American Journal of Biomedical and Life Sciences
2026, Vol. 14, No. 4, pp. 90–97
https://doi.org/10.11648/j.ajbls.20261404.14

Research Article

Resistance of *Klebsiella Sp* to Ceftazidime Associated with the Production of β-lactamase Enzymes at the Saint Camille Hospital in Ouagadougou (Hosco)

Pegdwende Rose Bonkoungou[1, *], Sidnooma Veronique Zongo[1], Damis Patrik Bouniounou[1], Amana Metuor Dabire[1, 2, 3], Mahoukede Theodora Zohoncon[1, 4]

[1]Faculty of Life and Earth Science, Joseph KI-ZERBO University, Ouagadougou, Burkina Faso

[2]Department of Microbiology-Molecular Biology, Pietro Annigoni Biomolecular Research Center (CERBA), Ouagadougou, Burkina Faso

[3]Training and Research Unit in Applied Sciences and Technologies, Daniel Ouezzin Coulibaly University, Dedougou, Burkina Faso

[4]Faculty of Medicine, Saint Thomas Aquinas University, Ouagadougou, Burkina Faso

Abstract

Enterobacteriaceae resistant to β-lactam include *Escherichia coli* and *Klebsiella sp*. Multiresistance to antibiotics in Enterobacteriaceae and in particular in *Klebsiella sp* is constantly evolving. *Klebsiella* is characterized by natural resistance to aminos and carboxypenicillins. Ceftazidime is a 3rd generation cephalosporin active on Gram-negative bacteria, strict aerobic or facultative anaerobic. Enterobacteriaceae resistance to β-lactam is more frequently mediated by the production of β-lactamases. The objective of this study was based on the resistance of *Klebsiella sp* to ceftazidime by the production of several enzymes at the Saint Camille Hospital in Ouagadougou. One hundred and sixty-two (162) strains of *Enterobacteriaceae* were collected at the Saint Camille Hospital in Ouagadougou, including 33 strains of *Klebsiella sp* and tested with antibiotics. The detection of resistance genes encoding Extended-spectrum beta-lactamases was performed at Laboratory of Molecular Biology and Genetics by conventional Polymerase Chain Reaction. The strains were mainly isolated from urine (29) and showed strong resistance to AMC penicillins (94.12%) and ceftazidime (72.72%). The Extended-spectrum β-lactamases phenotype was found in 42.85% of strains, which were positive by conventional PCR. The CTX-M genes (54,54%) followed by IMP (21.21%). Analysis of PCR products after agarose gel electrophoresis confirms the presence of resistance genes encoding the CTX-M and IMP genes. This study highlights the coexistence of resistance genes of bacterial strains encountered in the clinical environment, hence the need to set up and support an antibiotic resistance surveillance unit. The misuse or inappropriate use of antibiotics is mainly responsible for the emergence of antibiotic resistance. Our study confirmed the presence of the resistance genes *CTX-M-type Extended spectrum β-lactamase gene*, *Guiana Extended-spectrum β-lactamase* and *IMP-type metallo-β-lactamase gene* encoding the enzymes CTX-M, GES and IMP respectively in clinical strains of *Klebsiella* spp. isolated from the Saint Camille Hospital in Ouagadougou, Burkina Faso.

*Correspondence: Pegdwende Rose Bonkoungou (rose_bonkoungou@yahoo.com)

Received: 15 July 2026; **Accepted:** 28 July 2026; **Published:** 22 August 2026

Keywords

Gram Negative Bacteria, Extended-Spectrum B-Lactamases, Imp-Type Metallo-B-Lactamase Gene, Guiana Extended-Spectrum B-Lactamase, CtX-M-Type Extended Spectrum B-Lactamase Gene, Saint Camille Hospital in Ouagadougou

1. Introduction

Bacteria have become resistant and embody a global public health threat [1]. To combat this threat, the medical community has turned to antibiotics such as carbapenems as a first-aid treatment. Unfortunately, the misuse of these antibiotics has led to a more crucial problem, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) [2]. *Klebsiella pneumoniae* is a major cause of healthcare-associated infections and a cause of certain community-acquired infections, including severe bacterial infections [3]. *Klebsiella pneumoniae* is an opportunistic Gram-negative bacterium that often multidrug-resistant pathogen affects humans, causes severe infections worldwide, and is associated with a high mortality rate [4]. Enterobacteriaceae resistant to β-lactam include *Escherichia coli* and *Klebsiella sp*.[5]. Multidrug resistance in enterobacteriaceae and in particular in *Klebsiella spp* is constantly evolving [6]. *Klebsiella* is characterized by natural resistance to aminos and carboxypenicillins [7]. Ceftazidime is a 3rd generation cephalosporin active on strict aerobic or facultative anaerobic Gram-negative bacteria [8]. Enterobacteriaceae resistance to β-lactam is more frequently mediated by the production of β-lactamases. These resistance enzymes include extended-spectrum β-lactamases (ESBLs) of the CTX-M and GES types as well as IMP-type carbapenemases. The different mechanisms of resistance are encoded by these genes: decreased cell wall permeability, enzymatic inactivation and beta-lactamase production. Over the past two decades, an increase in the rate of resistance to certain antibiotics, including 3rd generation C3G cephalosporins and carbapenems, has been observed [4]. Klebsiella pneumoniae isolates have been identified by the World Health Organization (WHO) as a "critical concern" [9]. It is in this case that this study is based on the resistance of *Klebsiella sp* to ceftazidime by the production of several enzymes at the Saint Camille Hospital in Ouagadougou (HOSCO).

2. Materials and Methods

In the present study, the Saint Camille Hospital of Ouagadougou (HOSCO) served as the site for the collection of bacterial samples while molecular analyses were carried out at the Laboratory of Molecular Biology and Genetics (LABIOGENE). The isolates were included based on the following criteria after the hospital received samples—which could be pus, stool, urine, etc. A phase of bacterial identification and isolation was conducted before proceeding with data collection. The selection of these genes stems from the fact that antibiotic resistance is a major public health issue and a very serious concern, as it can sometimes lead to death because bacteria have developed resistance that prevents medications from being effective.

2.1. Bacterial Analyses

The study looked at Gram-negative strains of bacilli. These bacterial strains were isolated and identified from different types of biological samples, including urine, stool, pus, blood and vaginal swabs, at the Saint Camille Hospital in Ouagadougou.

After isolation and identification, the bacterial strains were stored with LABIOGENE at 4°C in a Luria-Bertani (LB) medium supplemented with 30% glycerol.

2.1.1. Collection

The collection of bacterial strains was carried out after confirmation of their isolation and identification. Pure colonies cultured on Mueller-Hinton (MH) agar at the collection site were taken to LABIOGENE and then incubated in the oven for 24 hours to verify their viability and purity.

A total of 162 bacterial strains were collected, including 33 strains belonging to the genus *Klebsiella spp.* A macroscopic reading of the colonies was carried out after 24 hours of incubation. An antibiogram was then performed for each bacterial strain according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [10].

2.1.2. Antibiotic Strain Susceptibility Testing

All isolates were tested for susceptibility to 6 different antibiotics by the Mueller Hinton II agar delivery method (Oxoid, England) in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology [10]. The results were interpreted according to CASFM/EUCAST criteria and the strains were classified into three categories: susceptible (S), high-dose susceptible (SFP) and resistant (R). The antimicrobial discs (Oxoid) used were ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), amoxicillin + clavulanic acid (30 µg) and

aztreonam (30 µg).

2.2. Molecular Analyses

2.2.1. DNA Extraction

The DNA extraction was done by the method described by Metuor *et al.,* in 2019 [11]. DNA concentration and purity were assessed using a NanoDrop spectrophotometer. A portion of the supernatant was used immediately for amplification reactions, while the remainder was retained at −80°C for subsequent analyses.

2.2.2. Polymerase Chain Reaction or PCR

The DNA extracted from the resistant strains was analyzed by conventional PCR to look for the CTX-M, GES and IMP genes using primer pairs specific (Table 2) to these resistance genes. PCR amplification reactions were performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, California, USA) in a 20 µL reaction mixture. This reaction mixture consisted of 4 µL of the 5X Firepol® Master Mix + 0.5 µL of sense primer + 0.5 µL of antisense primer + 14 µL of PCR water + 1 µL of bacterial DNA from each strain**.** The PCR program used is given in Table 1 and the primer sequences in Table 2.

Table 1. *PCR Program by Gene Type.*

Genes Settings	Condition / duration		
	***bla*IMP**	***bla*GES**	***bla*CTX-M**
Initial denaturation	96°C / 5 mn	96°C / 7 mn	96°C / 5 mn
Denaturation	96°C / 30s	96°C / 1mn	96°C / 1mn
Hybridization	54°C / 30s	60°C / 1mn	50°C / 1mns
Elongation	72°C / 30s	72°C / 1mn	72°C / 1mn
Final elongation	72°C / 7 mn	72°C /10 mn	72°C /10 mn
Number of cycles	30	35	35

Legend
This table shows the polymerization chain reaction steps for our genes with initial denaturation first followed by denaturation, hybridization, elongation and finally final elongation.

Table 2. *Nucleotide sequences of the different primers.*

Genes of interest	Sequences (5'-3')	Size (pb)	References
blaCTX-M	For: 5'GTTACAATGTGTGAGAAGCAG 3' Rev: 5' CCGTTTCCGCTATTACAAA 3'	1000	[12]
blaGES	For: 5' ATGCGCTTCATTCACGCAC 3' Rev: 5' CTATTTGTCCGTGCTCAGG 3'	863	[13]
blaIMP	For: 5' CATGGTTTGGTGCTTGT 3' Rev: 5' ATAATTTGGCGGACTTTGGC 3'	500	[14]

Legends
On the other hand, this table shows the different primers used for our genes of interest

2.2.3. Agarose Gel Electrophoresis

The PCR-amplified DNA fragments were separated by agarose gel electrophoresis (1.5%) prepared in a 1X EDTA 1X tribase - borate solution containing 8 µL of ethidium bromide. The 1000 bp molecular weight marker was used to assess the expected sizes of the strips. The migration was carried out for 30 minutes under a voltage of 110 V with an intensity of 65 mA. The resulting migration products were viewed under UV light with the transilluminator (Vilber E-Box) and the photos were recorded.

2.2.4. Statistical Analyses

The data collected was entered into Excel 2021. Antibiotic resistance rates were calculated according to the formula P= 100n/ N (with n the number of antibiotic-resistant strains and N the total number of strains studied). The same formula was used to calculate the prevalence of each gene as well as the prevalences of gene coexistence (with n the number of strains carrying the gene and N the total number of strains carrying resistance genes).

3. Results and Discussion

3.1. Bacterial Strains

Among the 162 Gram-negative resistant bacterial strains, we identified 33 species of the genus *Klebsiella spp*. These bacterial species of the genus *Klebsiella* were resistant to at least one of the antibiotics tested, which are: 3rd generation cephalosporins (ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX)), imipenem carbapenem (IMP), amoxicillin + clavulanic acid (AMC) and aztreonam (ATM).

These strains were isolated from various biological samples such as urine (n = 30) and pus (n = 3).

3.2. Resistance Profile

The isolated bacterial strains showed high resistance to amoxicillin + clavulanic acid (94.12%), cefotaxime (45.45%). Imipenem (IMI) is the only carbapenem used in this study. Of the 33 strains tested, 44.13% were resistant to this antibiotic. Our strains had the same resistance rate (44.13%) to cefotaxime and imipenem.

3.3. Molecular Characterization of Genes Coding for ESBL Production

Among the isolates from the 33 strains out of 162, a rate of 20.37% was observed. Analysis of the PCR products by agarose gel electrophoresis revealed that 24 isolates carried at least one of the genes being sought and that two (2) isolates harbored both the *CTX-M* and *IMP* genes. Of the 33 isolated strains, 18 carried the bla$_{CTX-M}$ gene, with amplicons of 1,000 bp (Figure 1); 7 strains tested positive for the bla$_{IMP}$ gene, detected at 500 bp (Figure 2); and 1 strain carried the bla$_{GES}$ gene (Figure 3). The Extended-spectrum β-lactamases phenotype was found in 72.72% of strains, which were positive by conventional PCR.

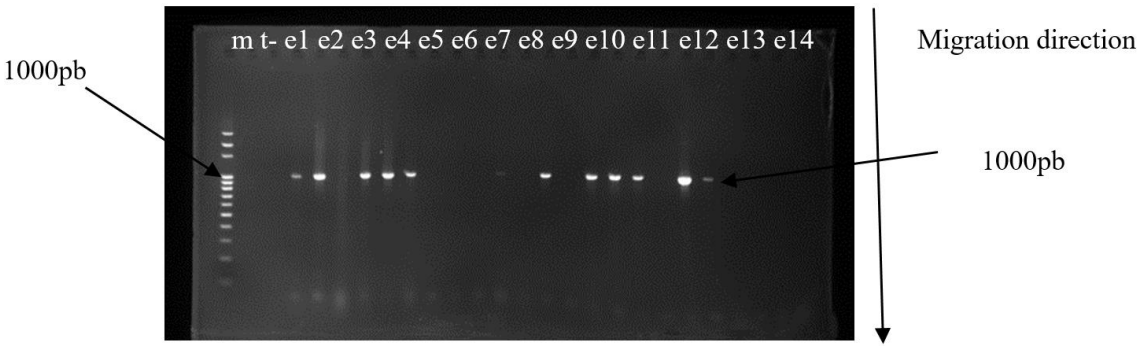

Figure 1. *Electrophoretic profile of the amplicons of the blaCTX-M gene at 1000bp.*

Legend:
blaCTX-M: Gene encoding the β-lactamase Cefotaximase-Munich
m: Molecular weight marker (100bp DNA scale),
t-: Negative control,
e1 – e14 represent the samples: e1 = urine; e2 = pus, e3: stool, e4: pus, e5: urine, e6: urine, e7: urine, e8: stool, e9: stool, e10: urine, e11: urine, e12: stool, e13: stool, e14: stool.
The direction of migration of electrophoresis is from top to bottom.

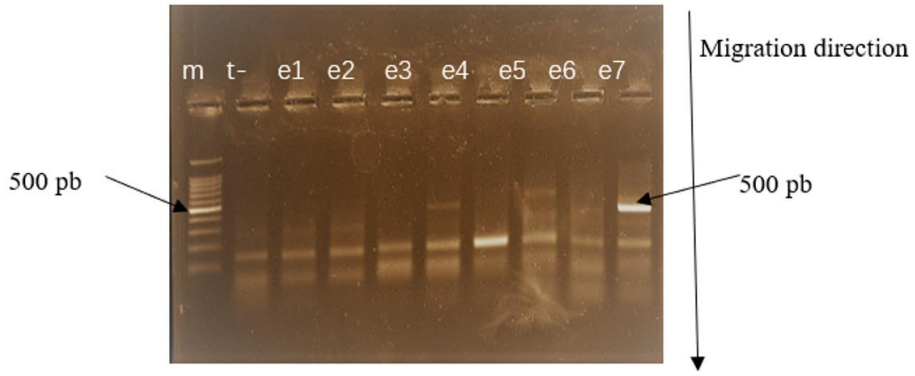

Figure 2. *Electrophoretic profile of the amplicons of the blaIMP gene at 500 bp.*

blaIMP: Gene enconding Imipenemase
m: Molecular weight marker (100bp DNA scale),
t-: Negative control, e1 – e8 represent the samples: e1 = urine; e2 = urine; e3 = urine; e4 = pus; e5 = urine; e6 = pus e7 = urine e8 = pus. The migration direction of electrophoresis is from top to bottom.

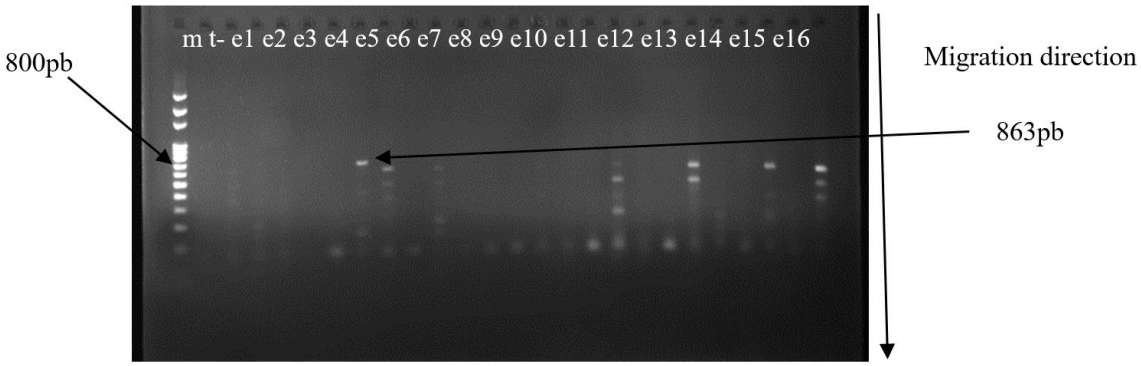

Figure 3. *Electrophoretic profile of the amplicons of the blaGES gene at 863 bp.*

Legend:
blaGES: Gene encoding Guyana Extended Spectrum β-lactamase
m: Molecular weight marker (100bp DNA scale).
t-: Negative control
The numbers e1 to e16 represent the samples: e1 = stool; e2 = stool; e3 = urine; e4 = urine; e5 = pus; e6 = urine, e7: stool, e8: pus, e9: pus, e10: urine, e11: urine, e12: stool, e13: stool, e14: urine, e15: pus, e16: urine. The direction of migration of electrophoresis is from top to bottom.

4. Discussion

Since their first description in the 1980s, especially after their detection in 1983, extended-spectrum β-lactamase-prs oducing Enterobacteriaceae (ESBL) have experienced a rapid global expansion, with variations in prevalence depending on geographical and hospital settings, [15]. Recent data confirm that these bacteria are widely involved in nosocomial and community-acquired infections, with a worrying increase in multidrug resistance, especially in resource-limited countries, [16].

The results of this study confirm the major role of bacteria of the genus *Klebsiella spp.* in resistant Gram-negative infections. The proportion observed (33 isolates out of 162 strains, or 20.37%) underlines their significant contribution, in line with data from the literature that identify *Klebsiella pneumoniae* as a major pathogen involved in urinary, respiratory and suppurative infections, particularly in hospital settings; [17-20].

The observed antibiotic resistance profile is of particular concern. The very high resistance to the amoxicillin/clavulanic acid combination (94.12%) can be explained not only by the production of ESBLs, but also by other mechanisms such as the hyperproduction of penicillinases, the modification of porins or the activation of efflux systems, [21, 22]. Similar rates have been reported in several studies in sub-Saharan Africa, reflecting frequent and often inappropriate use of this antibiotic combination, [23, 24].

Resistance to expanded spectrum cephalosporins in *K. pneumoniae* is usually mediated by the production of ESBLs belonging to the CTX-M families [25]. Resistance to cefotaxime (45.45%), a third-generation cephalosporin (C3G), is

strongly indicative of ESBL production. This observation is consistent with the global spread of CTX-M enzyme-producing Enterobacteriaceae, which have become the dominant ESBLs at the expense of TEM and HVS types [16, 26, 27]. The selection pressure exerted by the intensive use of C3G in the treatment of Gram-negative infections is a key factor promoting the emergence and spread of these resistances.

A particularly alarming result of this study is the high rate of imipenem resistance (44.13%). As carbapenems are considered antibiotics of last resort, such a level of resistance strongly suggests the presence of carbapenes. The detection of the bla_{IMP} gene in 21.2% of isolates confirms this hypothesis and indicates the circulation of metallo-β-lactamases capable of hydrolyzing carbapenems [28]. This rate is higher than those reported in some previous African studies, but is part of a recent trend of increasing resistance to carbapenems in resource-limited countries [29].

Molecular analysis reinforces these observations. The predominance of the bla_{CTX-M} gene (54.5%) confirms its central role in β-lactam resistance, in line with global epidemiological trends where these enzymes largely dominate among ESBLs [26, 27]. The presence of the bla_{IMP} gene in a significant proportion of isolates, as well as the detection of the bla_{GES} gene (3.03%), testifies to the diversity of resistance mechanisms circulating in the study population.

Of particular concern is the simultaneous detection of *the* bla_{CTX-M} and bla_{IMP} genes in some isolates. This phenomenon reflects the coexistence of several resistance mechanisms within the same strain, favored by the presence of mobile genetic elements such as plasmids, facilitating their horizontal dissemination, [30]. This results in an increase in the level of multidrug resistance and a drastic reduction in the therapeutic options available.

In addition, resistance to carbapenems may also be related to complementary mechanisms such as decreased membrane permeability (porin alteration) or activation of efflux pumps, often in association with β-lactamase production [31]. The emergence and spread of these resistances are strongly influenced by several factors, including the excessive and inappropriate use of antibiotics, inadequate hygienic conditions, weak infection control systems and the mobility of populations, facilitating the spread of resistant strains locally and internationally.

5. Conclusion

The misuse or inappropriate use of antibiotics is mainly responsible for the emergence of antibiotic resistance. The spread of resistant strains producing extended-spectrum β-lactamases poses a threat to public health by frustrating the treatment of serious infections. This public health problem requires careful monitoring and implementation of a policy on antibiotic use both in the community and in hospitals. Public education, banning the sale of antibiotics without a prescription, and reducing probabilistic antibiotic therapy in inpatient wards could be solutions to limit antibiotic resistance. The fight against this phenomenon does not necessarily involve the discovery of new antibacterial agents, but rather strict compliance with simple hospital hygiene measures and the more rational use of antibiotics.

Our study confirmed the presence of the resistance genes bla_{CTX-M}, bla_{GES} and bla_{IMP} encoding the enzymes CTX-M, GES and IMP respectively in clinical strains of *Klebsiella* spp. isolated from the Saint Camille Hospital in Ouagadougou, Burkina Faso.

Abbreviations

AMC	Amoxicillin + clavulanic Acid
ATM	Aztreonam
blaCTX-M	Gene Encoding the β-lactamase Cefotaximase-Munich
blaGES	Gene Encoding Guyana Extended Spectrum β-lactamase
blaIMP	Gene Encoding the β-lactamase Imipenemase
C3G	3rd Generation Cephalosporin
CASFM/EUCAST	Antibiogram Committe of the French Society of Microbiology
CAZ	Ceftazidime
CRE	Carbapenem-resistant Enterobacteriaceae
CTR	Ceftriaxone
CTX	Cefotaxime
DNA	Deoxyribonucleic Acid
ESBL	Extended-spectrum β-lactamase
HOSCO	Saint Camille Hospital in Ouagadougou
IMI	Imipenem
LABIOGENE	Laboratory of Molecular Biology And Genetics
LB	Luria Bertani
MH	Mueller-Hinton
PCR	Polymerase Chain Reaction

R	Resistant
S	Sensible
SFP	Sensitive at High Dosage
UV	Ultra-violet

Acknowledgments

We would like to thank all the staff of the Saint Camille Hospital in Ouagadougou (HOSCO) and the Laboratory of Molecular Biology and Molecular Genetics (LABIOGENE) who actively participated in the realization of this work.

Author Contributions

Pegdwende Rose Bonkoungou: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Sidnooma Veronique Zongo: Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Damis Patrik Bouniounou: Software, Visualization, Writing – original draft, Writing – review & editing

Amana Metuor Dabire: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Mahoukede Theodora Zohoncon: Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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