*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, namely 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* $_{TOHO\ genes}$ in 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 feces and urine of patients with internal and external CERBA 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% (23/09) of *Klebsiella sp* . and 4.35% (1/23) of Proteus sp. Among them, 60.87% (14/23) of the strains harbored the *bla* $_{Gene\ NDM}$ , 56.52% (13/23) *bla* $_{SHV}$ against 47.83% (23/11) of the strains carrying the *bla* $_{Gene\ TOHO.}$ The combinations of *bla* $_{SHV}$ + *bla* $_{NDM}$ , *bla* $_{SHV}$ + *bla* $_{TOHO}$ and *bla* $_{TOHO}$ + *bla* $_{NDM\ genes}$ were 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 the majority of these strains were isolated from urine cultures. This study revealed clinical ESBL-producing Enterobacteriaceae strains carrying the *bla* $_{NDM}$ , *bla* $_{SHV}$

and *bla* $_{\text{TOHO genes.}}$ The simultaneous carriage of two or three genes by certain strains suggests a spread that requires greater surveillance efforts and the rapid development of new therapeutic solutions.

**Keywords** : ESBL, antibiotic resistance, enterobacteria, *blah* $_{\text{SHV}}$ , *blah* $_{\text{TOHO}}$ , *blah* $_{\text{NDM}}$

**Introduction _**

Antibiotic resistance in bacteria constitutes a major challenge for medicine in the 21st century [1] . The increasing incidence of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBLSE) is the cause of serious infections and responsible for an increase in the prescription of broad-spectrum antibiotics [2 ] . Beta-lactamases are constitutional or bacterially acquired enzymes whose activity causes the opening of the beta-lactam ring and creates an unstable acyl-enzyme intermediate that is then degraded to an inactive acid [3, 4 ] .

The emergence and dissemination of new beta-lactamases, the first mechanism involved in the resistance of Gram-negative bacteria (GNB) to beta-lactams, are concomitant with the introduction into the therapeutic arsenal and the consumption of beta-lactams [5, 6 ] . In fact, the introduction of third-generation cephalosporins (C3G) into clinical practice at the beginning of the 1980s, allowing the 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 been described and their spread throughout the world represents 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 late 1990s, ESBL-producing Enterobacteriaceae were mainly so-called “hospital” species, spreading clonally among hospitalized patients [9] .

The beta-lactam family includes penicillins, cephalosporins, monobactams and carbapenems [10] . β-lactams constitute an important 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 hospitalization time, and increased hospitalization costs [6 ] .

The genes encoding ESBLs are diverse in nature and can be grouped into several families [9] . According to Ambler's 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 in its active site (NDM, IMP, VIM) [4, 9] . Although several class C carbapenemases have been described (ACT-1, CMY-2, CMY-10, CMY-19, CMY-37 and ADC-68), producing microorganisms generally show reduced sensitivity to carbapenems due to the low catalytic level. enzyme efficiency 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. , Ambler class D [15, 16] . The genes encoding these enzymes are carried by mobile genetic elements, explaining their significant dissemination [9, 11] .

carbapenemases are resistant to all β-lactams, including carbapenems, as well as to almost all other families of antibiotics used in the clinic (aminoglycosides, fluoroquinolones, sulfonamides, etc.), a multidrug resistance that severely limits therapeutic options [ 2 ] .

Previous studies by our research team have highlighted several ESBL-producing bacterial strains with multi-resistance in Burkina Faso [17-21] , Togo [22-24] and Niger [25] . In accordance with this work, the present study aims to detect the *coexistence* of the bla $_{NDM}$ , *bla* $_{SHV}$ and *bla* $_{TOHO\ genes}$ in clinical strains of gram-negative bacilli to provide information on their level of dissemination in the nosocomial environment.

**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) from August to December 2022. Bacterial culture and antibiogram were carried out at CERBA located in the Southeast zone . from 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 the Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso.

**Sampling**

The population of the present study consisted of bacteria isolated from diverse samples , including mainly feces and urine from patients from different departments. These were internal or external patients referred to CERBA by hospitals in the city of Ouagadougou.

**Isolation and identification of Gram-negative bacilli**

The isolation of bacteria was carried out using selective media. Urine and fecal samples were inoculated into common medium ( URI select, CLED, BCP, Hektoen, SS ) and then incubated for 24 hours at 37°C. Biochemical tests were performed on suspected colonies using Kligler Hajna medium, mannitol mobility, Simmons citrate medium, urea-indole, and peptone water.

The API 20E biochemical gallery (BioMérieux, France) 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 hours in Muller-Hinton medium and used for antibiogram and DNA extraction.

**Antibiotic susceptibility testing**

The Mueller-Hinton (MH) agar diffusion method was used to test the sensitivity of the strains to antibiotics in accordance with the recommendations of the Antibiograms Committee of the French Society of Microbiology (EUCAST/CA-SFM, 2021 ) . The bacterial noculum was prepared by placing a pure colony in 5 ml of physiological water. The suspension was then homogenized and calibrated to 0.5 McFarlane and then inoculated in narrow strips onto MH agar. The antibiotics were placed at a distance of approximately 20 mm from each other, at the rate of 4 disks per Petri dish. The different diameters of the inhibition zones obtained around the antibiotic disks were measured after 24 hours of incubation at 37°C and compared to the 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 synergy test**

Synergy testing allows the detection of broad-spectrum β-lactamases in a given strain. These enzymes can be demonstrated by the disc method, which consists of searching for an image called "champagne cork", which is a synergistic action between an antibiotic disc containing a β-lactamase inhibitor (Amoxicillin + Clavulanic Acid) and clavulanic acid of third generation. cephalosporin discs (Cefotaxime, Ceftazidime, Ceftriaxone) and Aztreonam (Jarlier et al., 1988) .

During the production of the antibiogram, the antibiotic discs were arranged to highlight the ESBL, seeking a synergy between clavulanic acid and third-generation cephalosporins and Aztéonam.

**Bacterial DNA extraction**

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 immersed in a water bath at 100°C for 15 minutes to release the bacteria's genetic material. Then, centrifugation for 10 min at 12,000 rpm; the supernatants containing the released DNA were transferred to a new Eppendorf tube. The quantity and purity of 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 specific primer pairs presented in Table 1. PCR was performed in a 20 µL reaction mixture comprising 4 µL of the 5X Firepol Master Mix ; 0.5 µL of sense and antisense primer, 14 µL of PCR water and 1 µL of DNA extract from each strain **.** Amplification was performed using the GeneAmp PCR System 9700 thermocycler (Applied Biosystems, California, USA) according to the following program: a first denaturation step at 96°C for 5 minutes, followed by 30 cycles for bla NDM or *35* $_{cycles}$ for *bla* $_{TOHO}$ and *bla* $_{SHV}$ each including denaturation at 96°C for 30 seconds for *bla* $_{NDM}$ and 1 minute for *bla* $_{TOHO}$ and *bla* $_{SHV}$ , hybridization at 62°C for 30 seconds for *bla* $_{NDM}$ , 50°C and 60°C for 1 min respectively for *bla* $_{TOHO}$ and *bla* $_{SHV}$ and stretching at 72°C for 30 seconds for *bla* $_{NDM}$ or 1 mm for *bla* $_{TOHO}$ and *bla* $_{SHV}$ . Finally, a final stretching step was performed at 72°C for 7 minutes for *bla* $_{NDM}$ or 10 minutes for *bla* $_{TOHO}$ and *bla* $_{SHV}$ .

**Agarose gel electrophoresis**

The DNA fragments amplified by PCR were separated by electrophoresis in 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 minutes. A 100 bp molecular weight marker was used to determine the size of amplicons visualized under UV light using the GeneFlash apparatus (Sygene, Bio-Imaging, UK).

**Statistical analyzes**

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

**Results _**

**Bacterial strains**

A total of thirty-seven (37) strains 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%, 37/12), *Proteus sp* (5.41%, 2/37) and *Salmonella typhi* (2.70 %, 1/37). Most bacterial species were isolated from urine samples (Figure 2).

**Resistance of bacterial strains to antibiotics**

The sensitivity test of the 37 enterobacteria isolates to different antibiotics showed that all 37 strains (100%) were resistant to Amoxicillin + clavulanic acid. Resistance rates of 45.9% (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* in 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 the strains carrying the *bla* $_{TOHO\ gene\ .}$ Strains carrying these different resistance genes, namely *E. coli* , *Klebsiella sp.* and *Proteus sp.* , were isolated mainly from urine with 47.83% (23/11), 43.48% (23/10) and 43.48% (23/10), respectively, for the *bla* $_{NDM}$ , *blah* $_{SHV}$ and *bla* $_{TOHO\ genes}$ against 13.04% (3/23), 13.04% (3/23) and 4.35% (1/23), respectively, in strains isolated from feces. *Blah* $_{NDM}$ was the most detected gene, while *E. coli* was the species that mainly harbored resistance genes (Table IV).

**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* $_{SHV}$ and *bla* $_{NDM}$ genes were found simultaneously in 30.43% (7/23) of the isolates, including 3 strains of *E* . *coli* , 03 strains of *Klebsiella sp* and 01 strain of *Proteus sp*, isolated mainly (57.43; 4/7) from urine . As for 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, simultaneous carriage of these three genes was recorded in 8.70% (2/23) of the strains including 1 strain of *E. coli* isolated from urine and 1 strain of *Proteus sp* from feces. Table IV shows the coexistence of resistance genes depending on the bacterial species.

**Discussion**

Due to the worrying increase in bacterial resistance to antibiotics and the scarcity of new products on the market, the latter, which have saved so many human lives, are at risk of becoming ineffective [26 ] . Thus, bacteria that previously caused mild infections have become multiresistant and can now cause serious infections with therapeutic failures. The emergence of multidrug-resistant bacteria (MRB) is now a worrying global phenomenon [27] . In the present study we detected the *bla* $_{\text{NDM,}}$ *bla* $_{\text{SHV}}$ and *bla* $_{\text{TOHO genes}}$ in clinical strains of Gram-negative bacilli isolated from urine cultures or stool cultures in 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 diseases. *Escherichia coli* (UPEC) is one of the main etiological factors [28] . In fact, 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.* it is also an important opportunistic urinary tract pathogen in debilitated individuals [30] . The production of urease allows the bacteria, which is a major cause of nosocomial enterobacterial infections, to survive the acidity of urine.

The antibiogram revealed that the strains in the present study were resistant to most β-lactam antibiotics. In fact, 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] . The pressure of medications in hospitals, poor adherence to

treatments, as well as the abusive use of antibiotics, sometimes without a medical prescription, are the main factors in the emergence and spread of multi-resistant pathogens [33 ] . Furthermore, acquired resistance has great dissemination power due to its 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 Enterobacteriaceae to Aztreonam in Burkina Faso [21] , while Aztreonam and Imipenem were 75% and 100% effective against ESBL-producing Enterobacteriaceae isolated from urine in Saint Louis, Senegal [34] . Bacteria use several resistance mechanisms, such as the inactivation of antibiotics by enzymes. The *bla* $_{\text{Gene NDM}}$ ( 60.87% ) was the most common in the present study, followed by the *bla* $_{\text{Gene SHV}}$ (56.52%) and the *bla* $_{\text{Gene TOHO}}$ (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 global health threat due to its spread in many environmental and animal reservoirs in Asia and the Middle East [35, 36] . . SHV-type beta-lactamases, including SHV-1 and at least twenty-three variants, generally exhibit broad-spectrum activity against newer broad-spectrum cephalosporins. Its likely ancestor is a chromosomal penicillinase from *Klebsiella pneumoniae* . SHV enzymes belong to the molecular class A of serine β-lactamases and share high functional and structural similarity with TEM b-lactamases [9] .

*Blah* $_{\text{SHV}}$ -1 has spread, via plasmids, to virtually all species of Enterobacteriaceae but is mainly found in *Klebsiella pneumoniae* . The *bla* $_{\text{SHV gene}}$ was identified in the genome of 17 isolates collected in the communes of Abomey-Calavi, Ouidah and Grandpopo 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 *bla* $_{\text{TOHO}}$ and bla $_{\text{SHV}}$ *genes* were found in 26.08% of the strains in our study with 13.04% of *E. coli* and 8.70% of

*Klebsiella sp* . Métuor-Dabiré et *al* . [19] reported the coexistence of *bla* $_{\text{TOHO}}$ and *bla* $_{\text{BES genes}}$ in *Escherichia coli* (34.4%) and *Klebsiella pneumoniae* (21.9%) at Saint Camille hospital in Ouagadougou. The coexistence of the *bla* $_{\text{SHV}}$ and bla $_{\text{NDM}}$ *genes* , as well as *bla* $_{\text{TOHO}}$ and *bla* $_{\text{NDM}}$ 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 coexistence of *bla* $_{\text{NDM-1}}$ (70.0%) and *bla* $_{\text{OXA-48 genes (52.0\%) in}}$ resistant isolates *of K. pneumoniae* . to carbapenems [40] . The coexistence of *bla* $_{\text{NDM}}$ , *bla* $_{\text{SHV}}$ and bla $_{\text{TOHO}}$ *genes* found in 02 (8.70%) strains in the present study is an aggravating factor of antibiotic resistance. The coexistence of *bla* $_{\text{CTX-M}}$ , *black* $_{\text{TEM}}$ and *bla* $_{\text{SHV genes}}$ has been reported in 6 ESBL-producing enterobacteria in Nigeria [41] . This suggests that these genes are transported by the bacterial chromosome and/or plasmids, favoring rapid dissemination both vertically and horizontally to bacteria of other species. Surveillance efforts as well as the development of new solutions or therapeutic combinations are necessary to maintain humanity's victory over pathogenic bacteria [42] .

**Conclusion**

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

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**Table I:** Sequence of primers for *bla* $_{SHV}$, *bla* $_{TOHO\ genes}$ and *bla* $_{NDM}$

| Genoa | Primers | Sequences (5'- 3') | Sizes | References |
|---|---|---|---|---|
| ***Blah*** $_{SHV}$ | Advance | ATG-CGT-TAT-ATT-CGC-CTG-TG | 875 bp | (Pagani et al., 2003) |
| | To reverse | TTA-GCG-TTG-CCA-GTG-CTC | | |
| ***Blah*** **TOHO** | Advance | ATGTGCAGTACCAGTAA | 876 bp | (Laurent et al. 1999) |
| | To reverse | TAGGTCACCAGAACCAG | | |
| | Advance | CCATGCGGGCCGTATGAGTGATT | 500 bp | (McGann et al., 2012) |

| | | |
|---|---|---|
| ***Blah*** **NDM** | To reverse | AAGCTGAGCACGCATTAGCCG |

| | Antibiotics | | | | | |
|---|---|---|---|---|---|---|
| **Bacterial strains** | **CAM n (%)** | **CAZ n (%)** | **CROn (%)** | **CTX n (%)** | **I'm in (%)** | **In 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 typhus*** | 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) |

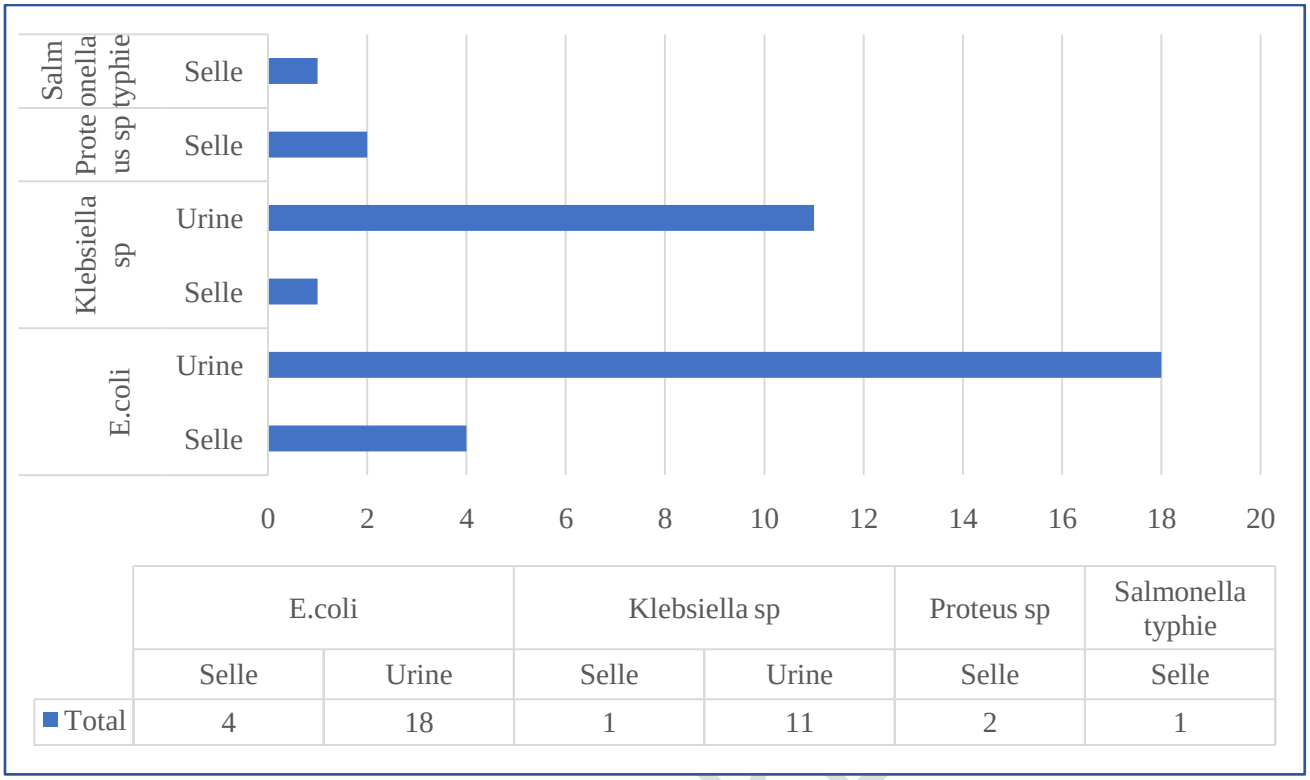
**Table II:** Resistance profile of bacterial strains

**Table III:** Distribution of genes according to bacterial strains

| | Genoa | | |
|---|---|---|---|
| **Bacteria** | ***Blah*** $_{\mathbf{NDM}}$ **n (%)** | ***Blah*** $_{\mathbf{SHV}}$ **, n (%)** | ***Blah*** $_{\mathbf{TOHO}}$ **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* | Genoa | | | |
|---|---|---|---|---|
| | ***Blah*** $_{SHV}$ **and** ***blah*** $_{TOHO}$ **n (%)** | ***Blah*** $_{SHV}$ ***and*** ***blah*** $_{NDM}$ **n (%)** | ***Blah*** $_{TOHO}$ **and** ***blah*** $_{NDM}$ **n (%)** | ***Blah*** $_{SHV}$ **,** ***blah*** $_{TOHO}$ **and** ***blah*** $_{NDM}$ **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 typhus*** | 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

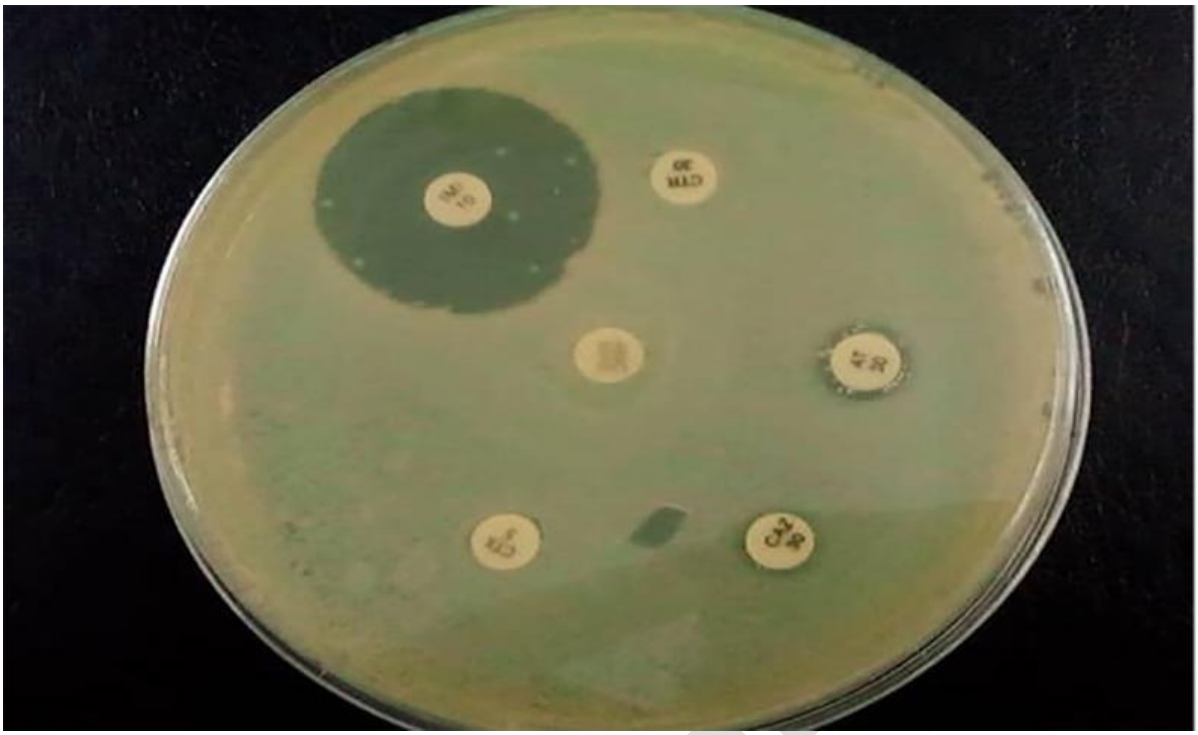


**Figure 2 :** *E. coli antibiotic* resistance phenotype in Petri dish

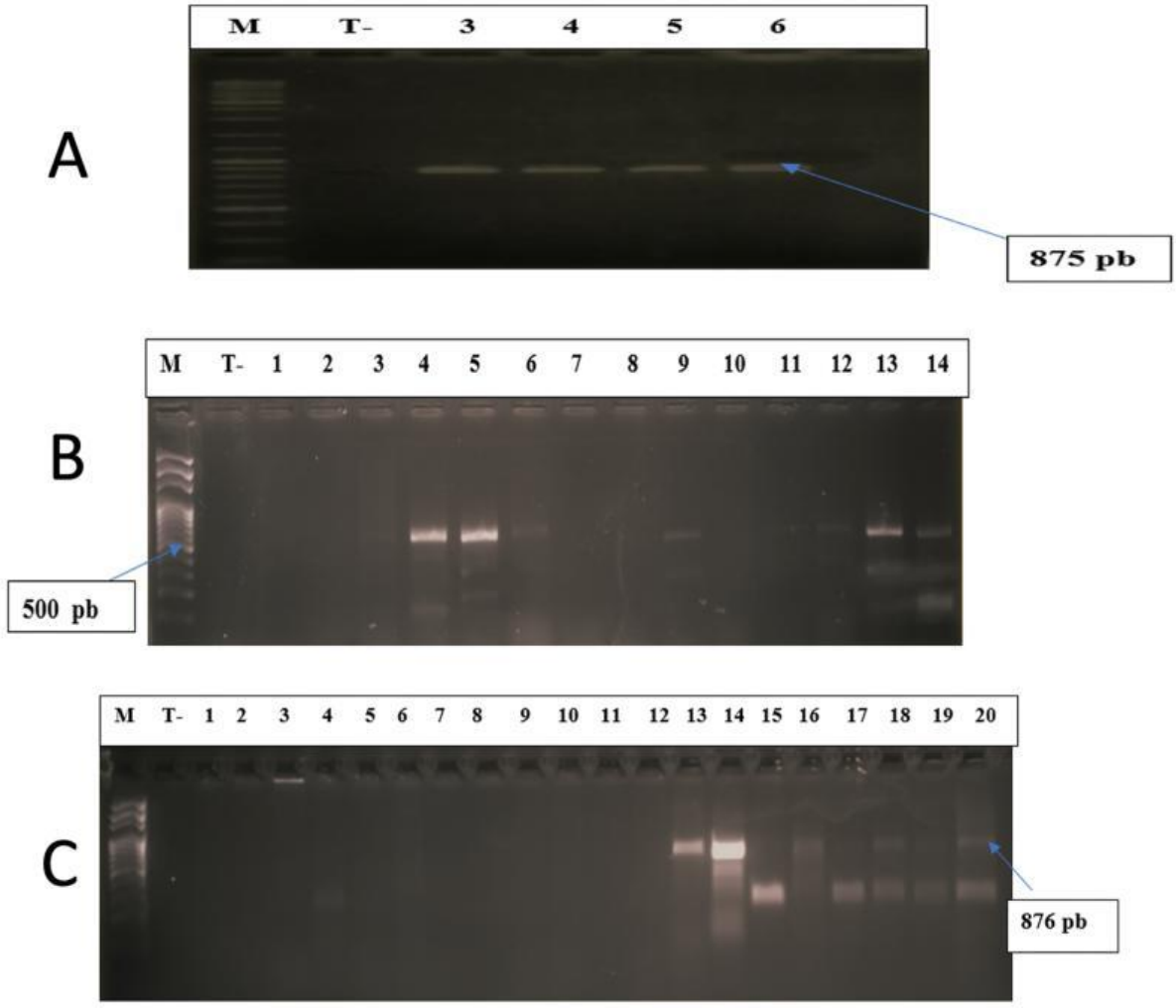



**Figure 3** : Electrophoretic profile of the different resistance genes.

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 $_{SHV}$ *blagene* . **B.** _ Samples 3 - 6, 9 and 11 - 14 are positive for the NDM *bla* $_{gene}$ . **C.** _ Samples 13, 14, 16 and 18 - 20 are positive for the $_{TOHO}$ *bla Gene* .