97)	(39) 100 (100-100)	NA

Cloxacillin	(104) 100 (47.4-100)	(157) 100 (100-100)	NA	NA
Colistin	(84) 16.3 (10.7-22)	(282) 0 (0-1.9)	NA	NA
Doxycycline	(576) 16 (7.5-24.6)	(590) 0 (0-2.6)	(727) 0 (0-0.1)	NA
Ertapenem	(431) 15.5 (8.3-43)	(730) 15 (13.5-16.5)	NA	NA
Erythromycin	(576) 76.9 (28.6-100)	(586) 84.5 (57.3-98)	(130) 75.8 (17-82)	(46) 37 (37-37)
Fosfomycin	(80) 7.5 (7.3-7.8)	NA	NA	NA
Gentamicin	(3588) 26.8 (5.5-52)	(2355) 0 (0-11.5)	(943) 0 (0-0)	(268) 18.9 (14.8-33.1)
Imipenem	(2120) 4.5 (0-16.7)	(778) 6.5 (3.8-8.9)	(9) 0 (0-0)	NA
Kanamycin	(100) 13 (6.5-41.5)	(843) 0 (0-13.4)	(39) 4.5 (0-4.5)	NA
Levofloxacin	(595) 20 (15.4-56)	(680) 1 (0.5-1.5)	NA	NA
Lincomycin	(98) 4.3 (2.2-6.5)	NA	NA	NA
Mecillinam	(55) 78 (78-78)	NA	NA	NA
Meropenem	(876) 3.4 (0-11)	(938) 0 (0-0)	(66) 0 (0-0)	NA
Metronidazole	(183) 90 (77.5-100)	NA	NA	NA
Minocycline	(74) 100 (100-100)	(182) 0.8 (0-6.2)	NA	NA
Moxifloxacin	(96) 18.1 (9-27.1)	NA	NA	NA
Nalidixic acid	(1847) 59.4 (28-83.3)	(2016) 10.4 (0-28.3)	(754) 0 (0-3)	(358) 26.4 (18.6-43.5)
Neomycin	(137) 1 (0.5-1.5)	(741) 0 (0-22.1)	(53) 0 (0-0)	NA
Netilmicin	(8) 100 (100-100)	NA	NA	NA
Nitrofurantoin	(1278) 20 (9.1-30.8)	(270) 8 (6-14)	(72) 20.5 (20.5-20.5)	(100) 30 (30-30)
Ofloxacin	(533) 72.1 (58.3-86)	(637) 0 (0-0)	NA	NA
Oxacillin	(121) 79.1 (43.8-100)	NA	NA	NA
Penicillin	(677) 87.1 (84-93.1)	(250) 51 (2.5-98)	NA	NA
Piperacillin	(642) 11.7 (0-79.3)	(278) 0 (0-0)	(39) 0 (0-0)	(76) 30.6 (30.6-30.6)
Piperacillin/tazobactam	(72) 20 (20-20)	NA	NA	NA
Polymyxin B	NA	(152) 2 (2-2)	NA	NA
Rifampicin	(127) 0 (0-29)	NA	NA	NA
Streptomycin	(944) 43 (29.8-85.5)	(1448) 22 (9.2-44.9)	(846) 1.9 (0-5.5)	NA
Sulfamethoxazole	(87)	(278)	NA	(149)

	59.4 (59.4-59.4)	80 (70-84.3)		55 (55-55)
Sulfizoxazole	(262)	(457)	(143)	NA
	86.5 (65.8-98.4)	12.5 (10.3-14.8)	14 (8-20.4)	
Tazobactam	(365)	NA	NA	NA
	56 (41.2-63.4)			
Temocillin	(166)	NA	NA	NA
	66.6 (54-79.1)			
Tetracycline	(2351)	(2615)	(943)	(417)
	70 (37.5-95)	36 (17.6-73)	0.8 (0-11.2)	30.2 (14.6-38.4)
Tigecycline	(80)	(52)	NA	NA
	23 (13.5-32.5)	0 (0-0)		
Tobramycin	(87)	NA	NA	NA
	50 (47-75)			
Trimethoprim	(280)	(557)	(66)	NA
	75 (30-96)	17.2 (17.1-17.3)	7.5 (4.8-16.8)	
Trimethoprim/sulfamethoxazole	(3261)	(2123)	(872)	(84)
	87.5 (69.9-98)	32.5 (12-60)	4.3 (0.4-15.2)	46.1 (44.1-48)
Vancomycin	(213)	(542)	NA	(84)
	0 (0-48.6)	100 (100-100)		94.7 (92-97.3)

4.2. Study 2:

Foodborne pathogens at the livestock-wildlife-human interface in rural western Uganda

EcoHealth (10.1007/s10393-023-01639-6)

Abstract

Foodborne pathogens are an important cause of morbidity and mortality worldwide. To assess the presence of *Salmonella*, *Campylobacter* and *Arcobacter* spp. in livestock (n = 255), wildlife (n = 209), and humans (n = 5) from different regions across western Uganda, 479 faecal samples were tested by PCR. *Salmonella* and *Campylobacter* spp. were more frequently detected in livestock (5.1% and 23.5%, respectively) compared to wildlife (1.9% and 16.8%, respectively). All samples analysed were negative for *Arcobacter* spp. Wildlife from remote areas showed lower *Salmonella* and *Campylobacter* spp. occurrence than in areas where interactions with livestock are common, suggesting that spill-over may exist from livestock or humans. Further studies are needed to better understand the transmission dynamics of these pathogens at the wildlife-livestock-human interface in western Uganda.

4.2.1. Introduction

Foodborne pathogens are an important cause of morbidity and mortality worldwide, threatening both human health and economic growth. *Salmonella* and *Campylobacter* spp. are important foodborne pathogens in developing countries, which are transmitted to humans through food contamination, but also by direct contact with animals, including wildlife, and by contaminated environment (Havelaar et al. 2015). In the recent years, *Arcobacter* species (Family *Campylobacteriaceae*) have been identified as emerging foodborne pathogens throughout the world in animals and humans (Ramees et al. 2017).

Despite important advances in the understanding of enteric pathogens' transmission, the presence of these infectious agents and their transmission dynamics in the African continent is still poorly understood (Penakalapati et al. 2017). In East-Africa, livestock is considered a common source of *Salmonella* and *Campylobacter* spp. to people (Thomas et al., 2020). However, the environmental reservoirs (soil and water sources) and transmission pathways of these pathogens among livestock, wildlife, and humans are poorly understood. Moreover, there are no studies in East Africa that assessed the presence and transmission of *Arcobacter* spp.

The aims of this study are to compare the presence of *Salmonella* spp., *Campylobacter* spp. and *Arcobacter* spp. among domestic animals, wildlife, and humans, in six rural areas from western Uganda with different levels of interaction.

4.2.2. Materials and Methods

Faecal samples were collected between 2015 and 2018 from six areas of western Uganda (Fig. 1.4.2), namely: Mgahinga Gorilla National Park (MGNP; 1°22′10″S, 29°38′25″E), Murchison Falls National Park (MFNP; 2°11′15″N, 31°46′53″E), Budongo Central Forest Reserve (BCFR; 1°43′27″N, 31°32′45″E), Queen Elizabeth National Park-Northern sector (QENP-N; 0°8′14″S, 30°02′28″E), Queen Elizabeth National Park-Southern sector (QENP-S; 0°33'00"S, 29°53'00"E), and Hoima District (HD; 1°25′55″N, 31°21′09″E). The areas sampled in this study are part of the

Albertine Rift and represent different levels of interaction between livestock and wildlife, classified as high, medium, low and no interaction (Annex 1). We defined high interaction when there is high density of human population in the area with settlements surrounding the park and there are no physical barriers separating protected areas (MFNP southern sector and QENP-N); medium interaction when density of human population is high but there are permeable barriers or when density of human population is medium to low but interactions occur regularly (MGNP, BCFR); low interaction when human population density is low but there are no physical barriers separating the protected area (QENP-S); no interaction when there is only presence of livestock (HD) or wildlife (MFNP northern sector) within or around the sampling area.

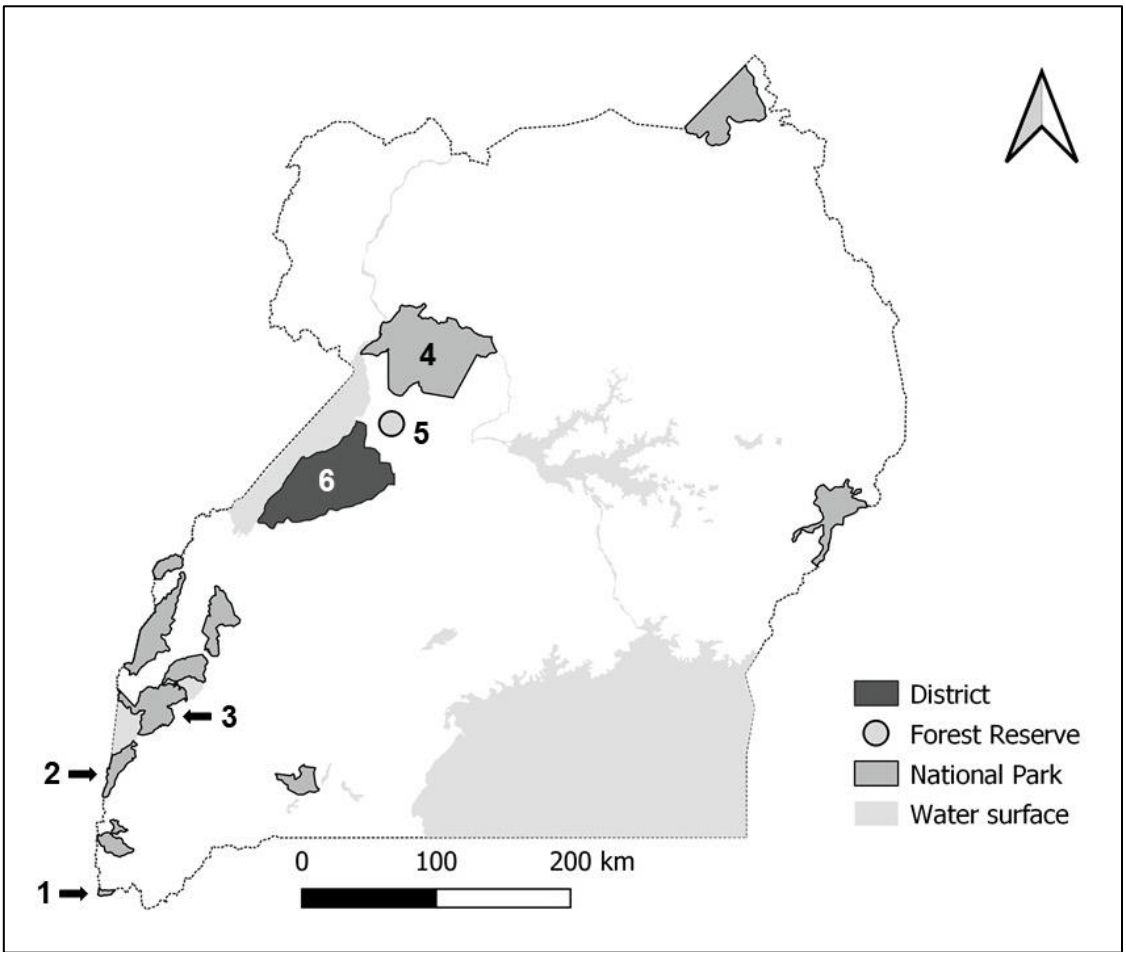


Figure 1.4.2. Natural protected areas in western Uganda and study sites: 1. Mgahinga National Park; 2. Queen Elizabeth National Park-Southern sector; 3. Queen Elizabeth National Park-Northern sector; 4. Murchison Falls National Park; 5. Budongo Central Forest Reserve; 6. Hoima District.

All the field and laboratorial procedures were authorized by the Uganda Wildlife Authority (UWA) (EDO/35/01 and COD/96/02), the Higher Degrees Research Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (SBLS/HDRC/20/011), and the School of Biomedical Sciences Research and Ethics Committee (SBS-REC-824). Overall, 479 fresh faecal samples were collected from domestic, wild animals, and humans: 139 cattle, 59 goat, 30 sheep, 27 chicken, 118 African buffalo (*Syncerus caffer*), 53 Uganda kob (*Kobus kob thomasi*), 19 African elephant (*Loxodonta africana*), 19 Anubis baboon (*Papio anubis*), and 15 humans (Table 1.4.2). Faecal samples were obtained by purposive sampling in both wildlife and livestock, selecting sampling points representative of each study area and rural communities surrounding natural protected areas. Finally, human stools were collected in QENP-N from herd owners who voluntarily provided the samples. Fresh faecal samples were collected immediately after defecation, using sterile sample bags. All samples were maintained at 4-8ºC for 24-48h during transportation and were stored at -20ºC at the laboratory until analyses.

Table 1.4.2. Samples and results from the molecular detection of *Salmonella* and *Campylobacter* spp., in faeces from animals and humans from western Uganda, 2015-2018. *Arcobacter* spp. was not detected.

	N	*Salmonella* spp.	*Campylobacter* spp.
Mgahinga NP (MGNP)	**114**	**3 (2.6%)**	**40 (35.1%)**
Cattle	35	2 (5.7%)	9 (25.7%)
Chicken	9	0	0
Goat	23	0	8 (34.8%)
Sheep	25	1 (4%)	15 (60%)
Buffalo	22	0	8 (36.4%)
Murchison Falls NP (MFNP)	**102**	**1 (0.9%)**	**12 (11.8%)**
Cattle	30	0	1 (3.3%)
Chicken	12	0	0
Goat	20	1 (5%)	9 (45%)
Sheep	5	0	1 (20%)
Buffalo	20	0	1 (5%)
Uganda kob	15	0	0
Queen Elizabeth NP N) (QENP-N)	**176**	**9 (5.1%)**	**38 (21.6%)**
Cattle	52	0	6 (11.5%)
Chicken	6	0	1 (16.7%)
Goat	16	4 (25%)	5 (31.2%)
Buffalo	41	1 (2.4%)	7 (17.1%)
Elephant	12	0	0
Uganda kob	34	3 (8.8%)	15 (44.1%)
Human	15	1 (6.7)	4 (26.7%)
Queen Elizabeth NP (S) (QENP-S)	**46**	**0**	**1 (2.2%)**
Buffalo	35	0	0

Elephant	7	0	0
Uganda kob	4	0	1 (25%)
Hoima District (HD)	**22**	**5 (22.7%)**	**5 (22.7%)**
Cattle	22	5 (22.7%)	5 (22.7%)
Budongo Central Forest Reserve (BCFR)	**19**	**0**	**3 (15.8%)**
Anubis baboon	19	0	3 (15.8%)
TOTAL	**479**	**18 (3.8%)**	**99 (20.7%)**

DNA extraction of stool specimens was performed using QIAamp DNA Stool Mini Kit® (QIAGEN, Germany), according to the manufacturer's instructions. The presence of *Salmonella*, *Campylobacter* and *Arcobacter* spp. was determined by PCR, using previously reported primers and PCR conditions (Table 2.4.2). *Campylobacter*-positive samples were screened to identify *Campylobacter* species using species-specific primers (Table 2.4.2). *Salmonella*-positive samples were further screened to identify serovars Enteritidis and Typhimurium (Table 2.4.2).

To assess differences of *Salmonella*, *Campylobacter* and *Arcobacter* spp. detection between study areas and animal species, and pathogen co-occurrence, Pearson's Chi-squared test χ^2 (with Yates' correction when appropriate) was performed using the R software (R Core Team 2022). Bonferroni post-hoc tests were also conducted to assess the relationship between study areas and *Campylobacter* and *Salmonella* spp. detection. *p*-Values lower than 0.05 were considered statistically significant. The map was done with QGIS (QGIS Development Team 2022).

4.2.3. Results

The sample prevalence was higher in livestock (*Salmonella* spp. 5.1%, $CI_{95\%}$ 2.7-8.6; and *Campylobacter* spp. 23.5%, $CI_{95\%}$ 18.5-29.2) compared with wildlife (*Salmonella* spp. 1.9%, $CI_{95\%}$ 0.5-4.8; and *Campylobacter* spp. 16.8%, $CI_{95\%}$ 12.0-22.5), but differences were not statistically significant. However, *Campylobacter*'s detection in wildlife samples was significantly lower in MFNP northern sector than in areas categorized with high wildlife-livestock interaction ($\chi^2 = 33.1$, df = 3, $p = 0.03$).

Table 2.4.2. Primer sequences used in this study. [a] Forward primers; [b] Reverse primer

Species	Primer	Nucleotide sequence (5' to 3')	Fragment length (bp)	Target gene
Salmonella spp.	invA-F[a]	GTG AAA TTA TCG CCA CGT TCG GGC AA	284	*invA* (Rahn et al. 1992)
	invA-R[b]	TCA TCG CAC CGT CAA AGG AAC C		*invA* (Rahn et al. 1992)
Salmonella Enteritidis	sefA-F[a]	TGT GCG AAT GCT AAT AGT TG	526	*sefABC* (Thomas 1994)
	sefA-R[b]	CTG CTG AAC GTA GAA GGT CG		*sefABC* (Thomas 1994)
Salmonella Enteritidis and Typhimurium	fljBA-F[a]	CTG GCG ACG ATC TGT CGA TG	250 and 1000	*fljB-fljA* (Echeita et al. 2001)
	fljBA-R[b]	GCG GTA TAC AGT GAA TTC AC		*fljB-fljA* (Echeita et al. 2001)
Campylobacter spp.	C412F[a]	GGA TGA CAC TTT TCG GAG C	857	16S rRNA (Linton et al. 1996)
	campR2[b]	GGC TTC ATG CTC TCG AGT T		16S rRNA (Katzav et al. 2008)
C. jejuni	lpxA-J[a]	ACA ACT TGG TGA CGA TGT TGT A	331	lpxA (Klena et al. 2004)
C. coli	lpxA-C[a]	AGA CAA ATA AGA GAG AAT CAG	391	lpxA (Klena et al. 2004)
C. lari	lpxA-L[a]	TRC CAA ATG TTA AAA TAG GCG A	233	lpxA (Klena et al. 2004)
C. upsaliensis	lpxA-C.Ups[a]	AAG TCG TAT ATT TTC YTA CGC TTG TGT G	206	lpxA (Klena et al. 2004)
C. jejuni, C. coli, C. lari, C. upsaliensis	lpxARKK2m[b]	CAA TCA TGD GCD ATA TGA SAA TAH GCC AT		lpxA (Klena et al. 2004)
C. lanienae	CLAN76F[a]	GTA AGA GCT TGC TCT TAT GAG	920	16S rDNA (Logan et al. 2000)
	CLAN1021R[b]	TCT TAT CTC TAA GAG GTT CTT A		16S rDNA (Logan et al. 2000)
C. fetus	JH0087[a]	TGA GGC TGT TAC AAG CGA GTT A	100	*cpn60* (Chaban et al. 2009)
	JH0088[b]	TGA GCT ATC GCT ATT TGC TGA A		*cpn60* (Chaban et al. 2009)
C. hyointestinalis	ChII-spBU8[a]	CCT AGT AGC GCT ACT TAG	215	*cdt* (Kamei et al. 2016)
	ChII-spBR8[b]	CAA ATA CCC TAC CTG TAG C		*cdt* (Kamei et al. 2016)
Arcobacter spp.	Arco I[a]	AGA GAT TAG CCT GTA TTG TAT C	1223	16S rDNA (Harmon and Wesley 1997)
	Arco II[b]	TAG CAT CCC CGC TTC GAA TGA		16S rDNA (Harmon and Wesley 1997)

Salmonella spp. was detected in 18 samples (3.8%; $CI_{95\%}$: 2.2-5.9) and was not associated with any specific host species (Table 1.4.1). However, HD had a significant highest percentage of samples positive to *Salmonella* spp. (22.7%; $CI_{95\%}$: 7.8-45.4) compared to areas with medium and low interaction ($\chi^2 = 27.9$, df = 5, $p < 0.001$). Among the *Salmonella*-positive samples, serovars could only be identified in two samples as *Salmonella enterica* serovar Typhimurium, belonging to one cow and a herder from HD and QENP-N, respectively.

Campylobacter spp. was detected in 99 samples (20.7%; $CI_{95\%}$ 17.1-24.6) from all the domestic and wild species sampled, except elephants. Significant higher sample frequency was found in sheep (53.3%), goats (37.3%), kobs (30.2%) and humans (26.7%), compared to Anubis baboon (15.7%), cattle (15.1%), buffalo (13.6%) and chicken (3.7%) ($\chi^2 = 48.9$, df = 8, $p < 0.001$). *Campylobacter* spp. detection was significantly different between study areas ($\chi^2 = 29.4$, df = 5, $p < 0.001$), being higher in MGNP (35.1%; $CI_{95\%}$: 26.4-44.6) (Table 1.4.1). Five *Campylobacter* species were identified from 17 *Campylobacter*-positive samples (n = 99): *C. jejuni* (6 cattle and 1 sheep), *C. lari* (1 cattle), *C. lanienae* (1 cattle and 6 goat), *C. fetus* (1 goat) and *C. hyointestinalis* (1 cattle).

Sixteen of the 18 *Salmonella*-positive samples were also positive to *Campylobacter* spp., but co-occurrence of both pathogens was not statistically significant. All samples analysed were negative for *Arcobacter* spp.

4.2.4. Discussion

The present study assesses a wide geographic distribution of both *Salmonella* and *Campylobacter* spp. in wildlife and livestock from remote areas in western Uganda at the Albertine Rift Valley. Agricultural changes and the expansion of livestock production, especially in proximity to wildlife habitats, creates attraction points for both domestic animals and wildlife, facilitating pathogens' spill-over and zoonotic disease emergence at the livestock-wildlife-human interface (Caron et al. 2013). In this sense, the study areas that had a more likely interaction between livestock and wildlife (MGNP, MFNP and QENP-N) also had a higher frequency of *Salmonella* and

Campylobacter spp. in wildlife. This result suggests that livestock might be the primary reservoir host for these foodborne pathogens, but also highlights that spill-over into wildlife probably occurs around natural protected areas in western Uganda.

The detection of *Salmonella* spp. in human wastewater treatment plants, slaughterhouses and farms has been widely reported in Uganda (Ikwap et al. 2014; Afema et al. 2016). The frequency detection of *Salmonella* in livestock samples from Uganda was lower compared with previous data on different African countries that ranged from 3.4 to 13.9% (Thomas et al. 2020). This may be caused by different factors such as differences in herd size (<50 individuals in our study), different contacts between herds or local differences in transmission due to presence and distribution of aggregation points such as water sources or grazing areas (Caron et al. 2013). The finding of the serovar Typhimurium in the present study, in one cattle and one human sample, together with the reports in pigs (Ikwap et al. 2014), and amongst severely malnourished children with bacteraemia in Uganda (Bachou et al. 2006), indicates a widespread occurrence of this zoonotic pathogen in Uganda and a potential public health threat in this country.

The frequency of *Campylobacter* spp.-positive samples we have found has also been reported in other areas within East-Africa (Hlashwayo et al. 2021), and its detection in multiple species supports the multi-host nature of this bacterial organism. The high proportion of *Campylobacter* spp.-positive samples in Uganda kob from QENP-N (n = 16/53) has not previously been reported in any other wild antelope in Africa. Despite sample size was low for stool samples from people (n = 15), *Campylobacter* spp. was more frequently detected (26.7%) than the pooled prevalence of this pathogen in West (10.7%) and South Africa (8.5%) where similar sample sizes were used (Hlashwayo et al. 2021). These results suggest that in rural areas, not only livestock but also wildlife, may harbour these zoonotic enteric bacteria, and control measures should be focused on both niches to mitigate the risk of transmission.

Although the most prevalent *Campylobacter* species detected in livestock from sub-Saharan Africa are *C. jejuni* and *C. coli*, especially in cattle and poultry (Hlashwayo et al. 2020), in this study *C. jejuni* has only been identified in cattle and sheep at a low frequency. Similar to other African regions, *C. lari* and *C. hyointestinalis* were detected in cattle (Hlashwayo et al. 2020). Despite the primary reservoirs of *C. fetus* are cattle and sheep, it has been described in domestic goats from Sudan (Elbrissi et al. 2017). In addition, the present study extends the known distribution range of *C. lanienae* within the African continent.

All samples were negative to *Arcobacter* spp. despite this pathogen has been detected in animal and human faecal samples from other sub-Saharan African countries (Adesiji et al. 2011). Additional studies are needed to clarify its distribution and pathogenic role in East-Africa.

4.2.5. Conclusions

In conclusion, *Salmonella* and *Campylobacter* spp. detection was higher in livestock than wildlife from western Uganda. Moreover, the frequency of detection of these bacteria in wildlife from remote areas was low compared to areas with high livestock-wildlife-human interaction. Molecular epidemiology and metagenomic studies are needed to better understand the transmission dynamics of these foodborne pathogens in wild hosts and their interface with livestock and humans in western Uganda.

4.3. Study 3:

Widespread of CTX-M variants among *Escherichia coli* and *Klebsiella pneumoniae* lineages of humans, livestock and wildlife origin from western Uganda

Abstract

Antimicrobial resistance (AMR) is considered one of the major global public health challenges. The burden is disproportionately higher in low- and middle-income countries (LMICs), where antimicrobials are widely available and inappropriately overused, leading to the emergence and spread of resistance. The expanding human population and increasing habitat fragmentation of natural areas force wildlife into greater contact with humans and livestock, facilitating AMR transmission. The aim of this study was to determine the occurrence of cephalosporin resistant Enterobacterales as an indicator of the burden of resistance across the human, animal and environmental interface in rural areas from western Uganda. In this study, we investigated the occurrence of extended spectrum cephalosporin (ESC) resistant *E. coli* and *Klebsiella pneumoniae* in samples from human (n = 65), domestic (n = 137) and wild animals (n = 301), and environmental sources (n = 52) in rural areas with varying levels of human-animal interaction. Phenotypic resistance was assessed using both, Kirby-Bauer disk diffusion method and minimal inhibitory concentration for several antimicrobials, to assess the resistance profiles of ESC-resistant *E. coli* and *K. pneumoniae* isolates. Whole genome sequencing was performed in ESC-resistant isolates (n = 62) to explore the lineages and resistant genes, and further compared with previously published sequences from Ugandan. We observed a high prevalence of the CTX-M-15 gene, in addition to the detection of other resistance genes such as CTX-M-27 and OXA-1. Multiple sequence types (STs) were detected for both *E. coli* and *K. pneumoniae*, with low frequencies of the predominant extraintestinal pathogenic *E. coli* (ExPEC) lineages described worldwide. The wide occurrence of ESC resistant bacteria in community settings and wildlife highlights their capacity to spread between anthropogenic and natural ecosystems, creating hotspots that facilitate the spread and evolution of AMR genes. An expanded One Health approach is needed to fully understand AMR transmission dynamics in rural areas from western Uganda.

4.3.1. Introduction

Antimicrobial resistance (AMR) is considered one of the major global public health challenges, threatening both, human and animal health (WHO 2014; Stapleton et al. 2017; Morrison and Zembower 2020). Currently, AMR causes approximately 700,000 deaths annually, but the burden of deaths associated to AMR could reach up to 10 million lives each year by 2050 (O'Neill 2016). The burden is disproportionately higher in low- and middle-income countries (LMICs), where antimicrobials are widely available and inappropriately overused, leading to the emergence and spread of resistance (Sulis et al. 2021).

The Enterobacterales is an order of Gram-negative bacteria that naturally inhabit the gastrointestinal tract of warm-blooded animals, but they also cause a wide range of diseases such as urinary tract infections (UTIs), gastroenteritis and septicaemia (Palmeira et al. 2021). Beta-lactams, including penicillins, cephalosporins, monobactams and carbapenems, are the most widely used antimicrobial agents worldwide to treat infections caused by these pathogens. Although overall consumption remains higher in high-income countries (HICs), antibiotic consumption rates are increasing in LMICs and are expected to surpass those in HICs due to the greater burden of infectious diseases (Klein et al. 2018). Since the early 2000s, there has been an increased trend of Enterobacterales, especially *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae*, resistant to third-generation cephalosporins, mainly due to the global spread of extended-spectrum beta-lactamases (ESBLs) (Pitout et al. 2005; Paterson 2006). Furthermore, these ESBL-producing organisms are often multi-drug resistant (MDR), leading to higher mortality rates, longer hospital stays, and increased costs compared to antibiotic-susceptible strains (Abban et al. 2023). CTX-M is the most disseminated ESBL type and has been widely reported in humans, domestic and wild animals, and environmental sources worldwide (Hassen et al. 2020; Tinoco Torres et al. 2022; Nakano et al. 2023; Guitart-Matas et al. 2024).

The expanding human population and increasing habitat fragmentation of natural areas force wildlife into greater contact with humans and livestock (Jones et al.

2008; Muylaert et al. 2021). This increased interaction substantially contributes to the spread of pathogens (Jori et al. 2021). Despite the substantial progress made in the study of the epidemiology of multi-host infections, the role of wildlife reservoirs in the dissemination of AMR in the natural environment is poorly understood (Greig et al. 2015; Huijbers et al. 2015). Wildlife often comes into contact with contaminated water sources and soil, which can harbour resistant bacteria from agricultural runoff and human waste (Vittecoq et al. 2016). Therefore, monitoring the burden of AMR bacteria in wildlife serves as a valuable tool to assess human impacts on the environment, and will provide insights into the pathways of resistance transmission to develop more effective strategies to mitigate the spread of AMR.

The aim of this study was to determine the occurrence of cephalosporin resistant Enterobacterales as an indicator of the burden of resistance across the human, animal and environmental interface. Isolates collected from water and faecal samples of livestock, wildlife and humans in different natural areas and neighbouring villages of western Uganda have been sequenced and compared to previously published sequences of human, and domestic and wild animal origin sequences from Uganda. The phylogenetic reconstruction intends to provide insights into the distribution of antimicrobial resistance lineages across the One Health settings to develop coordinated strategies to reduce the transmission of resistance.

4.3.2. Materials and Methods

4.3.2.1. Study area

This study was conducted from 2017 to 2019 in six areas of western Uganda (Fig. 1.4.3): Murchison Falls National Park-Northern sector (MFNP-N; 2°18′28″N, 31°33′40″E), Murchison Falls National Park-Southern sector (MFNP-S; 2°11′15″N, 31°46′53″E), Budongo Central Forest Reserve (BCFR; 1°43′27″N, 31°32′45″E), Queen Elizabeth National Park-Northern sector (QENP-N; 0°8′14″S, 30°02′28″E), Queen Elizabeth National Park-Southern sector (QENP-S; 0°33'00"S, 29°53'00"E), and Mgahinga Gorilla National Park (MGNP; 1°22′10″S, 29°38′25″E).

These areas are situated within the Albertine Rift and exhibit different degrees of interaction between livestock, wildlife, and humans (Annex 1). Briefly, MFNP-N and QENP-S are inhabited by wildlife, without any livestock presence. Then, interspecies contact is rare. In contrast, in MFNP-S and QENP-N domestic animals and human settlements are present, facilitating indirect interactions with wildlife. Similarly, in BCFR, contact between chimpanzees, baboons, domestic animals and humans is common since these primates forage outside the forest from croplands. Although a stone wall separates MGNP from inhabited areas, this wall is permeable for wildlife, facilitating indirect interactions with other species. Nevertheless, all these areas are constantly frequented by locals and tourists working or visiting the National Park (NP).

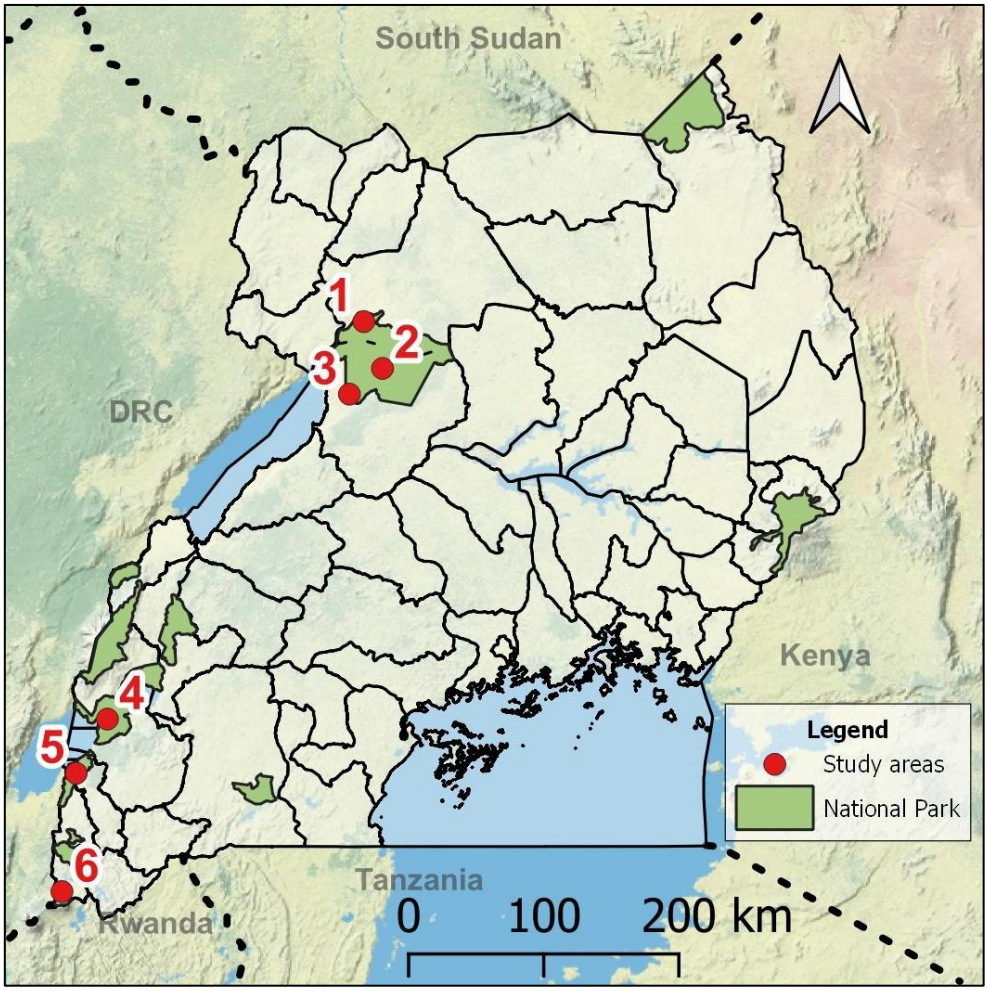


Figure 1.4.3. Map of Uganda and the areas of study: Murchison Falls NP-Northern sector (1), Murchison Falls NP-Southern sector (2), Budongo Forest (3), Queen Elizabeth NP-Northern sector (4), Queen Elizabeth NP-Southern sector (5) and Mgahinga Gorilla NP (6).

4.3.2.2. Sample collection

Overall, 52 environmental and 503 faecal samples from livestock (137), wildlife (301) and humans (65) were collected (Table 1.4.3). Human and livestock samples were collected from areas surrounding the NP previously mentioned. Faecal and water samples were collected aseptically using sterile plastic sample bags and BD Falcon tubes, respectively, and maintained at 4-8ºC until laboratorial analyses (<72h). All field and laboratorial procedures were approved by the Uganda Wildlife Authority (UWA) (EDO/35/01 and COD/96/02), the Higher Degrees Research Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (SBLS/HDRC/20/011), and the School of Biomedical Sciences Research and Ethics Committee (SBS-REC-824).

4.3.2.3. *Escherichia coli* isolation, identification and antimicrobial susceptibility testing

Faecal and water samples were collected for microbiological analysis. Water samples were filtered and resuspended in PBS prior to further processing. Faecal and water samples were inoculated onto both MacConkey agar (Oxoid, Basingstoke, United Kingdom) and MacConkey agar supplemented with 1 mg/L of ceftriaxone and incubated at 37ºC for 24 hours. One colony per positive sample morphologically compatible with *E. coli* or *Klebsiella* spp. was again subcultured on the same isolation agar. The inoculated agar plates were then incubated at 37 °C for 24 hours to obtain pure colonies. The colonies were biochemically identified, and isolates were stored at -80 ºC in brain heart infusion with 20% glycerol for further studies.

The Kirby-Bauer disk diffusion test (DDT) method was used to detect antibiotic susceptibility (Bauer et al. 1966). Six antibiotic disks were tested: ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), sulphonamide (240 µg) and tetracycline (30 µg). Minimal inhibitory concentration (MIC) was determined by microbroth dilution method (Sensititre®, ThermoFisher Scientific, Spain) following guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), in those isolates harbouring resistance to third-

generation cephalosporins obtained in the supplemented agar and confirmed by the Kirby-Bauer DDT method. Antimicrobials and concentrations tested were as follows: amikacin (4 to 128 µg/mL), ampicillin (1 to 32 µg/mL), azithromycin (2 to 64 µg/mL), cefotaxime (0.25 to 4 µg/mL), ceftazidime (0.25 to 8 µg/mL), ciprofloxacin (0.015 to 8 µg/mL), chloramphenicol (8 to 64 µg/mL), colistin (1 to 16 µg/mL), gentamicin (0.5 to 16 µg/mL), meropenem (0.03 to 16 µg/mL), nalidixic acid (4 to 64 µg/mL), sulfamethoxazole (8 to 512 µg/mL), tetracycline (2 to 32 µg/mL), tigecycline (0.25 to 8 µg/mL) and trimethoprim (0.25 to 16 µg/L). Isolates were considered wild type (susceptible) or non-wild type (resistant), based on epidemiological cut-off values (ECOFF) defined by EUCAST (https://www.eucast.org/).

4.3.2.4. Whole genome sequencing (WGS), *in silico* serotyping and identification of resistance profiles

Nucleic acid extraction of all extended-spectrum cephalosporin (ESC) resistant *E. coli* and *Klebsiella* spp. was extracted using the DNeasy Ultraclean Microbial Kit (Qiagen) following the manufacturer's instructions. Quality and concentration of the DNA were determined with the NanoDrop 2000 Spectrophotometer and a Qubit dsDNA BR Assay Kit (Fisher Scientific). Genomes were sequenced with the Illumina NovaSeq platform (Illumina Inc., San Diego, CA) at the Earlham Institute (2 × 250 bp paired-end chemistry). Sequencing reads were trimmed using Trimmomatic v0.39 with a four-base sliding window to cut when the average Phred quality scores dropped below 15 and reads shorter than 36 bp were excluded (Bolger et al. 2014). Draft genomes were generated *de novo* using the SPAdes v3.14.1 assembler performing a preliminary read error correction based on Hamming graphs and Bayesian subclustering and including the BWA mismatch corrector tool (Bankevich et al. 2012). QUAST v5.2.0 software was used to assess the quality of final assemblies (Gurevich et al. 2013).

Multilocus sequence typing (MLST) was determined *in silico* from assembled scaffolds against the PubMLST typing scheme of *E. coli* with the mlst v2.23.0 software (Jolley and Maiden 2010; Seemann 2022). Phylotypes were also analysed *in silico* using the Clermont PCR method of the ClermonTyping software (Beghain et

al. 2018). Resistance profiles were identified with Resfinder v4.3.0 using ResFinder and PointFinder databases for acquired genes and point mutations, respectively (Clausen et al. 2018; Bortolaia et al. 2020). Predicted location of beta-lactam resistance genes was determined from assemblies with VRprofile2 (M. Wang et al. 2022).

4.3.2.5. Phylogenetic analyses

To explore the phylogenetic relationship between the ESC resistant *E. coli* of this study and the assemblies of Uganda origin available at the Enterobase (https://enterobase.warwick.ac.uk/ ; last access 31/05/2024), phylogenetic analyses were performed by core genome alignments based on single nucleotide polymorphisms and insertions/deletions with the Snippy v4.6.0 software (Seemann 2015). A total of 124 assemblies with known source were downloaded: 108 from livestock, 14 from wildlife, and 2 from humans. The complete genome of *E. coli* strain K-12 substrain MG1655 (RefSeq Accession Number NC_000913.3) was used as reference. Gubbins v3.3.0 was implemented to analyse recombination in resultant alignments and phylogenetic trees were generated with IQ-TREE v2.2.2.3 with 10,000 bootstrap replicates and ascertainment bias correction (Croucher et al. 2015; Minh et al. 2020). The best-fit substitution model to build the tree was the transversion model with unequal base frequencies (TVM+F+ASC+R2). Final phylogenetic tree was visualized and edited with the iTOL v6.9.1 online tool (Letunic and Bork 2024).

4.3.2.6. Statistical analysis

To evaluate the differences in detection of ESC resistant *E. coli* across different study areas, Pearson's Chi-squared test ($\chi 2$) was used, with Yates' correction when appropriate. *p*-values less than 0.05 were interpreted statistically significant. Statistical analyses were conducted using the R version 4.3.0 (R Core Team 2022).

4.3.2.7. Data availability

The genome sequences of the 58 ESC resistant *E. coli* isolates of humans, livestock and wildlife from western Uganda have been deposited in the Enterobase (Accession numbers in Supplementary Table S1.4.3).

4.3.3. Results

4.3.3.1. Antimicrobial susceptibility testing and resistance genes

Out of 555 samples collected, 484 (87.1%) and 5 (0.9%) isolates were confirmed to be *E. coli* and *K. pneumoniae* respectively, each representing one individual per isolate, with varying proportions observed among the different study subjects (Table 1.4.3). Of them, 63 were selected in MacConkey supplemented with ceftriaxone, 58 *E. coli* and 5 *K. pneumoniae*, respectively. For *E. coli* isolates, antimicrobial resistance to ampicillin, sulfamethoxazole, tetracycline, ceftazidime and chloramphenicol was detected by Kirby-Bauer DDT method in livestock, wildlife and humans. However, AMR was not observed in environmental samples (Supplementary Table S2.4.3). The highest frequency of resistance observed was for sulfamethoxazole (24%), followed by ampicillin (21.9%), tetracycline (13.8%) and ceftazidime (12%). In all cases, resistance was higher in humans, followed by livestock and wildlife. All *K. pneumoniae* isolates, which belonged to human samples, were resistant to ampicillin, sulfamethoxazole, and ceftazidime. Isolates resistant to ceftriaxone and confirmed to exhibit resistance also to ceftazidime (Table 1.4.3) were further tested for several antibiotics using MIC. According to the study areas, MGNP exhibited significant higher levels of ESC resistant *E. coli* compared to BFCR and MFNP ($p = 0.004$). When comparing areas with frequent interaction (QENP-N, MGNP, MFNP-S and BCFR) to those with less interaction (QENP-S and MFNP-N), ESC resistant *E. coli* isolates were significantly more prevalent in areas with higher interaction ($p = 0.002$). However, when assessing the levels of ESC resistant *E. coli* isolates between study subjects across different areas, no statistical differences were observed.

Table 1.4.3. Total number of samples analysed, number and percentage of samples where *E. coli* and *K. pneumoniae* were detected, and number of isolates resistant to ceftazidime (CAZ-R).

	N samples	*E. coli* detection (%)	*Klebsiella* spp. detection (%)	CAZ-R
Environment	**52**	**17 (32.7)**	**0**	**0**
Water	52	17 (32.7)	0	0
Livestock	**137**	**122 (89.1)**	**0**	**14**
Cattle	57	51 (89.5)	0	6
Goat	58	49 (84.5)	0	8
Sheep	22	22 (100)	0	0
Wildlife	**301**	**288 (95.7)**	**0**	**10**
Baboon *(Papio anubis)*	43	41 (95.3)	0	8
Blue monkey *(Cercophitecus mitis)*	2	2 (100)	0	0
Buffalo *(Syncerus caffer)*	61	55 (90.2)	0	0
Bushback *(Tragelaphus scriptus)*	2	2 (100)	0	0
Chimpanzee *(Pan troglodytes)*	108	108 (100)	0	1
Duika *(Sylvicapra grimmia)*	2	2 (100)	0	0
Elephant *(Loxodonta africana)*	37	32 (86.5)	0	0
Giraffe *(Giraffa camelopardalis rothschildi)*	17	17 (100)	0	0
Uganda kob *(Kobus kob thomasi)*	29	29 (100)	0	1
Human	**65**	**57 (87.7)**	**5 (7.7)**	**39**
TOTAL	**555**	**484 (87.2)**	**5 (0.9)**	**63**

All the ESC resistant *E. coli* and *K. pneumoniae* isolates were resistant to ampicillin, sulfamethoxazole, cefotaxime, ceftazidime and trimethoprim, except for one *E. coli* human isolate that was susceptible to the latter and one wildlife isolate susceptible to ceftazidime (Table 2.4.3). Ciprofloxacin, tetracycline and nalidixic acid resistance were detected in isolates of all sources, with frequencies ranging from 80 to 100%, 35.7 to 80% and 20 to 41.1%, respectively. Resistance to gentamicin and amikacin was only present in human isolates, while all individuals were susceptible to meropenem and colistin. Multidrug resistance (MDR), defined as resistance to three or more classes of antibiotics (Schwarz et al. 2010), was present in all isolates. The number of resistances ranged from 5 to 11.

WGS detected 40 AMR genes associated with resistance to 9 different families of antibiotics, with a mean of 9.29 (SD = 3.39) resistance genes per sample in *E. coli* isolates (Supplementary Table S1.4.3). For *K. pneumoniae*, 28 AMR genes associated to 8 families of antibiotics, with a mean of 13 (SD = 2) genes per isolate were identified (Supplementary Table S3.4.3).

In the 58 *E. coli* isolates the presence of ESC resistance genes was confirmed in all isolates except for one, differently distributed across western Uganda

(https://microreact.org/project/qCA5iHmAHx7riG5GH2m77e-escherichiacoliuganda). The most prevalent beta-lactam resistance genes were CTX-M-15 (n = 53, 91.4%), OXA-1 (n = 6, 10.3%), SHV-12 (n = 5, 8.6%), CTX-M-27 (n = 3, 5.2%) and SHV-187 (n = 1, 1.7%). Additionally, TEM-1B (n = 31, 53.4%), TEM-104 (n = 1, 1.7%), and TEM-1C (n = 1, 1.7%) were also detected. OXA-1 was found in 6 isolates, all in combination with CTX-M-15, one with SHV-12 and one with SHV-187. Of those isolates, 5 belonged to humans and one to a chimpanzee. Regarding aminoglycoside-resistance genes, 49 harboured *aph*(6)-Id (84.5%), 37 *aph*(3")-Ib (63.8%), 12 *ant*(3")-Ia (20.7%), 8 *aadA2* (13.8%), 6 *aadA5* (10.3%), 5 *aac*(6')Ib-cr (8.6%), 3 harboured *aac*(3)-IIa (5.2%), one *aac*(3)-IId (1.7%) and one *ant*(2")-Ia (1.7%). For macrolides, *mph*(A) (n = 13, 22.4%), and *erm*(B) (n = 3, 5.2%) were detected. Fluoroquinolone resistance genes were identified, specifically *qnrS1* (*n* = 41, 70.7%) and *qnrB19* (*n* = 1, 1.7%). For phenicols, *catA1* (n = 7, 12.1%) was the most frequent, while *fosA* conferring resistance to fosfomycin was found in two human isolates. Resistance to tetracycline was conferred by *tet*(A) (*n* = 31, 53.4%) and *tet*(B) (*n* = 10, 17.2%). For trimethoprim, the most common genes encountered were *dfrA14* (*n* = 33, 56.9%), *dfrA1* (*n* = 9, 15.5%), *dfrA12* (*n* = 9, 15.5%), *drfA8* (*n* = 5, 8.6%), *dfrA7* (*n* = 3, 5.2%), *dfrA5* (*n* = 1, 1.7%), *dfrA15* (*n* = 1, 1.7%), *dfrA17* (*n* = 6, 10.3%), and *dfrA19* (*n* = 1, 1.7%), while in sulphonamides were *sul2* (*n* = 54, 93.1%), *sul1* (*n* = 18, 31%) and *sul3* (*n* = 2, 3.4%). Analyses of point mutations within the quinolone resistance-determining regions (QRDRs), which responsible for conferring resistance to this antibiotic class, detected mutations in the *gyrA*, *parC* and *parE* genes in 23 isolates. Eleven distinct combinations of mutations were identified (Table 3.4.3).

Table 2.4.3. Minimum inhibitory concentration (MIC) distribution for each antibiotic tested and epidemiological cut-off values (ECOFF) according to EUCAST in ESC resistant *E. coli* and *K. pneumoniae* isolates originating from faecal samples of humans, livestock and wildlife in western Uganda. White cells of the table represent tested concentrations for each antimicrobial. *Only 3 samples were analysed to these antibiotics.

Concentration mg/ml (*E. coli* humans N = 34)																		
Antimicrobials	ECOFF (≤)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024
Ampicillin	8							0.0	0.0	0.0	0.0	0.0	0.0	100				
Cefotaxime	0.25					0.0	0.0	0.0	0.0	100								
Ceftazidime	0.5					0.0	0.0	0.0	8.8	14.7	41.2	32.4						
Meropenem	0.125		97.1	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						
Ciprofloxacin	0.064		2.9	0.0	2.9	44.1	17.6	2.9	0.0	0.0	0.0	29.4						
Nalidixic acid	8									29.4	29.4	0.0	2.9	0.0	38.2			
Azithromycin	16								2.9	29.4	23.5	8.8	14.7	2.9	17.6			
Gentamicin	2						64.7	11.8	2.9	2.9	2.9	2.9	11.8					
Amikacin	8									91.2	5.9	2.9	0.0	0.0	0.0			
Chloramphenicol	16										79.4	2.9	2.9	0.0	14.7			
Tetracycline	8								20.6	2.9	0.0	0.0	2.9	73.5				
Tigecycline	0.5					94.1	5.9	0.0	0.0	0.0	0.0							
Colistin	2							100	0.0	0.0	0.0	0.0						
Sulfamethoxazole	64										0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Trimethoprim	2					0.0	0.0	2.9	0.0	0.0	0.0	0.0	97.1					
Concentration mg/ml (*E. coli* livestock N = 14)																		
Antimicrobials	ECOFF (≤)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024
Ampicillin	8							0.0	0.0	0.0	0.0	0.0	0.0	100				
Cefotaxime	0.25					0.0	0.0	0.0	0.0	100								
Ceftazidime	0.5					0.0	0.0	0.0	7.1	14.3	42.9	35.7						
Meropenem	0.125		100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						
Ciprofloxacin	0.064	7.1	0.0	0.0	14.3	35.7	14.3	7.1	0.0	0.0	0.0	21.4						
Nalidixic acid	8									42.9	21.4	0.0	0.0	14.3	21.4			
Azithromycin	16								0.0	35.7	35.7	14.3	0.0	7.1	7.1			
Gentamicin	2						85.7	14.3	0.0	0.0	0.0	0.0						
Amikacin	8									92.9	7.1	0.0	0.0	0.0	0.0			
Chloramphenicol	16										92.9	7.1	0.0	0.0				
Tetracycline	8								64.3	0.0	0.0	0.0	0.0	35.7				
Tigecycline	0.5					92.9	7.1	0.0	0.0	0.0	0.0							
Colistin	2							100	0.0	0.0	0.0	0.0						

Sulfamethoxazole	64											0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Trimethoprim	2					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100					

Concentration mg/ml (*E. coli* wildlife N = 10)

Antimicrobials	ECOFF (≤)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024
Ampicillin	8							0.0	0.0	0.0	0.0	0.0	0.0	100				
Cefotaxime	0.25					0.0	0.0	10	0.0	90								
Ceftazidime	0.5					1140	0.0	0.0	0.0	30	10	50						
Meropenem*	0.125		100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						
Ciprofloxacin	0.06	0.0	0.0	0.0	10	0.0	70	20	0.0	0.0	0.0							
Nalidixic acid	8									30	40	10	0.0	0.0	20			
Azithromycin*	16								0.0	33.3	33.3	33.3	0.0	0.0				
Gentamicin	2						70	20	0.0	0.0	0.0	0.0	10					
Amikacin*	8									100	0.0	0.0	0.0	0.0	0.0			
Chloramphenicol	16										80	0.0	0.0	20				
Tetracycline	8								20	0.0	0.0	0.0	30	50				
Tigecycline*	0.5					66.6	0.0	0.0	0.0	33.3	0.0							
Colistin	2							100	0.0	0.0	0.0	0.0						
Sulfamethoxazole	64											0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Trimethoprim	2					0.0	0.0	0.0	0.0	0.0	0.0	0.0	100						

Concentration mg/ml (*K. pneumoniae* humans N = 5)

Antimicrobials	ECOFF (≤)	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024
Ampicillin	8							0.0	0.0	0.0	0.0	0.0	0.0	100				
Cefotaxime	0.25					0.0	0.0	0.0	20	80								
Ceftazidime	0.5					0.0	0.0	0.0	20	0.0	40	40						
Meropenem	0.125		100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						
Ciprofloxacin	0.064	20	0.0	0.0	0.0	0.0	20	40	0.0	0.0	0.0	20						
Nalidixic acid	8									20	60	0.0	0.0	0.0	20			
Azithromycin	16								0.0	20	0.0	60	20	0.0				
Gentamicin	2						80	0.0	0.0	0.0	0.0	0.0	20					
Amikacin	8									100	0.0	0.0	0.0	0.0	0.0			
Chloramphenicol	16										60	0.0	0.0	0.0	40			
Tetracycline	8								40	0.0	0.0	0.0	0.0	60				
Tigecycline	0.5					40	40	0.0	20	0.0	0.0							
Colistin	2							100	0.0	0.0	0.0	0.0						
Sulfamethoxazole	64											0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Trimethoprim	2					0.0	0.0	0.0	0.0	0.0	0.0	0.0	100						

For *K. pneumoniae*, ESC genes were detected in all isolates. The most prevalent beta-lactam resistance genes were CTX-M-15 (n = 4, 80%) and TEM-1B (n = 3, 60%). OXA-1 was found in one isolate, in combination with CTX-M-15 and SHV-145. Regarding aminoglycoside-resistance genes, all isolates harboured *aph*(6)-Id and *aph*(3")-Ib. For fluoroquinolones, *qnrS1* was detected in 3 isolates (60%). Resistance to phenicol was conferred by *catA2* (n = 2, 40%) and *catA1* (n = 1, 20%), while *fosA* was the most frequent gene conferring resistance to fosfomycin (n = 3, 60%). For tetracyclines, *tet*(A) (*n* = 3, 60%) and *tet*(D) (*n* = 1, 20%) were detected. For trimethoprim, the most common gene encountered was *dfrA14* (*n* = 4, 80%), while for sulphonamides was *sul2* (*n* = 5, 100%). As for *E. coli* isolates, point mutations were analysed. Point mutations in the *ompK36*, *ompK37* and *acrR* genes were detected in all *K. pneumoniae* isolates, with four combinations of point mutations (Table 4.4.3).

Table 3.4.3. Mutations combination within the quinolone resistance-determining regions (QRDRs) conferring resistance to fluoroquinolones in ESC resistant *E. coli,* along with the origins of the samples exhibiting these mutations.

Genotype point mutations				
gyrA	parC	parE	N (%)	Study subject
p.S83A	-	-	1 (4.3)	Human
p.S83L	-	-	3 (13)	Human (2) and livestock (1)
p.S83L, p.D87N	p.S80I	-	2 (8.7)	Human
p.S83L, p.D87N	p.S80I	p.L416F	3 (13)	Livestock
p.S83L, p.D87N	p.S80I	p.S458A	5 (21.7)	Human (4) and wildlife (1)
p.S83L, p.D87N	p.S80I, p.E84G	-	1 (4.3)	Human
p.S83L, p.D87N	p.S80I, p.E84V	p.I529L	2 (8.7)	Human
p.S83L, p.D87N	p.S80I, p.S57T	p.S458A	1 (4.3)	Human
p.S83V	-	-	2 (8.7)	Human (1) and livestock (1)
-	-	p.I355T	2 (8.7)	Human
-	-	p.L416F, p.E460D	1 (4.3)	Human

Table 4.4.3. Point mutations combination in ESC resistant *Klebsiella pneumoniae*.

ompK36	acrR	ompK37	N (%)
p.N49S, p.L59V, p.N49S, p.L191S, p.F207W, p.A217S, p.N218H, p.D224E, p.Q227S, p.L228V, p.E232R, p.T254S, p.N304E	p.P161R, p.G164A, p.P161R, p.R173G, p.L195V, p.F197I, p.K201M	p.I70M, p.I128M	1 (20)

p.N49S, p.L59V, p.T184P	p.P161R, p.G164A, p.F172S, p.R173G, p.L195V, p.F197I, p.K201M	p.I70M, p.I128M	1 (20)
p.N49S, p.L59V, p.L191Q, p.A217S, p.N218H, p.Q227S, p.L229V, p.N304E	p.P161R, p.G164A - Frameshift, p.F172S, p.R173G, p.L195V, p.F197I, p.K201M	p.I70M, p.I128M	2 (40)
p.N49S, p.L59V, p.A190W, p.L191S, p.F207W, p.A217S, p.N218H, p.D224E, p.Q227S, p.L228V, p.E232R, p.T254S, p.N304E	p.P161R, p.G164A, p.F172S, p.R173G, p.L195V, p.F197I, p.K201M	p.I70M, p.I128M	1 (20)

Most genes associated with resistance to ESC in both *E. coli* and *K. pneumoniae* were predicted to be located on plasmids (Supplementary Tables S4.4.3 and S5.4.3). For *E. coli*, of the 101 genes detected conferring resistance to cephalosporins, 49 were predicted to be in plasmids (48.5%), 10 were located in the chromosome (9.9%), and 5 were found in viruses (5%). Additionally, 11 genes had an uncertain location (10.9%) and 26 were not found (25.7%). In the case of *K. pneumoniae*, of the 14 genes detected, 7 were located on plasmids (50%), 1 had an uncertain location (7.1%) and 6 were not found (42.9%).

4.3.3.2. Phylotyping and MLST

WGS data of the 58 *E. coli* and 5 *K. pneumoniae* isolates collected from humans, livestock and wildlife enabled the generation of genome assemblies, resulting in a mean genome coverage of 178.98 (SD = 53.58). The Clermont phylotyping scheme identified 20 isolates belonging to group B1 (34.5%), 16 to group A (27.6%), 12 to group D (20.7%), 3 to group U (5.2%), 2 to group B2 and E each (3.4%) and one to group C and F each (1.7%). One isolate could not be classified. Phylotypes A, B1 and D were found in wildlife samples. Furthermore, a remarkable range of ESC resistant *E. coli* lineages carrying different AMR genes were identified, comprising 37 different sequence types (STs) (Supplementary Table S1.4.3). ST6636 was the most common (*n* = 5, 9.4%), followed by ST2178 (*n* = 4, 7.5%), ST2852 (*n* = 3, 5.7%), ST10 (*n* = 2, 3.8%), ST224 (*n* = 2, 3.8%), ST295 (*n* = 2, 3.8%), ST720 (*n* = 2, 3.8%), ST1312 (*n* = 2, 3.8%), ST2705 (*n* = 2, 3.8%) and ST9138 (*n* = 2, 3.8%). ST6636 was detected in a Ugandan kob, while ST10, ST224 and ST2705 were found in baboon samples. ST131 was identified in one human sample. Four *E. coli*

strains did not have ST assignments as they did not meet the quality control standards of Enterobase. For *K. pneumoniae* isolates, 5 different STs were identified: ST16, ST414, ST841 ST5404 and ST1411.

4.3.3.3. Phylogenetic reconstruction

The phylogenomic tree (Fig. 2.4.3) included a total of 182 assemblies (58 from this study and 124 from public databases, all with Ugandan origin). The phylogenetic analysis revealed the presence of two clades, the main one containing all sequences except for two. The main clade clustered the *E. coli* isolates by ST and, to a lesser extent, by phylotype. Some clusters contained closely related isolates from different sources, according to the phylogenetic distance obtained from the core genome. The chimpanzee isolate UECR8A shared homology with isolates of human (A36316) and goat (A36279) origin. While both chimpanzee and goat samples were from BCFR, the human isolate belonged to an individual sampled in MGNP. Additionally, a livestock isolate from QENP (A36297) was closely related to an isolate of wildlife origin from central Uganda (VA3601AA). Genetically similar ST6636 isolates were observed between an Ugandan kob (A36308) from QENP, and a human and a cattle isolate from MGNP. Furthermore, homology was also observed among a human and cattle isolates (A36267 and A36304), harbouring CTX-M-15 predicted to be located in the same mobile genetic element.

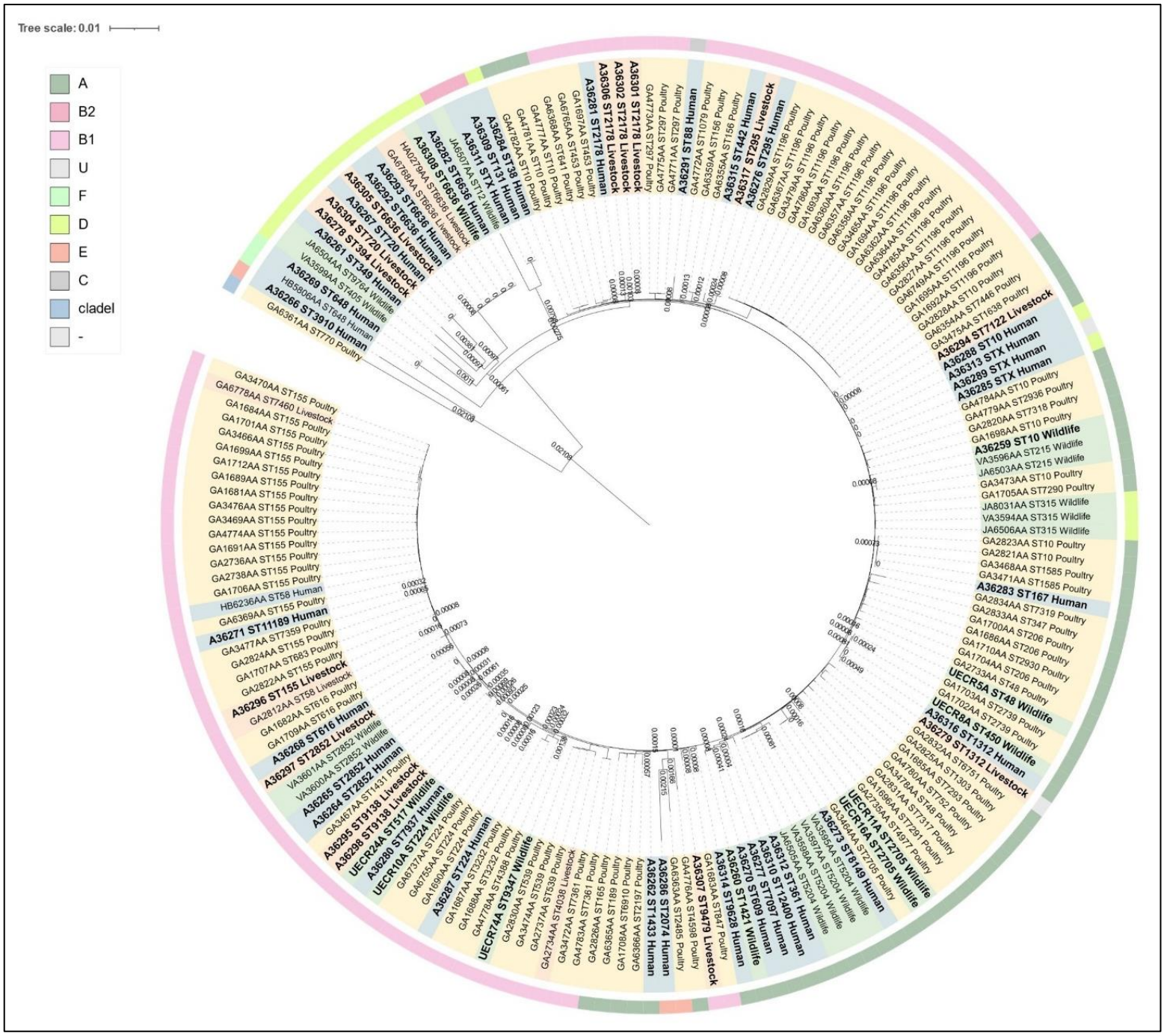


Figure 2.4.3. Phylogenetic reconstruction of *E. coli* genomic sequences isolated from humans, livestock and wildlife sampled in western Uganda, and *E. coli* genomic assemblies available at Enterobase from human, livestock and wildlife origin (https://enterobase.warwick.ac.uk/, last access 23/06/2024).

4.3.4. Discussion

Antimicrobial resistance (AMR) poses a significant challenge in Uganda, where limited resources and complex interactions between humans, wildlife, and livestock exacerbate the spread of resistant bacteria. In regions with constrained healthcare

infrastructure and diverse ecological landscapes, AMR threatens both, public health and biodiversity conservation efforts. This study addresses these challenges by investigating the occurrence, phenotypic and genotypic resistance, and potential origins of cephalosporin resistant Enterobacterales, including *E. coli* and *K. pneumoniae*, as indicators of AMR in humans, livestock and wildlife across western Uganda.

The antibiotic resistance profiles showed high percentage of isolates exhibiting resistance in humans and animals to commonly used antibiotics, such as ampicillin, sulfamethoxazole, and tetracyclines (Tadesse et al. 2017). However, isolates collected from water, representing environmental samples, were pansusceptible. In all cases, proportions of resistance were higher in humans, followed by livestock and wildlife. Levels of phenotypic resistance in *E. coli* isolated from humans were similar to other studies performed in Uganda (Mugerwa et al. 2021). While resistance frequency in cattle was lower than previously observed, (Weiss et al. 2018; Mugerwa et al. 2021) resistance in *E. coli* of goat and sheep origin, and wildlife isolates, were higher (Weiss et al. 2018). A high frequency of resistance to third-generation cephalosporins, which are widely prescribed in Uganda's hospitals (Kiggundu et al. 2022), has been observed in both humans and animals, including different wildlife species (see Study 1). Furthermore, all ESBL-producing isolates were MDR. The presence of these resistant isolates was significantly higher in areas with higher interaction between species, where rural interfaces could facilitate AMR transmission through direct contact among species, consumption of contaminated meat through game and home slaughter, and shared contaminated environments (Vittecoq et al. 2016; Jaja et al. 2020). However, despite significant differences were not observed in wildlife from areas with different levels of interaction, BCFR had the highest percentage of ESC resistant *E. coli* isolates from wildlife, which is mostly composed of non-human primates. This is concerning not only because of the close proximity of these species to human settlements, but also because of social interactions among non-human primates. Besides facilitating pathogen exposure, as previously reported in these species, these interactions can also promote the

transmission and maintenance of AMR within the same group (Lonsdorf et al. 2011; MacIntosh et al. 2012).

The characterization of ESC genes in Uganda is poorly documented, particularly in wild animals (see Study 1). In this study, we report the presence of MDR ESBL-producing *E. coli* harbouring different CTX-M genes in humans, livestock and wildlife, being CTX-M-15 the most common in all species. Resistance genes belonging to the CTX-M-1 group have been previously detected in humans in Uganda (Weil et al. 2020), while CTX-M-15, CTX-M-27 and OXA-1 have been detected in cattle (Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020; Bager et al. 2022). The acquisition of AMR in humans and livestock could be driven primarily by the selective pressure from the widespread and inadequate use of antibiotics in Uganda, which enables the rapid spread of resistance genes among bacterial populations (Iramiot, Kajumbula, Bazira, De Villiers, et al. 2020). Those resistance genes could be subsequently excreted and dispersed to the environment through agricultural and human sources, facilitating AMR dissemination to wildlife (Radhouani et al. 2014). The significance of AMR in Uganda is underscored by the close interactions between humans and animals, particularly in regions adjacent to national parks. The absence of effective barriers, along with the degradation of natural habitats due to agricultural expansion, facilitates these interactions, thereby enhancing the risk of pathogens and AMR transmission (Goldberg et al. 2007; Weiss et al. 2018). Despite CTX-M-15 being previously detected in wildlife, specifically in captive and wild chimpanzees from Uganda (Bager et al. 2022), this study constitutes the first report on the occurrence of MDR *E. coli* in an antelope (Uganda kob) producing CTX-M-15. CTX-M-15 is widely disseminated worldwide, mostly related to healthcare setting infections (Cantón et al. 2012). However, this gene has also been detected in wild birds and ungulates in different European and African countries, leading to the hypothesis that their presence in wildlife species could be attributed to anthropogenic activities (Bachiri et al. 2017; Tinoco Torres et al. 2022; Guitart-Matas et al. 2024). In addition, OXA-1 was detected in humans from different regions of Uganda and in a chimpanzee from BCFR, where encounters with human settlements are common as some chimpanzees leave the forest to obtain food from

croplands (Tweheyo et al. 2005). *Escherichia coli* and *K. pneumoniae* isolates also carried multiple AMR genes for other antibiotic families, such as aminoglycosides, trimethoprim and sulphonamides, with all being MDR. In addition, isolates from human, goat and a chimpanzee origins exhibited plasmid-mediated quinolone resistance (PMQR) determinants, which reduce the susceptibility of bacteria to quinolones facilitating the selection of quinolone-resistant mutants and treatment failure in Enterobacterales species (Kotb et al. 2019). The studied areas represent intricate ecosystems that facilitate the easy spread of resistant pathogens, posing significant challenges to public, animal and environmental health. Addressing AMR in these rural settings needs comprehensive strategies that integrate environmental, agricultural, and healthcare practices to mitigate the spread of resistance effectively.

Surprisingly, 38 different ST lineages were detected, with only six aligning with the predominant extraintestinal pathogenic *E. coli* (ExPEC) lineages commonly reported in humans globally: ST10, ST38, ST88, ST131, ST167 and ST648 (Manges et al. 2019). Each one of these lineages was represented in human isolates sequenced herein, while ST10 was also detected in a baboon isolate. Despite ST131 being a MDR lineage distributed worldwide and found in a wide host range (Rodríguez et al. 2021), in this study it was only detected in a human isolate. Furthermore, most resistance genes were predicted to be located in plasmids. In fact, the high diversity of *E. coli* lineages detected suggests that resistance is spreading through mobile genetic elements. This is of great concern as plasmid transfer allows for a much more rapid dissemination of resistance genes between diverse lineages and taxa (Marí-Almirall et al. 2021; J. Zhang et al. 2023), posing a significant challenge for the control and treatment of bacterial infections in Uganda. Additionally, plasmids can carry multiple resistance genes, enabling the propagation of MDR isolates (Castañeda-Barba et al. 2024). In the present study, some resistance genes detected in samples from humans, livestock and wildlife and predicted to be located on plasmids exhibited resistance not only to beta-lactams, but also to quinolones, aminoglycosides, and sulfonamides, posing a risk of MDR spread. Reports of *E. coli* ST6636 are rare. However, isolates A36282, A36292 and A36283 (human), A36305 (cattle) and A36308 (Uganda kob) exhibited 100% core genome homology

with Ugandan *E. coli* strains from dairy cattle described previously (Bioprojects PRJNA293225) on Enterobase (Ball et al. 2019). Furthermore, almost all isolates carried the CTX-M-15-encoding gene. Similarly, the core genome of a livestock isolate of lineage ST2852 B1 CTX-M-15 was identical to an isolate of chimpanzee origin from Ngamba Island Sanctuary, located in lake Victoria, which also carried the CTX-M-15-encoding gene (Bager et al. 2022). These findings not only indicate that resistance genes can spread across different species, but also underscore the significance of transmission pathways, including the influence of anthropogenic pressures in wildlife and the role of natural environmental contamination in the dissemination of antimicrobial resistance genes.

Despite their low frequency of detection, the identification of *E. coli* ExPEC lineages in human isolates underscores a critical concern. These strains contribute substantially to the global burden of infectious diseases and act as a reservoir for the development and dissemination of AMR (Manges et al. 2019). Particularly in LMICs, the impact of such pathogens is exacerbated by limited healthcare resources and higher rates of infectious diseases, posing the risk of them becoming untreatable.

In our study, MDR *K. pneumoniae* was detected in five human samples, each one representing a different ST lineage. ST16, which is considered an important emergent lineage carrying determinants to carbapenem-resistance and causing hospital outbreaks worldwide (Marcade et al. 2013; Tada et al. 2017; Kochan et al. 2023), was identified in one *K. pneumoniae* isolate harbouring CTX-M-15 and OXA-1 encoding genes. In addition, other three isolates carried the CTX-M-15-encoding gene. Point mutations that could alter the outer membrane porin OmpK36 were detected in all isolates, which play a critical role in mediating *K. pneumoniae* resistance to carbapenems (Wong et al. 2022). *K. pneumoniae* is considered an important reservoir of diverse AMR genes worldwide, most of which are plasmid-borne transmitted via conjugation, leading to a wider ecological distribution than other opportunistic bacteria and the emergence of extremely drug resistant strains (Wyres and Holt 2016; Navon-Venezia et al. 2017). While some bacteria originate

from human and animal sources, *K. pneumoniae* is ubiquitous in nature and can originate from various natural sources (Podschun and Ullmann 1998). Consequently, this pathogen plays a crucial role in AMR dissemination from environmental microbes to clinical critical pathogens. In fact, *K. pneumoniae* has been classified as an ESKAPE organism, which includes *Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *Enterobacter* species. This pathogens are the leading causes of nosocomial infections worldwide and are predominantly MDR (Rice 2008). Despite the alarming increase in MDR *K. pneumoniae* strains in humans reported over the last decades, its prevalence in domestic and wild animals, as well as in the environment, has not been significantly investigated. Therefore, further research is imperative to understand its ecological distribution and to develop effective measures to control its spread.

4.3.5. Conclusions

In conclusion, the wide occurrence of ESC resistant bacteria in community settings and wildlife highlights their capacity to spread between anthropogenic and natural ecosystems, creating hotspots that facilitate the spread and evolution of AMR genes in western Uganda. The detection of resistance genes in wildlife not only suggests that these animals may act as reservoirs but also highlights the potential risks to environmental health. Proximity to human settlements and livestock farming contributes significantly to this issue by increasing the selective pressure for AMR and facilitating its spread, highlighting the need to adopt an expanded One Health approach to fully understand AMR transmission dynamics in rural areas from Eastern Africa.

Supplementary material Study 3

Supplementary Table S1.4.3. List of the 58 cephalosporin-resistant *E. coli* isolates obtained from different sources, general genomic features, MLST, phylotyping and antimicrobial resistance genes for the different families of antimicrobials with the percentage of identity with published sequences.

Strain_ID	Enterobase_Accession_Number	Source	National Park	MLST	Phylotype	Genome_size	N50	GC_content	Coverage	Contig_number	Total_AMR
A36259	ESC_KB9213AA	Wildlife	Budongo	10	A	5062980	187450	50,69	244	421	9
A36260	ESC_KB9207AA	Wildlife	Budongo	1421	A	4500267	95537	50,83	198,3	119	4
A36261	ESC_KB9212AA	Human	Mgahinga NP	349	D	5016881	211028	50,64	196,9	73	8
A36262	ESC_KB9209AA	Human	Mgahinga NP	1433	A	5382496	121648	50,57	185,1	222	6
A36264	ESC_KB9208AA	Human	Mgahinga NP	2852	B1	4709203	236000	50,79	202,7	57	3
A36265	ESC_KB9216AA	Human	Mgahinga NP	2852	B1	4719859	217479	50,76	290,5	60	1
A36266	ESC_KB9215AA	Human	Mgahinga NP	3910	E	5076404	700660	50,27	232,2	41	7
A36267	ESC_KB9214AA	Human	Mgahinga NP	720	D	5243653	291711	50,41	223,2	55	5
A36268	ESC_KB9210AA	Human	Murchison Falls NP	616	B1	6310943	133544	50,58	197,9	1483	8
A36269	ESC_KB9211AA	Human	Murchison Falls NP	648	F	5485639	208081	50,5	184,3	117	15
A36270	ESC_KB9222AA	Human	Murchison Falls NP	609	A	4772445	83330	50,73	167,1	155	9
A36271	ESC_KB9231AA	Human	Murchison Falls NP	11189	B1	4914824	186017	50,49	250	105	7
A36273	ESC_KB9233AA	Human	Murchison Falls NP	8149	A	6516560	54716	50,55	302,3	1588	10
A36276	ESC_KB9219AA	Human	Murchison Falls NP	295	B1	5450805	139994	50,68	137,7	245	9
A36277	ESC_KB9220AA	Human	Murchison Falls NP	7097	A	4927880	117225	50,56	155,2	144	11
A36278	ESC_KB9217AA	Livestock	Budongo	394	D	5005864	407566	50,52	137,6	58	8
A36279	ESC_KB9230AA	Livestock	Budongo	1312	-	4629080	66568	50,77	214,7	190	7
A36280	ESC_KB9228AA	Human	Mgahinga NP	7937	B1	5171894	322658	50,72	188,3	79	6
A36281	ESC_KB9225AA	Human	Mgahinga NP	2178	B1	5117860	173590	50,45	171,7	115	7
A36282	ESC_KB9232AA	Human	Mgahinga NP	6636	D	4830597	208368	50,62	264,2	54	6
A36283	ESC_KB9223AA	Human	Mgahinga NP	167	A	4692131	132139	50,79	177,2	117	11
A36284	ESC_KB9226AA	Human	Mgahinga NP	38	D	5093078	208059	50,59	178,9	79	3
A36285	ESC_KB9218AA	Human	Mgahinga NP	-	D	8851505	3741	50,8	78,8	4234	9
A36286	ESC_KB9221AA	Human	Mgahinga NP	2074	E	5010978	503293	50,49	161,1	45	10
A36287	ESC_KB9227AA	Human	Mgahinga NP	224	B1	5134454	282294	50,85	176,4	104	14
A36288	ESC_KB9229AA	Human	Mgahinga NP	10	A	6210146	4881	50,43	151,8	2834	9
A36289	ESC_KB9224AA	Human	Mgahinga NP	-	U	9502888	13515	50,68	93,4	1973	12
A36291	ESC_KB9237AA	Human	Mgahinga NP	88	C	5235268	111356	50,64	156,7	185	16
A36292	ESC_KB9239AA	Human	Mgahinga NP	6636	D	5002168	241960	50,39	171,4	67	6
A36293	ESC_KB9238AA	Human	Mgahinga NP	6636	D	4861863	269360	50,6	178,2	56	6
A36294	ESC_KB9236AA	Livestock	Budongo	7122	A	4515557	93078	50,96	175,5	118	6
A36295	ESC_KB9247AA	Livestock	QENP	9138	B1	4786899	217899	50,75	210,7	70	3
A36296	ESC_KB9248AA	Livestock	QENP	155	B1	5565697	76745	50,57	196,7	504	11
A36297	ESC_KB9244AA	Livestock	QENP	2852	B1	4791868	217479	50,74	194,8	70	2
A36298	ESC_KB9249AA	Livestock	QENP	9138	B1	4783695	213326	50,74	237,5	66	3
A36301	ESC_KB9246AA	Livestock	Mgahinga NP	2178	B1	5020853	186271	50,42	201,1	84	5

A36302	ESC_KB9235AA	Livestock	Mgahinga NP	2178	B1	5020774	185632	50,42	159,5	87	5
A36304	ESC_KB9234AA	Livestock	Mgahinga NP	720	D	5280293	291394	50,41	128,1	55	5
A36305	ESC_KB9242AA	Livestock	Mgahinga NP	6636	D	5031854	242600	50,61	183,2	78	11
A36306	ESC_KB9240AA	Livestock	Mgahinga NP	2178	B1	5032830	248029	50,44	178,2	87	6
A36307	ESC_KB9243AA	Livestock	Mgahinga NP	9479	B1	4767586	661295	50,56	207,1	32	9
A36308	ESC_KB9245AA	Wildlife	QENP	6636	D	4873829	261145	50,6	205	57	6
A36309	ESC_KB9241AA	Human	QENP	131	B2	10401326	57042	54,02	169,4	607	17
A36310	ESC_KB9254AA	Human	QENP	12400	A	4975400	200921	50,61	190,8	76	9
A36311	ESC_KB9262AA	Human	QENP	-	B2	14410599	13623	54,53	130,1	2243	21
A36312	ESC_KB9255AA	Human	QENP	361	A	4735834	208759	50,73	208	63	3
A36313	ESC_KB9258AA	Human	QENP	-	D	11031422	5250	50,33	110,2	4225	17
A36314	ESC_KB9257AA	Human	QENP	9628	A	4789891	209847	50,67	219,6	68	5
A36315	ESC_KB9259AA	Human	QENP	442	B1	5126702	167193	50,69	227,5	82	9
A36316	ESC_KB9261AA	Human	QENP	1312	A	5817480	54287	48,04	256,5	1157	8
A36317	ESC_KB9256AA	Livestock	Mgahinga NP	295	B1	5289105	141907	50,73	200,4	138	9
UECR10A	ESC_KB9251AA	Wildlife	Budongo	224	B1	4863589	197810	50,79	115	63	8
UECR11A	ESC_KB9250AA	Wildlife	Budongo	2705	A	5005996	263304	50,5	90,6	86	11
UECR16A	ESC_KB9205AA	Wildlife	Budongo	2705	A	4983974	102301	50,48	28,4	149	10
UECR24A	ESC_KB9206AA	Wildlife	Budongo	517	B1	5011590	111622	50,66	34,1	129	5
UECR5A	ESC_KB9260AA	Wildlife	Budongo	48	A	4778509	106250	50,67	188	146	14
UECR74A	ESC_KB9252AA	Wildlife	Budongo	9347	B1	4803358	288450	50,69	130,6	64	3
UECR8A	ESC_KB9253AA	Wildlife	Budongo	450	A	4867901	85710	50,77	136,2	155	13

	Aminoglycoside									Beta-lactam								Phenicol				Macrolide	
Strain_ID	aac(3)-Iia	aac(3)-Iid	aac(6')-Ib-cr	aadA2	aadA5	ant(2'')-Ia	ant(3'')-Ia	aph(3'')-Ib	aph(6)-Id	blaCTX-M-15	blaCTX-M-27	blaOXA-1	blaSHV-12	blaSHV-187	blaTEM-104	blaTEM-1B	blaTEM-1C	catA1	catA2	catB3	cmlA1	erm(B)	mph(A)
A36259	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36260	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36261	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36262	.	.	.	.	.	.	82,51	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36264	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36265	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36266	.	.	.	.	.	.	82,51	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36267	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36268	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36269	100	.	100	.	100	.	.	100	100	100	.	100	.	.	.	100	.	100	.	.	.	.	100
A36270	.	.	.	97,92	.	.	.	100*	100*	.	100*	.	.	.	.	100*	.	100	100	.	.	.	100
A36271	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36273	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36276	.	.	.	.	.	.	82,3	100	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36277	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	100	100
A36278	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36279	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36280	.	.	.	97,92	.	.	.	.	.	100	.	.	.	.	.	.	.	100	.	.	.	.	.
A36281	.	.	.	.	.	.	82,51	.	.	100	.	.	.	.	.	.	.	100	.	.	.	.	.
A36282	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36283	.	.	.	97,92	.	.	.	.	100	100	.	.	.	.	.	.	.	100	.	.	.	.	100
A36284	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36285	.	.	.	.	.	.	82,51	100	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36286	.	.	.	.	.	.	.	100	100	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36287	.	.	.	97,92	.	.	.	100	100	.	100	.	.	.	.	100	.	100	.	.	.	100	100
A36288	.	.	.	.	.	.	.	100	100	100	.	.	100	.	.	100	.	.	.	.	.	.	.
A36289	.	100	.	.	100	.	.	100	100	100	100	.	.	.	.	100	.	.	.	.	.	.	100
A36291	.	.	.	.	.	100	82,82	100	100	100	.	100	.	.	.	100	.	100	.	100	.	.	.
A36292	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36293	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36294	.	.	.	.	100	.	.	.	.	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36295	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36296	.	.	.	.	.	.	.	100	83,39	100	.	.	100	.	.	100	.	.	.	.	.	.	.
A36297	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36298	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36301	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.

A36302	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36304	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36305	.	.	.	97,92	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	100	100
A36306	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36307	.	.	.	.	.	.	.	100	100	.	.	.	100	.	.	100	.	.	.	.	.	.	100
A36308	.	.	.	.	.	.	.	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
A36309	.	.	100	97,92	.	.	.	100	100	100	.	100	.	100	.	.	.	.	.	.	.	.	100
A36310	.	.	.	.	.	.	.	.	.	100	.	.	.	.	95,12	.	.	.	100	.	.	.	100
A36311	100	.	100	97,92	100	.	.	100	100	100	.	100	99,07	.	.	.	100	.	.	.	.	.	100
A36312	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36313	.	.	100	.	100	.	82,3	99,88	100	100	.	100	.	.	.	100	.	.	.	.	.	.	.
A36314	.	.	.	.	.	.	.	100	100	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
A36315	.	.	.	.	.	.	.	100	88,89	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36316	.	.	.	.	.	.	82,51	.	.	100	.	.	.	.	.	100	.	.	.	.	.	.	.
A36317	.	.	.	.	.	.	82,3	.	100	100	.	.	.	.	.	.	.	.	.	.	.	.	.
UECR10A	.	.	.	97,92	.	.	82,51	.	.	100	.	.	.	.	.	.	.	.	.	.	100	.	.
UECR11A	.	.	.	.	.	.	.	100	85,19	100	.	.	.	.	.	100	.	.	.	.	.	.	.
UECR16A	.	.	.	.	.	.	.	100	85,19	100	.	.	.	.	.	100	.	.	.	.	.	.	.
UECR24A	.	.	.	.	.	.	82,51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
UECR5A	.	.	.	.	.	.	82,51	100	100	.	.	.	100	.	.	100	.	.	.	.	100	.	100
UECR74A	.	.	.	.	.	.	.	100*	100*	100*	.	.	.	.	.	100*	.	.	.	.	.	.	.
UECR8A	100	.	100	.	100	.	.	100	100	100	.	100	.	.	.	.	.	.	.	.	.	.	100

	Trimethoprim									Fosfomycin	Efflux pumps			Quinolone		Sulphonamide			Tetracycline	
Strain_ID	dfrA12	dfrA14	dfrA15	dfrA17	dfrA19	dfrA1	dfrA5	dfrA7	dfrA8	fosA	mdf(A)	oqxA	oqxB	qnrB19	qnrS1	sul1	sul2	sul3	tet(A)	tet(B)
A36259	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36260	.	100	.	.	.	.	.	.	.	.	100	.	.	100	100*	.	100*	.	97,8	.
A36261	.	.	.	.	.	.	.	100	.	.	100	.	.	.	.	90,89	100	.	.	.
A36262	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36264	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
A36265	.	.	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	.	.
A36266	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36267	.	100	.	.	.	.	.	.	.	.	100	.	.	.	.	.	100	.	.	.
A36268	.	.	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36269	.	.	.	100	.	.	.	.	.	.	100	.	.	.	.	100	100	.	.	100
A36270	100	100	.	.	.	.	.	.	.	.	100	.	.	.	.	100	100*	.	.	100
A36271	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36273	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	92,39	100	.	97,8	.
A36276	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	100	.
A36277	.	.	.	.	.	.	.	100	.	.	100	.	.	.	.	90,89	100	.	97,8	.
A36278	.	.	.	.	.	.	.	100	.	.	100	.	.	.	.	.	100	.	97,8	.
A36279	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36280	100	.	.	.	.	.	.	.	.	.	100	.	.	.	.	100	.	.	.	.
A36281	.	.	.	.	.	100	.	.	.	.	100	.	.	.	.	100	.	.	97,8	.
A36282	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36283	100	100	.	.	.	.	.	.	.	.	100	.	.	.	.	100	100	.	.	100
A36284	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
A36285	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36286	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	97,8	100
A36287	100	100	.	.	.	.	.	.	.	.	100	.	.	.	.	100	100	.	.	100
A36288	.	100	.	.	.	.	.	.	.	.	.	.	.	.	100	.	100	.	97,8	.
A36289	.	.	.	100	.	.	.	.	.	.	100	.	.	.	.	.	100	.	97,8	.
A36291	.	.	.	.	100	.	.	.	100	.	100	.	.	.	100	100	100	.	.	100
A36292	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36293	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36294	.	.	.	100	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36295	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
A36296	.	100	.	.	.	.	100	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36297	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	.	.
A36298	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
A36301	.	100	.	.	.	.	.	.	.	.	100	.	.	.	.	.	100	.	.	.
A36302	.	100	.	.	.	.	.	.	.	.	100	.	.	.	.	.	100	.	.	.
A36304	.	100	.	.	.	.	.	.	.	.	100	.	.	.	.	.	100	.	.	.
A36305	100	100	.	.	.	.	.	.	.	.	100	.	.	.	100	100	100	.	.	.

A36306	.	100	.	.	.	.	.	.	.	.	100	.	.	.	.	100	100	.	.	.
A36307	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36308	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100	.	100	.	.	.
A36309	100	.	.	.	.	.	.	.	.	100	100	100	100	.	100	100	100	.	96,86	.
A36310	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	97,8	.
A36311	100	.	.	100	.	.	.	.	.	100	100	100	100	.	100	100	100	.	96,86	.
A36312	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
A36313	.	.	.	100	.	100	.	.	100	.	96,03	.	.	.	100	100	100	.	97,8	100
A36314	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100	.	97,8*	.
A36315	.	.	.	.	.	.	.	.	100	.	100	.	.	.	100	.	100	.	.	100
A36316	.	.	100	.	.	.	.	.	.	.	100	.	.	.	100	100	.	.	97,8	.
A36317	.	100	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	100	.
UECR10A	100	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	.	100	97,8	.
UECR11A	.	100	.	.	.	.	.	.	100	.	100	.	.	.	100	.	100	.	97,8	100
UECR16A	.	100	.	.	.	.	.	.	100	.	100	.	.	.	100	.	100	.	.	100
UECR24A	.	.	.	.	.	100	.	.	.	.	100	.	.	.	100	.	100	.	.	.
UECR5A	100	.	.	.	.	.	.	.	.	.	100	.	.	.	100	100	100	100	97,8	.
UECR74A	.	100	.	.	.	.	.	.	.	.	100	.	.	.	100*	.	100*	.	97,8	.
UECR8A	.	.	.	100	.	.	.	.	.	.	100	.	.	.	.	100	100	.	97,8	.

Supplementary Table S2.4.3. Frequency and percentage of resistance observed to ampicillin (AMP), ceftazidime (CAZ), gentamicin (GEN), tetracycline (TET), chloramphenicol (CHL) and sulfamethoxazole (SMX) by Kirby-Bauer DDT method, in the different studied species.

	AMP (%)	CAZ (%)	GEN (%)	TET (%)	CHL (%)	SMX (%)
Environment	**0/17 (0)**	**0/17 (0)**	**0/17 (0)**	**0/17 (0)**	**0/17 (0)**	**0/17 (0)**
Livestock	**28/122 (23)**	**14/122 (11.5)**	**0/122 (0)**	**17/122 (13.9)**	**2/122 (1.6)**	**32/122 (26.2)**
Cattle	10/51 (19.6)	6/51 (11.8)	0/51 (0)	2/51 (3.9)	0/51 (0)	6/51 (11.8)
Goat	14/49 (28.6)	8/49 (16.3)	0/49 (0)	11/49 (22.4)	2/49 (4.1)	16/49 (32.7)
Sheep	4/22 (18.2)	0/22 (0)	0/22 (0)	4/22 (18.2)	0/22 (0)	10/22 (45.5)
Wildlife	**30/288 (11.4)**	**10/288 (3.5)**	**5/288 (1.7)**	**14/288 (4.9)**	**4/288 (1.4)**	**38/288 (13.2)**
Baboon	10/41 (24.4)	8/41 (19.5)	1/41 (2.4)	11/41 (26.8)	4/41 (9.8)	12/41 (29.3)
Blue monkey	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Buffalo	6/55 (10.9)	0/55 (0)	3/55 (5.5)	0/55 (0)	0/55 (0)	11/55 (20)
Bushback	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	1/2 (50)
Chimpanze	4/108 (3.7)	1/108 (0.9)	1/108 (0.9)	3/108 (2.8)	0/108 (0)	5/108 (4.6)
Duika	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Elephant	3/32 (9.4)	0/32 (0)	0/32 (0)	0/32 (0)	0/32 (0)	5/32 (15.6)
Giraffe	1/17 (5.9)	0/17 (0)	0/17 (0)	0/17 (0)	0/17 (0)	2/17 (11.8)
Uganda kob	6/29 (20.7)	1/29 (3.4)	0/29 (0)	0/29 (0)	0/29 (0)	2/29 (6.9)
Human	**48/57 (84.2)**	**34/57 (59.6)**	**6/57 (10.5)**	**36/57 (63.2)**	**8/57 (14)**	**46/57 (80.7)**
TOTAL	**106/484 (21.9)**	**58/484 (12)**	**11/484 (2.3)**	**67/484 (13.8)**	**14/484 (2.9)**	**116/484 (24)**

Supplementary Table S3.4.3. List of the 5 cephalosporin resistant *Klebsiella pneumoniae* islotes obtained from humans, general genomic features, MLST, phylotyping and antimicrobial resistance genes for the different families of antimicrobials with the percentage of identity with published sequences.

									Aminoglycoside						Phenicol	
Strain_ID	Source	National Park	MLST	Genome_size	N50	GC_content	Contig_number	Total_AMR	aac(3)-Iia	aac(3)-Iid	aac(6')-Ib-cr	ant(3")-Ia	aph(3")-Ib	aph(6)-Id	catA1	catA2
A36263	Human	Mgahinga NP	1411	5348081	80229	57,41	162	10	100*	.	.	82,51*	95,9	100	100	.
A36272	Human	Murchison Falls NP	5405	5209663	32944	57,43	376	12	.	100	.	.	100	100	.	100
A36274	Human	Murchison Falls NP	841	5275217	41211	57,8	243	5	.	.	.	.	100*	100*	.	.
A36275	Human	Murchison Falls NP	414	5337269	56803	57,72	215	6	.	.	.	.	100*	100*	.	.
A36290	Human	Mgahinga NP	16	8886151	16402	54,63	2851	14	.	.	100	.	100	100	.	100

	Beta-lactam									Trimethoprim			Fosfomycin			Efflux pumps		Quinolone	Sulphonamide		Sulphonamide	
Strain_ID	blaCTX-M-15	blaOKP-B-15	blaOKP-B-8	blaOXA-1	blaSCO-1	blaSHV-145	blaSHV-172	blaSHV-2	blaTEM-1B	dfrA14	dfrA15	dfrA17	fosA5	fosA6	fosA	oqxA	oqxB	qnrS1	sul1	sul2	tet(A)	tet(D)
A36263	100*	.	.	.	100	.	100	.	100*	.	100	.	.	.	98,33	100	100	.	100*	100	97,8*	.
A36272	.	.	.	.	.	.	.	100	.	100	.	.	.	97	.	100	100	100	.	100	.	100
A36274	100*	99,88	.	.	.	.	.	.	100*	100	.	.	.	.	100	100	100	100*	.	100*	.	.
A36275	100*	.	100	.	.	.	.	.	100*	100	.	.	.	.	100	100	100	100*	.	100*	97,8	.
A36290	100	.	.	100	.	100	.	.	.	100	.	86,5	99,05	.	.	100	100	.	.	100	97,8	.

Supplementary Table S4.4.3. Predicted location of identified beta-lactam resistance genes and mobile genetic elements associated for the 58 cephalosporin-resistant *E. coli* isolates obtained from different species. PL: plasmid, C: chromosome, V: virus, U: uncertain, NF: not found.

Strain_ID	Species	blaCTX-M-15	blaCTX-M-27	blaOXA-1	blaSHV-12	blaSHV-187	blaTEM-104	blaTEM-1B	blaTEM-1C
A36259	Wildlife	PL	.	.	.	.	.	PL	.
A36260	Wildlife	PL	.	.	.	.	.	PL	.
A36261	Human	V	.	.	.	.	.	U	.
A36262	Human	PL	.	.	.	.	.	.	.
A36264	Human	PL	.	.	.	.	.	PL	.
A36265	Human	PL	.	.	.	.	.	PL	.
A36266	Human	PL	.	.	.	.	.	.	.
A36267	Human	V	.	.	.	.	.	.	.
A36268	Human	PL	.	.	.	.	.	NF	.
A36269	Human	C	.	U	.	.	.	NF	.
A36270	Human	.	PL	.	.	.	.	PL	.
A36271	Human	PL	.	.	.	.	.	.	.
A36273	Human	PL	.	.	.	.	.	NF	.
A36276	Human	PL	.	.	.	.	.	.	.
A36277	Human	C	.	.	.	.	.	NF	.
A36278	Livestock	PL	.	.	.	.	.	NF	.
A36279	Livestock	PL	.	.	.	.	.	.	.
A36280	Human	V	.	.	.	.	.	.	.
A36281	Human	V	.	.	.	.	.	.	.
A36282	Human	C	.	.	.	.	.	.	.
A36283	Human	NF	.	.	.	.	.	.	.
A36284	Human	PL	.	.	.	.	.	PL	.
A36285	Human	PL	.	.	.	.	.	.	.
A36286	Human	PL	.	.	.	.	.	PL	.
A36287	Human	.	PL	.	.	.	.	NF	.
A36288	Human	PL	.	.	NF	.	.	PL	.
A36289	Human	C	PL	.	.	.	.	NF	.
A36291	Human	NF	.	PL	.	.	.	NF	.
A36292	Human	C	.	.	.	.	.	.	.

Strain_ID	Species	blaCTX-M-15	blaCTX-M-27	blaOXA-1	blaSHV-12	blaSHV-187	blaTEM-104	blaTEM-1B	blaTEM-1C
A36293	Human	C	.	.	.	.	.	.	.
A36294	Livestock	C	.	.	.	.	.	.	.
A36295	Livestock	PL	.	.	.	.	.	PL	.
A36296	Livestock	PL	.	.	NF	.	.	U	.
A36297	Livestock	PL	.	.	.	.	.	PL	.
A36298	Livestock	PL	.	.	.	.	.	PL	.
A36301	Livestock	PL	.	.	.	.	.	.	.
A36302	Livestock	PL	.	.	.	.	.	.	.
A36304	Livestock	V	.	.	.	.	.	.	.
A36305	Livestock	C	.	.	.	.	.	.	.
A36306	Livestock	PL	.	.	.	.	.	.	.
A36307	Livestock	.	.	.	NF	.	.	NF	.
A36308	Wildlife	C	.	.	.	.	.	.	.
A36309	Human	U	.	U	.	NF	.	.	.
A36310	Human	NF	.	.	.	.	NF	.	.
A36311	Human	U	.	U	NF	.	.	.	PL
A36312	Human	PL	.	.	.	.	.	PL	.
A36313	Human	U	.	U	.	.	.	NF	.
A36314	Human	PL	.	.	.	.	.	PL	.
A36315	Human	PL	.	.	.	.	.	NF	.
A36316	Human	NF	.	.	.	.	.	PL	.
A36317	Livestock	PL	.	.	.	.	.	.	.
UECR10A	Wildlife	C	.	.	.	.	.	.	.
UECR11A	Wildlife	U	.	.	.	.	.	NF	.
UECR16A	Wildlife	U	.	.	.	.	.	NF	.
UECR24A	Wildlife	.	.	.	.	.	.	.	.
UECR5A	Wildlife	.	.	.	NF	.	.	NF	.
UECR74A	Wildlife	PL	.	.	.	.	.	PL	.
UECR8A	Wildlife	PL	.	NF	.	.	.	.	.

Supplementary Table S5.4.3. Predicted location of identified beta-lactam resistance genes and mobile genetic elements associated for the 5 cephalosporin-resistant *Klebsiella pneumoniae* isolates obtained from human samples. PL: plasmid, U: uncertain, NF: not found.

Strain_ID	Species	CTX-M-15	OKP-B-15	OKP-B-8	OXA-1	SCO-1	SHV-145	SHV-172	SHV-2	TEM-1B
A36263	Human	PL	.	.	.	NF	.	NF	.	PL
A36272	Human	.	.	.	.	.	.	.	NF	.
A36274	Human	PL	NF	.	.	.	.	.	.	PL
A36275	Human	PL	.	NF	.	.	.	.	.	PL
A36290	Human	PL	.	.	U	.	NF	.	.	.

4.4. Study 4:

Pathogenic bacterial communities in the gut of chimpanzees, humans and goats from western Uganda

Abstract

Anthropogenic alterations are disrupting tropical ecosystems, creating exceptional opportunities for pathogen exchange between animals and humans. Budongo Central Forest Reserve (BCFR) is a forest located in the Albertine Rift in western Uganda, inhabited by wild chimpanzees. The anthropogenic pressure on the forest and chimpanzee communities is high, which may facilitate the exchange of pathogens among humans, livestock, and chimpanzees. This study aims to investigate the presence of potentially pathogenic bacteria from faecal samples in two chimpanzee communities from BCFR (Sonso and Waibira), as well as in humans and goats from the surrounding areas. Between 2021 and 2022, 87 faecal samples were collected and sequenced through full-length 16S rRNA metabarcoding using the nanopore long-read sequencer MinION™. *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the most abundant phyla across all species analysed. Twenty-one potential pathogenic bacterial genera and 181 bacterial species were identified. *Clostridium perfringens, Escherichia coli*, *Salmonella enterica*, *Shigella* and *Streptococcus* species were detected in all the species studied. Although gut microbiota of human and goat samples exhibited greater similarity compared to chimpanzees, chimpanzees from BCFR still harbour potentially pathogenic bacteria similar to humans. This suggests that increasing contact between people and chimpanzees enhances conditions for the transmission of bacterial pathogens in BCFR and its surrounding areas. Longitudinal studies involving genomic analyses of potential pathogenic microorganisms may play a crucial role in the surveillance and conservation efforts of chimpanzees from BCFR.

4.4.1. Introduction

Several anthropogenic alterations such as deforestation, farming practices, agriculture expansion and oil extraction are disrupting tropical ecosystems (Laurance et al. 2014). Expanding agricultural activities are the main cause of tropical forest loss in Africa (Pendrill et al. 2022), bringing wildlife populations at risk of decline (Brearley et al. 2013; Crooks et al. 2017). In the case of non-human primates, approximately 60% of all the world's primates are threatened with extinction due to habitat loss and fragmentation (IUCN 2024). In turn, habitat degradation and the close relationship between humans and primates create exceptional opportunities for pathogens exchange between wildlife, livestock and humans (Cooper and Nunn 2013; Olivero et al. 2017). Primates in degraded forests have proved to face nutritional deficiencies and, in consequence, an impoverished gut microbiome diversity. These primates also show an increased prevalence of parasites and pathogens (Calvignac-Spencer et al. 2012; Bublitz et al. 2015; Parsons et al. 2015).

Budongo Central Forest Reserve (BCFR) is a moist semi-deciduous forest located at the top north of the Albertine Rift in western Uganda. It is the largest forest reserve in Uganda, covering about 853 km^2, and is inhabited by different species of diurnal primates, including the Eastern chimpanzee (*Pan troglodytes schweinfurthii*), the colobus monkey (*Colobus guereza*), the baboon (*Papio anubis*), the blue monkey (*Cercopithecus mitis*) and the red-tailed monkey (*Cercopithecus ascanius*) (Sandbrook et al. 2018). In fact, one of the main goals of BCFR is the conservation of local chimpanzee populations. Land use and cover change around BCFR have changed significantly since 1988, primarily due to sugarcane plantations and agriculture expansion (Mwavu and Witkowski 2008). Bujenje county is one of the populated regions bordering BCFR, where the main economic activities are agriculture and subsistence farming. Due to a shortage of available food within the forest during the dry season (Tweheyo et al. 2005), some chimpanzees leave BCFR to forage for food in croplands, leading to frequent encounters with people and domestic animals, and the potential interaction with livestock and/or human pathogenic bacteria (Dunay et al. 2018; Bager et al. 2022). The "Status Survey and

Conservation Plan 2010-2020" for the Eastern Chimpanzee by the IUCN SSC Primate Specialist Group identified, as an objective, the improvement of understanding and halting the decline in chimpanzee populations due to human-transmitted diseases in Chimpanzee Conservation Units (CCUs) (Plumptre et al. 2010).

Microbial exchange between humans and non-human primates poses a risk to primate health and conservation. Studying the dynamics of infectious agents is crucial in areas with high chimpanzee-human-livestock interaction to predict the emergence of epidemics that could endanger the conservation of great apes. This is exemplified by the transmission of respiratory pneumoviruses and paramyxoviruses of human origin, which have caused disease in wild apes across Sub-Saharan Africa, including Uganda (Grützmacher et al. 2016; Emery Thompson et al. 2018; Weary et al. 2024). However, there has been limited research over the last two decades on the ecology, surveillance and monitoring of the impact of infectious diseases, particularly bacterial diseases, on chimpanzees in East Africa.

Due to the difficulty of capturing and sampling live chimpanzees safely and accessing fresh post-mortem carcasses, the few studies on chimpanzee health assessment have relied on non-invasive samples and conventional molecular techniques such as PCRs, which only detected a selected number of pathogen species. In contrast, the recent development of next-generation sequencing (NGS)-based methods allows researchers to identify the complete diversity of bacteria in a sample by amplifying and sequencing universal bacterial genetic targets, such as the 16S rRNA gene (Johnson et al. 2019). Nowadays, the emergence of 3rd generation sequencing methods such as nanopore-based sequencing by Oxford Nanopore Technologies allows longer read lengths that permit sequencing the entire 16S rRNA gene, providing an improved taxonomic resolution, as well as cost-efficient (M.Q. Zhang et al. 2023). Full-length 16S rRNA gene sequencing has been performed for environmental samples and clinical diagnostics (Catozzi et al. 2020; Matsuo et al. 2021; Huggins et al. 2022) but, to our knowledge, it has never been used to monitor pathogenic bacteria in wild chimpanzee communities. Therefore,

the aim of this study is to use the full-length 16S rRNA gene sequencing as a broad screening tool for potentially pathogenic enteric bacteria in humans, goats and wild chimpanzee communities in an area with high chimpanzee-human-livestock interaction.

4.4.2. Materials and Methods

This study was conducted in 2021 and 2022 in Budongo Central Forest Reserve (BCFR; 1°43′27″N, 31°32′45″E) and Bujenje county (BC; 1º31′48″N, 31º37′28″E), located in the Rift Valley, Western Uganda. The forest is home to several chimpanzee communities. Two of these, Sonso and Waibira, are habituated to human presence and have estimated population sizes of approximately 70 and 110 individuals, respectively (Hobaiter et al. 2017). These communities are observed daily, with a variable percentage of their members individually identified. However, chimpanzees from Sonso and Waibira communities exhibit different levels of interaction with humans and livestock. During periods of low food availability, Sonso chimpanzees venture outside the forest to forage in croplands, whereas Waibira chimpanzees do not engage in such behaviour (Villioth 2018).

Overall, 87 faecal samples were collected from goats (n = 21), chimpanzees (n = 41) and humans (n = 25). Regarding wild chimpanzees, 21 and 20 samples were collected from Sonso and Waibira communities, respectively. Faecal samples were collected in sterile plastic sample bags and stored at 4-8ºC for up to 72 hours until laboratory analyses. All the methodologies were authorized by the Uganda Wildlife Authority (UWA) (EDO/35/01 and COD/96/02), and the Uganda National Council for Science and Technology (UNCST) (A127ES).

Faecal DNA extraction was carried out using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's instructions. The DNA concentrations of the samples were quantified using the Qubit Fluorometric Quantification High Sensitivity Assay (Invitrogen, California, USA). 16S rRNA was selectively amplified from genomic DNA by the polymerase chain reaction (PCR) with universal bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and

1492R (5'-GGT TAC CTT GTT ACG ACT T-3'), using SQK-RAB201 rapid kit from Oxford Nanopore Techlonogies (ONT, Oxford, United Kingdom). Four sequencing runs of 24 multiplexed samples were carried out on a MinION sequencer (ONT) using a brand new R9.6 flow cell. The sequencing data were processed using the Min-KNOW software suite (Lu et al. 2016). Reads adapters and barcodes were trimmed with qcat-1.1.0 (ONT). Taxonomic assignment at genus level was conducted with Centrifuge 10.3-beta (Kim et al. 2016), and at species level with EMU v3.2.0 (Curry et al. 2022), using Silva 132 database (Quast et al. 2013) based on a 95 % of identity threshold. Finally, relative abundance tables were obtained from counts tables.

Alpha diversity of each samples' whole microbiome and pathogenic fraction of interest for this study was estimated by Observed, Shannon, Inverse Simpson, and Pielou indices. Differences between alpha diversity indices were assessed using the Kruskal–Wallis test (Kruskal and Wallis 1952) with a significance threshold set at $p < 0.05$. Additionally, beta diversity was determined using Bray–Curtis distances and significant differences assessed with multivariate homogeneity of groups dispersion (Beta disper) and non-parametric multivariate statistical permutation (PERMANOVA) tests with a significance threshold set at $p < 0.05$. Alpha diversity boxplots, non-metric multidimensional scaling (NMDS) plots and Bar Plots were obtained using the R package vegan (Oksanen et al. 2007) at phylum, genus and species levels. Core (taxa shared by 100% of samples), accessory (taxa shared by samples from 2 or 3 scenarios) and exclusive microbiomes (taxa found exclusively in one scenario) were identified and the Venn diagram was obtained using the VennDiagram package (Chen and Boutros 2011). All statistical analyses were performed using the R version 4.3.0 (R Core Team 2022).

4.4.3. Results

Firmicutes, *Bacteroidetes* and *Proteobacteria* were the most abundant phyla across all species analysed (Fig. 1.4.4).

Fig. 1.4.4. Stacked bar plot of relative abundances of phyla from goats, humans and Sonso and Waibira chimpanzees.

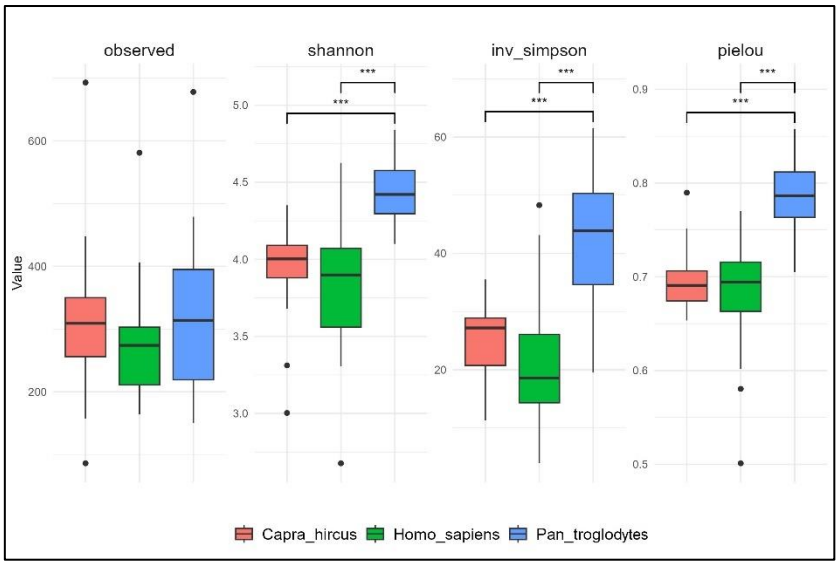


Fig. 2.4.4. Alpha diversity boxplot. Significant differences were observed in chimpanzee samples compared to both goat and human samples for Shannon, Inverse Simpson, and Pielou's alpha diversity indices.

Despite no significant differences have been observed within chimpanzee communities, or between humans and goats, alpha diversity analyses revealed distinct patterns of bacterial diversity among the studied species (Fig. 2.4.4). Beta diversity obtained by Bray-Curtis distance also showed statistical differentiation of community structure across species (PERMANOVA's R: 0.43, p = 0.001). Core community analysis indicated that 232 genera were shared by all species. Notably, bacterial species composition differed significantly between study subjects, with chimpanzees exhibiting a notably more diverse microbiome (Fig. 3.4.4).

Regarding potentially pathogenic bacterial genera, twenty-one out of the thirty-two genera studied were identified. *Bacillus*, *Campylobacter*, *Clostridium*, *Escherichia*, *Enterobacter*, *Enterococcus*, *Haemophilus*, *Mycoplasma*, *Salmonella*, *Shigella,* *Streptococcus* and *Treponema* spp. were detected in all species. *Helicobacter* and *Citrobacter* spp., and *Klebsiella* and *Staphylococcus* spp. were detected in humans and chimpanzees, and humans and goats, respectively. In contrast, *Listeria* spp. was only present in humans, and *Acinetobacter*, *Brucella*, *Pseudomonas* and *Serratia* spp. in chimpanzees (Table 1.4.4). *Aeromonas*, *Arcobacter*, *Bordetella*, *Coxiella*, *Legionella*, *Leptospira*, *Moraxella*, *Mycobacterium*, *Neisseria*, *Vibrio* and *Yersinia* spp. were not detected.

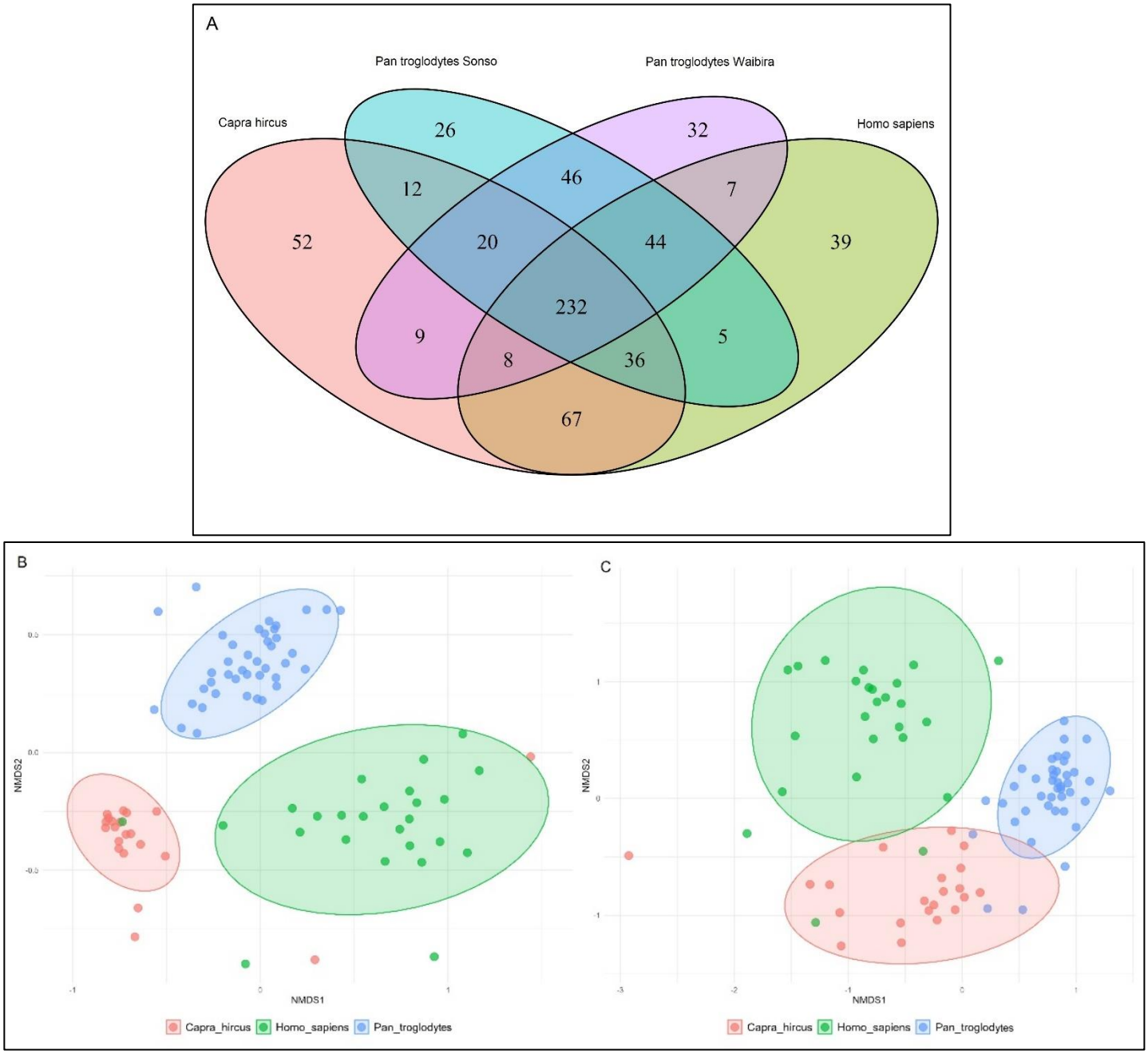


Fig. 3.4.4. (A) VennDiagram of genera shared across species. Non-metric multidimensional scaling (NMDS) plot comparing bacterial communities from different species (goats, humans, and chimpanzees) of whole microbiome species (B) and the potential pathogens of interest fraction (C).

Table 1.4.4. Occurrence of the different bacterial genera detected according to each species studied and 95% confidence intervals (CI) between parentheses.

	Goats	Humans	Chimpanzees		
			Sonso	Waibira	Total
Acinetobacter spp.	0	0	25% (11.2-46.9)	31.6% (14.3-55.5)	28.2% (16.4-44)
Bacillus spp.	95% (76.4-99.1)	25% (11.2-46.9)	15% (5.2-36)	26.3% (11.6-49.1)	20.5% (10.4-36.1)
Brucella spp.	0	0	5% (0.8-23.6)	0	2.6% (0.3-13.8)
Campylobacter spp.	90% (69.9-97.2)	60% (38.7-78.1)	100%	100%	100%
Citrobacter spp.	0	5% (0.9-23.6)	5% (0.8-23.6)	21.1% (8.5-43.4)	12.8% (5.5-26.9)
Clostridium spp.	65% (43.3-81.9)	60% (38.7-78.1)	40% (21.9-61.4)	47.4% (27.3-68.3)	43.6% (28.9-54.9)
Escherichia spp.	100%	80% (58.4-92)	50% (16.3-59.1)	73.7% (48.6-90)	61.5% (45.4-75.5)
Enterobacter spp.	15% (5.2-36)	20% (8.1-41.6)	35% (18.1-56.7)	31.6% (14.3-55.5)	33.3% (20.4-49.3)
Enterococcus spp.	60% (38.7-78.1)	20% (8.1-41.6)	15% (5.2-36)	42.1% (21.5-65.6)	28.2% (0.1-15.1)
Haemophilus spp.	10% (2.8-30.1)	85% (64-94.8)	0	36.8% (18.5-59.7)	17.9% (8.9-32.7)
Helicobacter spp.	0	5% (0.8-23.6)	25% (11.2-46.9)	21.1% (8.5-43.4)	23.1% (12.6-38.4)
Klebsiella spp.	10% (2.8-30.1)	20% (8.1-41.6)	0	0	0
Listeria spp.	0	10% (2.8-30.1)	0	0	0
Mycoplasma spp.	20% (8.1-41.6)	15% (5.2-36)	35% (18.1-56.7)	36.8% (35.4-91.9)	35.9% (22.7-51.6)
Pseudomonas spp.	0	0	25% (11.2-46.9)	42.1% (23.1-63.7)	33.3% (20.4-49.4)
Salmonella spp.	65% (43.3-81.9)	30% (14.5-51.9)	20% (8.1-41.6)	36.8% (19.1-59)	28.2% (16.4-44
Shigella spp.	40% (21.9-61.3)	25% (11.2-46.9)	10% (2.8-30.1)	5.3% (0.9-24.7)	7.7% (2.5-20.7)
Serratia spp.	0	0	15% (5.2-36)	36.8% (35.4-91.9)	25.6% (14.3-41.5)
Staphylococcus spp.	5% (0.9-23.6)	5% (0.9-23.6)	0	0	0
Streptococcus spp.	55% (34.2-74.2)	90% (70-97.2)	30% (23.8-68)	36.8% (35.4-91.9)	33.3% (20.4-49.4)
Treponema spp.	85% (64-94.8)	15% (5.2-36)	100%	100%	100%

Regarding pathogenic genus abundance across goats, humans and chimpanzees, *Clostridium* and *Streptococcus* were identified as the most prevalent genera. *Bacillus* showed high dominance in goat and human samples, while *Treponema* was notably abundant in chimpanzees (Fig. 4.4.4). Comparing the chimpanzee communities, Sonso and Waibira, *Acinetobacter, Bacillus, Campylobacter*, *Citrobacter, Clostridium*, *Escherichia, Enterobacter, Enterococcus, Helicobacter*, *Mycoplasmia, Pseudomonas*, *Salmonella, Serratia, Shigella*, *Streptococcus* and *Treponema* spp. were detected in both groups at different frequencies (Table 1.4.4) while *Brucella* and *Haemophilus* spp. were only detected in Sonso and Waibira respectively.

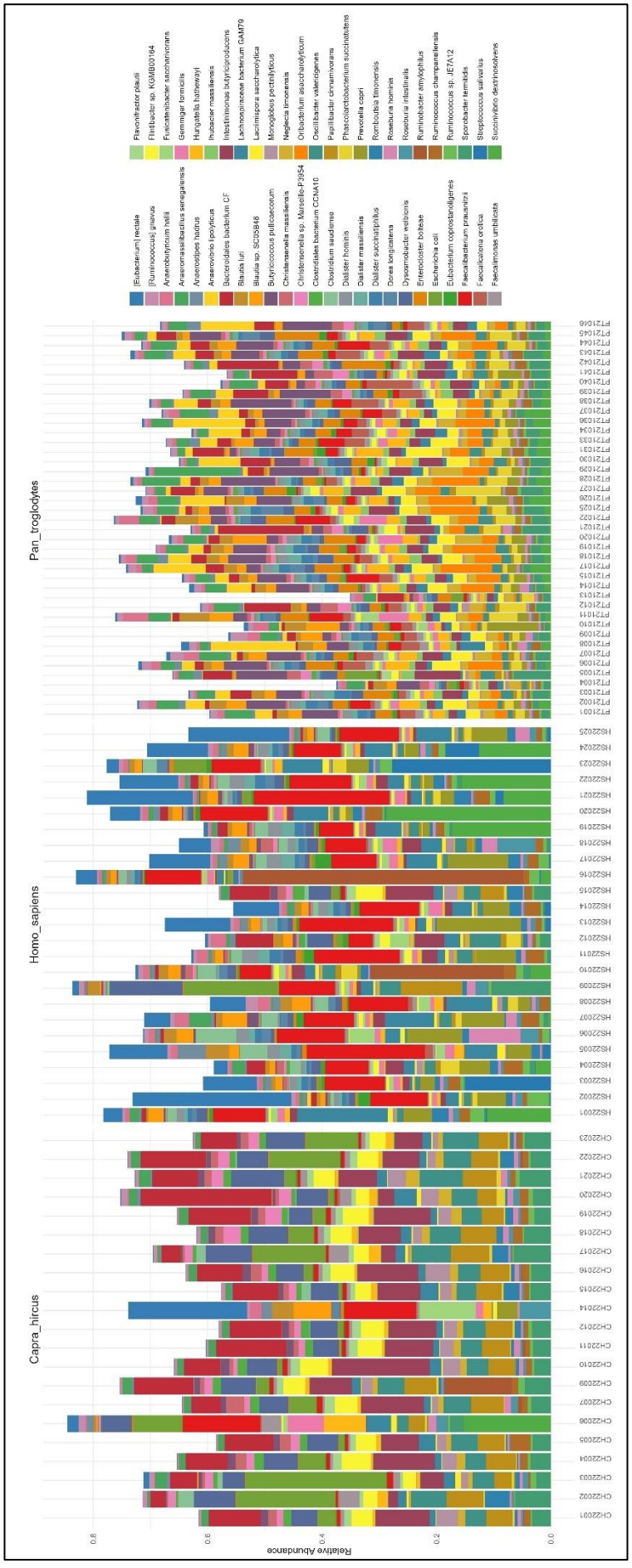


Fig. 4.4.4. Stacked bar plot showing the relative abundances of the 50 most common species across goats, humans and chimpanzees.

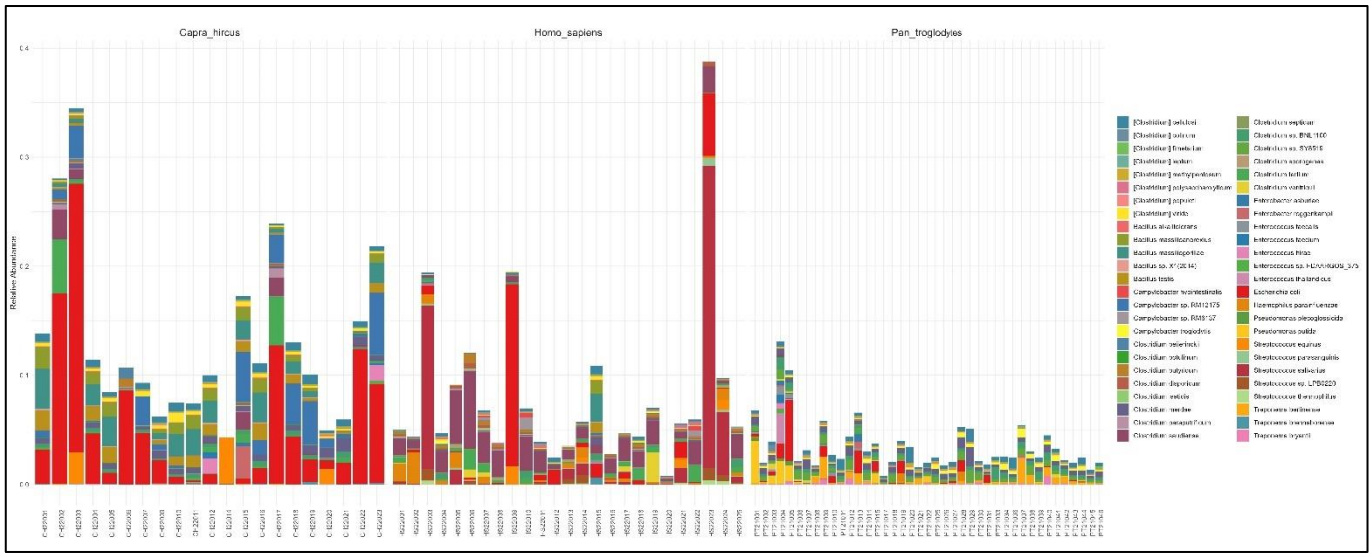


Figure 5.4.4. Stacked bar plot showing the relative abundances of the 50 most common pathogenic species across goats, humans and chimpanzees.

According to the same criteria, several bacterial species primarily associated with gastrointestinal (GI) and respiratory diseases were further investigated. The relative abundance of each bacterial species was notably lower in chimpanzees compared to goats and humans, where such species can constitute nearly 40% of the total microbial community. In contrast, in chimpanzees, no sample exceeds 15-20% in relative abundance (Fig. 5.4.4). From the 169 bacterial species identified, *Clostridium perfringens, E. coli*, *Salmonella enterica*, *Shigella boydii, Shigella flexneri* and *Streptococcus (Strep.) suis* were detected in all the species studied. *Campylobacter lanienae* was found in goats and humans, while *Campylobacter troglodytis* and *Helicobacter fennelliae* were detected in humans and chimpanzees. However, several bacterial species were identified in one single source. *Campylobacter coli* and *C. jejuni* were exclusively present in goats, whereas *Strep. pneumoniae* and *K. pneumoniae* were only detected in human samples. Finally, *Campylobacter troglodytis*, *Haemophilus influenzae* and various species of the genus *Pseudomonas* were exclusively detected in chimpanzees.

4.4.4. Discussion

According to previous research, the gut microbiota of all species in our study mainly comprises by *Firmicutes*, *Bacteroidetes* and *Proteobacteria* (Nishida and Ochman 2019; Zhang et al. 2019). While *Clostridium* and *Streptococcus* are the most abundant genera across all species, *Bacillus* is more abundant in human and goat samples, whereas *Treponema* is more abundant in chimpanzees. The present study shows that chimpanzees from BCFR harbour potentially pathogenic enteric bacteria similar to those found in goats and humans from a nearby region in western Uganda. This finding is consistent with previous research indicating that agricultural and farming expansion, particularly in proximity to wildlife habitats, promotes the circulation of bacterial pathogens at the livestock-human-chimpanzee interface (Bengis et al. 2004).

Despite the similarities observed, chimpanzees exhibited more diverse microbiomes and a lower abundance of pathogenic bacteria compared to humans and goats. This greater microbial diversity is generally associated with a healthier gut ecosystem, providing resilience against infections and disruptions (Clemente et al. 2012). The lower abundance of pathogenic bacteria suggests that chimpanzees may be less susceptible to certain diseases, potentially due to their natural environment and diet, which supports a more balanced and robust microbial community (Baümler and Sperandio 2016). However, the proximity and interaction with human settlements pose a significant risk of introducing harmful pathogens into chimpanzee populations. These findings emphasize the importance of protecting chimpanzees from human-related impacts that could disrupt this beneficial microbiome balance.

Respiratory diseases are the primary cause of morbidity and mortality in human-habituated wild apes (Kaur et al. 2008; Palacios et al. 2011). However, information about the impact of enteric bacteria on the health of these species is limited. In Uganda, enterobacterial pathogens, including *Campylobacter* spp., *E. coli*, *Salmonella* spp. and *Shigella* spp. have been reported in non-human primates from human-impacted habitats, with transmission attributed to habitat sharing (Nizeyi et al. 2001; Goldberg et al. 2007; McLennan et al. 2018). In this study, the detection

of *Clostridium perfringens*, *E. coli*, *Salmonella enterica* and *Shigella* spp. in the three species of study suggests a potential bacterial sharing between humans, livestock and chimpanzees. Even though chimpanzee individuals from our study were apparently healthy, *Shigella* and *Salmonella* spp. infections have been previously attributed to captive chimpanzees' deaths (Ocholi et al. 1987; Enurah et al. 1988). Therefore, more studies on the potential pathogenicity of enteric bacteria and their impact on the conservation of wild chimpanzees are needed.

Bacterial communities from goats and humans were more similar to each other than those from chimpanzees, with highly abundant *Campylobacter* spp. and *Staphylococcus* spp. in both species. On the other hand, chimpanzees shared *Campylobacter troglodytis* and *Helicobacter fennelliae* with humans. *Campylobacter* species are a major cause of gastroenteritis worldwide, and despite poultry being the main source of transmission of campylobacteriosis to humans, members of this genus naturally colonize a wide range of hosts (Man 2011). *Campylobacter troglodytis* was first detected in 2011 in asymptomatic, human-habituated chimpanzees from Tanzania (Kaur et al. 2011), but also in faeces of infants prone to enteric infectious diseases living in developing countries (Platts-Mills et al. 2014). *Helicobacter* species have been reported to cause gastrointestinal and hepatic disease in humans (Cover and Blaser 2009; Sumida et al. 2015). *Helicobacter fennelliae*, detected in chimpanzees from our study, is a relatively new species isolated from asymptomatic and symptomatic people, especially immunocompromised individuals (Totten et al. 1985; O'Rourke et al. 2001). Additionally, it has also been detected in asymptomatic gorillas and chimpanzees from different African countries (Flahou et al. 2014). Various species of *Streptococcus* and *Pseudomonas* have been identified in chimpanzees in this study. Notably, *Strep. pneumoniae* of human origin has been previously detected in wild chimpanzees, posing a serious threat to wild chimpanzee populations conservation (Köndgen et al. 2017). This finding underscores the potential for pathogenic *Streptococcus* species to impact chimpanzee populations' health. While *Pseudomonas aeruginosa* has been detected in nasopharyngeal samples from asymptomatic semi-captive chimpanzees in Uganda (Mugisha et al. 2014), none of

the *Pseudomonas* species detected in our study have been previously reported in free-ranging chimpanzees.

Contrary to expectations, no differences were observed in the detection and abundance of enteric bacteria between Sonso (with interaction with human settlements) and Waibira chimpanzees (with no interaction with human settlements). During periods of food scarcity, chimpanzees extend their ranging patterns to access croplands, typically occurring in the dry season (Tweheyo et al. 2005). As the sampling was conducted at the end of the wet season, the restricted range of both chimpanzee communities inside the forest could explain the similarity in pathogen detection. Increased interaction with human settlements in periods of low food availability could alter the enteric bacterial patterns in the Sonso community. Thus, further sampling in different seasons and years is needed to shed light on the implications of a wide movement range and food availability on chimpanzees' enteric bacteria. Understanding how factors such as diet and seasonality influence the microbiome is crucial, as dietary variations and changes in ranging behaviour can significantly impact the composition and diversity of gut bacteria (Hicks et al. 2018). This is essential for effective conservation and health management of chimpanzee populations, enabling better strategies to mitigate the risks of pathogen transmission and maintain the overall health of these primates.

Since 1988, land cover around BCFR has changed significantly, mainly attributed to agricultural expansion (Mwavu and Witkowski 2008). This forest habitat loss facilitates pathogens' direct and indirect transmission through common environmental sources between chimpanzees and human populations (Tweheyo et al. 2005). Interdisciplinary research focusing on both landscape and disease ecology is needed to comprehend the consequences of land degradation on chimpanzee populations' health. Furthermore, integrated policy and management measures are essential to minimise pathogen spillover, protecting both chimpanzees and public health. In addition to habitat degradation, the growing number of great apes habituated for research and tourism increases the risk of anthropozoonotic disease transmission (Glasser et al. 2021; Weary et al. 2024). This poses a threat

to non-human primates' conservation, as observed in various research and tourism sites in Africa that have experienced viral, bacterial and parasitic infection transmission from humans (Goldberg et al. 2007; Hanamura et al. 2008; Kaur et al. 2008; Grützmacher et al. 2018; Negrey et al. 2019). While the habituation of chimpanzees from BCFR for research has a significant positive impact on their conservation, it also presents significant health challenges. The close proximity of human populations around the forest and the activities involved in habituating and studying chimpanzees introduce several health risks that need to be identified and investigated thoroughly. Collaborative efforts between researchers, conservationists, local communities, and governments are vital to balancing the benefits of habituation and research with the need to protect chimpanzees and their habitats.

4.4.5. Conclusions

Increasing contact between people and chimpanzees enhances conditions for the transmission of bacterial pathogens in BCFR and its surrounding areas. As human populations encroach into new habitats, pathogens' spillover between humans and wildlife becomes increasingly likely. Since emerging infectious diseases pose a serious threat to endangered primates, longitudinal studies involving genomic analyses may play a crucial role in the surveillance and conservation efforts of chimpanzees from BCFR through early detection and identification of novel pathogens.

5. General discussion

This thesis aims to provide a comprehensive investigation into the presence of various pathogens and the emergence of AMR in western Uganda, where the intersection between human, animal, and environmental health is of particular concern. By gaining a deeper understanding of the interplay between pathogens and antimicrobial resistance in the different regions under study, this research aims to contribute with evidence-based data to the development of strategies for mitigating AMR transmission and safeguarding human, animal, and environmental health.

One Health is an integrated approach that recognizes the interconnection between human, animal, and environmental health, making it crucial for addressing health challenges worldwide. This approach is critical in developing countries due to the high burden of zoonotic diseases and antimicrobial resistance dissemination, which can significantly impact public health, food security, and economic development. Agricultural practices and poor animal husbandry practices increase the risk of pathogen transmission from animals to humans. Additionally, increased human-wildlife contact and water and soil contamination serve as reservoirs of pathogens, affecting both animal and human populations. Furthermore, inadequate sanitation facilities and improper waste disposal contribute to food and water contamination. Coupled with the lack of proper food safety measures and inadequate cooking practices, these conditions facilitate the spread of foodborne pathogens. Globally, the most prominent foodborne pathogens include *Salmonella*, *Campylobacter*, and *E. coli*, major causes of gastrointestinal illness and foodborne outbreaks (WHO 2022). These pathogens pose significant public health risks due to their widespread prevalence and varied transmission sources. Study 2 examines the distribution of *Campylobacter*, *Salmonella* and *Arcobacter* spp. in humans, livestock and wildlife from areas with different levels of interaction in rural western Uganda. Despite previous studies have reported a greater prevalence of *Salmonella* spp. in Uganda than the one observed in our study, the detection of serovar Typhimurium in cattle and humans represents a significant public health threat, as this pathogen is considered is an important cause of bacteraemia in Africa, especially in immunocompromised individuals (Uche et al. 2017). In Uganda, the burden of HIV,

malaria and tuberculosis significantly tensions the healthcare system and exacerbates vulnerability to other infectious diseases (UNAIDS 2023; WHO 2023a; WHO 2023b), amplifying the impact of foodborne pathogens. Additionally, *S.* Typhimurium is often antibiotic-resistant, complicating treatment strategies (Marchello et al. 2020). Study 2 also revealed a high diversity and a wide host range of *Campylobacter* spp. in western Uganda by its detection in both livestock and wildlife, reflecting the complex ecology of these pathogens. The high frequency observed in Ugandan kob and the significant occurrence in humans underscores the zoonotic potential of *Campylobacter* spp. Even though wildlife can act as an important carrier of this pathogen, especially wild birds (Olvera-Ramírez et al. 2023), poultry and cattle are considered to act as the main reservoirs of *Campylobacter* human infection (Mughini-Gras et al. 2021). Our findings suggest that while *Campylobacter* is prevalent in certain wildlife species, the lower detection rates in remote areas underscore the role of livestock as the main reservoirs for these foodborne pathogens, suggesting that pathogen spillover into wildlife likely occurs, particularly in and around protected natural areas in western Uganda. Landscape fragmentation can contribute to *Campylobacter*'s dynamics, influencing wildlife reservoirs, as previously reported in banded mongooses living in urbanized areas from Botswana (Medley et al. 2020). To gain a clearer understanding of how these pathogens exchange between hosts, genomic analyses are needed. Additionally, it is crucial to investigate environmental sources as potential reservoirs of foodborne pathogens, such as contaminated water, soil and wildlife habitats, to better identify and mitigate the sources of infection.

Like other sub-Saharan African countries, Uganda is facing the escalating crisis of AMR (Antimicrobial Resistance Collaborators 2022). The situation is worsened by the inappropriate use and overprescription of antibiotics in human medicine and animal husbandry (Mukasa et al. 2012; Nabaweesi et al. 2021). Additionally, poverty, inadequate sanitation, international travel and the rising need for healthcare interventions for an increasingly vulnerable population contribute to this crisis (Alividza et al. 2018; Frost et al. 2019). Recent surveillance reports indicate a high prevalence of resistant strains of common pathogens affecting humans and livestock

(Obakiro et al. 2021; Kakooza et al. 2023). However, an examination of relevant databases revealed a lack of information on antimicrobial resistance detection in animals and environmental sources in Uganda. By reviewing grey literature from the Faculty of Veterinary Medicine at Makerere University, it was evidenced that valuable information on these subjects had not been published. For that reason, data from 2001 to 2022 were retrieved from both relevant databases and grey literature dissertations to make a systematic literature review about antimicrobial resistance in *E. coli* isolates, which is considered an ideal microorganism for research purposes due to its ease cultivation in laboratory settings, its ability to rapidly develop of resistance to antibiotics, and its significant role in clinical infections (WHO 2021b). While incorporating grey literature can pose challenges, such as variability in quality and lack of standardization, it is crucial for a comprehensive assessment of AMR in Uganda. Grey literature often provides critical data missing from peer-reviewed, commercially published sources (Mahood et al. 2013). This inclusion is essential given the limited publication on AMR in animals, especially wildlife and environmental sources (Berendonk et al. 2015; Vittecoq et al. 2016). By integrating grey literature, we can address publication bias, capture essential data on AMR in veterinary and environmental contexts, and include studies with negative results that might be overlooked. This approach ensures a holistic understanding of the AMR landscape, vital for developing effective interventions in Uganda. Study 1 encompassed this comprehensive literature review and a questionnaire survey conducted with farmers in rural areas of western Uganda to evaluate antimicrobial use and disposal patterns, revealing a high rate of antibiotic administration and consumption among livestock and farmers. The high usage of antibiotics predominantly involved readily available and inexpensive options, such as penicillins, tetracyclines, and sulphonamides, as evidenced by the reviewed literature and the survey conducted with farmers. In addition to the widespread use of these antibiotics, including self-medication and incorrect administration to livestock, the survey revealed a significant failure to adhere to withdrawal periods for consuming milk and meat derived from treated animals, as well as improper disposal practices for these antibiotics. This agrees with previous reports of antibiotic residues detection in meat in Uganda (Basulira et al. 2019). These practices are similar to those observed in neighbouring countries such

as Rwanda and Tanzania (Manishimwe et al. 2017; Kimera, Frumence, et al. 2020). Failure to adhere to withdrawal times for antibiotics in animals can lead to the presence of drug residues in meat and milk, posing significant risks to human health, such as AMR transfer to humans through the food chain and other adverse health effects in consumers (Bacanli and Başaran 2019). In addition, improper disposal of antibiotics can also have significant health implications as residues can contaminate soil and water sources, leading to AMR bacteria proliferation in the environment. This behaviour is not unique to Uganda; it has also been observed in eastern and western African countries (Julius et al. 2021; Karungamye et al. 2022; Karimi et al. 2023). Implementing proper waste management systems and educating farmers on respecting withdrawal times and safe disposal practices is crucial for mitigating risks and protecting public health.

In Study 3, the phenotypic resistance of *E. coli* and *Klebsiella* spp. isolates from human, livestock and wildlife samples collected from the same regions as the questionnaires were assessed. Initially, a high prevalence of *E. coli* was observed across humans, livestock, wildlife and water samples. The detection of *E. coli* is particularly significant as it serves as both an indicator of resistant bacterial phenotypes globally and a marker of faecal contamination (Anjum et al. 2021). Higher proportions of phenotypic resistance to beta-lactams, sulphonamides and tetracyclines were observed in human, livestock and wildlife samples, aligning with the frequent and improper antimicrobial use practices identified in the survey from Study 1, as this misuse of readily available antibiotics could facilitate the emergence and spread of resistance (WHO 2019b). Surprisingly, despite improper antibiotic dispensing practices, no AMR was detected in *E. coli* strains from water samples across the different national parks. Although AMR detection reports in environmental sources in Africa are limited (Kimera, Mshana, et al. 2020), the extensive use of antibiotics has led to their widespread presence in both natural and artificial environments worldwide (Gothwal and Shashidhar 2015). The absence of *E. coli* resistant strains could be attributed to several factors. Environmental samples generally have a lower and more dispersed microbial load than human or animal samples. Hence, random sampling may not fully capture the diversity and

prevalence of *E. coli* resistance strains in the environment (Van Elsas et al. 2011). Furthermore, culturing methods may not be sensitive enough due to their generally low throughput, which can be effective for monitoring AMR in clinical infections but challenging in environmental contexts (Delgado-Blas et al. 2021). Therefore, to obtain a comprehensive understanding of AMR in water sources, it may be necessary to employ more sensitive techniques, such as molecular or sequencing-based methods (Anjum et al. 2021), which enable a genomic examination offering a thorough perspective on AMR present in various microbial reservoirs.

A high frequency of resistance to third-generation cephalosporins, widely described in Uganda's hospitals (Kiggundu et al. 2022), was observed in humans, livestock and wildlife from the rural areas of our study. Those isolates resistant to third-generation cephalosporins, which were also MDR, were further analysed by WGS to investigate lineages and resistance genes. The widespread presence of the CTX-M ESBL enzymes in *E. coli* and *K. pneumoniae* isolates across human and animal species is reported in Study 3, being CTX-M-15 the most common gene detected. This resistance gene was detected not only in human and livestock populations, but also in wildlife, including baboons and a chimpanzee from the BCFR, which is a mosaic of dense tropical rainforest, and a kob from QENP, characterized for a savanna landscape. Overall, the villages surrounding BFCR are predominantly agricultural, with sugarcane, tobacco and maize cultivation as the main economic activities (Mwavu and Witkowski 2008; Kusiima et al. 2022). In QENP, the surrounding villages are diverse, engaging primarily in agriculture, fishing, and small-scale trade, benefiting from their proximity to the park through tourism and natural resources. However, this proximity to human settlements presents various challenges, such as human-wildlife conflicts, where animals from the park can damage crops or threaten livestock and people (Webber et al. 2007; Braczkowski et al. 2020). Habitat overlap has already been reported as a risk for pathogen spillover between humans, livestock and wildlife in Uganda (Goldberg et al. 2007; Rwego et al. 2008). Despite conservation efforts and community-based tourism initiatives aimed to balance these challenges by providing sustainable livelihoods and fostering positive relationships between protected natural areas and their

neighbouring communities (Sabuhoro et al. 2021; Joseph et al. 2022), the detection of CTX-M-15 and other common resistance genes in these diverse hosts underscores the interconnectivity between human, animal, and environmental health in these ecologically sensitive areas. The presence of such resistant strains in wildlife is particularly alarming as it indicates potential transmission pathways and reservoirs of AMR, complicating efforts to manage and treat bacterial infections. Moreover, many resistance genes were located in plasmids, facilitating rapid dissemination and often harbour multiple resistance genes (J. Zhang et al. 2023; Castañeda-Barba et al. 2024). Previous reports documenting the emergence of plasmid-associated resistance genes encoding ESBLs in wildlife suggested the exchange of genetic material among human, livestock, and environmental reservoirs (Mohsin et al. 2017; Donato and Baron 2022; Tinoco Torres et al. 2022). Although no resistance was detected in environmental samples from our study, the presence of CTX-M genes in wildlife underscores the pivotal role of environmental factors in the spread of AMR. Studies in neighbouring regions and comparable ecological settings have reported its detection in soil, water, and agricultural runoff, highlighting the broader environmental dissemination of ESBL-producing Enterobacterales (Subbiah et al. 2020; Geuther et al. 2023). Additionally, the improper use of antibiotics in livestock, as observed in Study 1, exacerbates the problem by fostering the development and spread of resistance genes. This highlights the urgent need for integrated management strategies that address environmental and agricultural practices to combat AMR effectively. Furthermore, it is vital to enhance education about AMR among farmers, veterinarians and veterinary technicians. Strengthening the knowledge of these animal health professionals is crucial, as their expertise directly influences farmers' practices and understanding of responsible antibiotic use.

A high diversity of *E. coli* and *K. pneumoniae* lineages was detected in Study 3, which aligns with previous findings of resistance gene detection in mobile genetic elements. Although detected at low frequency, lineages of public health significance were identified in both *E. coli* and *K. pneumoniae* strains. Multidrug-resistant *E. coli* ST131 clonal group has been widely reported worldwide as a cause of extraintestinal

infections in humans, posing significant challenges for infection control and acting as a reservoir for the development and spread of AMR (Manges et al. 2019). Furthermore, *K. pneumoniae* ST16, detected in one human isolate, is an important carrier of resistance determinants causing outbreaks worldwide. The detection of these critical clonal groups in remote areas of western Uganda, a country already struggling with a high burden of communicable and non-communicable diseases (WHO 2018; UNAIDS 2023; WHO 2023a; WHO 2023b), underscores the urgent need for targeted surveillance and infection control. This situation highlights the risk of exacerbating AMR in regions with limited healthcare resources and emphasizes the importance of both local and global efforts to address resistance in such vulnerable settings.

The presence of similar enteric bacteria, including *Clostridium perfringens*, *E. coli* and *Salmonella enterica*, in chimpanzees, humans and livestock from BCFR and surrounding regions, as reported in Study 4, underscores a possible cross-species pathogen exchange. These findings align with previous research highlighting the role of agricultural expansion near wildlife habitats in promoting pathogen circulation (Bengis et al. 2004). Although no significant differences in bacterial abundance were observed between chimpanzee communities with varying degrees of human interaction -likely due to their restricted ranging patterns at the end of the wet season-, Study 3 revealed the presence of ESC-resistant *E. coli* isolates in wildlife within BCFR. These results could be attributed to multiple factors, including proximity to human settlements and interaction with livestock, antibiotic misuse as it was reflected in Study 1, combined with environmental contamination. This proximity facilitates pathogen transmission and creates exceptional conditions for resistant bacteria spread. Despite the detection of enteric bacteria using 16S rRNA sequencing provides valuable insights, advanced metagenomic studies are crucial to fully understand the genetic relatedness and potential for pathogenic exchange. These studies will offer a more comprehensive view of the genetic makeup of these pathogens and their potential similarities across species, which is essential for developing targeted interventions to manage and mitigate pathogen and AMR transmission in BCFR.

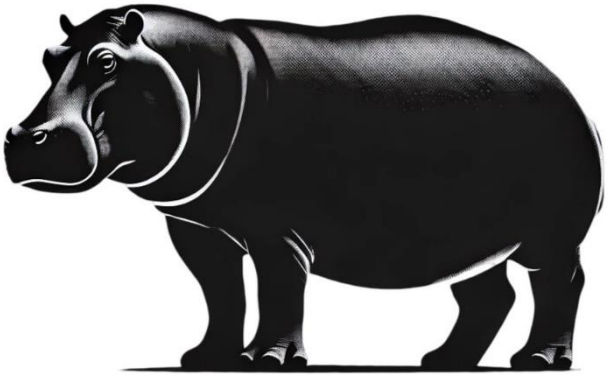
In Uganda, significant actions are being developed to combat infectious diseases and AMR through the concerted efforts of various national and international bodies. The Integrated Disease Surveillance and Response (IDSR) system plays a pivotal role in early detection and prompt response to disease outbreaks (Ministry of Health 2021), enhancing the country's capacity to manage public health threats. Complementing these efforts, the National Action Plan on Antimicrobial Resistance (NAP-AMR) outlines a comprehensive framework for mitigating the spread of AMR through strategic interventions in surveillance, infection prevention and control, and antimicrobial stewardship (Government of Uganda 2018). Additionally, organizations such as the Uganda National Institute of Public Health (UNIPH), the Uganda National Academy of Sciences (UNAS), and the Infectious Disease Institute (IDI), alongside partnerships with the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (OMSA), further support these initiatives. However, Uganda faces significant limitations that hinder the effectiveness of measures against AMR and infectious diseases (Mremi et al. 2021; Matee et al. 2023). These limitations include resource constraints, inadequate human resources, regulatory and enforcement challenges, cultural and behavioural factors, and regional dynamics. Resource constraints, such as limited funding and infrastructure, impede the implementation of comprehensive strategies for managing AMR and controlling infectious diseases. Inadequate human resources, including shortage of trained healthcare professionals and laboratory technicians, further exacerbate the challenge, limiting the detection and management of infectious diseases and AMR. Regulatory and enforcement challenges also pose significant barriers, with gaps in legislation and enforcement mechanisms weakening the control of antibiotic use, distribution, and infection prevention measures. Cultural and behavioural factors, such as the misuse of antibiotics in both human and veterinary medicine, a lack of awareness about AMR, and practices that contribute to the spread of infectious diseases, play a substantial role in the persistence and spread of resistant strains and pathogens. Additionally, regional dynamics, including cross-border movements and trade, facilitate the spread of AMR and infectious diseases across national boundaries, complicating containment efforts. Addressing these limitations requires sustained investment,

robust regulatory frameworks, enhanced surveillance capacity, and ongoing public and professional education to achieve meaningful progress in the fight against infectious diseases and AMR in Uganda.

Looking ahead, future research should focus on several key areas to further advance the understanding and management of pathogen transmission and AMR in Uganda within the One Health framework. Detailed studies on the environmental reservoirs of pathogens and AMR genes, including soil, water, and agricultural runoff, are needed to better understand the pathways of spread and their potential impact on human and animal health. These studies will provide crucial insights for designing and implementing effective interventions. Crucial interventions include improving antibiotic use practices among farmers and enhancing education on the risks of AMR, which should involve developing and promoting guidelines for responsible antibiotic use and exploring community-based programs to raise awareness. Additionally, strengthening surveillance systems to monitor AMR trends and pathogen prevalence across human, animal and environmental domains will provide a more comprehensive picture of their drivers, guiding future interventions.

The research conducted in western Uganda underscores the urgent need for coordinated efforts to address the growing threat of infectious disease and AMR spread. Future studies should build on these findings by focusing on environmental monitoring, improving antibiotic use practices, and enhancing surveillance systems, all within the framework of One Health. These actions are essential to fostering a sustainable and holistic approach to managing pathogen transmission and AMR dynamics, ultimately safeguarding public and environmental health in Uganda and contributing to planetary health.

6. Conclusions



1. There is a notable lack of data on the impact of AMR in Uganda, particularly in domestic animals, wildlife and the environment.

2. There is a significant lack of knowledge and awareness regarding proper antibiotic use and disposal among farmers in rural western Uganda, significantly associated with low levels of education.

3. Wildlife from remote areas exhibit lower prevalence of foodborne pathogens than those from regions with increased livestock-wildlife-human interaction. This suggests that the intensification of livestock farming, particularly near wildlife habitats, may facilitate pathogen spillover.

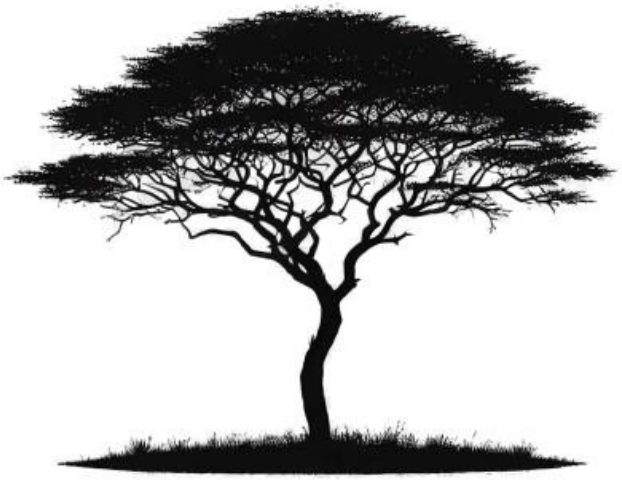
4. High resistance to commonly used antibiotics was observed in humans, livestock and wildlife. Additionally, third-generation cephalosporin resistance, with all isolates being MDR, was detected. Resistant isolates were more prevalent in areas with increased interspecies interaction, suggesting rural interfaces from western Uganda may facilitate AMR transmission.

5. The widespread detection of the CTX-M-15 resistance gene in ESBL-producing *E. coli* and *K. pneumoniae* across humans, livestock and wildlife in Uganda, particularly in remote and pristine areas, underscores the extensive dissemination of multidrug-resistant genes and suggests significant anthropogenic impact on wildlife.

6. The detection of *E. coli* and *K. pneumoniae* lineages with significant public health implications, underscores a critical concern regarding the global burden of diseases and their role in AMR dissemination.

7. Increased interactions among humans, livestock and chimpanzees in BCFR, driven by human activity and seasonal variations in animal behaviour, result in the sharing of potential pathogenic bacterial populations. While direct transmission cannot be confirmed, the presence of similar species suggests possible spillover.

7. Annex 1: Study areas description in rural western Uganda



Study areas' description according to the levels of interaction between livestock and wildlife, based on local human population, the presence of wildlife and livestock, physical barriers, and knowledge on local wildlife-livestock interactions.

Study area	Level of interaction	Population density*	Wildlife/livestock presence	Physical barriers presence	Wildlife incursions	References
MGNP	Medium interaction	402	Yes/Yes	A stone wall separates MGNP from inhabited areas.	The wall is permeable for wildlife and indirect contact with livestock still happens.	(Babaasa et al. 2013)
MFNP northern sector	No interaction	29	Yes/No	The Nile River separates MFNP northern sector from MFNP southern sector.	There are no incursions as only wildlife is present.	(Tweheyo et al. 2005; Dowhaniuk et al. 2018; Scoon 2021)
MFNP southern sector	High interaction	250	Yes/Yes		Livestock and wildlife may interact indirectly as there are no geographical barriers separating the distribution areas of these animals and human settlements. This area also covers a large part of BFCR, which harbours chimpanzees and baboons.	
QENP-N	High interaction	909	Yes/Yes	QENP northern and southern sectors are separated by more than 100 km by road, the Kazinga channel and Maramagambo forest. Non-physical barriers are present.	Sharing of grazing areas and water sources between livestock and wildlife is common in the border of QENP-N.	(Meunier et al. 2017; Fernandez Aguilar et al. 2020)

QENP-S	Low interaction	198	Yes/Yes	Non-physical barriers.	Interactions between humans and elephants are common due to human pressure and uncontrolled resource use outside the protected areas.	(Keigwin 2001)
BCFR	Medium interaction	74	Yes/Yes	Non-physical barriers.	There are no livestock grazing areas within BCFR, but baboon and chimpanzee incursions are common in human settlements, with a high rate of crop-raiding incidents.	(Tweheyo et al. 2005)
HD	No interaction	156	No/Yes	Non-physical barriers.	There are no incursions as wildlife is not present in this study area.	

* Population density (number of people/km^2) per district was estimated in 2014 (UBOS 2016).

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