

Research Article

Multidrug Resistance of *Escherichia Coli* by Production of TOHO, NDM and SHV Type Enzymes at the Pietro Annigoni Research Centre (CERBA)

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Abstract

Antibiotics are drugs used to fight infections caused by bacteria. They are one of the most important discoveries in medicine that has saved and continues to save millions of lives each year, but their effectiveness is threatened because bacteria can adapt and resist treatment. The present study aimed to identify the *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes in *Escherichia coli* strains isolated from patients at the Pietro Annigoni Biomolecular Research Center (CERBA) in Ouagadougou. The isolation and purification of bacterial strains from the stools and urine of internal and external patients of CERBA were carried out on selective media and Muller Hinton (MH) medium. The antibiogram was carried out according to the disc diffusion method. The API 20E biochemical gallery (Bio M érieux, France) was used for the identification of enterobacteria and the *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes were detected by conventional Polymerase Chain Reaction (PCR). A total of one hundred and twenty-two (122) strains of Gram-negative bacilli were isolated, including 85 (69.67%) strains of *E. coli*. All *E. coli* strains were resistant to at least one of the antibiotics studied. Conventional PCR showed that 48.23% (41/85) of *E. coli* strains carried at least one gene studied. Among the 85 *E. coli* strains, 35.30% (30/85) of the strains harbored the *bla*_{NDM} gene, 18.82% (16/85) *bla*_{SHV} and 8.23% (7/85) of strains carrying the *bla*_{TOHO} gene. Among the 85 strains, the coexistence of *bla*_{SHV} genes + *blah*_{NDM}, *blah*_{SHV} + *blah*_{TOHO}, *blah*_{TOHO} + *bla*_{NDM}, and *bla*_{SHV} + *bla*_{NDM} + *bla*_{TOHO} were found in 16.47% (14/85) in total. This study has highlighted the multi-resistance of ESBL-producing *E. coli* strains carrying the *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes. The strong coexistence of genes in certain *E. coli* strains requires efforts to find new therapeutic solutions to combat bacterial resistance.

Keywords

Resistance to Antibiotics, Enzymes, Enterobacteria, *E.coli*, SHV, NDM and TOHO Genes

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1. Introduction

Resistant bacteria cause infections in humans or animals that are more difficult to treat than those caused by non-resistant bacteria. The choice of antibiotics that can be prescribed is indeed more limited. Bacteria can be resistant to one or more antibiotics; in which case we speak of multi-resistant bacteria (MDR). In extreme cases, fortunately still very rare, a bacterium can be resistant to all antibiotics that can be used in humans. It is then called pan-resistant and can lead to a therapeutic impasse with no further treatment possible. The most worrying MDRs are multi-resistant enterobacteria; enterobacteria such as *Escherichia coli* are bacteria in the digestive tract responsible for a very large number of infections.

Beta-lactam antibiotics remain the most widely used class of antibiotics worldwide. This is primarily due to the number of available products covering a relatively broad bacterial spectrum, the mechanism of defense the more widespread and the more developed by bacteria against effects of these beta-lactams are produced by enzymes (beta-lactamases) that chemically modify the beta-lactams to make them inactive [1].

Broad-spectrum β -lactamases (ESBL) are enzymes that confer resistance to oxymino-cephalosporins and aztreonam [2]. In fact, they result from mutations in the genes of common plasmid β -lactamases (notably SHV, TOHO and NDM) that alter the configuration of the enzyme at its active site to increase the affinity and hydrolytic capacity of the β -lactamase for oxymino compounds. Organisms producing these ESBLs are clinically relevant and remain an important cause of treatment failure with oxymino cephalosporins [3]. In effect, the researchers have found bacteria that can express up to eight β -lactamase genes at the same time (Bush, 2013). ESBLs are mainly produced by Enterobacteriaceae, particularly *Klebsiella pneumoniae* (Ahmadi *et al.*, 2022) and *Escherichia coli*. [4].

ESBL-carrying bacteria can also acquire and most often exhibit additional resistance to other classes of antibiotics such as monobactams (aztreonam), quinolones, tetracyclines, aminoglycosides (Tiemtoré *et al.*, 2022). Our study focused on the NDM, SHV and TOHO genes present in *E. coli*. Our objective was to determine the presence of these resistance genes in *E. coli* isolated at the Pietro Annigoni Research Centre (CERBA).

2. Materials and Methods

2.1. Framework of the Study

The collection of our isolates was carried out at the Pietro Annigoni Biomolecular Research Center (CERBA) in Ouagadougou.

In-depth studies on the various isolates collected; such as identification, antibiotic sensitivity testing, and the search for ESBL genes using conventional PCR techniques were carried

out at the Laboratory of Molecular Biology and Genetics (LABIOGENE). LABIOGENE is a research laboratory attached to the Doctoral School of Sciences and Technologies (ED/ST) of Joseph KI-ZERBO University. It has received the label of Center of Excellence from the West African Economic and Monetary Union (UEMOA).

2.2. Type of Study

This is a cross-sectional study of various ESBL-producing bacterial strains isolated at the Pietro Annigoni Biomolecular Research Center (CERBA) from March 1, 2024 to June 1, 2024.

2.3. Sampling

The samples selected were those received at the CERBA laboratory for bacteriological examinations and which, after analysis, allowed the detection of the presence of enterobacteria. Pus, urine, stool, vaginal and vulvar samples were included in our study.

The examination reports that were compliant included, among other things, the identity of the patient, the nature of the sample, the examination requested by the prescriber, the consultation and/or hospitalization service.

Samples collected in non-sterile equipment or suspected of any external soiling were excluded, as were samples collected the day before or days before for stool and urine samples.

2.4. Isolation and Identification of Gram-negative Bacilli

Bacterial isolation was performed using selective media. Urine and stool samples were inoculated onto standard media (URI select, CLED, BCP, Hektoen, SS) and incubated for 24 h at 37 °C. Biochemical tests were performed on suspect colonies using Kligler Hajna, mannitol-mobility, Simmons citrate, urea-indole and peptone water media.

The API 20E biochemical gallery (BioMérieux, France) was then used for the identification of Enterobacteriaceae according to the manufacturer's recommendations. The selected colonies were purified by culture at 37 °C for 24h on Muller-Hinton medium and used for antibiogram and DNA extraction.

2.5. Antibiotic Sensitivity Test

The Mueller-Hinton (MH) agar diffusion method was used for the antibiotic susceptibility test of strains according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [5]. The inoculum Bacterial suspension was prepared by placing a pure colony in 5 ml of physiological saline. The suspension was then homogenized and calibrated to 0.5 McFarlane and then inoculated by tight

streaks on the MH agar. The antibiotics were placed at a distance of approximately 20mm from each other, at a rate of 4 discs per Petri dish. The different diameters of the inhibition zones obtained around the antibiotic discs were measured after 24h of incubation at 37 °C and compared to the CA-SFM standards to determine the sensitive (S), intermediate (I) and resistant (R) phenotypes. The antibiotics Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Imipenem (IMP), Amoxicillin + Clavulanic Acid (AMC) and Aztreonam (AT) were tested.

2.6. Extraction of Bacterial DNA

An isolated pure colony was picked from the MH Petri dishes and suspended in 200µl of sterile distilled water in Eppendorf tubes. The tubes were then soaked in a 100 °C water bath for 15 minutes to release the genetic material from

the bacteria. After centrifugation for 10 min at 12000 rpm, the supernatants containing the released DNA were transferred to new Eppendorf tubes. The quantity and purity of the DNA extracts were determined spectrophotometrically using the NanoDrop. The DNA was stored at -20 °C until PCR analyses.

2.7. Gene Amplification

*Bla*_{TOHO}, *bla*_{SHV}, *bla*_{NDM} genes were detected by conventional PCR using the specific primer pairs presented in (Table 1). The PCR was carried out in a 20µL reaction mixture including 4µL of 5X Firepol Master Mix; 0.5µL of sense and antisense primer, 14µL of PCR water and 1µL of DNA extract from each strain. Amplification was carried out using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, California, USA) according to an appropriate program (Table 2).

Table 1. Primer sequence for *bla*_{SHV}, *bla*_{TOHO} genes and *bla*_{NDM}.

Genoa	Primers	Sequences (5' - 3')	Sizes	References
<i>Bla</i> _{SHV}	Forward	ATG-CGT-TAT-ATT-CGC-CTG-TG	875 bp	(Pagani et al., 2003)
	Reverse	TTA-GCG-TTG-CCA-GTG-CTC		
<i>Bla</i> _{TOHO}	Forward	ATGTGCAGTACCAGTAA	876 bp	(Laurent et al., 1999)
	Reverse	TAGGTCACCAGAACCAG		
<i>Bla</i> _{NDM}	Forward	CCATGCGGGCCGTATGAGTGATT	500 bp	(Mc Gann et al., 2012)
	Reverse	AAGCTGAGCACGCATTAGCCG		

Table 2. PCR program.

Genoa	Condition / duration		
Settings	<i>Bla</i> _{TOHO}	<i>Bla</i> _{SHV}	<i>Bla</i> _{NDM}
Initial denaturation	96 °C / 5 min	96 °C / 5 min	96 °C / 5 min
Denaturation	96 °C / 1 min	96 °C / 1 min	96 °C / 30s
Hybridization	50 °C / 1 min	60 °C / 1 min	62 °C / 30s
Elongation	72 °C / 1 min	72 °C / 1 min	72 °C / 30s
Final elongation	72 °C / 10 min	72 °C / 10 min	72 °C / 7 min
Number of cycles	35	35	30

2.8. Agarose Gel Electrophoresis

PCR-amplified DNA fragments were separated by electrophoresis on a 1% agarose gel prepared in a 1X tris

base-borate-EDTA solution containing 0.5µg/mL ethidium bromide. Migration was performed at 110mV and 850mA for 30 minutes. A 100 bp molecular weight marker was used to determine the size of the amplicons visualized under UV light using the GeneFlash device (Syngene, Bio-Imaging, UK).

2.9. Statistical Analyses

The collected data were entered into Excel 2019 and then analyzed using the standard Statistical Package for Social Sciences (SPSS) version 22 software. The results were described in terms of percentage (%) and frequency for categorical variables.

3. Results

3.1. Bacterial Strains

We obtained a total of 122 strains of Gram-negative bacteria. Among these 122 strains, 69.67% (85/122) of *E. coli* strains were all resistant to at least one of the beta-lactams.

3.2. Distribution Strains of *E. Coli* in Function of the Samples Organic

Among the 85 strains of *E. coli* we found 91.76% (78/85) in

urine, 4.70% (04/85) in stool, 2.35% (02/85) in pus, and 1.17% (01/85) in a vaginal sample (VS). (figure 1).

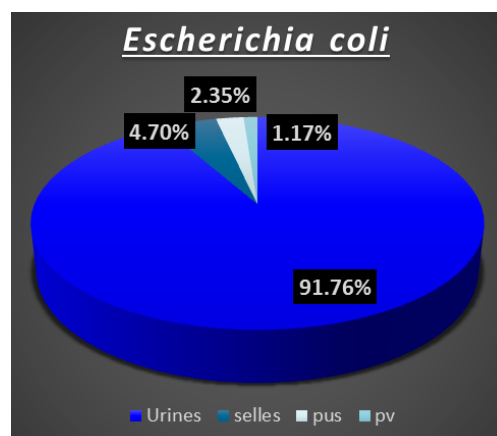


Figure 1. Distribution of strains bacterial in function of the samples organic.

3.3. Resistance of *E. Coli* Strains to Antibiotics

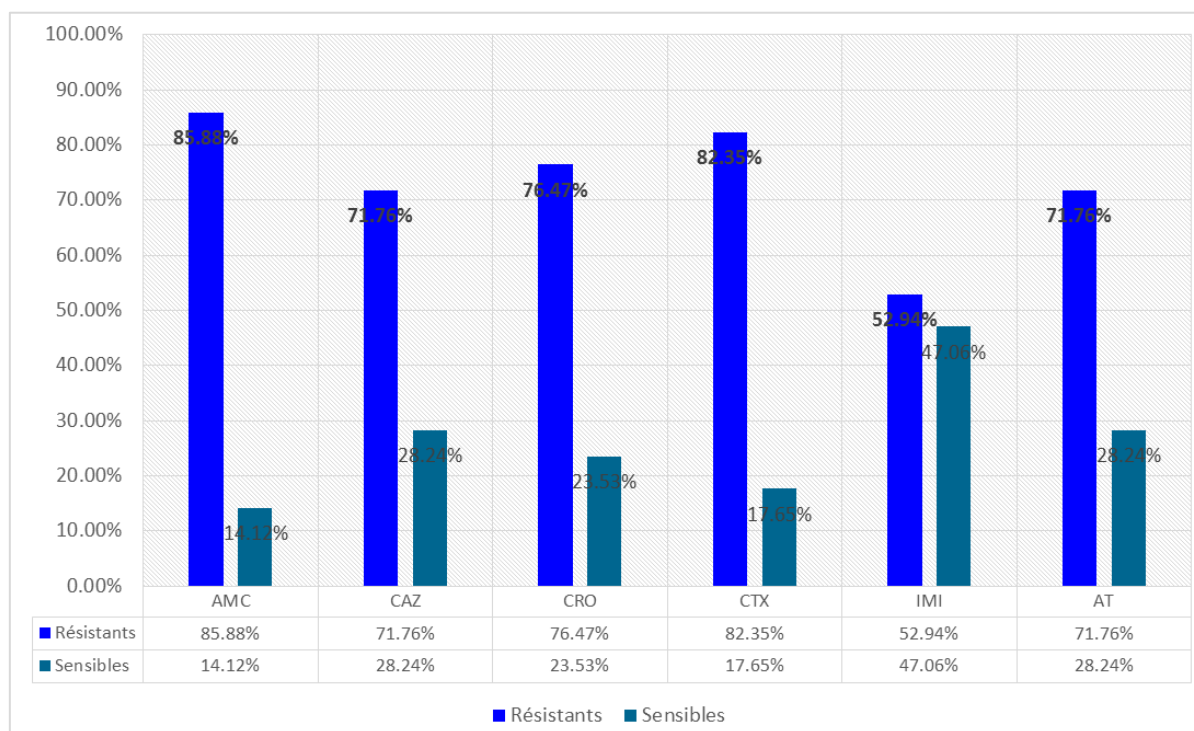


Figure 2. Resistance profile of *Escherichia coli* strains.

The antibiogram carried out on each strain of *E.coli* allowed to study their resistance profiles with respect to the antibiotics tested. The results of the study on the resistance of the 85 isolates to the different antibiotics show that 85.88% were resistant to Amoxicillin + clavulanic acid, 71.76% were

resistant to Cefotaxime, 76.47% were resistant to Ceftriaxone, 82.35% were resistant to Cefotaxime, 52.94% were resistant to Imipenem, 71.76% were resistant to Aztreonam (figures 2 and 3).

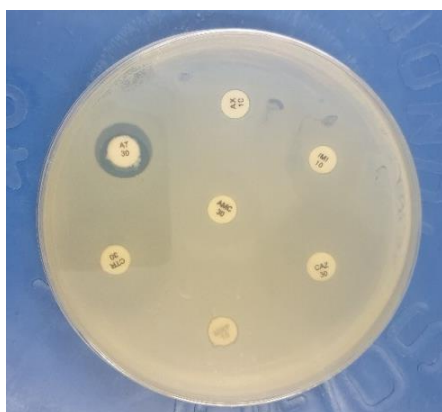


Figure 3. Resistance phenotype *E. coli* on Petri dishes.

3.4. Molecular Characterization of Resistance Genes

Detection of resistant *E. coli* strains by conventional PCR. Among them, 35.30% (30/85) of the strains harbored the *bla*_{NDM} gene, 18.82% (16/85) *bla*_{SHV} and 8.23% (7/85) strains carried the *bla*_{TOHO} gene. *Bla*_{NDM} was the most detected gene in *E. coli* (figure 4).

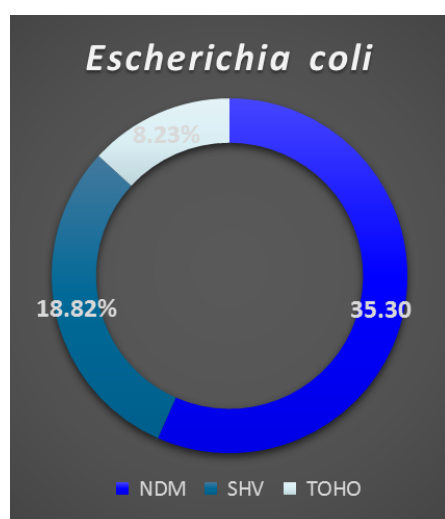


Figure 4. Distribution of genes according to *E.coli* strains.

3.5. Distribution of Genes According to Biological Samples

Urine was the sample that contained much more beta-lactamase-producing *E.coli* strains (Table 3). In our study, we found in urine 35.90% (28/78) of strains carrying the NDM gene, 19.23% (15/78) of strains carrying the SHV gene, 8.97% (7/78) of strains carrying the TOHO gene. For stool, we found 25% (1/4) of strains carrying the NDM gene and

also 25% (1/4) carrying the SHV gene. Regarding pus, we found 50% (1/2) of strains carrying NDM.

Table 3. Distribution of genes according to biological samples.

Genoa	<i>Bla</i> _{NDM}	<i>Bla</i> _{SHV}	<i>Bla</i> _{TOHO}
Samples	n (%)	n (%)	n (%)
Urine	28/78 (35.90)	15/78 (19.23)	7/78 (8.97)
Saddles	1/4 (25)	1/4 (25)	0 (0)
Pus	1/2 (50)	0 (0)	0 (0)
Pv	0 (0)	0 (0)	0 (0)

3.6. Coexistence of Resistance Genes

PCR analysis also showed that among the 85 *E. coli* strains, the combinations of *bla*_{SHV} + genes *blah*_{NDM}, *blah*_{SHV} + *blah*_{TOHO}, *blah*_{TOHO} + *bla*_{NDM} and *bla*_{SHV} + *bla*_{NDM} + *bla*_{TOHO} were found in 16.47% (14/85) in total including 57.14% (8/14), 21.14% (3/14), 14.29% (2/14) and 7.14% (1/14) respectively. Figure 5 shows the coexistence of resistance genes in *E.coli* strains.

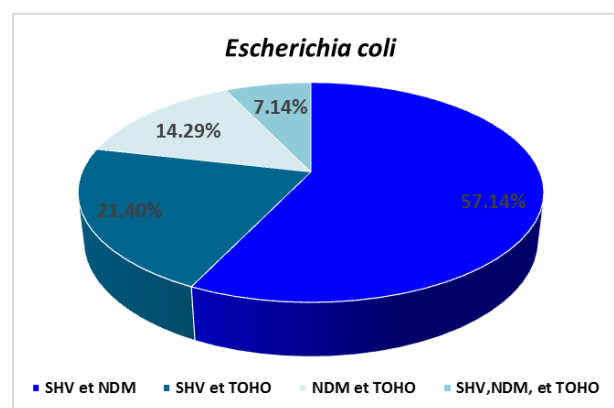


Figure 5. Coexistence of genes depending on *E. coli* strains.

3.7. Coexistence of the Genoa in Function of the Samples Organic

Strains carrying several genes were almost all found in urine, of which 21.42% (3/14) harbored both SHV and TOHO genes, 50% (7/14) SHV and NDM, 14.29% (2/14) TOHO and NDM, 7.14% (1/14) SHV, NDM and TOHO. In stools there were only 7.14% (1/14) strains that harbored both SHV and NDM (Table 4).

Table 4. Coexistence of the Genoa in function of the samples organic.

Genoa	<i>Bla</i> _{SHV} and <i>bla</i> _{TOHO}	<i>Bla</i> _{SHV} and <i>bla</i> _{NDM}	<i>Bla</i> _{TOHO} and <i>bla</i> _{NDM}	<i>Bla</i> _{SHV} , <i>bla</i> _{TOHO} and <i>bla</i> _{NDM}
Samples	n (%)	n (%)	n (%)	n (%)
Urine	3/78 (3.84)	7/78 (8.97)	2/78 (2.56)	1/78 (1.28)
Saddles	0	1/4 (25)	0	0

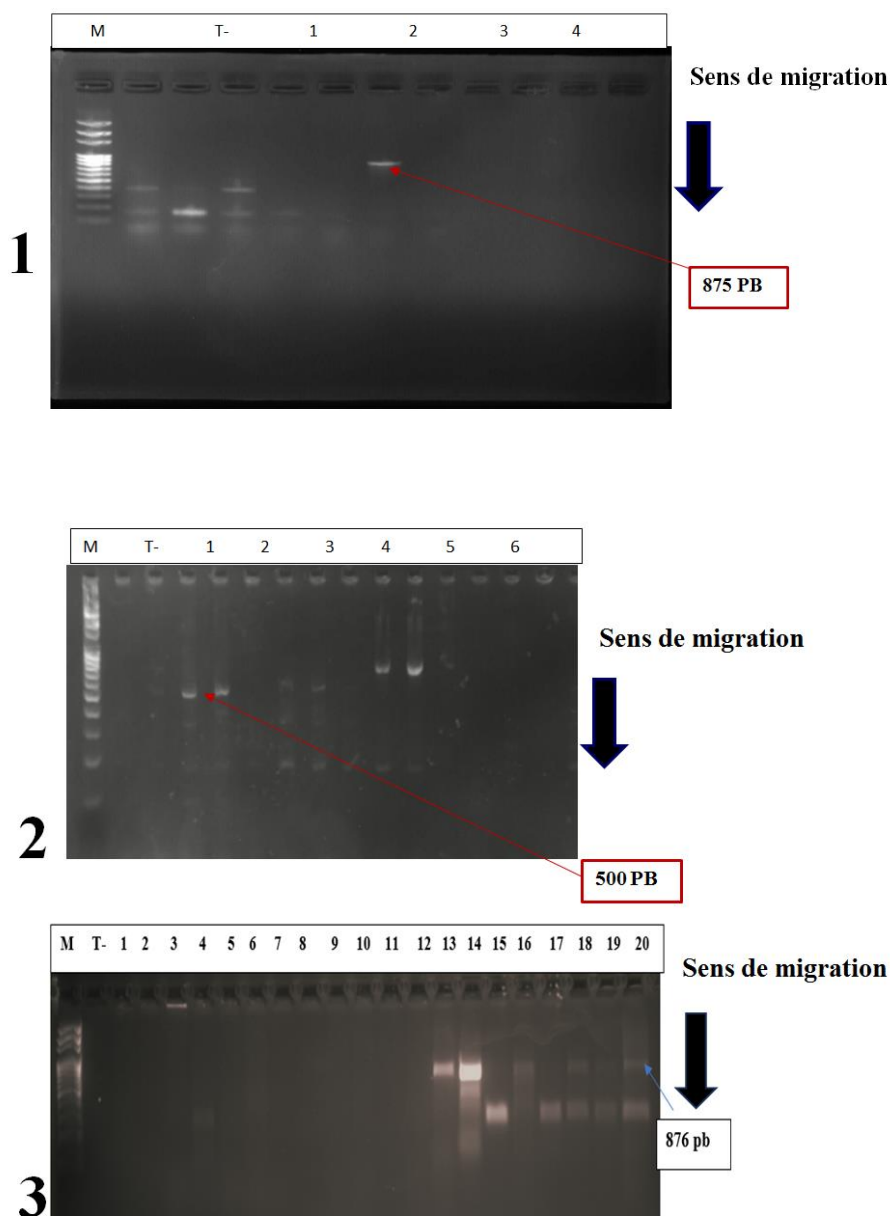


Figure 6. Electrophoretic profiles of the different resistance genes present on *E.coli* samples.

The direction of migration is from top to bottom. M = 100 bp DNA Ladder; T-: Negative control. 1. Sample 5 is positive for the *bla* SHV gene. 2. Samples 2, 3, 5 and 6 are positive for the *bla* NDM gene. 3. Samples 13, 14, 16 and 18, 20 are positive for the *bla* TOHO gene.

4. Discussion

Bacteria that once caused mild infections have become multidrug-resistant and can now cause serious infections that complicate therapeutic strategies. The emergence of multidrug-resistant bacteria (MDR) is now a worrying global phenomenon [6]. In our study, we found *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes in many *Escherichia coli* strains isolated from urine or stool cultures at CERBA, Ouagadougou, Burkina Faso. Most of the resistant *E. coli* strains (91.76%) in the present study were isolated from urine, similar to previous studies by our research team in Burkina Faso [21] and Togo [7]. *Escherichia coli* strains represent the most important enterobacteria in bacterial infections, especially urinary tract infections [8]. Indeed, it is estimated that 53.33% of women and 46.67% of men experience at least one episode of symptomatic urinary tract infection during their lifetime, and that 27 to 48% of affected women suffer from recurrent urinary tract infections.

E. coli strains (85.88%) were resistant to amoxicillin, a semi-synthetic penicillin, in combination with clavulanic acid which is a β -lactamase inhibitor [9]. Our results are higher than those of [10] with a resistance rate to amoxicillin-clavulanate at 43% against a resistance rate of 13.7% in the city of El Jadida [11]. These high rates of resistance of *E. coli* to amoxicillin justify that aminopenicillins are no longer currently recommended in the probabilistic treatment of urinary tract infections [10]. Resistance rates to third-generation cephalosporins (C3G) of 71.76%, 76.47% and 82.35% were recorded respectively for Cefotaxime, Ceftriaxone, and Cefotaxime in the present study. Resistance to C3G has also been reported in previous studies in Burkina Faso. In the study by Zahir *et al.*, in Morocco, *Escherichia coli* was resistant to amoxicillin in 80% of cases, to the amoxicillin-clavulanic acid combination in 59% of cases, and to third-generation cephalosporins. Drug pressure in hospitals, poor treatment control and the overuse of antibiotics, sometimes without medical prescription, are the main causes of the emergence and spread of multi-resistant pathogens. In addition, acquired resistance has a high power of dissemination due to its plasmid determinism. The resistance rate to Aztreonam (monobactams) was 71.76% compared to 52.94% for Imipenem in the present study. Contrary to the results of our study All isolated strains had high sensitivity to imipenem (100%), cefoxitin (81.25%) and sensitivities averages to ceftriaxone (53.12%) and cefotaxime (53.12%) in [12]. The results of the sensitivity of *E. coli* to imipenem are comparable to those obtained by [13] in Chad which were (100%). This demonstrates the absence of carbapenemase-producing *E. coli* strains [12]. Resistance to aztreonam could be better explained by the presence of other resistance mechanisms, including AmpC β -lactamase or a combination of permeability defects and efflux mechanisms. Bacteria use various resistance mechanisms such as inactivation of antibiotics by enzymes.

*Bla*_{NDM} gene (35.30%) was the most common in the pre-

sent study, followed by the *bla*_{SHV} gene (18.82%) and the *bla*_{TOHO} gene (8.23%). New Delhi metallo- β -lactamase-1 (NDM-1) is an enzyme capable of hydrolyzing most β -lactam antibiotics. The prevalence of NDM-1-producing bacteria is receiving increasing attention as a global health threat given their spread in many environmental and animal reservoirs in Asia [14], the Middle East [15], and India [16]. The *bla*_{NDM1} gene is located on plasmids harboring multiple resistance determinants, thus conferring widespread drug resistance, leaving few or no therapeutic options [16]. NDM1 has been identified primarily in *Escherichia coli* [16]. However, the usefulness of carbapenems is seriously threatened by the emergence of carbapenemases, notably the newly characterized NDM1. SHV-type beta-lactamases, including SHV-1 and at least twenty-three variants, generally exhibit broad-spectrum activity against newer broad-spectrum cephalosporins. SHV enzymes belong to the molecular class A of serine β -lactamases and share high functional and structural similarity with TEM β -lactamases [17].

*Bla*_{SHV} -1 has spread via plasmids to virtually all species of enterobacteria but is most common in *E. coli*. TOHO-2 has high catalytic activity against cephalothin, cephaloridine, cefotaxime, and piperacillin [18]. TOHO-2 has been reported in Tokyo, Japan, in *E. coli* isolated from the urine of a patient treated with β -lactams [18].

There were two types of TOHO enzymes (TOHO1 and TOHO2) and their prevalence precise has never been reported in an epidemiological study and the veracity of the sequence was questioned because it was very closely related to CTX M2 [19]. TOHO1 was an ESBL that achieved effective activity not only against penicillins but also against third-generation cephalosporins [20]. Coexistence of *bla*_{TOHO} and *bla*_{SHV} genes was found in 21.4% of *E. coli* strains in our study. M'auor-Dabiré *et al.* reported the coexistence of *bla*_{TOHO} and *bla*_{BES} genes in *Escherichia coli* (34.4%) at Saint Camille Hospital in Ouagadougou in 2019 [21]. Coexistence of *bla*_{SHV} and *bla*_{NDM} genes as well as *bla*_{TOHO} and *bla*_{NDM} was found in 57.14% and 14.29% of the isolates in our study. The coexistence of *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes found in 01 (7.14%) of the *E. coli* strains in the present study is a factor aggravating antibiotic resistance. This suggests that these genes are carried by the bacterial chromosome and/or plasmids promoting rapid spread both vertically and horizontally to bacteria of other species. However, the development of therapeutic combinations is necessary to maintain humanity's victory over multi-resistance of pathogenic bacteria.

5. Conclusion

Multidrug resistance of *E. coli* through enzyme production represents a major challenge for the treatment of bacterial infections. This study identified the *bla*_{NDM}, *bla*_{SHV} and *bla*_{TOHO} genes in clinical strains of ESBL-producing Enterobacteriaceae isolated mainly from uropathogenic *E. coli*. The coexistence of the three genes in the *E. coli* strain suggests a rapid

spread of multidrug resistance. Rigorous surveillance, prudent use of antibiotics, infection control strategies, sequencing and rapid development of new antibiotic combinations or therapeutic solutions at the local level are essential to prevent and combat bacterial multidrug resistance in Burkina Faso.

Abbreviations

CERBA	Pietro Annigoni Research Center
MH	Muller Hinton
PCR	Polymerase Chain Reaction
SHV	Sulphydryl Variable
NDM	New Delhi m ϵ Tallo-b ϵ ta-lactamase
KPC	<i>Klebsiella pneumoniae</i> Co-producing KPC
ESBLs	Extended Spectrum Beta-lactamases
CLED	Cystine Lactose Electrolyte Deficient
BCP	BromoCr ϵ sol Pourpre
SS	Salmonella-Shigella
DNA	Deoxyribonucleic Acid
CRO	Ceftriaxone
CAZ	Ceftazidime
CTX	Cefotaxime
IMP	Imipenem
AMC	Amoxicillin + Clavulanic Acid
AT	Aztreonam (AT)
TEM	Temoneira
EUCAST/	European Committee on Antimicrobial
CASFM	Susceptibility Testing / Antibigram Committee of the French Society of Microbiology

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Rabi ϵ ou Niki ϵ na: Formal Analysis, Investigation, Methodology

Bambara Eliada Lionel: Formal Analysis, Investigation, Methodology

Abdoul Karim Ouattara: Formal Analysis, Investigation, Methodology, Supervision, Visualization

Th ϵ dora Mahouk ϵ l ϵ Zohoncon: Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Validation

Jacques Simpor ϵ Conceptualization, Investigation, Supervision

Conflicts of Interest

The authors declare no conflicts of interest.

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