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Research Article

Prevalence of Extended-Spectrum β-Lactamases in *E. coli* of Rats in the Region North East of Gabon

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Antibiotic resistance occurs in the environment by multiplication and the spread of multidrug-resistant bacteria that would be due to an improper and incorrect use of antibiotics in human and veterinary medicine. The aim of this study was to establish the prevalence of *E.coli* producing Extended-Spectrum beta-Lactamase (ESBL) antibiotics from rats and gregarious animals in a semirural area of Gabon and to evaluate the origin of a resistance distribution in the environment from animal feces. The bacterial culture was carried out, and the identification of *E. coli* strains on a specific medium and the antibiotic susceptibility tests allowed establishing the prevalence. Characterization of resistance genes was performed by gene amplification after DNA extraction. On 161 feces collected in rats, 32 strains were isolated, and 11 strains of *E. coli* produced ESBL with a prevalence of 34.37%. Molecular tests showed that CTX-M genes 214 bp were identified in rats. The presence of CTX-M genes could have a human origin. So, the rats can carry ESBL-producing *Enterobacteriaceae* which poses a risk to human health and pets in this region of Gabon.

1. Introduction

The beta-lactamase family of antibiotics is widely used in the clinic. These molecules, by binding penicillin-binding protein (PBP), inhibit the synthesis of petidoglycan, an essential component the bacterial wall [1]. The first beta-lactamase plasmid (TEM-1/2, SHV-1) was initially described in the 60s in *Escherichia coli* and *Klebsiella pneumoniae* and quickly spread among other species such as *Enterobacteriaceae* [1]. But, CTX-M (Céfotaximase-Munich) diffusion mechanisms seem more complex compared to TEM (Temoneira)/SHV (sulfhydryl variable) ESBLs which is the diffusion of plasmids or other mobile genetic elements [2]. The use of extended-spectrum cephalosporin in clinical practices is at the origin of the emergence of Extended-spectrum Beta-lactamases (ESBLs) [3]. They are the consequence of theurapeutic failures [4, 5]. Antibiotic resistance has become a public health problem and has led to an increased mortality in the human population [6]. In 2018, the World Health Organization estimated that 500,000 people had been suspected of bacterial infections in 22 countries [7].

Enterobacteriaceae producing ESBL are a major cause of resistance to penicillin, cephalosporin, carbapenenes, and aztreonam [8]. *Enterobacteriaceae* in the gut of humans and animals acquire resistance through the selection pressure of antibiotics and their ability to exchange genetic material [9]. Thus, the spread of ESBL strains of Enterobacteria in the environment and wildlife has been observed [10–12].

Some authors around the world, and also, in Africa and many other countries around the world, have studied the

spread of antibiotic-resistant bacteria in wildlife [10, 11] and urban environment [13]. Some studies consider wildlife as a potential reservoir of antibiotic resistance [14–18]. Furthermore, the prevalence of antibiotic resistance in wildlife is thought to decrease progressively with increasing distance from humans [10, 11].

Rats are known to be vectors of a variety of zoonotic pathogens responsible for significant human morbidity and mortality in cities around the world [19, 20]. Also, antibiotic-resistant *Enterobacteriaceae* have been isolated from urban rats [21–26]. Rats and other animals constitute a sentinel of choice for studies on the spread of resistance in community and wilderness [23, 27].

Gabon has a very diverse fauna, rats are well represented on the territory, and several studies on the carriage of different pathogens have already been carried out [28–30]. There are no data on the prevalence of antibiotic-resistant bacteria in rats in Gabon. The aim of this study is to evaluate the prevalence of ESBLs in Enterobacteria isolated from rat feces in the urban area of Makokou (Ogooue Ivindo province, Gabon).

2. Materials and Methods

2.1. Sampling Period and Site. The sampling was performed in agreement with the recommendations of the Gabonese National Ethics Committee (Authorization NPROT/0020/2013I/S G/CNE).

The capture of the rats was carried out from April to September 2018 inside the Makokou Regional Hospital and from the outpatient houses near the hospital. The rodents (rats) were captured using live traps (Tomahawk and Sherman) as described by Duplantier [31]. To capture the rats, traps have been installed from 17 h to 18 h in the small forest of the hospital and in some external houses next to the hospital. All the traps were recovered from 6 : 00 to 7 : 00 a.m. and transported to our laboratory. A cotton swab was turned inside the rat rectum and immediately discharged into 2 ml of a sterile mixture of phosphate buffered saline (PBS) and glycerol (80%/20%) in an Eppendorf tube and was kept at 4°C pending for further analysis.

In the bacteriology laboratory of the International Center for Medical Research of Franceville (CIRMF), each fecal sample was enriched with heart-brain broth (BHB) and streaked on Methylene Blue Eosin ((EMB) (bioMérieux, France) supplemented with 2 mg/L cefotaxime and incubated at 37°C for 24 h. After incubation, each colony, differentiated by structure and color, was picked and transferred by the same means and incubated in the same conditions. The purified colonies were subjected to biochemical identification by using the VITEK® 2 Compact 15 (bioMérieux, Marcy l'étoile, France).

Antibiotic resistance was assessed by the diffusion disc method [32] and inhibition diameters were interpreted using Clinical Laboratory Standard Institute (CLSI) guidelines [33]. Extended-spectrum beta-lactamase production was tested with the double-disc synergy test. The comparative study of the results of a set of beta-lactam antibiotics tested simultaneously on the same antibiogram was carried out to determine the acquired or intrinsic phenotype [34–37].

2.2. Determination of Gene Resistance by the Polymerase Chain Reaction. The primer pair SHV-F (5′-3′) and SHV-R (5′-3′) was used from reference [38] and the primer pair CTX- M-F (5′-3′) and CTX-M-R (5′-3′) were used from reference [39] for the characterization of gene resistance.

The amplification of the genes was carried out using a thermal cycler (T100 Thermal Cycler, BIO-RAD). The PCR steps were composed of denaturation for 5 minutes at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C, and a final extension of 7 min at 72°C. Then, the amplicons were analyzed by agarose gel migration. The revelation was made by previously preparing a 1.5% agarose migration gel stained with ethidium bromide (1 μl/ml) for 30 min under 100 V in 1X TAE buffer and subjected to a 264 nm UV lamp. The software Statistical Package for Social Science (IBM SPSS Statistics 20) was used for the statistical analysis.

3. Results

3.1. Prevalence of ESBL E.coli in Rats. Of 161 feces of rats, all collected in and around the hospital, 32 MBE agar green color colonies were identified as *E. coli* after confirmation and were ESBL-producing *E. coli* with a prevalence of 32/161 (20%).

3.2. Prevalence of Resistance to Different Antibiotic Families: Case of Rats. As shown in Figure 1, among the antibiotics of the beta-lactam family, the most resistant was amoxicillin, followed cefotaxim and cefepim. In the aminoglycoside family, streptomycin and gentamycin were the most prevalent. In the fluoroquinolone family, ciprofloxacin was the most prevalent. The families of phenicol (chloramphenicol) and phosphonic acid (fosfomycin) were the least resistant.

Aztreonam (TM), Imipenem (IMP), Ertapenem (ERT), Piperacillin-Tazobactam (TPZ), Amakacin (AK), Netilmicin (NET), Tobramycin (TOB), Gentamicin (GEN), Colistin (CT), Tetracycline (TE)), Chloramphenicol (CHL), Fosfomycin (FOS), Levofloxacin (LEV), Ciprofloxacin (CIP), Ofloxacin (OFX), Nalidixic acid (NA), Streptomycin (S), Kanamycin (KAN), Trimethoprim + Sulphamethoxazole (SXT)), Cefoxitin (FOX), Ticarcillin + Clavulanic acid (TIM), Ceftazidime (CAZ), Cefepime (CEF), Erythromycin (E), finally antibiotics considered to be more resistant Amoxicillin (AML), Cephalexin (CL), Ticarcillin (TIC), Cefotaxime (CTX) Amoxicillin + Clavulanic acid (AMC) Cefpodoxime (CPD), Piperacillin (PIR) Ampicillin (AM), Beta-lactam (BL), Aminoglycoside (AMG), Fluoroquinolone-Quinolone (FLQ), Polymixin (PM), Macrolid (MA), Sulfamid (SUL), Tetracyclin (TET), Phenycol (PHEN), and Phosphonic acid (PHOS).

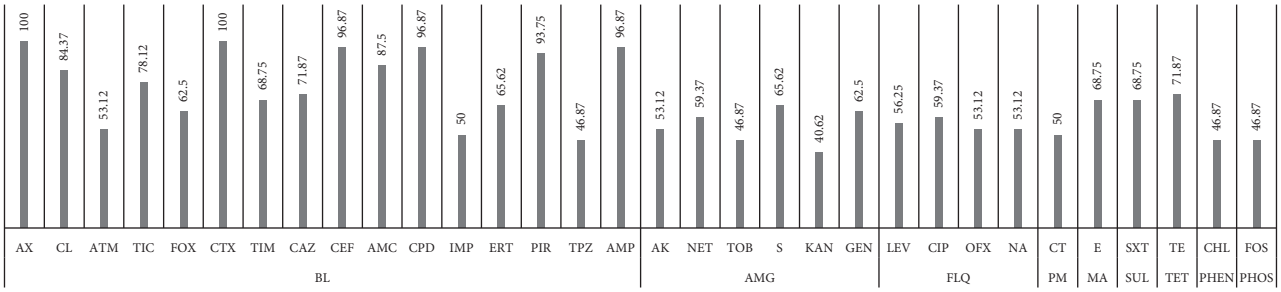

Figure 1: Prevalence of antibiotic resistance. *Y*-axis (%); *X*-axis (antibiotics and families).

3.3. Resistance Genes Identified in Rats. Of the 32 strains of *E. coli* with phenotypic ESBL production, 11 (34.37%) expressed the CTX-M gene.

4. Discussion

The purpose of this study was to determine the prevalence of *E. coli* producing ESBL in rat feces collected in the town of Makokou.

Mammals such as rats occupying various ecological niches and adapting to different feeding patterns may harbor and contribute to the spread of antimicrobial-resistant bacterial species [39–42]. Several authors worldwide have conducted antimicrobial resistance studies of *E.coli* producing BLSE [43–45].

This study revealed the presence of 20% of *E. coli* producing ESBL in fecal samples of rats. The prevalence of ESBL-producing *E.coli* in rats is similar in the study from Kenya (20%) [27] and from Conakry (Guinea, West Africa) (20%) [13].

The most resistant antibiotic family in rats is beta-lactamines followed by aminoglycosides (netilmycin, amikacin, gentamycin, kanamycin, streptomycin, and tobramycin), tetracyclines, and fluoroquinolones (nalidixic acid, ofloxacin, ciprofloxacin, and levofloxacin). The rats were collected from hospitals and surrounding homes. Third-generation cephalosporins are widely used in hospitals in Gabon and are considered to be responsible for the emergence of extended-spectrum beta-lactamase (ESBL) [42]. These antibiotics (beta-lactam, aminoglycosides, and quinolones) are used in the first line in the treatment of human infections of the bacterial origin [46, 47]. Hence, the prevalence of resistance to urban wild mammals depends on the antibiotics consumed by human populations [48–50]. Imipenem is the molecule that has shown the greatest susceptibility in the majority of bacteria isolated. Its relatively high cost is an advantage, and it reduces the risk of excessive use and, thus, the development of resistance [51]. This may explain the low prevalence of resistance to carbapenems, which are antibiotics used as a last resort in the treatment of infections in human medicine [52].

The prevalences of resistance to streptomycin (65.6%), cefotxime (100%), and tetracycline (71.8%) were higher in our study compared to other studies [15, 27]. The prevalences of resistance to nalidixic acid (53%) and amoxicillin + clavulanic acid (87.5%) in our study were lower compared to those in other studies conducted.

Rats in sewer tunnels showed more resistance than captured rats in other areas of the city, probably because these rats' sewer tunnels were in contact with human sewage [39].

These results showed the presence of CTX-M in rats. ESBL gene type CTX-M-15 is the most common among the isolates of *Enterobacteriaceae* of humans and animals [53, 54]. The spread worldwide of ESBL *Enterobacteriaceae* clinical isolates is a serious problem for the treatment of infectious diseases, in particular the emergence of *E. coli* producing CTX-M-15 [16]. CTX-M-15 is probably the most widespread ESBL gene in humans worldwide [55] and the most detected in human clinical contexts [56]. In addition, the CTX-M-15 and SHV-11 genes are recognized as plasmid-mediated resistance genes [42, 57]. In Gabon, similar studies conducted at the Omar Bongo Ondimba Military Hospital in Libreville and the Albert Schweitzer Hospital in Lambaréné revealed the presence of CTX-M and VHS in hospitalized patients [42, 45]. CTX-M-15 seems to have a particular capacity for dissemination [58]. This could explain the presence of these genes in the population of rats [42, 45].

5. Conclusions

This study provided an inventory of antibiotic resistance in the urban wildlife. The presence of multidrug-resistant *E.coli*, in particular those producing ESBL, has been demonstrated in rats that are gregarious mammals, close to humans. As is the case around the world, CTX-M family enzymes predominate, regardless of the bacterial species involved or the compartment in which the gene has been identified. However, these CTX-M genes could be CTX-M-15. In summary, our results show the presence of ESBL-producing *E.coli* from rat population of Makokou. Given the health conditions in this region, rats carrying ESBL-producing Enterobacteria pose a risk to human health and domestic animals.

Data Availability

Our information is currently restricted. They can be shared at the request of researchers who are interested in our work.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Collection of samples and experiment were performed by Mr Arsène Mabika Mabika; conceptualization and design were made by Mbehang Nguema Pierre Philippe and Onanga Richard. Materials/analysis tools coordination was performed by Mabika Mabika Arsène, Tonda Leslie Wed, Obague Mbeang Jean Constant, and Mbehang Nguema Pierre Philippe. Ndong Atome Guy Roger and Onanga Richard prepared the draft, wrote the manuscript, and agreed to be accountable for all aspects of the work, Supervision was conducted by Onanga Richard and Mbehang Nguema Philippe. The manuscript was read and corrected by Lebibi Jacques. All the authors approved the final manuscript.

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