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Antibiotic Resistance Profile in *Klebsiella pneumoniae* and *Klebsiella oxytoca* Isolated from Urine in the Republic of Congo

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A B S T R A C T

The emergence and spread of antibiotic resistance means an increased risk of therapeutic failure. They lead to longer hospital stays, higher treatment costs and increased morbidity and mortality, compromising the fight against infectious diseases. A total of 2,876 urine samples were collected and analyzed using conventional microbiology techniques. Bacteria were identified on the basis of biochemical characteristics. Sensitivity testing was carried out using the standard Mueller-Hinton agar diffusion method. It should be noted that 1484 (51.6%) samples were positive out of 2876 after culture. After identification, 256 (17.25%) bacteria were from the *Klebsiella* genus, compared with 1228 from other genera. The 256 *Klebsiella* strains were made up of 104 (40.62%) *K. pneumoniae* and 152 (59.38%) *Klebsiella oxytoca*, mostly isolated from urine collected from women. Both species were more responsive in patients over 40 years of age. The sensitivity test showed that *K. pneumoniae* were more sensitive to norfloxacin, gentamicin and netilmicin, with rates of 18.26%, 21.11% and 22.15%, versus 91.34% for cotrimoxazole and amoxicillin + clavulanic acid. Other rates were also observed: Ceftriaxone (38.46%), Ciprofloxacin (48.07%), Ofloxacin (49.03%), Cefotaxim (64.42%), Nalidixic Acid (74.03%) and Chloramphenicol (77.88%). In *K.oxytoca*, netilmicin and norfloxacin were more active, with rates of 10.52% and 22.36% respectively. High rates of resistance in *K.oxytoca* were observed with cotrimoxazole (78.94%) and amoxicillin + clavulanic acid (78.98%). Some antibiotics showed rates such as 42.76% for Cefotaxime, 52.63% for Ceftriaxone, 36.18% for Gentamicin 33.55% for Ciprofloxacin, 59.86% for Nalidixic acid, 46.05% for Ofloxacin and 67.10% for Chloramphenicol. Quinolones were more active, with rates of 21.63% in *K. pneumoniae and* 23.35% in *K.oxytoca*. Over half of *Klebsiella* strains also showed resistance to at least two antibiotics. This increase and spread of resistance could be due to unjustified antibiotic use, antibiotic selection pressure and genetic exchange. The above-mentioned antibiotics can be used as first-line treatment for *Klebsiella* infections.

Introduction

Klebsiella pneumoniae (*K. pneumoniae)* is a common cause of nosocomial infections and has also emerged as an agent of serious community-acquired infections, including pyogenic liver abscesses, pneumonia, and meningitis (Shon *et al.,* 2013). Increasing antimicrobial resistance in *K. pneumoniae*, a member of the *ESKAPE* (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*) group of pathogenic bacteria (Bialek-Davenet *et al.,* 2014), poses serious therapeutic challenges. Similarly, *Klebsiella oxytoca* (*K.oxytoca*) belongs to a complex of nine species of Gram-negative bacilli of the order *Enterobacterales*, including (Yang *et al.,* 2022) *Klebsiella michiganensis* (Saha *et al.,* 2013), *Klebsiella grimontii* (Passet and Brisse *et al.,* 2018) and *Klebsiella pasteurii* (Merla *et al.,* 2019). This complex, called KoSC (*Klebsiella oxytoca* species complex), is the second most frequently identified group of *Klebsiella* as the agent responsible for clinical infections in humans, just after the *Klebsiella pneumoniae* species complex (KpSC) (Stewart *et al.,* 2022). The emergence and spread of antibiotic resistance expose patients to an increased risk of therapeutic failure. They lead to longer hospital stays, increased treatment costs, high morbidity and mortality compromising the fight against infectious diseases (Ouedraogo *et al.,* 2017).

The European Parliament has warned of the development and spread of Antimicrobial Resistance (AMR) associated with a considerable cumulative economic cost by 2050, mainly in developing countries (Shrivastava *et al.,* 2018). In February 2017, WHO published its first list of antibiotic-resistant "priority pathogens", featuring *Enterobacteriaceae* resistant to 3rd-generation Cephalosporins (C3G) and Carbapenems including *Klebsiella pneumoniae* strains (Shrivastava *et al.,* 2018). Antibiotic resistance in *Klebsiella* has been the subject of several countries worldwide, including those of Bialek-Davenet *et al.,* (2014) in France on multiresistance and hypervirulence in *K.pneumomiae*; Pérez-Vazquez *et al.,* (2019) in Spain worked on the characterization of betalactamases in *K.oxytoca*; Wang *et al.,* (2020) in China on the characterization of virulence, biofilm and antibiotic resistance in *K.pneumoniae*. Stewart *et al.,* (2022) and Hawkey *et al.,* (2022) in Australia respectively on epidemiology and genomic analysis in *K.oxytoca* and plasmid ESBLs in *K.pneumiae*). These have also been carried out in several African countries, including Kenya (Taitt *et al.,* 2017), Tunisia (Maamar *et al.,* 2019) and Togo (Salah *et al.,* 2021). In Congo, few works absorb antibiotic resistance in *Klebsiella* except those of Moyen *et al.,* in 2014 conducted on the production of betalactamases in beta-lactam-resistant Enterobacteriaceae isolated at the CHUB. The study is part of an effort to increase data on the resistance of *Klebsilella* strains, particularly *K. pneumoniae* and *K. oxytoca* in the Republic of Congo, in order to improve the management of infections caused by this bacterial genus. These data could be used by doctors in the initial treatment of Klebsiella infections.

Materials and Methods

Urine samples were collected at the National Public Health Laboratory and forwarded to the bacteriology department. These samples were taken between January and December 2015.

Isolation and purification of colonies

Isolation was performed on Mac Conkey culture medium preceded by streaking and incubation in an oven at 37 oC for 18 to 24 hours.

Identification

Strains were identified by API 20 E gallery according to the BioMerieux manufacturer's recommendations.

Antibiotic sensitivity testing

Quality control of antibiotic discs was done using the reference strain of *K.pneumoniae* ATCC 700603 as recommended by the Antibiogram Committee of the French Society for Microbiology CASFM (2015). The antibiotic resistance profile of the bacterial strains was evaluated by the standard Kirby-Bauer disc diffusion method (Prats *et al.,* 2000). The inoculum was prepared by suspending of a well-isolated colony of a young; pure bacterial culture (24 hours on agar medium) in 5 ml normal saline (NaCl at 0, 9%) and the turbidity of the suspension was adjusted at 0.5 Mac Farland using Vitex Densichek. The culture medium, Mueller-Hinton agar, was inoculated using the swab as recommended by CLSI (Clinical and Laboratory Standard Institute) on performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2010). The following antibiotics were tested: Amoxicillin + clavulanic acid (AMC, 20/10µg), Chloramphenicol (C, 30µg), Ciprofloxacin (CIP, 5µg), Ceftriaxone (CRO, 30µg), Cefotaxime (CTX, 30µg),

Cotrimoxazole (Cot, 1, 25/23, 75), Gentamicin (GEN, 10µg), Nalidixic acid (NA, 30µg), (NOR, 10µg), Netilmicin (Net, 10µg), Ofloxacin (OfX, 5µg).). These antibiotic discs were then applied to the inoculated Mueller Hinton agar medium. The plates were incubated at 37˚C for 18 - 24 h.

The diameter of bacterial growth inhibition area around the disc after incubation were measured and the antibiotics susceptibility was interpreted based on the breakpoint values published by the Antibiogram Committee of the French Society of Microbiology (CA-SFM). The strains were categorized as either: sensitive, intermediate or resistant against the antibiotics.

Results and Discussion

Isolation

Of 2,876 urine samples received at the laboratory during the study period, 1,484 (51.6%) were positive, compared with 1,392 (48.4%) negative.

Identification

Of the 1484 positive samples, 256 (17.25%) were of the *Klebsiella* genus, compared with 1228 of other genera (figure 1). Of the 256 *Klebsiella*, 104 (40.62%) were *K. pneumoniae* and 152 (59.38%) were *Klebsiella oxytoca* (figure 2).

Of the 256 *Klebsiella* strains isolated, 164 strains (64.07%) were isolated from urine collected from women, and 92 strains (35.93%) from men. The sex ratio (M/F) was 0.56 in favor of women. Both *Klebsiella* species were predominantly isolated from patients over 40 years of age (43.77%), i.e. 71 strains (46.71%) for *K. oxytoca* and 41 strains (39.24%) for *K. pneumoniae*, compared with the low rate of isolation 7 (5.85%) observed in patients aged 10 to 20 years (figure 3).

Sensitivity test

In *K. pneumoniae*, the lowest rates were observed for, gentamycin and netilmicin 18.26%, 21.15% and 22.11% versus 91.34% for cotrimoxazole and amoxicillin + clavulanic acid. On the other hand, in *K.oxytoca*, netilmicin and were more active, with rates of 10.52% and 22.36% respectively. High rates of resistance in *K.oxytoca* were observed with amoxicillin + clavulanic acid (78.98%) and cotrimoxazole (78.94%). According to the families of antibiotics tested, aminoglycosides were more active, with rates of 21.63% in *K. pneumoniae* and 23.35% in *K.oxytoca* (Table 1).

One hundred and eighty-six (186) (72.65%) strains were resistant to at least two antibiotics, including 82 strains (44.08%) for *K. pneumoniae* and 118 strains (55.92%) for *K.oxytoca*.

Of the 186 strains, 68 strains (36.55%) were isolated from men and 118 strains (63.45%) from women.

The study included 256 *Klebsiella* sp strains isolated during a one-year period (January-December 2015). During this period, the frequency of isolation of *Klebsiella* strains was around 17.25% of the total number of bacteria isolated from our samples. This frequency is close to that reported by the Public Health Agency of Canada (the *Klebsiella* genus is responsible for around 17% of urinary tract infections) (Baudrand *et al.,* 2009); but far from that obtained by Tlamçani *et al.,* in 2009 in Rabat, where *Klebsiella* was responsible for 28% of urinary tract infections (Boni-Cisse *et al.,* 2007). However, of the 256 strains isolated in this study, *K. pneumoniae* accounted for 40.62% and *K.oxytoca* for 59.38%, contrary to the data reported by Tlamçani *et al.,* (2009). In that study, the author reported a *K. pneumoniae* isolation rate of 86%, compared with just 14% for *K. oxytoca* (Ben *et al.,* 2022). This predominance of *K.oxytoca* strains in clinical isolates seems to be a peculiarity. Indeed, studies carried out in other countries have shown that *K. oxytoca* is less frequent in this type of sample (Ben *et al.,* 2022).

The isolation rate by gender was 64.07% for women and only 35.93% for men; the sex ratio (M/F) was 0.56. Overall, these results corroborate those of other authors, such as Belbel *et al.,* (2014) in Algeria, who reported a sex ratio of 0.85, with 54% of women and 46% of men. This high tendency in women could be due to anatomical causes (shortness of urethra, proximity of anal and vaginal orifices), poor hygiene habits, sexual intercourse, pregnancy, use of spermicidal gel, uterine and bladder prolapse. In this study, the age group most affected was that of patients aged over 40 (43.77%), followed by patients aged 20-30 (20.70%).

Patients aged 0-9 accounted for 10.54%. Age, which is related to patients' immune status, is indeed one of the risk factors for *Klebsiella* infection (Tosson and Speer, 2011), given that it is, more often than not, an

opportunistic pathogen (Belbel *et al.,* 2014). The high prevalence of *Klebsiella* in patients aged over 40 could be partly explained by the frequency of chromosomal genetic mutations. These could be due to antibiotic selection pressure. Genetic exchanges are also responsible.

This thoughtfulness could also be due to excessive consumption of antibiotics following repeated infections. *Klebsiella* play an important role in the infectious pathology of the newborn (Tlamçani *et al.,* 2009), and have a high colonization rate in infants (Baudrand *et al.,* 2009; Samanta *et al.,* 2011). Although we did not assess the immune competence of our study population, it is nonetheless known that immune incompetence, alongside other factors including urinary tract catheterization, poor hygiene conditions and the frequency of hand-carried transmission by nursing staff, may be responsible for this prevalence for nosocomial patients (Belbel *et al.,* 2014).

In terms of antibiotic resistance, *Klebsiella* resistance to Aminosides varies between 21.63% and 23.35% for *K. pneumoniae* and *K.oxytoca* respectively, compared with 30% reported by Tlamçani *et al.,* in Rabat in 2009. This is quite low, as *Klebsiella*, like most *Enterobacteriaceae*, are naturally sensitive to Aminosides (Clave *et al.,* 2013). The more or less preserved efficacy of these molecules could be explained by the fact that their parenteral administration limits their frequent use or self-medication. Resistance to quinolones in *Klebsiella* was over 47%, compared with 33% reported by Tlamçani *et al.,* (2009) in Rabat. It was 20-30% in Tunisia in 2012 reported by Elhani *et al.,* (2012) and 54% in Algeria in 2014 reported by Belbel *et al.,* (2014). This difference could be explained by the fact that acquired resistance is evolutionary, changing with time, antibiotic use and geographical location.

Although *Klebsiella* is naturally susceptible to quinolones, acquired resistance to these molecules is the result of a combination of several mechanisms (Tlamçani *et al.,* 2009) and is very often linked to resistance to third-generation cephalosporins (C3G) (Clave *et al.,* 2013). Strains producing extended-spectrum beta-lactamases could carry plasmids coding for ESBLs and for quinolone resistance (Ben *et al.,* 2022). The rate of resistance to Cotrimoxazole was 83.90%, which is close to those reported by Ben *et al.,* in Tunisia (80.1%) (Ben *et al.,* 2022) and substantially equal to those reported by Belbel *et al.,* (2014) (84%). Indeed, the very frequent use of these molecules in the treatment of certain conditions would be at the origin of the increase in the percentage of resistant strains. Resistance to chloramphenicol was 71.84%, which is notably higher than the 51.5% reported in Tunisia in 2002 and by Farah *et al.,* in 2007 (26.9%) in *K.pneumoniae* producing ESBL in hospitals in the city of Annaba in Algeria (Farah *et al.,* 2007).

This high resistance rate could be explained by the abusive use of these molecules and by self-medication. The beta-lactam resistance rate in this study was higher than 50%, which could be linked to the selection pressure exerted by the widespread and excessive use of antibiotics from this family in both outpatient and hospital settings (Skumik and Andremont, 2006). Boni-Cisse *et al.,* in 2007 in Abidjan and Meite *et al.,* (2010) in Abidjan in 2010 showed that beta-lactam antibiotics were among the most prescribed antibiotics in Africa in both human and veterinary medicine. Amoxycilin + clavulanic acid was the antibiotic with the highest resistance rate (91.34%), compared to 50% reported by Tlamçani *et al.,* (2009) in Rabat and 75.2% from 2005 to 2012 for *Enterobacteriaceae* reported by Okalla *et al.,* in (2015) in Douala.

They believe that the low sensitivity of *Klebsiella* strains to Amoxicillin + Clavulanic acid would be due to the partial restoration of the activity of Amoxicillin by clavulanic acid (Okalla *et al.,* 2015). Moyen *et al.,* (2014) in Brazzaville, working on the antibiotic resistance of beta-lactamase-producing bacteria isolated from different biological products at the Brazzaville University Hospitals found a resistance rate in *Klebsiella* of 66.6% resistance to Amoxicillin + Clavulanic acid which is lower than 78.98% and 91.34%, rates revealed by our study respectively for *K.oxytoca* and *K.pneumoniae*.

The same study reported a resistance rate of 83.3% to cefotaxime and 63.6% to ceftriazone compared to those of our study, whose rates varied between 42.76% to 64.42% and 38.46% to 52.63%. Our study also showed that 186 strains (72.65%) were resistant to at least two antibiotics, including 82 strains (44.08%) for *K. pneumoniae* and 118 strains (55.92%) for *K. oxytoca*. This increased resistance could be explained by the presence of mobile genetic elements such as plasmids (Skumik and Andremont, 2006). Of the 186 strains that were resistant to at least two antibiotics, 68 strains (36.55%) were isolated from men and 118 strains (63.45%) were isolated from women. This high rate in women could be explained by repeated infections.

Table.1 Antibiotics resistance profile in *K. pneumoniae* and *K.oxytoca*

Antibiotics families	Antibiotics tested	Species	
		***K. Pneumoniae* (%) (n=104)**	***K. oxytoca* (%) (n= 152)**
Beta-lactams	**AMC, 20/10µg**	95(91.34)	120(78.98)
	CTX, 30µg	67(64.42)	65(42.76)
	CRO, 30µg	40 (38.46)	80(52.63)
Global resistance		**202 (64.74)**	**265 (58.11)**
Aminosides	**Net, 10µg**	23 (22.11)	16(10.52)
	GEN, 10µg	22(21.15)	55(36.18)
Global resistance		**45(21.63)**	**71 (23.35)**
Quinolones	**CIP, 5µg**	50(48.07)	51(33.55)
	NOR, 10µg	19 (18.26)	34(22.36)
	NA, 30µg	77(74.03)	91(59.86)
	OfX, 5µg	51(49.03)	70 (46.05)
Global resistance		**197 (47.35)**	**246 (59.13)**
Phenicols	**C, 30µg**	81(77.88)	102(67.10)
Global resistance		**81(77.88)**	**102(67.10)**
Sulfonamide	**Cot, 1,25/23,75 µg**	95 (91.34)	120(78.94)
Global resistance		**95 (91.34)**	**120(78.94)**

Legend: AMC: Amoxicillin + clavulanic acid ; CTX: Cefotaxim ; CRO: Ceftriaxone; Net: Netilmicin; GEN: Gentamicin ; CIP: Ciprofloxacin; NOR: Norfloxacin; NA: Nalidixic Acid, OfX : Ofloxacin; C : Chloramphenicol ; Cot: Cotrimoxazole

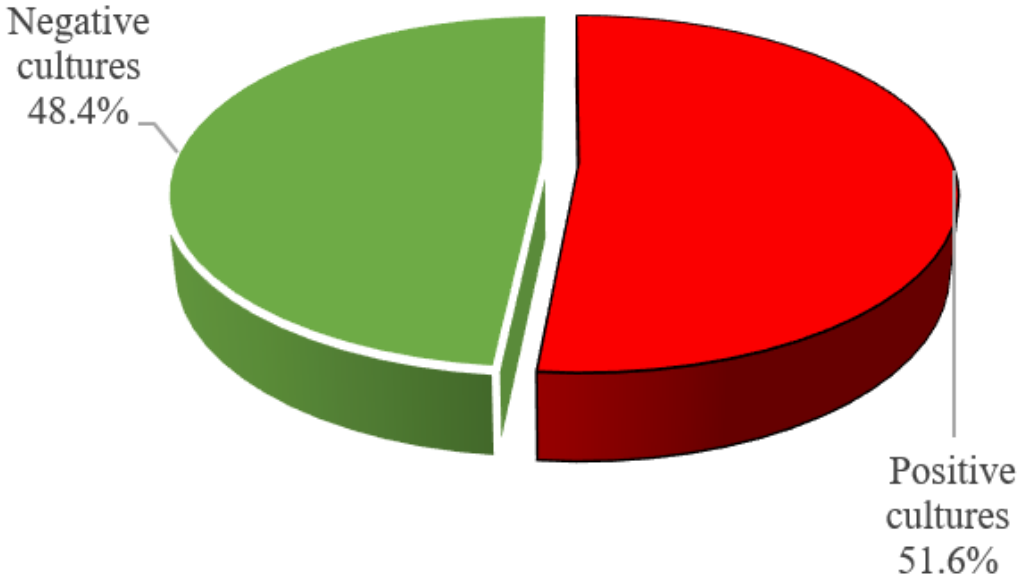

Figure.1 Frequency of positive cultures

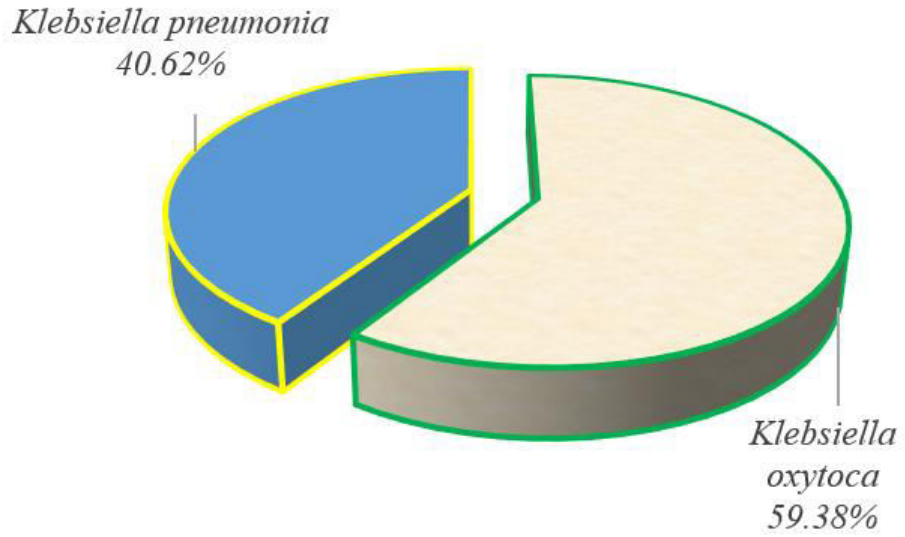

Figure.2 Distribution of *Klebsiella* strains after identification

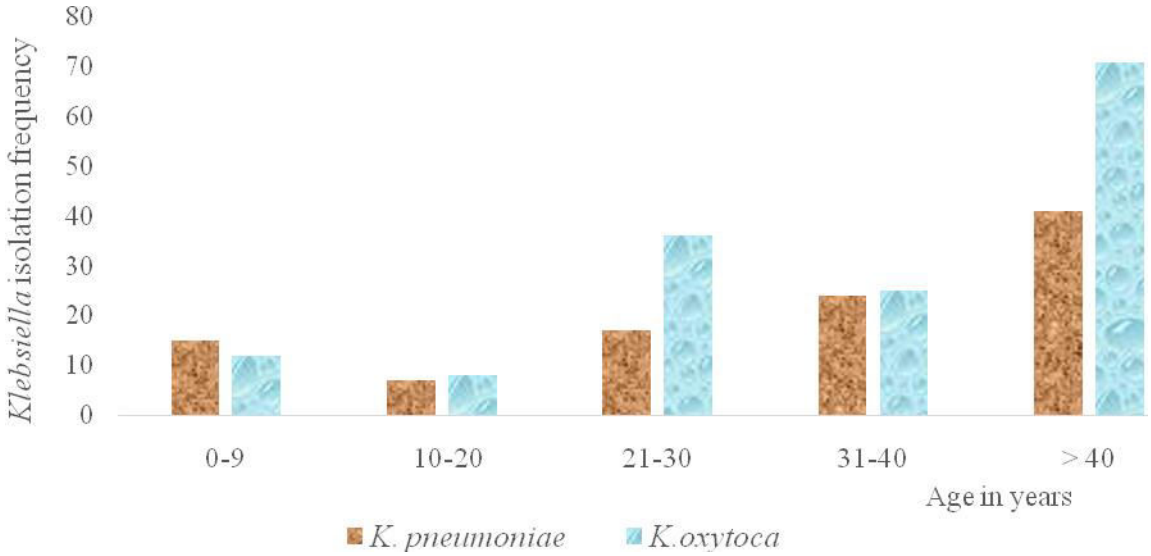

Figure.3 Frequency of isolation of *K. pneumoniae* and *K. oxytoca* strains according to age.

The prevalence and antibiotic resistance of the *Klebsiella* strains reported in this study are reaching alarming proportions. Given the magnitude and complexity of the problem, it is now imperative to curb the development of resistance by changing our behaviors and prescribing antibiotics only when necessary and rationally. Our study shows that only two *Klebsiella* strains were isolated among the bacteria responsible for urinary tract infections: *K. oxytoca* and *K. pneumoniae*, with *K. oxytoca* predominating. Susceptibility testing showed high resistance rates to amoxicillin + clavulanic acid and cotrimoxazole for both species. Netilmicin and gentamicin were the most active antibiotics against both species, followed by for *K. pneumoniae*. Of all the families tested, quinolones were the most active. More than half of *Klebsiella* strains also showed resistance to at least two antibiotics. This increase and spread of resistance could be due to unjustified use of antibiotics, antibiotic selection pressure, and genetic exchange. The antibiotics mentioned above can be used as first-line treatment for *Klebsiella* infections. This work highlights other perspectives which could allow us to understand the causes of this resistance but also the mechanisms involved in the transfer of resistance genes.

Author Contributions

Prisca Nicole Nieko Dangui Makaya: Investigation, formal analysis, writing—original draft. Aimé Christian Kayath: Validation, methodology, writing—reviewing. Tarcisse Baloki Ngoulou:—Formal analysis, writing—review and editing. Etienne Nguimbi: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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