

Original Research Article

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Molecular Characterization of *bla* Genes in Pathogenic *Enterobacteria* Isolated From Urinary Catheter Users

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ABSTRACT

Enterobacteriaceae are frequently implicated in urinary tract infections and pose a significant public health concern. The objective of this study was to detect beta-lactam resistance genes in *Enterobacteriaceae* communities isolated from the urine of patients with urinary catheters. 27 bacterial strains were collected and identified using standard bacteriological and biochemical tests. Phenotypic characterization to antibiotics was determined by the antibiotic disk diffusion method. The production of extended-spectrum beta-lactamases was determined by the double synergy method. Finally, the detection of *bla* genes was performed by polymerase chain reaction. Of the collected *Enterobacteriaceae*, 37% came from the outpatient department and 29.69% from the Nephrology department ($\chi^2 = 6.6000$; $p = 0.472$). Regarding bacterial resistance to antibiotics, the results indicate that isolates treated with imipenem were highly susceptible (85.19%). However, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* exhibited resistance rates ranging from 45% to 80% against third-generation cephalosporins. Of the 27 strains, 14 produced extended-spectrum beta-lactamases (44.26%). Genotyping revealed that the *bla*_{SHV} gene was detected in 13 out of 20 strains (65%). The *bla*_{TEM} gene was detected in 14 out of 20 strains (70%), and the *bla*_{CTX-M1} gene in 11 out of 20 strains (55%). This study highlights the involvement of *Enterobacteriaceae* harboring beta-lactam resistance genes. Faced with this situation, it is wise to pay particular attention to curbing the spread of such strains.

Keywords

Urinary tract infections, *Enterobacteriaceae*, antibiotic resistance, *bla* gene

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Introduction

Urinary tract infection (UTI) is an inflammation of the bladder resulting from the abnormal proliferation of infectious agents in the urinary system (Trześniewska-Ofiara *et al.*, 2025). UTIs represent a major public health

problem and are primarily caused by bacteria of the *Enterobacteriaceae* family. Among these, *Escherichia coli* is the predominant agent, responsible for 70 to 90% of community-acquired UTIs, while other *Enterobacteriaceae* such as *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., and *Citrobacter* spp. are frequently

implicated, especially in nosocomial infections and in immunocompromised patients (Wanke-Rytt, 2023). These bacteria, present in the intestine, colonize the urinary tract through ascending contamination.

For the treatment of urinary tract infections, medicine relies on antibiotics (Halimi *et al.*, 2019). However, self-medication and the irrational use of antibiotics, including among healthcare professionals, have contributed to an increase in antimicrobial resistance, making the treatment of bacterial infections more difficult (Ibrahim *et al.*, 2025). Indeed, the excessive production of cephalosporinase (AmpC) and extended-spectrum beta-lactamases (ESBLs) in certain Enterobacteriaceae leads to increased resistance to third-generation cephalosporins and extended-spectrum beta-lactams (Vodovar *et al.*, 2013). This production can be induced by environmental factors and by the acquisition of resistance genes, thus contributing to the spread of multidrug-resistant bacteria in hospital and community settings (Tamma *et al.*, 2019). Horizontal spread of *bla* genes is primarily mediated by transferable plasmids, often associated with a class 1 integron or transposon, which explains the speed and extent of dissemination (Shropshire *et al.*, 2022). In a clinical study in Ethiopia, approximately 75% of CTX-M-producing isolates carried transferable plasmids, providing quantitative evidence of plasmid transferability (Negeri *et al.*, 2023).

Beta-lactam resistance in Enterobacteriaceae typically manifests as a reduction in target affinity due to either altered permeability of the bacterial outer membrane, activation of an active efflux pump, or, most importantly, enzymatic inactivation (Zhao and Hu, 2013). Alarming, antibiotic resistance is responsible for approximately 700,000 deaths annually worldwide, including 50,000 in Europe and the United States. Without preventive measures, this number could reach 10 million deaths per year by 2050, according to estimates from the World Health Organization (WHO, 2019). In Côte d'Ivoire, the antibiotic resistance rate of Enterobacteriaceae increased from 9% in 2002 to 46% in 2018 (Traoré, 2019). Furthermore, several studies in the country have revealed that the production of extended-spectrum beta-lactamases (ESBLs) by these bacteria has become a significant public health challenge (Tahou *et al.*, 2022; Afran *et al.*, 2023; Cissé *et al.*, 2025). Other studies have also reported the presence and spread of ESBL strains in Enterobacteriaceae of animal and environmental origin, as well as in human strains (Yao *et al.*, 2010; Cissé *et al.*, 2017; Ouattara *et al.*, 2014). To address the progression

of antibiotic resistance and the lack of local data in Côte d'Ivoire, studies focusing on bacterial resistance in urinary catheter users appear essential for effectively implementing surveillance and prevention policies. The aim of this work is to detect beta-lactam resistance genes in enterobacterial communities isolated from the urine of urinary catheter users.

Materials and Methods

Collection of uropathogenic Enterobacteria

A sample of 27 bacterial strains, comprising *Escherichia coli* (9), *Citrobacter koseri* (1), *Proteus mirabilis* (2), *Enterobacter cloacae* (4), *Klebsiella pneumoniae* (10), and *Enterobacter aeruginosa* (1), was collected at the biobank of the Biological Resource Center (CeReB) of the Pasteur Institute of Côte d'Ivoire (IPCI).

These enterobacteria were isolated from urine collected from catheter tips during 2022 at the Clinical Bacteriology Unit (UBC) of the IPCI. Strain identifiers were determined based on information related to the hospital department (nephrology, outpatient, neurology, intensive care, pediatrics, pulmonology, emergency), the patient's age, and sex.

Revivification of collected Enterobacteriaceae

The bacterial strains were revived after incubation at 37°C for 24 h in brain-heart broth (BHC). They were then successively isolated on Mueller-Hinton agar, cetrimide agar, and Eosin Methylene Blue (EMB) agar. The plates were then incubated at 37°C for 24 h.

Determination of β -Lactamines susceptibility of Enterobacteriaceae

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was determined by the Mueller-Hinton agar disk diffusion method, according to the recommendations of the French Society for Microbiology's Antibiotic Susceptibility Testing Committee (EUCAST-CASFM, 2023). The antibiotic disks tested were cefotaxime (5 μ g), cefepime (30 μ g), ceftazidime (10 μ g), ceftazidime (30 μ g), imipenem (10 μ g), amoxicillin/clavulanic acid (30/10 μ g), ticarcillin/clavulanic acid (75/10 μ g), and aztreonam (30 μ g). The reference strain *Escherichia coli* ATCC

25922 was used in the antibiograms to perform the positive control.

Detection of extended-spectrum β -Lactamases production

The double synergy method was used to detect extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* according to Jarlier *et al.*, (1988). This method involves placing third-generation cephalosporin (cefotaxime, ceftriaxone, and ceftazidime) and aztreonam disks 30 mm around a central amoxicillin-clavulanic acid disk according to EUCAST-CASFM (2023). The presence of ESBLs is indicated by a distortion of the inhibition zone, creating a "champagne cork" pattern.

Detection of β -Lactam resistance genes

DNA was extracted from the collected strains and the reference strains *Escherichia coli* ATCC 29522 and *Klebsiella pneumoniae* ATCC 70603 using alkaline lysis with phenolization. Conventional Polymerase Chain Reaction (PCR) was used to detect beta-lactam resistance genes (*bla_{CTM-X}*, *bla_{SHV}*, and *bla_{TEM}*). Specific primer pairs listed in Table I were used to amplify the genes. PCR amplification was performed in a 25 μ L volume using a thermocycler (Perkin® Elmer Gen Amp Lapped Biosystems 9700). The amplification conditions consisted of an initial DNA denaturation step for 5 min at 95 °C. This step was followed by 35 amplification cycles, including denaturation at 95 °C for 1 min, hybridization at 56 °C for 1 min, elongation at 72 °C for 1 min 30 s, and a final elongation step at 72 °C for 10 min. The reaction medium consisted of 12.5 μ L of Master Mix, 1 μ L of forward and antisense primers, and 6.5 μ L of ultrapure water. To each well of the PCR plate, pre-filled with the mixture, 5 μ L of bacterial DNA was added. A separate reaction mixture without DNA was used as the negative control. The amplified products were analyzed by electrophoresis on 1.5% agarose gel (Invitrogen) stained with ethidium bromide. The reading was taken on an ultraviolet (UV) plate (Gel doc).

Results and Discussion

Distribution of collected strains by department

The bacterial strains originated from several hospital departments: Outpatient, Nephrology, Pediatrics,

Pulmonology, Intensive Care, Rheumatology, Emergency, and Neurology. Of these departments, 37% of the collected bacteria came from the Outpatient department and 29.69% from the Nephrology department ($\chi^2 = 6.6000$; $p = 0.472$; Figure 1). Other departments were represented in small proportions.

Distribution of *Enterobacteriaceae* according to patient sex and age

The results indicate an 85.20% (n?) isolation rate of *Enterobacteriaceae* in men versus 14.80% (n?) in women, with a sex ratio of 5.75. The analysis indicates a difference in *Enterobacteriaceae* infection rates according to patient sex ($\chi^2 = 27.92$; $p = 0.000$). Regarding age, the analysis showed that the collected strains came from patients aged between 56 and 73 years, representing a rate of 43% ($\chi^2 = 10.557$; $p = 0.014$; Figure 2).

Resistance profile of tested *Enterobacteriaceae* to beta-lactams

Analysis of the results indicated that isolates in the presence of imipenem were highly susceptible (85.19%). However, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* showed resistance rates ranging from 45% to 80% to the third-generation cephalosporins (3GCs) cefepime, ceftazidime, and ceftazidime (Table II). *Citrobacter koseri* and *Enterobacter aerogenes* were resistant (100%) to the tested antibiotics.

Detection of extended-spectrum beta-lactamases

Of the 27 strains, 14 were positive in the synergy test (44.26%). Analysis of the results revealed the presence of a champagne cork-shaped image. ESBL-producing strains were predominant in *Klebsiella pneumoniae*, representing 6 out of 27 strains (Table II).

β -Lactam Resistance Genes Detected

Beta-lactam resistance genes were detected in 20 strains, 14 of which tested positive for the synergy assay and 6 of which were resistant to cephalosporins. All of these strains harbored the *bla* genes, specifically *bla_{CTX-M1}*, *bla_{TEM}*, and *bla_{SHV}*. Figures 3 and 4 show the electrophoretic profiles on 1.5% agarose gel. The *bla_{SHV}* gene was detected in 13 strains, representing a rate of 65%. The *bla_{TEM}* gene was found in 14 strains at a rate of

70%, and the *bla*_{CTX-M1} gene was found in 11 strains at a rate of 55%. Of the 14 ESBL-producing strains, 13 harbored both the *bla*_{TEM} and *bla*_{SHV} genes. The *bla*_{CTX-M1} and *bla*_{TEM} genes were simultaneously expressed in 11 strains. Regarding the *bla*_{SHV} and *bla*_{CTX-M1} genes, they were detected in 5 *Klebsiella pneumoniae* strains, 3 *E. coli* and finally one *Enterobacter cloacae* strain (Figure 3).

In the present study, the results showed that males were more frequently infected (85%) by *Enterobacteriaceae* than females. This male dominance was reported by Sanou *et al.*, (2021). These authors showed a male predominance of 55.9% (n) with a sex ratio of 1.26. However, Yao *et al.*, (2011) reported in a study conducted at the Treichville University Hospital in Côte d'Ivoire that 93% of subjects with urinary tract infections were women. The observed male predominance could be explained by several factors. Biologically, hormonal and immune differences between the sexes appear to play a role. Androgens, being associated with a less effective innate immune response than that modulated by estrogen in women, could promote bacterial colonization (Woerther *et al.*, 2013). Epidemiological and contextual biases may contribute to this imbalance, such as later attendance at healthcare facilities or the predominance of comorbidities, including diabetes, prostate pathologies and HIV, which promote complicated infections in men (Sanou *et al.*, 2021).

Analysis of the results showed that the outpatient department recorded a high rate of *Enterobacteriaceae* strain isolation (62%). This result is similar to that reported by El Bouamria *et al.*, (2024).

These authors obtained 86% of uropathogenic *Enterobacteriaceae* primarily from patients in the outpatient department. This result could be explained by the fact that in outpatient settings, many patients consult specifically because they have urological problems. This increases the likelihood of a positive sample. In addition, some outpatients arrive after having already tried empirical treatments, which can select for more resistant or more difficult-to-eradicate strains, thus promoting more frequent isolation.

Regarding age groups, the most represented in this study was the 56-73 age group, with a rate of 43%. This predominance in the elderly was also reported by Tahou *et al.*, (2018). In their study, all age groups were infected

with *Klebsiella pneumoniae*, with a higher prevalence in the 51-95 age group, at 30.77%. In Burkina Faso, the work carried out by Sanou *et al.*, (2021) also reported a predominance in elderly patients, with a mean age of 66.91 years.

Indeed, the male predominance could be correlated with the patients' age. Studies reveal that after age 50, men are most likely to suffer from benign prostatic hyperplasia (BPH), which can lead to urinary problems and retention. These conditions often necessitate the insertion of a urinary catheter, which also increases the risk of urinary tract infections (Esomonu *et al.*, 2024). In addition to advanced age, individuals with weakened immune systems, poor hygiene, or transmission via hand-to-mouth contact by healthcare personnel (iatrogenic acts and nosocomial infections) can also contribute to infection (Maltezou *et al.*, 2013).

Regarding antibiotic resistance, it should be noted that the majority of tested strains expressed resistance rates ranging from 45% to 80% in the presence of third-generation cephalosporins (3GCs). Similar results were also reported by Warjri *et al.*, (2015) in India, with resistance rates of ESBL-producing *Enterobacteriaceae* to cefotaxime and ceftriaxone of 97.2% and 94.9%, respectively. Mathlouthi *et al.*, (2016), in studies conducted in hospitals in Algeria, also reported high resistance rates to cefotaxime and ceftriaxone, with rates of 97% and 84%, respectively. The high resistance rates observed in *Enterobacteriaceae* can be explained by several synergistic factors.

First, the inappropriate and excessive use of antibiotics, whether through empirical prescriptions not guided by an antibiogram or self-medication, is the main driver of antibiotic resistance (O'Neill, 2016). This situation is exacerbated in many developing countries where access to antibiotics without a prescription is common, leading to inappropriate use in terms of dosage and treatment duration (Auta *et al.*, 2019). Furthermore, inadequate hospital hygiene and infection prevention measures promote the cross-spreading of resistant strains, particularly in overcrowded settings or when hygiene protocols are not followed (Launay *et al.*, 2012).

In addition to these factors, the massive use of antibiotics in agriculture and livestock farming contributes to the selection of resistant strains transmissible to humans via the food chain (Van Boeckel *et al.*, 2015).

Figure.1 Distribution of enterobacteria according to hospital departments

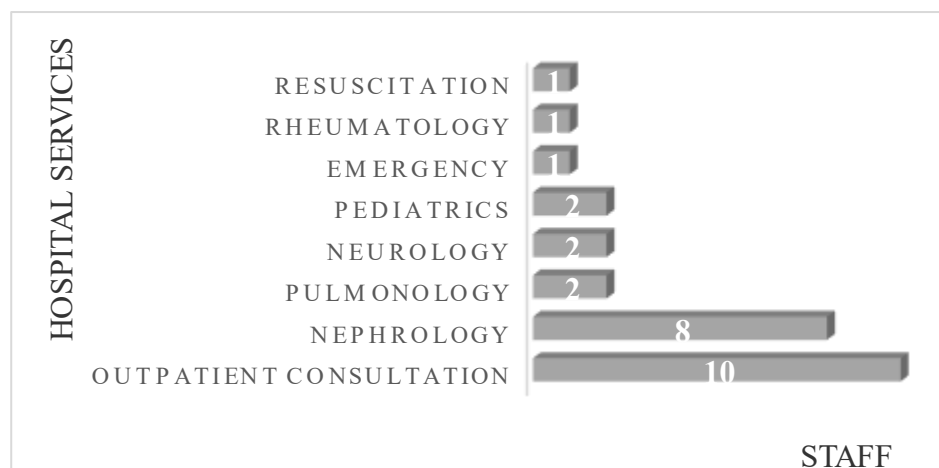


Figure.2 Distribution of collected strains according to age groups

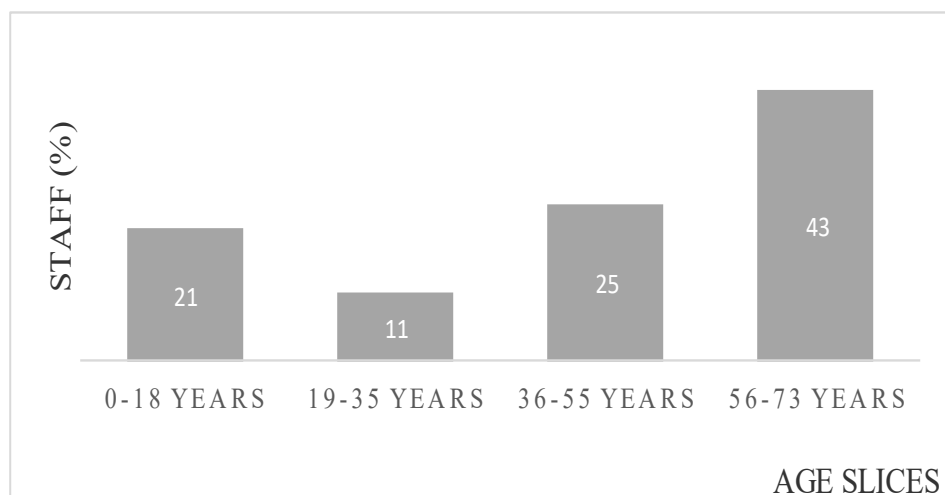
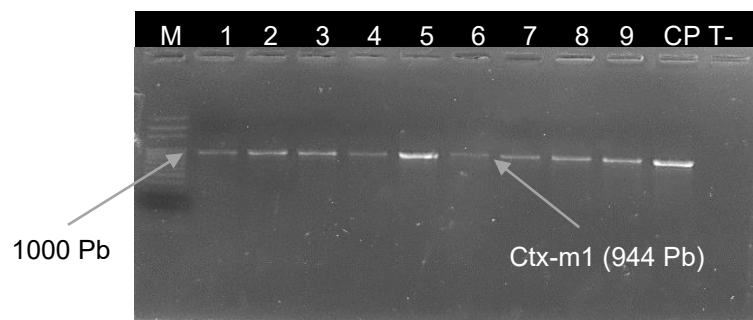


Figure.3 Electrophoretic profiles on 1.5% agarose gel of the *bla*_{CTX-M1} genes



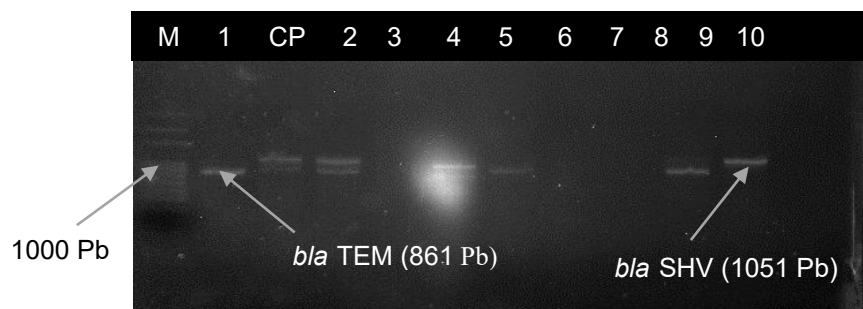
Lane M: molecular weight marker (Solis Biotec 100 Pb), Lane CP: positive control, Lane T-: negative control, Lanes 1 to 9: samples positive for *bla*_{CTX-M1}

Table.1 Resistance rates of enterobacteria in the presence of antibiotics

Strains (N)	AMC N (%)	TIC N (%)	FEP N (%)	COX N (%)	CAZ N (%)	IMP N (%)	ATM N (%)	FOX N (%)	BLSE N (%)
<i>E. coli</i> (09)	4(45)	8 (89)	7 (78)	7 (78)	7 (78)	2 (22)	4 (45)	4 (45)	5 (55)
<i>K. Pneumoniae</i> (10)	5 (50)	10 (100)	8 (80)	8 (80)	7 (70)	1 (10)	6 (60)	5 (50)	6 (60)
<i>E. aerogenes</i> (01)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
<i>E. cloacae</i> (04)	3 (75)	3 (75)	2 (50)	3 (75)	3 (75)	0 (0)	3 (75)	1 (25)	2 (50)
<i>C. koseri</i> (01)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)
<i>P. mirabilis</i> (02)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)

N = number of strains; cefotaxime: COX, cefepime: FEP, ceftazidime: CAZ, cefoxitin: FOX, imipenem: IMP, amoxicillin/clavulanic acid: AMC, ticarcillin/clavulanic acid: TIC, and aztreonam: ATM

Figure.4 Electrophoretic profiles on 1.5% agarose gel of the *bla*_{TEM} and *bla*_{SHV} genes



Lane M: molecular weight marker (Solis Biodyne 100 Pb), Lane CP: positive controls, Lane 10-: negative control, Lanes 1, 2, 3, 5, and 9: samples positive for *bla*_{TEM}, Lanes 2, 3, 5 and 9: samples positive for *bla*_{SHV}

Table.2 Primers used for the detection of β -Lactam resistance genes

Gene name	Primer	5'-3' primer sequences	Amplicons (pb)
<i>bla</i> _{TEM}	F	ATGAGTATTCAACATTTCCG TG	861
	R	TTACCAATGCTTAATCAGTG AG	
<i>bla</i> _{CTX-M-1}	F	CCCATGGTTAAAAAATCAC TGC	944
	R	CAGCGCTTTTGCCGTCTAAG	
<i>bla</i> _{SHV}	F	ATTTGTCGCTTCTTTACTCG C	1051
	R	TTTATGGCGTTACCTTTGAC C	

Table.3 Distribution of beta-lactam resistance genes detected in the tested strains

Souches	Genes			Co-expression		
	TEM N (%)	SHV N (%)	CTX-M-1 N (%)	TEM /SHV N (%)	TEM /CTX M-1 N (%)	SHV/CTX M-1 N (%)
<i>E. coli</i>	5 (35,71)	3(23,08)	5(45,45)	3(25)	5(45,45)	3(33,33)
<i>K.pneumoniae</i>	6 (42,86)	7 (53,85)	5(45,45)	6(50)	5(45,45)	5(55,56)
<i>C. koseri</i>	1(7,14)	1(7,69)	0(0)	1(8,33)	0	0
<i>E. cloacae</i>	2(14,29)	2(15,38)	1 (9,1)	2(16,67)	1(9,1)	1(11,11)
Total	14	13	11	12	11	9
Taux (%)	(70)	(65)	(55)	(65)	(55)	(45)

Finally, the genetic plasticity of bacteria, particularly through horizontal gene transfer via plasmids, integrons, and transposons, accelerates the spread of resistance determinants such as the *bla* or *qnr* genes (Partridge *et al.*, 2018).

The spread of such bacteria constitutes a public health threat because the available arsenal of antibiotics is struggling to be effective. This raises enormous health concerns, especially given the lack of prospects for the arrival of new molecules in the coming years.

The β -lactam resistance genes detected after analysis of the results were *bla*_{CTX-M-1} (55%), *bla*_{TEM} (70%), and *bla*_{SHV} (65%). These results are significantly lower than those obtained by Bebel *et al.*, (2014) in Algeria. They reported respective resistance rates of 92.5%, 95%, and 91.25% for *bla*_{TEM}, *bla*_{CTX-M-1}, and *bla*_{SHV}. Hou *et al.*, (2015) also obtained lower results than those in this study, with 57.89% for *bla*_{SHV}, 26.31% for *bla*_{TEM}, and 18.42% for *bla*_{CTX-M-1}. β -lactam resistance genes are widespread in *Enterobacteriaceae* due to their frequent location on conjugative plasmids and other mobile genetic elements. These carriers facilitate horizontal transfer between bacterial species, promoting rapid dissemination in the community and in hospital settings (Partridge *et al.*, 2018). In addition, the massive use of third-generation cephalosporins and penicillins in the management of urinary and enteric infections exerts strong selection pressure which enriches strains producing extended-spectrum β -lactamases (ESBL) (Bush and Bradford, 2020).

The emergence of *bla*_{CTX-M-1} family genes, and in particular *bla*_{CTX-M-1}, is strongly linked to their dissemination via epidemic plasmids, often associated with other resistance genes (aminoglycosides, fluoroquinolones), which promotes co-selection (Cantón *et al.*, 2012). As for the *bla*_{TEM} and *bla*_{SHV} genes,

historically among the first identified, their high persistence is explained by their capacity for adaptation and mutation, giving rise to multiple variants with broad activity against cephalosporins (Bradford, 2001).

However, four *Enterobacteriaceae*, including two strains of *Klebsiella pneumoniae*, one *Escherichia coli*, and one *Enterobacter cloacae*, received particular attention because, in addition to being resistant to the majority of antibiotics tested, they harbored all the targeted *bla* genes. The co-expression of several *bla* genes in the same *Enterobacteriaceae* strain is primarily explained by the presence of multidrug-resistant plasmids and class 1 and 2 integrons, which can group different resistance genes onto a single mobile element (Partridge *et al.*, 2018). This allows bacteria to simultaneously express several β -lactamases, conferring broad resistance to penicillins, cephalosporins, and sometimes β -lactamase inhibitors. The co-existence of these genes thus provides a major evolutionary advantage to bacteria, rendering several classes of antibiotics ineffective at once, and contributes to the global spread of multidrug-resistant *Enterobacteriaceae* (Bush and Bradford, 2020).

In conclusion, this study included 27 strains isolated from the urine of patients with urinary catheters from various hospital departments. Male patients aged 56–73 years were the most frequently infected with *Enterobacteriaceae* (43%). Of all the strains collected, *Klebsiella pneumoniae* was the most prevalent species (37%). The antibiotic resistance profile of the strains showed high resistance rates to third-generation cephalosporins. Molecular characterization revealed a wide diversity of resistance genes, with a predominance of *bla*_{TEM} (70%) and *bla*_{SHV} (65%) genes. Simultaneous expression of β -lactam resistance genes was detected in 14 *Enterobacteriaceae* strains.

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Authors' Contributions

This work was a collaborative effort by all authors. Eric Joël TAHOU and Fougoutin Hamidou COULIBALY designed the study, planned the study, and collected the data. Yves-Nathan T. TIAN-BI analyzed and interpreted the results. All co-authors contributed to the content, revision, and final version of the manuscript.

Data Availability

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

Declarations

Competing Interests

Authors have declared that no competing interests exist.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Ethical Approval

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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