Original Research Article

Detection of antibiotic resistance genes *bla*$_{SHV}$, *bla*$_{TOHO}$ and *bla*$_{NDM}$ in pathogenic gram-negative bacilli at the Pietro Annigoni Biomolecular Research Center

Abstract

Gram-negative bacilli, notably Enterobacteriaceae, are increasingly resistant to antibiotics thanks to the acquisition and dissemination of extended-spectrum β-lactamases (ESBLs). The present study aims to identify the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO}$ genesin clinical strains of Gram-negative bacteria isolated from patients at the Pietro Annigoni Biomolecular Research Center (CERBA), Ouagadougou. The isolation and purification of bacterial strains isolated from the stools and urine of internal and external CERBA patients were carried out respectively on selective and Muller Hinton (MH) media. The antibiogram was performed using the disk diffusion method. The API 20E biochemical gallery (BioMérieux, France) was used for the identification of Enterobacteriaceae while the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO}$ genes were detected by conventional PCR. A total of thirty-seven (37) strains of Gram-negative bacilli were included in the present study. The antibiogram showed that 62.16% (23/37) of them were ESBL producers with 56.52% (13/23) of *Escherichia coli*; 39.13% (9/23) of *Klebsiella sp*. and 4.35% (1/23) of Proteus sp. Among them, 60.87% (14/23) of the strains harbored the *bla*$_{NDM}$ gene, 56.52% (13/23) *bla*$_{SHV}$ against 47.83% (11/23) of the strains carrying the *bla*$_{TOHO}$ gene. The combinations of *bla*$_{SHV}$ + *bla*$_{NDM}$, *bla*$_{SHV}$ + *bla*$_{TOHO}$ and *bla*$_{TOHO}$ + *bla*$_{NDM}$ geneswere found in 30.43% (7/23), 26.08 (6/23) and 17.39% (4 /23) strains, respectively against 8.70% (2/23) of strains carrying *bla*$_{SHV}$ + *bla*$_{NDM}$ + *bla*$_{TOHO}$. It should also be noted that most of these strains were isolated in urine cultures. This study revealed clinical strains of ESBL-producing Enterobacteriaceae carrying the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO}$ genes. The

simultaneous carriage of two or three genes by certain strains suggests dissemination which requires increased surveillance efforts and the rapid development of new therapeutic solutions.

Keywords: ESBL, antibiotic resistance, enterobacteria, *bla*$_{SHV}$, *bla*$_{TOHO}$, *bla*$_{NDM}$

Introduction _

Antibioticresistance in bacteriarepresents a significant challenge for medicine in the 21 st century [1] .The increased incidence of extended-spectrumß-lactamase-producingEnterobacteriaceae (ESBLSE) has been associatedwithserious infections and has led to an increase in the prescription of broad-spectrumantibiotics[2] . ß-lactamases are constitutional or bacterially acquired enzymes whose activity causes the opening of the ß-lactam ring and creates an unstable acyl-enzyme intermediate which is then degraded to an inactive acid

[3,4] .

The emergence and dissemination of new ß-lactamases, which are the main mechanism of resistance in Gram-negativebacteria (GNB) to ß-lactams, have coincidedwith the introduction and use of theseantibiotics [5,6] . Indeed, the introduction of third generation cephalosporins (C3G) into clinical practice at the beginning of the 1980s, making it possible to fight against infections caused by penicillinase-producing pathogens, was followed, in 1983, by the description of the first ESBL in *Klebsiella pneumonia* in Germany [7] .More than 200 ESBLs have now been described and their spread throughout the world poses a real public health problem [8,9] . ESBLs are broad-spectrum enzymes, conferring resistance to almost all beta-lactams, except cephamycins (difficult to use therapeutically) and carbapenems. Until the end of the 1990s, ESBL-producing enterobacteria were mainly so-called “hospital” species, spreading clonally between hospitalized patients [9] .

The ß-lactam family includes penicillins, cephalosporins, monobactams and carbapenems [10] . β-lactams constitute a major family of antibiotics widely used in the clinic [11] . These molecules act by inhibiting the synthesis of the bacterial wall by binding to penicillin-binding proteins (PLP), enzymes involved in the synthesis of peptidoglycan [12] .

In Gram-negative bacilli (GNB), there are three types of resistance mechanisms to β-lactams: low affinity for PLP, impermeability and efflux phenomena and enzymatic inactivation by β-lactamases (ESBL) [11,12] . Infections caused by ESBL-producing strains are associated with high morbidity and mortality, prolonged length of hospitalization, and increased hospitalization costs [6] .

The genes encoding ESBLs are diverse in nature and can be grouped into several families [9] . According to the Ambler classification, carbapenemases can be divided into classes A, BC and D. Class A carbapenemases are serine β-lactamases (TEM, SHV, TOHO) while those of class B are metallo-β-lactamases (MBL), characterized by the need for zinc ions on their active site (NDM , IMP, VIM) [4,9] .Although several of the class C carbapenemases have been described (ACT-1, CMY-2, CMY-10, CMY-19, CMY-37 and ADC-68), the producing microorganisms generally exhibit reduced sensitivity to carbapenems due to the low catalytic efficiency of the enzyme and a permeability defect [13] . Common class D carbapenemases, serine β-lactamases, include OXA-type enzymes (OXA-48, OXA-23, OXA-40, OXA-58 and OXA-143, etc.)[14] . The most widespread carbapenemases in the world are the KPC type enzymes (Ambler class A), the metallo-β-lactamases types NDM, VIM and IMP (Ambler class B), and the OXA-48 type oxacillinases, class D of Ambler [15,16] . The genes that code for these enzymes are carried by mobile genetic elements, explaining their significant dissemination [9,11] .

Carbapenemase-producing bacilli are resistant to all β-lactams, including carbapenems, as well as almost all other families of antibiotics used in the clinic (aminoglycosides,

fluoroquinolones, sulfonamides, etc.), a multi-resistance which strongly limits therapeutic options [2] .Previous studies have highlighted several ESBLproducing and multi-resistant bacterial strains in Burkina Faso [17-21], Togo and [22-24] and Niger [25]. The present study aims to detect the coexistence of bla genes. NDM, bla SHV and bla TOHO in clinical strains of Gram-negative bacilli in order to better understand their level of dissemination in Burkina Faso.

Material and methods

Type and period of study

This was a prospective study involving bacterial samples collected at the Pietro Annigoni Biomolecular Research Center (CERBA) during the period from August to December 2022. The culture of the bacteria as well as the antibiogram were carried out at CERBA located on the South-East side of the city of Ouagadougou. DNA extraction as well as detection of resistance genes by conventional PCR were carried out at the Laboratory of Molecular Biology and Genetics (LABIOGENE) of Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso.

Sampling

The population of the present study consisted of bacteria isolated from various samples including mainly the stools and urine of patients from different departments.

Isolation and identification of Gram-negative bacilli

Isolation of bacteria was carried out using selective media. The urine and stool samples were inoculated on ordinary media (URI select, CLED, BCP, Hektoen, SS) then incubated for 24 h at 37°C. Biochemical tests were performed on suspect colonies using Kligler Hajna media, mannitol-mobility, Simmons citrate medium, urea-indole and peptone water.

The API 20E biochemical gallery was then used for the identification of enterobacteria according to the manufacturer's recommendations. The selected colonies were purified by

cultivation at 37°C for 24 h on Muller-Hinton medium and used for antibiogram and DNA extraction.

Antibiotic Susceptibility Test

The diffusion method on Mueller-Hinton (MH) agar was used for testing the sensitivity of strains to antibiotics according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (EUCAST / CA-SFM, 2021) . The inoculum bacterial was prepared by putting a pure colony in 5 ml of physiological water. The suspension was then homogenized and calibrated at 0.5 McFarlane then inoculated in tight streaks onto MH agar. The antibiotics were placed at a distance of approximately 20 mm from each other, at the rate of 4 discsper Petri dish. The different diameters of the inhibition zones obtained around the antibiotic disks were measured after 24 h of incubation at 37°C and compared to CA-SFM standards to determine the sensitive (S), intermediate (I) and resistant (R). The antibiotics Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Imipenem (IMP), Amoxicillin + clavulanic acid (AMC) and Aztreonam(AT) were tested.

Detection of ESBLs by the synergy test

The synergy test allows the detection of broad-spectrum β-lactamases in a given strain. These enzymes can be demonstrated by the disk method, which consists of searching for a so-called "champagne cork" image, which is a synergistic action between an antibiotic disk containing a β-lactamase inhibitor (Amoxicillin + Acid clavulanic) and third-generation cephalosporin discs (Cefotaxime , Ceftazidime , Ceftriaxone) and Aztreonam.

During the production of the antibiogram the antibiotic disks were arranged in such a way as to highlight the ESBL by looking for a synergy between clavulanic acid and third generation cephalosporins and Aztreonam.

Extraction of bacterial DNA

An isolated colony was collected from the MH Petri dishes and suspended in 200 µl of distilled water in Eppendorf tubes. The tubes were then soaked in a water bath at 100°C for 15 min to release the genetic material of the bacteria. Afterwards, centrifugation for 10 min at 12,000 rpm; the supernatants containing the released DNA were transferred to new Eppendorf tube. The quantity and purity of the DNA extracts were determined spectrophotometrically using NanoDrop. DNA was stored at -80°C until PCR analyses.

PCR amplification

bla$_{TOHO}$, *bla*$_{SHV}$, *bla*$_{NDM}$ genes were detected by conventional PCR using the pairs of specific primers presented in Table1. The PCR was carried out in a 20 µL reaction mixture comprising 4 µL of the 5X FirepolMaster Mix; 0.5 µL of sense and antisense primer, 14 µL of PCR water and 1 µL of DNA extract from each strain**.** The amplification was carried outusing the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems , California, USA) according to the following program: a first denaturation step at 96°C for 5 min, followed by 30 cycles for *bla*$_{NDM}$ or 35 cycles for *bla*$_{TOHO}$and *bla*$_{SHV}$ each including denaturation at 96°C for 30 s for *bla*$_{NDM}$and 1 minute for *bla*$_{TOHO}$and *bla*$_{SHV}$, hybridization at 62°C for 30 s for *bla*$_{NDM}$, 50°C and 60°C for 1 min respectively for *bla*$_{TOHO}$and *bla*$_{SHV}$ and elongation at 72°C for 30 s for *bla*$_{NDM}$ or 1 mm for *bla*$_{TOHO}$and *bla*$_{SHV}$. Finally, a final elongation step was carried out at 72°C for 7 min for *bla*$_{NDM}$ or 10 min for *bla*$_{TOHO}$and *bla*$_{SHV}$.

Agarose gel electrophoresis

The DNA fragments amplified by PCR were separated by electrophoresis on a 1% agarose gel prepared in a 1X tris base - borate - EDTA solution containing 0.5 µg/mL of ethidium bromide. Migration was performed at 110 mV for 30 min. A 100 bp molecular weight marker was used to determine the size of amplicons visualized under UV light using the GeneFlash apparatus (Syngene, Bio-Imaging, UK).

Statistical analyzes

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

Results

Bacterial strains

A total of thirty-sevenstrains of Gram-negative bacilli resistant to at least one beta-lactam (AMC, CRO, CAZ, CTX, IMI, AT) were isolated during the study period. These were the species *E. coli* (59.46%, 22/37), *Klebsiella sp*(32.43%, 12/37), *Proteus sp*(5.41%, 2/37) and *Salmonella typhi* (2.70%, 1/37). The majority of bacterial species were isolated from urine samples (Figure 2).

Resistance of bacterial strains to antibiotics

The sensitivity test of the 37 enterobacteria isolates to the different antibiotics showed that all 37 strains (100%) were resistant to Amoxicillin + clavulanic acid. Resistance rates of 45.90% (17/37), 43.24% (16/37), 37.84% (14/37), 35.14% (13/37) and 24.32% (9/37) were observed respectively for Cefotaxime, Ceftazidime, Ceftriaxone, Aztreonam and Imipenem. Figure 3 shows a resistance phenotype of *E. coli* on a Petri dish while Table II presents the resistance profile of the different bacterial strains in the present study.

Molecular characterization of resistance genes

Conventional PCR gene detection of bla_{SHV}, bla_{NDM} and bla_{TOHO} (Figure 3) revealed that 62.16% (23/37) of the bacterial strains carried at least one of the three resistance genes. Among them, 60.87% (14/23) of the strains harbored the bla_{NDM} gene,56.52% (13/23) the $bla_{SHV\ gene}$ against 47.83% (11/23) of strains carrying the $bla_{TOHO\ gene.}$ The strains carrying these different resistance genes, namely *E. coli*, *Klebsiella sp*and *Proteus sp*, were mainly isolated from urine with 47.83% (11/23), 43.48% (10/23) and 43.48 % (10/23) respectively

for the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO}$ genes against 13.04% (3/23), 13.04% (3/23) and 4.35% (1/23) respectively, in strains isolated from stools. *Bla*$_{NDM}$ was the most detected gene while *E. coli* was the species mainly harboring the resistance genes.

The coexistence of resistance genes

PCR analysis also showed that 26.08% (6/23) of the strains, of which 83.33% (5/6) were isolated from urine, carried both the *bla*$_{SHV}$ and *bla*$_{TOHO}$ genes. These were 3 strains of *E. coli,* 2 strains of *Klebsiella sp*and 1 strain of *Proteus sp*. *Bla*$_{SHV}$ genesand *bla*$_{NDM}$ were simultaneously found in 30.43% (7/23) of the isolates, including 3 strains of *E. coli* 03 strains of *Klebsiella sp,* and 01strain of *Proteus sp,* mainly isolated (57.43; 4/7) from urine. As for the double carriage of *bla*genes $_{TOHO}$and *bla*$_{NDM,}$ it was observed in 17.39% (4/23) of the isolates including 02 strains of *E. coli*, 1 strain of *Klebsiella sp*and 1 strain of *Proteus sp*and 75% (3/4) of them isolated from urine.Furthermore, the simultaneous carriage of these three genes was recorded in 8.70% (2/23) of the strains including 1strain of *E. coli* isolated from urine and 1 strain of *Proteus sp*from stools.The coexistence of resistance genes depending on the bacterial species.

Discussion

Due to a worrying increase in bacterial resistance to antibiotics and the scarcity of new products on the market, the latter, which have saved so many humans lives, risk becoming ineffective [26].Bacteriathatused to cause mild infections have nowbecomemulti-resistant, increasing the risk of serious infections withtherapeutic limitations. The emergence of multidrug-resistantbacteria (MRB) is an urgent global concern[27] . In the present study we detected the *bla*$_{NDM,}$ *bla*$_{SHV}$ and *bla*$_{TOHO}$ genesin clinical strains of Gram-negative bacilli

isolated from urocultures or stool cultures at CERBA, Ouagadougou, Burkina Faso. Most of the resistant strains 78.38% (29/37) in the present study were isolated from urine like previous studies by our research team in Burkina Faso [19,20] and Togo [22 ,24] . The uropathogenic species were mainly *E. coli* (59.46%) and *Klebsiella sp*(32.43%). Urinary tract infections mainly affect women and uropathogenic *Escherichia coli* (UPEC) is one of the main etiological factors [28] . Indeed, it is estimated that 40% of women and 12% of men experience at least one episode of symptomatic UTI during their lifetime, and that 27 to 48% of affected women suffer from recurrent UTIs [29] . *Klebsiella sp*is also an important opportunistic pathogen of the urinary tract in debilitated individuals [30] . The production of urease allows the bacteria, which is one of the main causes of nosocomial enterobacterial infections, to survive the acidity of urine.

The results of the antibiogramdemonstratedthat the strainsanalyzed in thisstudyshowedsignificantresistance to the majority of beta-lactam antibioticstested. Indeed, all strains were resistant to amoxicillin, a semi-synthetic penicillin, in combination with clavulanic acid which is a β-lactamase inhibitor [31] .

Third generation cephalosporin (C3G) resistance rates of 45.9%, 43.24% and 37.84% were recorded for Cefotaxime, Ceftazidime, Ceftriaxone, respectively, in the present study. High resistance to C3G has also been reported in previous studies in Burkina Faso [19-21] , Togo [23, 24, 32] and Nigeria [33] . Drug pressure in hospitals, poor compliance with treatments as well as the abusive use of antibiotics sometimes without medical prescriptions constitute the main factors in the emergence and spread of multi-resistant pathogens [33] . In addition, acquired resistance has a great power of dissemination due to their plasmid determinism. The resistance rate to Aztreonam (monobactams) was 35.14% compared to 24.32% for Imipenem in the present study. A previous study reported a 77% resistance rate of enterobacteria to Aztreonam in Burkina Faso [21] while Aztreonam and Imipenem were 75% and 100%

effective against ESBL-producing enterobacteria isolated fromurine in Saint Louis in Senegal [34] . Bacteria use various resistance mechanisms such as inactivation of antibiotics by enzymes. The *bla*$_{NDM}$ gene(60.87%) was the most frequent in the present study, followed by the *bla*$_{SHV}$ gene(56.52%) and the *bla*$_{TOHO\ gene}$(47.83%). 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 threat to global health in light of their spread in many environmental and animal reservoirs in Asia and the Middle East [35,36]. SHV-type ß-lactamases, including SHV-1 and at least twenty-three variants, generally exhibit broad-spectrum activity against newer broad-spectrum cephalosporins. Their probable ancestor is a chromosomal penicillinase from *Klebsiella pneumoniae*. SHV enzymes belong to molecular class A of serine β-lactamases and share high functional and structural similarity with TEM b-lactamases [9] .

Bla$_{SHV}$ -1 has spread, via plasmids, to virtually all species of Enterobacteriaceae but is mainly found in *Klebsiella pneumoniae*. The *bla*$_{SHV}$ genewas identified in the genome of 17 isolates collected in the communes of Abomey-Calavi, Ouidah and Grand- popo in Benin [37] . TOHO-1 is an ESBL with effective activity not only against penicillins but also against 3rd generation cephalosporins [38] . TOHO-2 has high catalytic activity against cephalothin, cephaloridine, cefotaxime and piperacillin [39] . The coexistence of the *bla*$_{TOHO}$ and *bla*$_{SHV}$ genes was found in 26.08% of the strains in our study with 13.04% of *E. coli* and 8.70% of *Klebsiella sp*. Mètuor-Dabiré et *al* . [19] reported the coexistence of the *bla*$_{TOHO}$ and *bla*$_{BES}$ genes in *Escherichia coli* (34.4%) and *Klebsiella pneumoniae* (21.9%) at Saint Camille hospital in Ouagadougou. The coexistence of the *bla*$_{SHV}$ and *bla*$_{NDM}$ genes as well as *bla*$_{TOHO}$and *bla*$_{NDM}$ was demonstrated in 30.43% and 17.39% of the isolates in our study. A recent study in Egypt reported a high incidence of multidrug resistance with the emergence of the coexistence of *bla*$_{NDM-1}$ (70.0%) and *bla*$_{OXA-48\ (52.0\%)}$ genes $_{in}$ resistant *K. pneumoniae*

isolates. to carbapenems [40] . The coexistence of the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO}$ genes found in 02 (8.70%) strains in the present study is a factor aggravating antibiotic resistance. The coexistence of *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes has been reported in 6 ESBL-producing enterobacteria in Nigeria [41] . This suggests that these genes are carried by the bacterial chromosome and/or plasmids favoring rapid dissemination both vertically and horizontally towards bacteria of other species. Surveillance efforts as well as the development of new therapeutic solutions or combinations are necessary to maintain humanity's victory over pathogenic bacteria [42] .

Conclusion

The present study made it possible to highlight the *bla*$_{NDM}$, *bla*$_{SHV}$ and *bla*$_{TOHO\ genes}$ in clinical strains of ESBL-producing enterobacteria isolated mainly from *E. coli* uropathogenic. The simultaneous carriage of two or three resistance genes by certain strains suggests rapid spread of multi-resistance. Increased surveillance efforts through sequencing and the rapid development of new combinations or therapeutic solutions at the local level are necessary to prevent or combat multi-resistant bacteria, especially in the context of Burkina Faso.

Through this study, we will provide research perspectives:

- ✓ To carry out the sequencing of the genes found (TOHO, SHV and NDM) in order to complete the results obtained;
- ✓ To search for inhibitors based on local medicinal plants which can inhibit several enzymes found in the same bacterial strain at the same time.
- ✓ Identify the genetic support of these enzymes

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Table I: Sequence of primers for the $bla_{SHV,}$ bla_{TOHO} and bla_{NDM} genes

Genoa	Primers	Sequences (5'- 3')	Sizes	References
***Bla*$_{SHV}$**	Forward	ATG-CGT-TAT-ATT-CGC-CTG-TG	861 bp	(Pagani et al.,2003)
	Reverse	TTA-GCG-TTG-CCA-GTG-CTC		
***Bla*$_{\mathbf{TOHO}}$**	Forward	ATGTGCAGTACCAGTAA	584 bp	(Laurent et al.,1999)
	Reverse	TAGGTCACCAGAACCAG		
***Bla*$_{\mathbf{NDM}}$**	Forward	CCATGCGGGCCGTATGAGTGATT	763 bp	(McGann et al.,2012)

	Reverse	AAGCTGAGCACCGCATTAGCCG

Table II: Resistance profile of bacterial strains

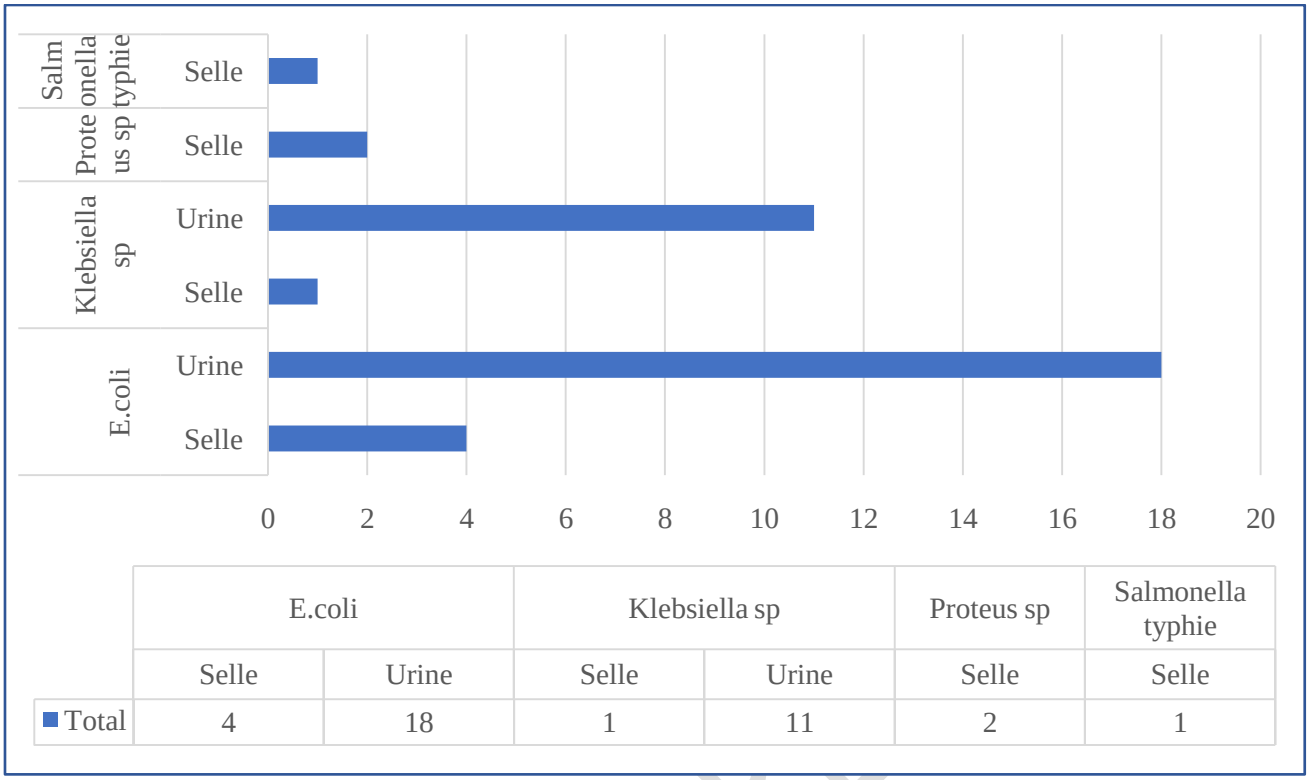
	Antibiotics					
Bacterial strains	**AMCn (%)**	**CAZ n (%)**	**CROn (%)**	**CTX n (%)**	**IMP (%)**	**AT n (%)**
E.coli	22 (100)	10 (45.45)	10 (45.45)	11 (50)	3 (13.63)	10 (45.45)
Klebsiella sp.	12 (100)	4 (33.33)	2 (16.66)	3 (25)	4 (33.33)	1 (8.33)
Protea sp.	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)
Salmonella typhi	1100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
Total	37 (100)	16 (43.24)	14 (37.84)	17 (45.9)	9 (24.32)	13 (35.14)

Table III: Distribution of genes according to bacterial strains

	Genoa		
Bacteria	***Bla*$_{NDM}$ n (%)**	***Bla*$_{SHV}$, n (%)**	***Bla*$_{TOHO}$ n (%)**
E.coli	7 (30.43)	6 (26.09)	7 (30.43)
Klebsiella sp	6 (26.09)	6 (26.09)	3 (13.04)
Proteus sp	1 (4.35)	1 (4.35)	1 (4.35)
Salmonella Typhi	0 (0)	0 (0)	0 (0)
Total	14 (60.87)	13 (56.52)	11 (47.83)

Table IV: Coexistence of genes depending on bacterial strains

Bacteria	**Genes**			
	***Bla*$_{SHV}$ and *bla*$_{TOHO}$ n (%)**	***Bla*$_{SHV}$ *and* *bla*$_{NDM}$ n (%)**	***Bla*$_{TOHO}$ and *bla*$_{NDM}$ n (%)**	***Bla* $_{SHV}$, *bla* $_{TOHO}$ and*bla* $_{NDM}$ n (%)**
E. coli	3 (13,04)	3 (13,04)	2 (8,70)	1 (4,35)
Klebsiella sp.	2 (8,70)	3 (13,04)	1 (4,35)	0 (0)
Proteus sp	1 (4,35)	1(4,35)	1 (4,35)	1(4,35)
Salmonella typhi	0 (0)	0 (0)	0 (0)	0 (0)
Total	6 (26.08)	7 (30.43)	4 (17.39)	2 (8.70)



	E.coli		Klebsiella sp		Proteus sp	Salmonella typhie
	Selle	Urine	Selle	Urine	Selle	Selle
Total	4	18	1	11	2	1

Figure 1: Distribution of bacterial strains according to biological samples

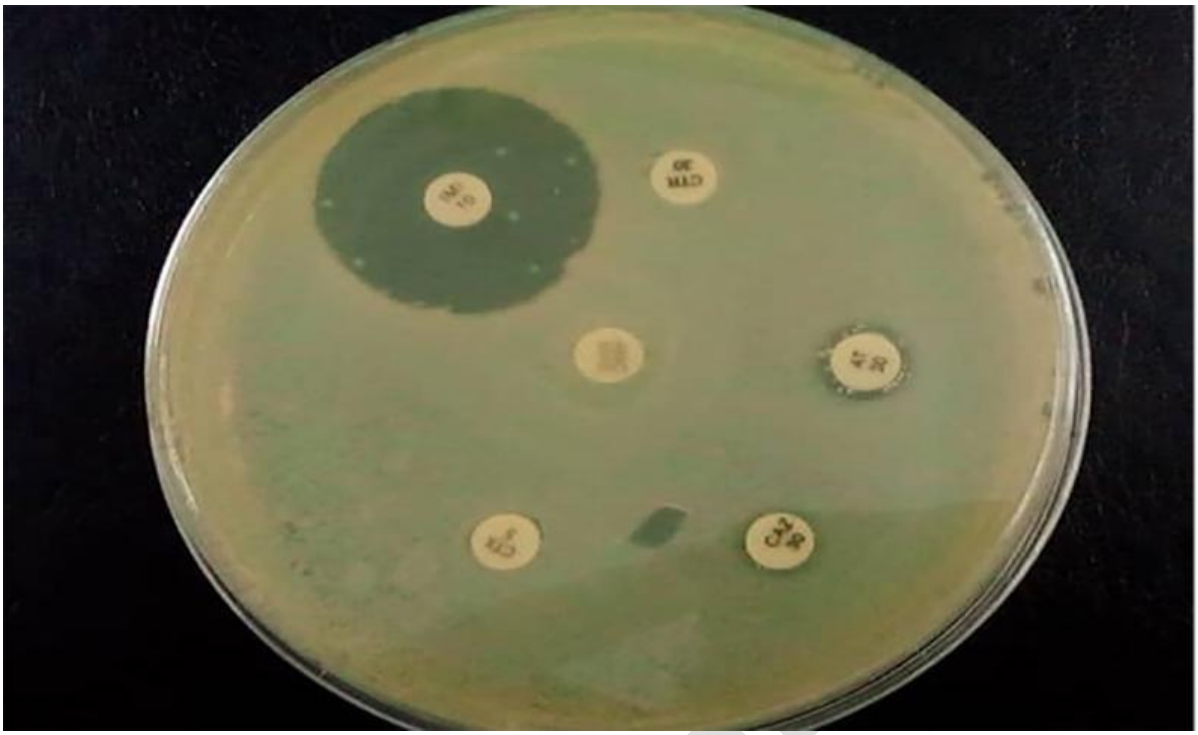


Figure 2: Resistance phenotype *with* antibiotics *E. coli* on petri dish

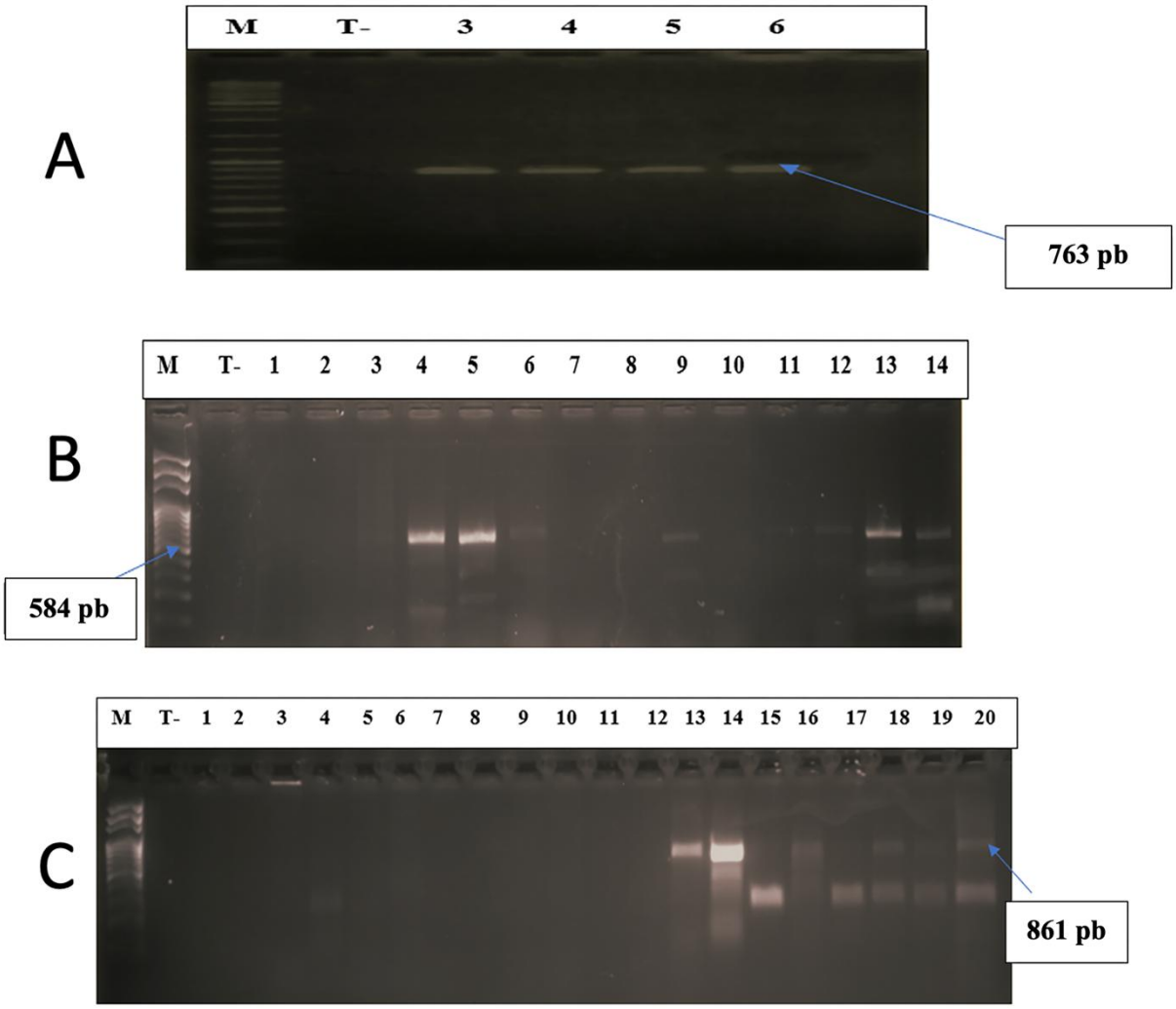


Figure 3: Electrophoretic profile of the different resistance genes.

The direction of migration is from top to bottom. M = 100 bp DNA Ladder; T-: Negative control. **A.** _ Samples 3 to 6 are positive for the *bla*$_{SHV}$ gene. **B.** _ Samples 3 - 6, 9 and 11 - 14 are positive for the *bla*$_{NDM}$ gene. **C.** _ Samples 13, 14, 16 and 18 - 20 are positive for the *bla*$_{TOHO}$ gene.