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Original Article

Detection of CTX-M and SHV Genes in Extended Spectrum Beta-Lactamase Producing Klebsiella Pneumoniae and Pseudomonas Aeruginosa in a Tertiary Hospital in North-central Nigeria

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Abstract

Background: Antimicrobial resistance (AMR) is an emerging threat to global health security. Globally, an estimated 700,000 deaths are attributed to AMR annually. Annual deaths due to AMR are projected to reach 10 million by 2050 if current trends persist. Extended Spectrum β-Lactamases (ESBLs) have the ability to hydrolyse penicillins, cephalosporins up to the third generation, and monobactams, but not β-lactamase inhibitors such as clavulanic acid. ESBLs undergo continuous mutation, leading to the development of new enzymes with over 400 different ESBL variants described. This study aimed to detect selected CTX-M genes, SHV,and TEM genes in Extended Spectrum Beta-Lactamase producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Jos, Nigeria.

Methodology: A total of 110, non-replicated isolates of *Klebsiella pneumonia* and 125 isolates of *Pseudomonas aeruginosa* were identified phenotypically from clinical specimens of patients at a tertiary hospital in Jos, North-central Nigeria. The isolates were screened for ESBL production using the disk diffusion method of the Clinical Laboratory Standard Institute (CLSI) breakpoints. Phenotypic confirmation of ESBL production was done using the double-disc synergy test. Multiplex PCR was used to detect ESBL genes.

Results: Fifty (45.5%) of the 110 isolates of *Klebsiella pneumoniae* and 9(7.2%) of the 125 isolates of *Pseudomonas aeruginosa* were ESBL-positive. Typing of 20 representative ESBL isolates (17 *Klebsiella* and 3 *Pseudomonas spp)* showed the presence ofblaCTX-M1, blaCTX-M9, and blaSHV genes in these isolates. All 20 (100%) isolates had the blaCTX-M1 gene. The blaSHV gene was detected in 16(80%) while CTX-M9 was detected in 6(30%) of the isolates studied.

Conclusion: The study showed that there is a high prevalence of ESBL genes among isolates of *Klebsiella pneumoniae* and *Pseudomonas* aeruginosa in North-central Nigeria. This emphasizes the need for continuous surveillance and coordinated infection prevention and control to curtail its spread.

Keywords: ESBL genes; Jos; *Klebsiella pneumoniae; Pseudomonas aeruginosa*.

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Quick Response Code:

Introduction

Beta-lactam antibiotics are commonly used to treat bacterial infections. The increased use of these agents has been associated with the emergence of beta-lactamase-mediated bacterial resistance, and subsequent development of extended-spectrum beta-lactamase (ESBL) producing bacteria.[1]The selective pressure exerted by the third-generation cephalosporins, especially in critical care centers leads to amino acid substitutions by plasmid-determined beta-lactamases. These amino acid substitutions result in the production of ESBLs that are capable of inactivating oxyimino-beta-lactam antibiotics.[2]

Both Gram-positive and Gram-negative organisms produce beta-lactamases, but it is a more common feature of the latter.[3] While the Gram-positive organisms release beta-lactamase into the extracellular space, the Gram-negative release it into the periplasmic space of the organism.[3]

ESBLs are a rapidly evolving group of beta-lactamases that can hydrolyze penicillins, cephalosporins up to the third generation, and monobactams but are inhibited by beta-lactamase inhibitors such as clavulanic acid.[4] ESBLs under continuous mutation, causing the development of new enzymes showing expanded substrate profiles. The first plasmid-mediated ESBL in Gram-negative bacteria was discovered in the early 1960s and was later detected in *Klebsiella* in Europe, Germany, and France in 1980, 1983, and 1985 respectively.[4]At present, more than 400 different ESBL variants have been described.[5] Temoniera (TEM) and Sulphydryl variables (SHV) were predominantly the major types, however, CTX-M (cefotaximase) type is increasingly becoming important.[4]

Beta-lactamase-mediated antibiotic resistance is the predominant mechanism for resistance to beta-lactam antibiotics in Gram-negative organisms. In recent years, antimicrobial resistance has become a significant human health issue.[5] Several organisms in both hospitals and communities thwart treatment because they are resistant to many antibiotics.[6] *Klebsiella pneumoniae* is widely reported as a common ESBL-producing organism and is an important causative agent of hospital-acquired infections.[7]*Pseudomonas aeruginosa* is notorious for multidrug resistance causing widespread infections and leading to substantial morbidity and mortality especially in immune-compromised patients.[8]

Antibiotic resistance varies according to geographic locations and is directly proportional to the use and misuse of antibiotics.[9]It is a major problem for health care delivery, particularly in developing countries where apart from a high level of poverty, ignorance, and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation coupled with poor hospital infection prevention and control measures.[10]This study aimed to identify the molecular subtypes of ESBL-producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in a tertiary hospital in Jos, North-central Nigeria.

Materials and Methods

Study Area

The study was conducted at a tertiary health institution with a bed capacity of 600 and a major referral center serving many states in North-central Nigeria.

Study Design

This was a prospective hospital based cross-sectional study.

Clinical Isolates

A total of 110 and 125 isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively, isolated from clinical specimens of patients between May 2016 and May 2017 were used for this study.

Sampling Method
A convenience sampling technique was employed in this study.

Screening Test for ESBL
The isolates were screened for ESBL production using the disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines. All isolates with reduced susceptibilities to ceftazidime (30 µg) disk (zone of inhibition ≤22mm), and ceftriaxone (30 µg) disk (zone inhibition ≤25 mm) were considered positive for ESBL production.[11]

Confirmatory Test for ESBL
A phenotypic confirmatory test for ESBL was conducted using the double-disc diffusion test (DDDT) according to the CLSI guidelines.[11]Briefly, a disc of ceftazidime (30 µg) and a disc of ceftazidime plus clavulanic acid combination (30/10µg) were placed 25mm apart from center to center on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37^0C for 24 hours. Any isolate that showed a ≥5 mm increase in zone diameter of growth inhibition for ceftazidime/clavulanic acid compared to the zone diameter of growth inhibition of ceftazidime alone was considered ESBL positive.[11]*Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative control respectively.

DNA Extraction from Isolates
This was done using Phenol-Chloroform Method based on Silva and Silva with little modifications.[12]Bacterial cell lysates were prepared from 0.5 mL of overnight cultures on Tryptic Soy Broth (TSB). After centrifugation at 12000*g* for 10 minutes, the bacterial pellets were washed with 500µL of Tris-hydrochloride-ethylene diamine tetra-acetic acid (EDTA) (TE) buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA) and centrifuged again. The pellets were resuspended in 200 µL of TE buffer and incubated at 37°C for one hour. Fifteen microliter of proteinase K, 20 mg/mL, was added and the suspension was incubated at 56°C for one hour. The suspension was then heated at 95°C for 15minutes to inactivate the proteinase K. An equal volume of phenol-chloroform was added, and the mixture was centrifuged at 12000*g* for 10 minutes. The supernatant was extracted with an equal volume of phenol-chloroform and then chloroform. The DNA in the supernatant was mixed with 2 volumes of 95% ethanol and stored overnight at -20°C. The mixture was then centrifuged at 12000g for 5minutes. The DNA pellet was washed with ice-cold 70% ethanol, recentrifuged, and dried by tube inversion. The DNA was suspended in 100µL of sterile TE, pH 7.5, quantified in a spectrophotometer (at 260 nm), and kept frozen at -20°C until used for PCR.[12]

Amplification of TEM, SHV, CTX-M1, CTX-M2, and CTX-M9 Genes
The Multiplex PCR was done to identify bla TEM, blaSHV, and blaCTX-M genes simultaneously.[13] The sequences of primers used for detection of the bla TEM gene were: 5′-TCGGGGAAATGTGCGCG-3′ as a forward primer and 5′-TGCTTAATCAGTGAGGCACC-3′ as a reverse primer. The two primers included a 950bp fragment. For the detection of the blaSHV gene, the sequences of primers used were 5′-TATCTCCCTGTTAGCCACC-3′ as a forward primer and 5′-GATTTGCTGATTTCGCTCGG-3′ as a reverse primer. The two primers included an 800bp fragment.

For the detection of the blaCTX-M1 gene, the sequences of primers used were 5′-AAAAATCACTGCGCCAGTTC-3′ as aforward primer and 5′-AGCTTATTCATCGCCACGTT-3′ as a reverse primer. The two primers included a 415bp fragment.

For the detection of the bla CTX-M2 gene, the sequences of primers used were 5′-CGACGCTACCCCTGCTATT-3′ as a forward primer and 5′-CCAGCGTCAGATTTTTCAGG-3′ as a reverse primer. The two primers included a 552bp fragment.

For the detection of the bla CTX-M9 gene, the sequences of primers used were 5′-CGACGCTACCCCTGCTATT-3′ as a forward primer and 5′-ATTGGAAAGCGTTCATCACC-3′ as a reverse primer. The two primers included a 205bp fragment.[14]

Amplification Reactions

The amplification reaction mixture consisted of 1µlof template DNA mixed with PCR mixture, 0.5µl of each primer of bla TEM, blaSHV, bla CTX-M1, CTX-M2, CTX-M9 genes, and 10.5µl of nuclease-free water. The conditions for PCR amplification were set according to the following thermal and cycling conditions starting with a pre-denaturation step at 94°C for 5 min followed by a denaturation step at 94ºC for 30seconds, then followed by 30 repeated cycles of denaturation at 94°C for 45 sec, annealing step at 55°C for 45 sec and extension step at 72°C for 60 seconds and finally one extension step at 72°C for 5 min.[15]

Gel Electrophoresis and Visualization under UV Lights by Transilluminator

The PCR products, standard molecular weight ladder, and negative control were loaded into the agarose gel and the electrodes connected appropriately and electrophoresis was allowed to run for one hour. This was followed by visualization of the DNA bands to detect specific amplified products under UV lights by transilluminator and comparing them with a standard molecular weight marker.[16]

Ethical Consideration

The ethical approval for this study was obtained from the Institution's Ethics Committee.

Data Analysis

The data entry and analysis were performed using Statistical Package for Social Sciences (SPSS) version 21 (IBM SPSS Inc, USA) and presented in tables and charts.

Results

This study included 235 isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* recovered from clinical specimens. The overall prevalence of ESBL among the clinical isolates was 25.1% (59/235). Out of the 110 isolates of *K. pneumoniae* studied, 50(45.5%) were ESBL positive while, 9(7.2%) of the 125 isolates of *P. aeruginosa*, were ESBL positive. This was statistically significant at p<0.0001 (Table 1) ($\chi 2 = 45.538$, $P < 0.0001$).

Out of the 59 ESBL producing isolates, 20 randomly selected isolates (17 *Klebsiella* and 3 *Pseudomonas*) were subjected to Multiplex-PCR to determine the possible resistant genes (bla TEM, blaSHV, and CTX-M) responsible for the production of ESBL (Figure 1 - Plate 1a and Figure 2 - Plate 1b). The isolates were found to harbor one or more resistant genes. The most prevalent gene was bla CTX-M1 present in all the 20 selected isolates followed by the blaSHVgene that was present in 16 isolates (15 isolates of *K. pneumoniae* and 1 isolate of *P. aeruginosa*). Seven of the isolates were positive for bla CTX-M1, bla CTX-M9, and blaSHV genes, while bla TEM and CTX-M2 were not detected in any of the selected isolates (Figure 3).
Table 1:

Table 1: Prevalence of ESBL producing *K. pneumoniae* and *P. aeruginosa* in a Tertiary Hospital in Jos North-Central Nigeria.

Organism	ESBL Producing (%)	Non-ESBL producing (%)	Total
Klebsiella pneumoniae	50(45.5)	60(54.5)	110
Pseudomonas aeruginosa	9(7.2)	116(92.8)	125
Total	**59(25.1)**	**176(74.9)**	**235**

$\chi 2 = 45.538$
P< 0.0001
df = 1

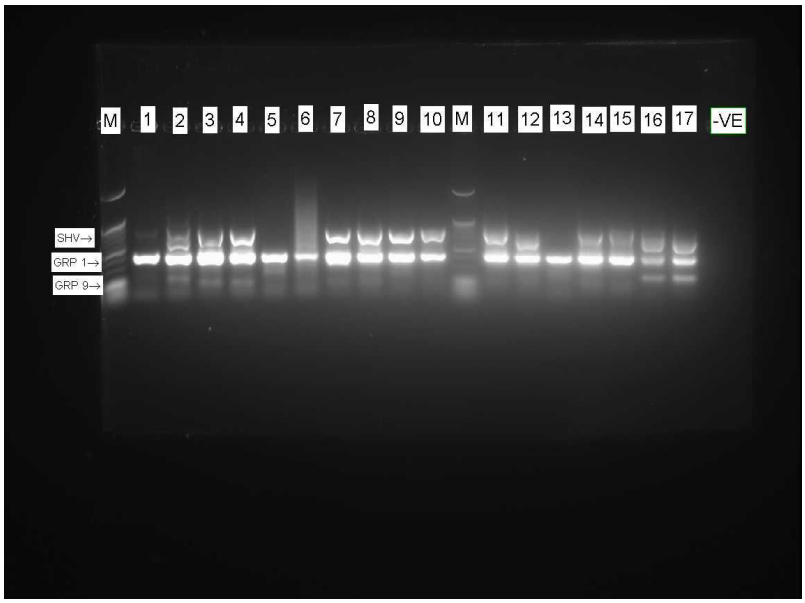

Figure 1: Plate 1a: The gel electrophoresis of the Multiplex PCR products shows amplification of SHV, CTX-M1, and CTX-M9 genes.

Key:
M = ladder which was 100 base pairs (100bp)
SHV = Lane 2, 3, 4, 7-12, and lane 14-20)
GRP 1 = CTX-M1 (Lane 1-17)
GRP 9 = CTX-M9 (Lane 2, 3, 4, 7, 8, 16, and lane 17)
-VE = Negative control

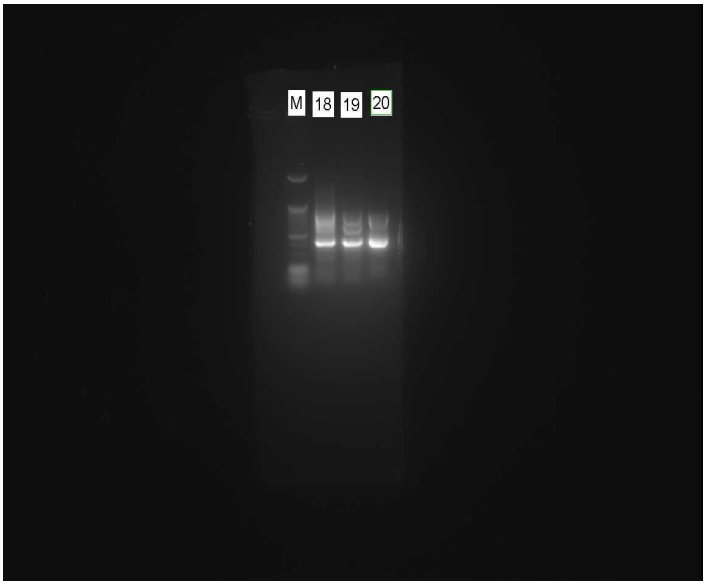

Figure 2: Plate 1b: The gel electrophoresis of the Multiplex PCR products shows amplification of SHV and CTX-M1 gene.

Key:
M = ladder which was 100 base pairs (100bp)
SHV = Lane 18, 19, and 20
GRP 1 = CTX-M1 (Lane 18, 19 and 20)

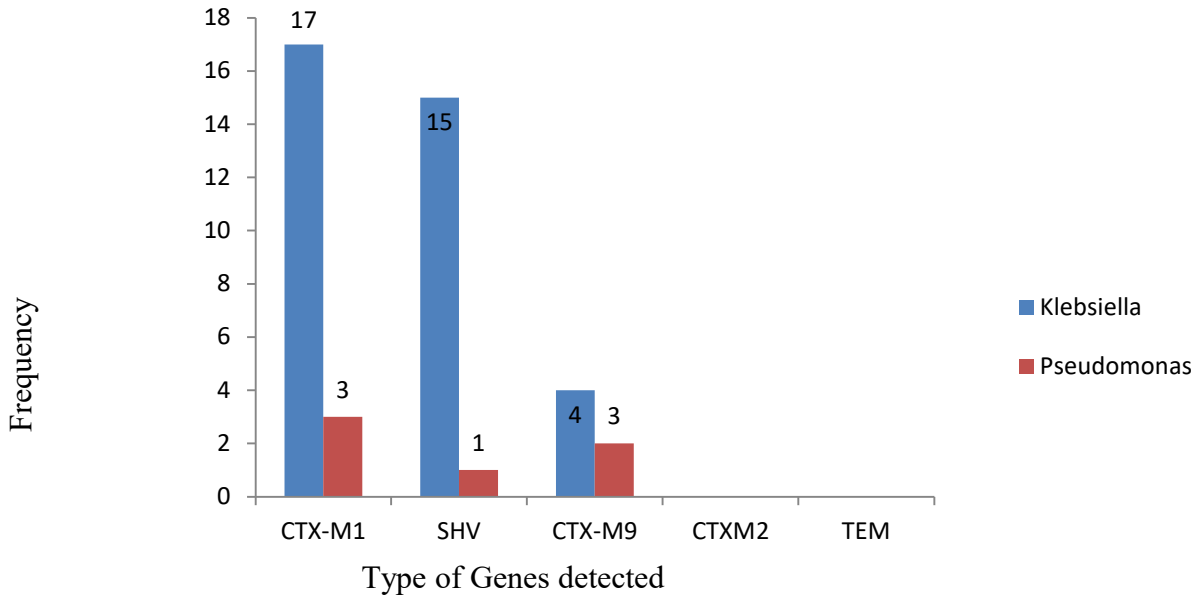

Figure 3: Frequency of ESBLgenes from isolates of *K. pneumoniae* and *P. aeruginosa* from a Tertiary Hospital in Jos, North-Central Nigeria.

Discussion

This study was conducted to detect selected CTX-M genes, SHV and TEM genes in Extended Spectrum β-Lactamase producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Jos, Nigeria. The prevalence of ESBL among the studied isolates was 25.1%. This was higher than the prevalence of 0.87% reported among *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in a recent study in Kaduna State, North-western, Nigeria.[17] In our study, it was observed that the prevalence of ESBL was higher in *Klebsiella pneumoniae* (45.5 %) than in *Pseudomonas aeruginosa* (7.2%) which was consistent with observations made by Chourasia and colleagues in a previous study.[18] However, a higher ESBL prevalence of 61.2% and 62.9% among *Klebsiella* isolates was reported in South-east and North-western Nigeria in 2009 and 2017 respectively.[7],[19] Similarly, previous reports from other studies across the world demonstrated a high prevalence of ESBL among clinical isolates.[20],[21],[22]

Although a general increase in the prevalence of ESBL-producing bacteria is being reported worldwide, there is a considerable variation in the specific patterns and rates across countries and geographical regions.[23],[24],[25] The variations could be due to the differences in the sources and number of the isolates studied. For instance, the study in Iran was a surveillance study where isolates were pooled from seven major teaching hospitals across the country, unlike this study that was conducted in one tertiary hospital.

The implication of the high prevalence of multidrug-resistant bacteria is enormous and of great public health concern especially as it regards healthcare delivery. Infections with these superbugs are associated with limited therapeutic options, appreciable cost of care, and prolonged hospital stay.[26]In addition to therapeutic challenges, multidrug-resistant pathogens also have a high potential for acquiring additional resistance and being widely disseminated within the hospital, posing a higher threat to the control of infections.[27]These organisms are associated with bloodstream infections, hospital-associated pneumonia, surgical site infections, and other nosocomial infections increasing intensive care unit (ICU) admissions, morbidity, and mortality.[28]

The most common resistant gene in this study was the CTX-M1 detected in all the isolates. Other resistant genes detected were CTX-M9, and SHV, while TEM and CTX-M2 were not detected. These findings were similar to a recently conducted research in Chad where CTX-M1 was reported as the most common resistant gene among ESBL-producing Enterobacteriaceae.[29] In contrast to this finding, a multi-center study from tertiary care hospitals in India reported TEM as the most common ESBL gene among clinical isolates of

Escherichia coli and *Klebsiella pneumoniae.*[30]In addition, a recent study in North-eastern Nigeria identified the SHV gene as the most prevalent.[14] This variation in the distribution of ESBL resistant genes across different geographical regions emphasizes the need for regular surveillance to guide the choice of antimicrobial therapy. These ESBL genes are plasmid-mediated and pose a serious risk for sharing of resistant genes among bacteria. Outbreaks of ESBL-producing organisms are a great challenge to contain as there are limited therapeutic options in most developing nations. Notably, poor practice of infection prevention and control (IPC) in addition to the lack of antibiotic stewardship within most health institutions could promote the occurrence and spread of resistant bacteria globally.[31]

The risk of the spread of resistant genes can be halted by using appropriate infectioncontrol interventions and antimicrobial stewardship programme.

Conclusion

There is a high prevalence of ESBL-producing *Klebsiella pneumoniae* with blaCTX-M1 as the most common type of circulating ESBL gene in Jos, North central Nigeria. This poses great difficulty to the effective treatment of infections caused by these multidrug-resistant organisms. A coordinated national policy on infection prevention and control with antimicrobial stewardship would reduce the spread and limit mutations in these bacterial species.

Limitation of the Study

Lack of positive control strains with the requisite ESBL genes for the multiplex PCR and inability to type all 59 phenotypically determined ESBL isolates.

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There was no financial support nor sponsorship for this research.

Conflicts of Interest

There are no conflicts of interest among the authors.

Reference

1. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. *Antimicrob Resist Infect Control*. 2019; **8**:39.
2. El-Masry EA, Melake NA, Taher IA. Phenotypic and molecular characterization of extended-spectrum β-lactamase producing klebsiella spp. From nosocomial infections in Egypt. *Int Med J.* 2019; **26**:376–80.
3. Alekshun MN, Levy SB. Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell*. 2007; **128**:1037–50.
4. Rawat D, Nair D. Extended-spectrum ß-lactamases in gram negative bacteria. *J Glob Infect Dis.* 2010; **2**:263–74.
5. Jacoby GA, Munoz-Price LS. The New β-Lactamases. *N Engl J Med*. 2005; **352**:380–91.
6. Ventola L. The Antibiotics Resistance Crisis. *Compr Biochem*. 2015; **40**:181–224.
7. Iroha I, Ezeifeke G, Amadi E, Umezurike C. Occurrence of ESBL producing Resistant E. coli and K. pneumoniae in clinical isolates and associated risks factors. *Res J Biol Sci*. 2009; **4**:588–92.
8. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. *Clin Microbiol Rev*. 2019; **32**:e00031-19.
9. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathog Glob Health.* 2015; **109**:309–18.
10. Sasirekha B. Prevalence of ESBL, AmpC B- lactamases and MRSA among uropathogens and its antibiogram. *EXCLI* J. 2013;**12**:81–8.

11. CLSI. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-ninth informational Suppl M100-S29. 2018; **32**.
12. da Silva ER, da Silva N. Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in southeastern Brazil. *Can J Vet Res*. 2005; **69**:260–4.
13. Mohamed Hamed Al-Agamy. Genetic basis of cefotaxime resistant isolates of *Klebsiella pneumoniae* from Cairo. *African J Microbiol Res.* 2012; **6**:20–7.
14. Yarima A, Haroun AA, Bulus T, Manga MM. Occurrence of Extended Spectrum Beta Lactamase Encoding Genes among Urinary Pathogenic *Escherichia coli* and *Klebsiella pneumoniae* Isolates Obtained from a Tertiary Hospital in Gombe Nigeria. *J Biosci Med.* 2020; **8**:42–55.
15. Xu L, Ensor V, Gossain S, Nye K, Hawkey P. Rapid and simple detection of blaCTX-M genes by multiplex PCR assay. *J Med Microbiol*. 2005; **54**:1183–7.
16. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp*. 2012; **62**:3923.
17. Ibtihaji K, Dadah A, Abdulfatai K. Prevalence of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*among women with urinary tract infections attending antenatal care in Kaduna, Nigeria. *Sci World J.* 2021; **16**:312–8.
18. Chourasia E, Singh KP, Kher SK. Extended Spectrum Beta Lactamases in clinical isolates of Gram-negative bacilli in Ajman, United Arab Emirates. *Gulf Med J.* 2015; **4**:14–21.
19. Ibrahim Y, Sani Y, Saleh Q, Saleh A, Hakeem G. Phenotypic Detection of Extended Spectrum Beta lactamase and Carbapenemase Co-producing Clinical Isolates from Two Tertiary Hospitals in Kano, North West Nigeria. *Ethiop J Health Sci.* 2017; **27**:3–10.
20. Vasaikar S, Obi L, Morobe I, Bisi-Johnson M. Molecular characteristics and antibiotic resistance profiles of *Klebsiella* isolates in Mthatha, Eastern Cape province, South Africa. *Int J Microbiol.* 2017; **5**:1–7.
21. Dalela G. Prevalence of extended spectrum beta lactamase (ESBL) producers among Gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. *J Clin Diagnostic Res*. 2012; **6**:182–7.
22. Poorabbas B, Mardaneh J, Rezaei Z, Kalani M, Pouladfar G, Alami MH, et al. Nosocomial infections: Multicenter surveillance of antimicrobial resistance profile of*Staphylococcus aureus* and Gram negative rods isolated from blood and other sterile body fluids in Iran. *Iran J Microbiol.* 2015; **7**:127–35.
23. Odumosu BT, Ajetunmobi O, Dada-Adegbola H, Odutayo I. Antibiotic susceptibility pattern and analysis of plasmid profiles of *Pseudomonas aeruginosa* from human, animal and plant sources. *Springerplus*. 2016; **5**:1381.
24. Ahmed OB, Omar AO, Asghar AH, Elhassan MM. Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella spp* urinary isolates from Sudan with confirmed ESBL phenotype. *Life Sci J*. 2013; **10**:191–5.
25. Begum S, Salam MA, Alam KF, Begum N, Hassan P, Haq JA. Detection of extended spectrum β-lactamase in *Pseudomonas* spp. isolated from two tertiary care hospitals in Bangladesh. *BMC Res Notes*. 2013; **6**:7.
26. Adegoke AA, Faleye AC, Singh G, Stenström TA. Antibiotic resistant superbugs: Assessment of the interrelationship of occurrence in clinical settings and environmental niches. *Molecules*. 2017; **22**:29.
27. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. *Infect Control Hosp Epidemiol.* 2016; 37:1288–301.
28. Cairns S, Reilly J, Booth M. Prevalence of healthcare-associated infection in Scottish intensive care units. *J Hosp Infect*. 2010; **76**:308–10.
29. Mahamat OO, Lounnas M, Hide M, Dumont Y, Tidjani A, Kamougam K, et al. High prevalence and characterization of Extended-spectrum ß-lactamase producing Enterobacteriaceae in Chadian hospitals. *BMC Infect Dis*. 2019;**19**:205.

30. Gautam V, Thakur A, Sharma M, Singh A, Bansal S, Sharma A, et al. Molecular characterization of extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and*Klebsiella pneumonia*e: A multi-centric study from tertiary care hospitals in India. *Indian J Med Res. 2019;* **149**:208–15.
31. Gilbert GL, Kerridge I. Hospital Infection Prevention and Control (IPC) and Antimicrobial Stewardship (AMS): Dual Strategies to Reduce Antibiotic Resistance (ABR) in Hospitals. 2020;**89**–108.