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Phenotypic Characterisation of Antibiotic-Resistant *Enterococcus faecium* in Bats from Lambaréné (Gabon)

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ABSTRACT
Enterococci are gram-positive, non-spore-forming bacteria commonly found in the intestinal tracts of mammals, including humans. The emergence of antibiotic-resistant *Enterococcus* spp., such as vancomycin-resistant Enterococcus (VRE), in wild mammals is a growing concern, as these animals can act as reservoirs and vectors for resistant bacteria, facilitating their spread in the environment and increasing the risk of infections in humans and other species. Research on the microbiome of bats, particularly concerning antibiotic resistance, has gained attention in recent years, although no studies on Enterococcus resistance have been conducted in Gabonese bats. The aim of this study was to assess the prevalence of antibiotic-resistant enterococci in bats from Lambaréné, Gabon. Bat faeces were collected and cultured to isolate *Enterococcus* spp. *Colonies*, which was identified using the Api 20 Strept galleries and antibiotic susceptibility testing. The antibiotics tested included amoxicillin, vancomycin, teicoplanin, tetracycline, and tobramycin. The study found that 54.3% of isolates were resistant to tobramycin, 33.7% to tetracycline, 25% to vancomycin, and 20.7% to teicoplanin. Multidrug resistance (MDR) was observed in 2.2% of the isolates, with two distinct resistance phenotypes: Van B (resistant only to vancomycin) and Van A (resistant to both vancomycin and teicoplanin), both of which are monitored by the WHO. This study is the first to report the presence of vancomycin-resistant Enterococcus faecium in bats from Gabon. While vancomycin-resistant Enterococci are more common in humans (38.5%) than in bats (15%), the findings highlight a significant concern for antibiotic resistance in wild mammals, particularly in urban environments like Lambaréné.

Keywords : Vancomycin-resistant *Enterococcus,* Bats, Lambaréné

Introduction
Enterococci are Gram-positive, non-sporulating bacteria that appear as isolated cocci or are arranged in pairs or chains [1]. These bacteria are ubiquitous and can be found in various ecological niches, including the intestinal tract of animals and humans [2]. They are also present in other animal species such as reptiles, birds, and even insects. Furthermore, they can be found in soil, surface waters, plants, and vegetation due to their ability to grow and thrive under hostile environmental conditions.
Vancomycin-resistant enterococci (VRE) represent a major concern in public health due to their ability to cause nosocomial infections that are difficult to treat. These bacteria, which naturally inhabit the gastrointestinal tract of humans and animals, have acquired resistance to vancomycin, an antibiotic often used as a last resort [3]. The presence of VRE in bats raises critical questions regarding the spread of antibiotic resistance in natural environments and its potential impact on wildlife as well as human health [3] [4] .It suggests that bats may serve as reservoirs for pathogens carrying antibiotic-resistance genes. However, bats are not well characterized as reservoirs of multidrug-resistant strains [5]. The study of VRE in wild mammals is crucial for several reasons. Firstly, wild animals can act as reservoirs and vectors of resistant bacteria,

facilitating their dissemination in the environment and increasing the risk of infections for humans and other animals [6]. Secondly, the consumption of wild mammals contaminated with VRE poses a direct risk to public health, particularly for rural communities that rely on these animals as a protein source [7]. Lastly, understanding the prevalence and distribution of VRE in wild mammal populations is essential for developing conservation strategies that minimise hazardous interactions between wildlife and resistant pathogens [8].

In recent years, research has been conducted to investigate the gut microbiome of bats comprehensively, taking into account species, diet, and even seasonal variations [9] [10] [11]. These studies have advanced knowledge regarding the significance of bats as potential vectors of pathogenic microorganisms affecting humans and other animals. To date, analyses of the susceptibility of the bat microbiome have primarily focused on members of the Enterobacteriaceae family [12] [13], while another group of indicator bacteria, namely *Enterococcus* spp., has been largely neglected and underestimated.

In Gabon, the only study conducted to date has demonstrated the presence of *Escherichia coli* and *Klebsiella*, both of which are Gram-negative bacilli belonging to the Enterobacteriaceae family, producing extended-spectrum β-lactamases (ESBL) [14]. No study has been carried out on antibiotic resistance in other bacterial groups within Gabonese bats, despite their suspected role as vectors or reservoirs of pathogens [15]. Consequently, the present study is particularly relevant in the current context, where the spread of antibiotic resistance poses an increasing threat to global health and biodiversity conservation [16].

Materials and Methods

Study Framework

Faecal samples from bats were obtained as part of a collaboration between researchers from the Institute of Tropical Medicine at Nagasaki University and researchers from the Institute for Research in Tropical Ecology (IRET). The collection of these samples was conducted over three sampling campaigns organised in March and August 2023, and October 2024.

Sample collection site

Bats were captured under the bridge in the Adouma district and an abandoned bar beyond the Adouma district in the city of Lambaréné, province of moyen-Ogooué.

Sampling Method

These samples were collected during two four-day sampling campaigns: the first in April 2024 and the second in October 2024. Bat capture typically commenced at 8 a.m., to obtain 20 to 30 specimens per day. To capture the bats under the Adouma bridge, two teams of three individuals, wearing protective suits, gloves, visors, and masks, were formed. One team remained stationed under the bridge, dislodging the bats clinging to the bridge using small stones thrown into the water. The second team, which was more mobile, moved on the water in a large canoe, with some members throwing stones to dislodge the bats and others collecting them using a telescopic nylon net with an aluminium alloy handle (POPETPOP brand, Germany). The second team also retrieved bats dislodged by the first team.

To capture bats in the abandoned nightclub, nets were first installed inside a room along an open doorway. Two teams of three individuals, equipped with protective suits, gloves, visors, masks, and torches, were responsible for this capture: one team inside and the other outside. The team inside frightened the bats, prompting them to fly chaotically around the room, with some clinging to the walls or the net. These were then captured using long-handled nets. The bats were dissected in a temporary laboratory located within the premises of the Centre de Recherche Médicale de Lambaréné (CERMEL). Faecal samples or tissue from the rectal area of each bat were collected and stored in 1 ml of PBS solution (70%) + glycerol (30%). These tubes were kept in a cooler, protected from sunlight. At the IRET Microbiology Laboratory, these samples were stored in a freezer at -18°C pending further analysis.

Culture, Isolation, and Identification of Colonies

For this study, fecal samples were collected from 450 bats. These samples underwent a preliminary

enrichment step, wherein approximately 1 g of feces was mixed in 1 ml of bile-esculin broth (Millipore, Spain) and incubated at 37°C for 48 hours. A colour change from brown to black in the broth indicated bacterial growth.

A 100 µl aliquot of each enriched broth was streaked onto bile-esculin agar (Millipore, Spain) and incubated at 37°C for 24 hours. Following incubation, only grey colonies with a black background were selected, as these were presumed to be *Enterococcus* spp. The identification of these colonies was carried out using the Api 20 Strept galleries (BioMérieux,Lyon, France), and results were interpreted using the Api Web software (BioMérieux, Lyon, France).

Antibiotic Susceptibility Testing

Antibiotic resistance was assessed by the disk-diffusion test method [17] on MH agar (BioMérieux, Lyon, France) and the clinical breakpoints recommended the Clinical Laboratory Standards Institute (CLSI) guidelines (2023 version). The antibiotics tested included Amoxicillin (AML, 30 µg), Teicoplanin (TEI, 30 µg), Tetracycline (TET, 30 µg), Tobramycin (TOB, 30 µg), and Vancomycin (VAN, 5 µg).

Briefly, a colony of *Enterococcus* was taken from each Petri dish using a sterile inoclic and suspended in 700 µl of NaCl (0.9 %) solution, according to a protocol already established by Mbehang et al (2020) [14]. The suspension was homogenised using a vortex mixer (Thermo Fisher Scientific, France), achieving turbidity equivalent to 0.5 McFarland units. The mixture was then streaked onto MH agar plates using sterile swabs. Antibiotic discs were placed on the MH agar at a spacing of 3 cm between the discs and 1.5 cm from the edge of the Petri dish. The plates were incubated at 37° C for 24 hours.

Statistical analysis

Data on bacterial species identification and antibiotic susceptibility testing were collected, cleaned, and entered into the Statistical Package for Social Sciences (SPSS) 20.0 software (SPSS Inc., Chicago, IL, USA). These data were analysed using descriptive statistics, frequency analyses, and bivariate analyses (cross-tabulations). The diameter of the inhibition zone around each antibiotic disc was measured using a digital calliper (Carbon Fiber Composite Digital Caliper, France), and qualitative interpretation was performed by comparing the inhibition diameters obtained. The results were classified as susceptible (S), intermediate (I), or resistant (R). However, in the context of this study, both susceptible and intermediate results were considered as susceptible.

Results

Bacterial Culture

Out of 450 faecal samples cultured, 276 yielded black colonies, representing a prevalence of 61.3%. Api 20 Strept gallery readings confirmed that all black colonies belonged to *Enterococcus faecium*.

Antibiotic Susceptibility

Overall Antibiotic Susceptibility

Among the 92 *Enterococcus faecium* isolates, antibiotic resistance was observed as follows: Tobramycin (150 isolates; 54.3%), Tetracycline (93 isolates; 33.7%), Vancomycin (69 isolates; 25%), and Teicoplanin (57 isolates; 20.7%) (Table 1). Table 1 also indicates that susceptibility appeared to be higher than resistance, particularly for amoxicillin (AML), vancomycin (VAN), teicoplanin (TEIC) and tetracyclin (TET).

Table 1: Overall Prevalence of Antibiotic Susceptibility

	Amoxicillin (AML)	Vancomycin (VAN)	Teicoplanin (TEIC)	Tetracyclin (TET)	Tobramycin (TOB)
S	264 (95.7%)	207 (75 %)	219 (79.3 %)	183 (66.3 %)	126 (45.7 %)
R	12 (4.3 %)	69 (25 %)	57 (20.7 %)	93 (33.7 %)	150 (54.3 %)

R : Resistant, S : Sensitive

Antibiotic Resistance Profiles and Phenotypes

Our results illustrate the presence of multiple resistance phenotypes. These phenotypes range from *Enterococcus faecium* strains that are susceptible to all tested antibiotics to those exhibiting resistance to one, two, three, or four antibiotics. Only two isolates demonstrate resistance to three antibiotic classes, specifically AML (Amoxicillin), TET (Tetracycline), TEIC (Teicoplanin), and VAN (Vancomycin). AML belongs to the β-lactam class, TET to the tetracycline class, while TEIC and VAN

are glycopeptides. Consequently, the multidrug resistance (MDR) rate is 2.2% (6/276).

Furthermore, these results highlight two distinct vancomycin resistance phenotypes: 5.4% (15/276) of the isolates are resistant exclusively to vancomycin, corresponding to the Van B phenotype, whereas 19.6% (54/276) exhibit resistance to both teicoplanin and vancomycin, corresponding to the Van A phenotype. These two resistance phenotypes are monitored by the World Health Organization (WHO). Our findings indicate that 25% of *Enterococcus* isolates are vancomycin-resistant (VRE)

Discussion

Prevalence of *Enterococcus faecium* in Bats

Our study identified *Enterococcus faecium* in bats collected in Lambaréné. This marks the first instance of a Gram-positive coccus of the *Enterococcus* genus being isolated from bat faeces in Gabon. However, enterobacteria such as *E. coli* and *Klebsiella* have previously been isolated from the faeces of frugivorous bats in Makokou, Gabon [14]. These findings align with global trends, which indicate that enterobacteria are the most commonly studied bacterial group in bats [18] [19].

Studies specifically investigating the *Enterococcus* genus in bats remain scarce [20]. Furthermore, *Enterococcus faecalis* is generally the predominant species identified in bats [12] [21], whereas studies on *E. faecium* are infrequent [22]. Nevertheless, a study on livestock in Gabon by Ekore et al. [23] also identified this bacterial species. *E. faecium* is most commonly associated with human hosts [24]. The presence of this bacterium in bats residing in highly anthropised urban environments likely indicates human-to-bat transmission. Our results are in accordance with this hypothesis, which stipulates that the prevalence of antibiotic resistance in wildlife is higher in animals living in urban than in non-urban areas [25, 26].

Antibiotic Susceptibility

Our study demonstrated resistance to amoxicillin, vancomycin, teicoplanin, tetracycline, and tobramycin. The highest resistance rates were observed for tobramycin (54.3%), tetracycline (33.7%), and vancomycin (25%). In most studies on antibiotic-resistant enterococci, whether in animals or humans, resistance has been most frequently reported for tetracycline, vancomycin, and aminoglycosides [8] [27]. However, multidrug-resistant (MDR) vancomycin-resistant *Enterococcus* (VRE) isolates are more prevalent in humans (38.5%) [28] than in bats (approximately 15%) [8]. Among human isolates, vancomycin and tetracycline resistance rates are approximately 46.5% and 76.9%, respectively [28], whereas in bats, these rates are around 49% and 30% [25]. Our findings appear consistent with those reported by Monroe [25]. However, the prevalence of VRE in our study also seems comparable to previous findings.

The presence of VRE in bats may be explained by two possible transmission routes: indirect airborne transmission via tourists, who could introduce resistant bacterial strains both inside and outside bat caves [4], thereby contributing to the spread of antimicrobial resistance (AMR) in ecosystems [26]; or contamination through aquatic environments (rivers, lakes), which frequently serve as reservoirs for MDR bacteria and antibiotic resistance genes [27]. Indeed, rivers are often exposed to wastewater discharges containing microbial contaminants from industrial, agricultural, hospital, and domestic sources [29] [30].

Consequently, our study, in conjunction with previous research, suggests that bats harbour a highly diverse intestinal bacterial community (microbiota), making them one of the most significant animal reservoirs of pathogens and antibiotic resistance [30] [31] .

Conclusion

The digestive tract of bats harbours multiple pathogenic species, including members of the *Enterococcus* genus. In humans, two *Enterococcus* species are commonly identified: *E. faecalis* and *E. faecium*. Our results confirm the presence of *E. faecium*, which appears to be of anthropogenic origin. Additionally, bats may act as reservoirs for *Enterococcus* species resistant to clinically relevant antibiotics, such as vancomycin and tetracycline. Human isolates typically exhibit higher antibiotic

resistance prevalence than those found in bats. Our study highlights the presence of VRE in bats, albeit at lower prevalence rates compared to human cases. Waterborne transmission appears to be the most plausible contamination route. A key finding of our research is the identification of vancomycin-resistant *Enterococcus faecium* in bats inhabiting the urban area of Lambaréné. Our study highlights the danger of bat feces and their capacity to pollute the waters of the Ogooué, the river crossed by the bridge under which we sampled the bats. The feces of these bats fall directly into the Ogooué. This river could potentially be contaminated by the feces of these bats. It could compromise the health of Lambaréné residents who use this water.

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References:

[1]. H.A.D.Ruwandeepika, D.N. Madushanka, and T.S.P. Jayaweera, Foodborne organisms: General characteristics, virulence factors, and clinical manifestations, in Food Microbial and Molecular Biology, *Apple Academic Press*., pp. 213-285, 2023.

[2]. V. Cattoir, The multifaceted lifestyle of enterococci: genetic diversity, ecology and risks for public health, *Current Opinion in Microbiology*, vol. 65, pp. 73-80, 2022.

[3]. A.M. Almansour, et al., The silent threat: Antimicrobial-resistant pathogens in food-producing animals and their impact on public health. *Microorganisms*, vol. 11, n° 9, pp. 2127, 2023.

[4]. A.Garcês, and I. Pires, European wild carnivores and antibiotic resistant bacteria: a review. *Antibiotics*, vol. 12, n°12, pp. 1725, 2023.

[5]. A. Nowakiewicz, et al., Analysis of the occurrence and molecular characteristics of drug-resistant strains of Enterococcus faecalis isolated from the gastrointestinal tract of insectivorous bat species in Poland: A possible essential impact on the spread of drug resistance? *Environmental Pollution*, vol. 269, pp. 116099, 2021.

[6]. M.Vittecoq, et al., Antimicrobial-resistant bacteria carriage in rodents according to habitat anthropization. *EcoHealth*, vol. 20, n°1, pp. 84-92, 2023.

[7]. E. Guerrero-Ramos, et al., Antimicrobial resistance and virulence genes in enterococci from wild game meat in Spain. *Food microbiology*, vol. 53, pp. 156-164, 2016.

[8]. M.Dec, et al., Antibiotic susceptibility and virulence genes in *Enterococcus* isolates from wild mammals living in Tuscany, Italy. *Microbial Drug Resistance*, vol. 26, n°5, pp. 505-519, 2020.

[9]. M.Vengust, T. Knapic, and J.S. Weese, The fecal bacterial microbiota of bats; Slovenia. *PLoS One*, vol. 13, n°5, pp. e0196728, 2018.

[10]. J.Wolkers-Rooijackers, K. Rebmann, T. Bosch, and W.C. Hazeleger, Fecal bacterial communities in insectivorous bats from the Netherlands and their role as a possible vector for foodborne diseases. *Acta Chiropterologica*, vol. 20, n° 2, pp. 475-483, 2018.

[11]. G. Xiao, et al., Seasonal changes in gut microbiota diversity and composition in the greater horseshoe bat. *Frontiers in Microbiology*, vol.10, pp. 2247, 2019.

[12]. A.Nowakiewicz, G. Ziółkowska, P. Zięba, and A. Kostruba, Undomesticated animals as a reservoir of multidrug-resistant Enterococcus in eastern Poland. *Journal of wildlife diseases*, vol. 50, n°3, pp. 645-650, 2014.

[13]. J.Benavides, et al., Extended-spectrum beta-lactamase-producing *Escherichia coli* in common vampire bats Desmodus rotundus

and livestock in Peru. *Zoonoses and public health*, vol. 65, n°4, pp. 454-458, 2018.

[14]. P.P. Mbehang Nguema, et al., Characterization of ESBL-Producing Enterobacteria from Fruit Bats in an Unprotected Area of Makokou, Gabon. *Microorganisms*, vol. 8, n°1, pp. 138, 2020.

[15]. A.C.Fagre, and R.C. Kading, Can bats serve as reservoirs for arboviruses? *Viruses*, vol.11, n°3, pp. 215, 2019.

[16]. D.S.Schmeller, F. Courchamp, and G. Killeen, Biodiversity loss, emerging pathogens and human health risks, *Springer,* pp. 3095-3102, 2020.

[17]. A. Bauer, W. Kirby, J.C. Sherris, and M. Turck, Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, vol. 45, n° 4_ts, pp. 493-496, 1966.

[18]. A.C.R.Ferreira, et al., Zoonotic bacterial pathogens in bats samples around the world: A scoping review. *Preventive Veterinary Medicine*, vol.225, pp. 106135, 2024.

[19]. F. McDougall, W. Boardman, M. Gillings, and M. Power, Bats as reservoirs of antibiotic resistance determinants: A survey of class 1 integrons in Grey-headed Flying Foxes (*Pteropus poliocephalus*). *Infection, Genetics and Evolution*, vol.70, pp. 107-113, 2019.

[20]. V.G. Javůrková, et al., Unveiled feather microcosm: feather microbiota of passerine birds is closely associated with host species identity and bacteriocin-producing bacteria. *The ISME journal*, vol.13, n°9, pp. 2363-2376, 2019.

[21]. J.A.Benavides, et al., Long-term maintenance of multidrug-resistant *Escherichia coli* carried by vampire bats and shared with livestock in Peru. *Science of the Total Environment*, vol. 810, pp. 152045, 2022.

[22]. F. Lebreton, R.J. Willems, and M.S. Gilmore, *Enterococcus* diversity, origins in nature, and gut colonization. Enterococci: from commensals to leading causes of drug resistant infection [Internet], 2014.

[23]. D.Otsague Ekore, et al., The antibiotics used in livestock and their impact on resistance in *Enterococcus faecium* and *Enterococcus hirae* on farms in Gabon. *Antibiotics*, vol. 11, n°2, pp. 224, 2022.

[24]. S.S. Banawas, et al., Prevalence of multidrug-resistant bacteria in healthcare-associated bloodstream infections at hospitals in Riyadh, Saudi Arabia. *Pathogens*, vol.12, n°9, pp. 1075, 2023.

[25]. Monroe, B., Using a culturable approach and metagenomic analysis to uncover gut bacterial diversity of gray Bats In Kansas. 2023.

[26]. S.Skok, et al., Antimicrobial resistant *Escherichia coli* from karst waters, surfaces and bat guano in Slovenian caves. *Acta Carsologica*, vol. 49, n°2-3, 2020.

[27]. N.Vezeau, and L. Kahn, Current understanding and knowledge gaps regarding wildlife as reservoirs of antimicrobial resistance. *American Journal of Veterinary Research*, vol. 85, n°6, 2024.

[28]. A.G. Mubarak, et al., Phenotypic and genotypic characterization of *Enterococcus faecalis* and Enterococcus faecium isolated from fish, vegetables, and humans. *Scientific Reports*, vol.14, n°1, pp. 21741, 2024.

[29]. K.Y. Lim, and K.Y. Foo, Hazard identification and risk assessment of the organic, inorganic and microbial contaminants in the surface water after the high magnitude of flood event. *Environment international*, vol.157, pp. 106851, 2021.

[30]. L.Federici, M. Masulli, V. De Laurenzi, and N. Allocati, An overview of bats microbiota and its implication in transmissible diseases. *Frontiers in Microbiology*, vol. 13, pp. 1012189, 2022.

[31]. J.D. Soto-López, M. Diego-del Olmo, P. Fernández-Soto, and A. Muro, Bats as an important source of antimicrobial-resistant bacteria: a systematic review. *Antibiotics*, vol. 14, n°1, pp. 10, 2024.

