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Molecular Identification of Extended Spectrum Beta Lactamase Producing Organisms from Students in a University Community

Afunwa Ruth Asikiya[1*], Nwofia Martin Chukwunonso[1], Ugwu Precious Chisom[1], Gbadamosi Francis Ayodele[2], Ikegbune Chidozie[1], Ezebialu Chinenye Uzoamaka[3] and Ezemba Constance Chinyere[1]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria
[2]Department of Biological Sciences, Godfrey Okoye University, Enugu State, Nigeria
[3]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria

[*]**Corresponding author:** Afunwa Ruth Asikiya, Department of Pharmaceutical Microbiology and Biotechnology, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria, Tel: 2348027341073; E-mail: drruthafunwa@yahoo.com

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Abstract

Antibiotic therapeutic failures and the surge in resistance remains a global concern. Extended Spectrum Beta Lactamase (ESBL) producing bacteria are bacteria enzymes that hydrolyze and confer resistance to newest cephalosporin antibiotics. They constitute the major mechanism of resistance especially in developing countries. Random use of antibiotics by teens and young adults for prophylaxis and treatment of infections is on the rise due largely to the "antibiotic can cure it all" mindset. This study is aimed at determining the prevalence of ESBL producing bacteria among students at Chukwuemeka Odumegwu Ojukwu university, Igbariam, Anambra state, Nigeria. The study included seventy students of both genders between ages 18-24. Clean catch midstream urine samples were collected by non-repetitive random sampling method. Seventy five isolates from the Enterobacteriaceaes were identified. The isolates were cultured on MacConkey, Cetrimide and *Salmonella Shigella* agar and identified using standard biochemical tests. The isolates were exposed to multi-antibiotic discs; cephalexin, levofloxacin, ofloxacin, ampicillin, augmentin, gentamicin and co-trimoxazole and phenotypically tested as ESBL producing bacteria using the Double Disc Synergy Test (DDST). Molecular identification of these isolates was also determined using forward and reverse primers of SHV, CTX and BLAOXA. The seventy five isolates obtained include; (*Proteus* spp. 32 (43%), *E. coli* 28 (37%) and *Klebsiella* spp. 15 (20%). Results of antimicrobial susceptibility tests for the isolates on multi antibiotic gram negative disc were interpreted using the EUCAST 2021 standard breakpoints and 75% of the isolates showed resistance to the antibiotics used. An increase in inhibition zone diameters of ≥ 5 mm confirmed ESBL production among the isolates. The results of the molecular studies confirmed the presence of *BlaSHV* gene after PCR and a gene size of above 1000 bp. This study identified organisms within the Enterobacteriaceaes which are listed in the WHO global priority antibiotic resistant bacteria of critical concern. The incidence of ESBL producing bacteria among the study group in the University community may be as a result of low drug knowledge and uncontrolled use of antibiotics. Antimicrobial stewardship programs are required to correct the mindset and may also break the cycle of antibiotic abuse.

Keywords: Antibiotics; Enterobacteriaceae; Resistance; Extended Spectrum β-Lactamase (ESBLs)

Introduction

Escherichia coli, a member of the Enterobacteriaceae family, has been reported to be one of the most predominant organisms causing Urinary Tract Infections (UTIs) which are very common reasons for consultation and antibiotic prescription in current practice [1,2]. Massive and usually inappropriate use of antibiotics for treatment of UTIs generates a selective pressure that is followed by the rapid emergence and spread of multi-drug resistant bacterial strains. Nowadays, resistance of uropathogenic *E. coli* to many antibiotic classes is a very common finding in human medicine and is usually associated with increased medical costs, prolonged hospital stays and frequent therapeutic failure [3].

The production of Extended Spectrum β-Lactamases (ESBL) by urinary *E. coli* strains is a major public health concern in both hospital and community settings. These ESBL-producing strains represent a significant therapeutic challenge as they are resistant to all currently available β-lactam antibiotics but cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g. imipenem and ertapenem) [4].

Resistance to expanded-spectrum cephalosporins by ESBL production is mainly due to members of the TEM and SHV families of enzymes. The distribution of ESBLs has evolved to a predominance of CTX-M enzymes, mainly with *E. coli* as one of the major carriers of ESBL-encoding genes. Nowadays, the class-A ESBLs, TEM, SHV and CTX-M types, are the most widespread and clinically relevant [5].

Materials and Methods

Study design

The study was conducted in faculty of pharmaceutical sciences, Chukwuemeka Odumegwu Ojukwu university, Igbariam campus Anambra state from October 2021 to February 2022. Urine samples were obtained by simple random sampling method from students within the faculty. The study included 70 students of both genders between ages 18-24. The samples were collected using the clean catch midstream urine sampling technique.

Bacterial isolates

Urine samples were inoculated into 5 ml of nutrient broth and left for 24 hours. A loopful from the nutrient broth was cultured on three different selective agar; MacConkey agar, Cetrimide agar and *Salmonella Shigella* agar plates. The plates were incubated for 24 h at 37°C. Urine samples with positive cultures with a colony count ≥ 105 colony forming units per milliliter (CFU/mL) were selected. Out of 70 non repetitive samples included in the study, 75 isolates of Enterobacteriaceae were identified. Enterobacteriaceae isolates were identified by the standard biochemical tests including indole, oxidase test, catalase test and citrate utilization tests.

Antibiotic susceptibility testing

Disk difusion method was used for identification of antibiotic susceptibility of the Enterobacteriaceae isolates to different antibiotics according to EUCAST guidelines. The used discs were; ofloxacin (10 µg), pefloxacin (10 µg), ciprofloxacin (10 µg), amoxicillin-clavulanic acid (30 µg), gentamicin (10 µg), streptomycin (30 µg), cephalexin (10 µg), nalidixic acid (30 µg), co-trimoxazole (30 µg), ampicillin (30 µg). Resistance to three or more classes of antimicrobial agents is defined as Multiple Drug Resistance (MDR) [6].

Test for ESBL production

The isolates were tested for ESBL production using the Double Disc Synergy Test (DDST) method as previously established procedure [7]. Briefly; a combination disc (amoxicillin 20 µg and clavulanic acid 10 µg) was placed at the centre of the Petri dish and antibiotics (cefotaxime 30 µg and ceftriaxone 30 µg) were placed 15 mm apart on both sides of the plates. It was incubated at 37˚C for 24 hours after which the various inhibition zone diameters were measured. Positive result is identified when the zone of inhibition is extended towards AMC (20 µg/10 µg) disc 5 mm [8,9].

DNA extraction

Genomic DNA from the isolates was extracted using Zymo research quick-DNA mini prep plus Kit (D4068). 20 mg of the bacteria cells were re-suspended in 200 ul of molecular grade water in a micro centrifuge tube. 200 µ of bio fluid and 20 ul of proteinase K were added to the re suspended cells to enable lysing of the cells. The samples were vortexed for 10-15 seconds after which, they were incubated at 55˚C for 10 minutes. 420 ul of the genomic binding buffer was added to the digested samples in the tubes (containing the samples) after incubation. They were thoroughly mixed by vortexing for 15 seconds. The mixture was transferred to a Zymo-spinTM IIC-XLR column in a collection tube. After this, the tubes were placed in a micro centrifuge and centrifuged at 12,000 rpm for 1 minute. The flow through and collection tube from this process was discarded. 400 µl of DNA pre-wash buffer was added to the spin column in a new collection tube and centrifuged at 12,000 rpm for 1 min, then the flow through was discarded. 700 µl of g-DNA wash buffer was added to the spin column and centrifuged at 12,000 rpm for 1 minute, then, the flow through was discarded. Again, 200 µl of g-DNA wash buffer was added to the spin column and centrifuged at 12,000 rpm for 1 minute, then, the flow through and collection tubes were discarded. The spin column was transferred to a clean micro centrifuge tube. 50 µl of DNA Elusion buffer was added directly to the spin column matrix and incubated at room temperature for 5 minutes, then centrifuged at maximum speed for 1 minute. The resulting flow through is the DNA which was stored at 200˚C.

Polymerase chain reaction for the evaluation of ITS gene region in the bacteria samples: The primers were synthesized in a DNA synthesizer (applied bio systems, UK) at InqabaBiotec company, Pretoria (South Africa). PCR was carried out in a total volume of 25 µl containing 2.0 µl of genomic DNA, 12.5 µl of 1 × PCR master mix (New England Biolabs, New York, NY, USA), 1.0 µl each of forward and reverse primer (10 mM) and 8.5 µl of H_2O. The PCR protocols for BlaSHV and BlaCTX are as follows; 1 min denaturation (95˚C followed by 30 cycles of 96˚C for 30 s, 62˚C for 30 s, and 72˚C for 30 seconds and final extension of 72˚C for 10 min. Conditions were identical for both assays except the annealing temperatures of BlaCTX, which is 55˚C [10]. For BlaOXA, initial denaturation step at 96°C for 5 min, followed by 35 cycles consisting of denaturation at 96°C for 1 min, annealing temperature at 56°C, at 1 min, primer extension at 72°C for 1 min and final extension for 10 min. Electrophoresis of the PCR products was carried out for 30 min at 100 V on 1% (DNA samples) and 2% (for PCR amplicons) agarose gel.

Primer	Sequence	Gene(s)	Base pair	Annealing temperature	Reference
BlaSHV-	BlaSHV-F-GCGAAAGCCAGC TGTCGGGC	Antibiotic resistance gene	>1000 BP	62°C	Ayodele, et al.

	(forward primer) BlaSHV-R-GATTGGCGGCGC TGTTATCGC (revers e)				
BlaCTX	BlaCTX-F-GTGCAGTACCAGT AAAGTTATGG (forward) BlaCTX-R CGCAATATCATTG GTGGTGCC (reverse)	Antibiotic resistance gene	>1000 BP	55°C	Ayodele, et al. [19]
BlaOXA	BlaOXA'-F-GCGCGATCTGGTT CACTCG (forward) BlaOXA-R-AGTCGACAGTTGC GCCGGC (reverse)	Antibiotic resistance gene	>1000 BP	56°C	Iroha, et al. [20]

Results

Out of the seventy (70) samples collected, 32 (43%) isolates of *Proteus* spp., 28 (37%) isolates of *E. coli* and 15 (20%) *Kebsiella* spp. were isolated.

Klebsiella spp. showed percentage resistance of >60% from ofloxacin, ciprofloxacin, augmentin, perfloxacin and ceporex while ampicillin and co-trimoxazole has 7% and 53% respectively.

Proteus spp. showed percentage resistance of >60% to ofloxacin, pefloxacin, ciprofloxacin, augmentin, gentamicin, ceporex and ampicillin while co-trimoxazole recorded 38% resistance. *E. coli* showed >60% resistance to ofloxacin, pefloxacin, augmentin, gentamicin, ceporex and ampicillin while ciprofloxacin and co-trimoxazole showed 53% and 31%resistance (Tables 1-3).

Table 1: Percentage frequency of isolates.

Isolate	Frequency of isolate
Proteus spp.	43% (32)
E.coli	37% (28)
Klebsiella spp.	20% (15)
Total	100% (75)

Table 2: Antibiotics susceptibility test result (inhibition zone diameter measured in mm).

Probable organism	OFX	PEF	CPX	AU	CN	CEP	SXT	PN
Proteus spp.	0	0	0	0	0	0	0	0
E. coli	23	0	17	13	18	0	21	17
Proteus spp.	20	26	25	26	18	0	21	16
Proteus spp.	0	0	0	0	0	0	0	0
E. coli	15	14	17	15	16	14	20	11
E. coli	23	27	22	19	15	0	20	16

E. coli	20	16	24	15	16	0	17	16
Proteus spp.	24	21	28	25	27	20	24	16
Proteus spp.	12	0	0	0	0	0	0	0
Proteus spp.	33	31	24	16	28	16	26	0
Klebsiella spp.	26	20	22	22	26	20	22	20
Proteus spp.	26	24	22	20	23	20	24	20
Proteus spp.	30	30	28	16	20	0	16	0
E. coli	20	20	28	14	25	0	24	0
Proteus spp.	16	0	15	16	15	18	18	16
Klebsiella spp.	20	0	26	19	20	22	18	0
Proteus spp.	0	15	22	10	0	11	21	19
Proteus spp.	0	0	0	0	0	0	0	0
E. coli	26	22	26	10	30	20	28	0
E. coli	12	28	30	24	12	0	24	0
E. coli	16	20	22	26	20	16	0	0
E. coli	16	19	18	19	16	0	13	16
Proteus spp.	22	18	27	13	0	10	24	10
E. coli	16	22	20	0	0	0	18	0
E. coli	15	0	0	14	16	0	0	0
E. coli	0	0	0	0	0	0	0	0
Proteus spp.	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0
E. coli	15	18	0	15	0	0	22	0
Proteus spp.	20	17	25	19	25	0	28	0
Proteus spp.	20	19	22	22	25	0	19	0
E. coli	20	20	25	16	16	0	15	0
E. coli	0	0	0	0	0	0	0	0
Proteus spp.	25	16	16	16	18	0	25	0
E. coli	22	21	26	15	30	0	32	0

Klebsiella spp.	23	22	28	13	22	0	18	0
E. coli	19	21	28	19	16	0	18	0
E. coli	19	21	21	20	14	0	18	0
E. coli	20	16	29	20	21	0	25	0
Proteus spp.	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0
Proteus spp.	18	0	20	18	16	0	0	0
E. coli	19	18	27	19	23	0	16	0
E. coli	0	20	0	0	27	0	0	0
E. coli	19	25	20	0	14	0	12	14
Proteus spp.	19	16	18	12	10	0	20	12
E. coli	20	19	20	13	23	0	27	16
Klebsiella spp.	20	26	0	14	0	12	20	0
E. coli	0	15	16	14	13	0	21	16
Klebsiella spp.	19	25	18	22	20	0	18	0
Proteus spp.	30	30	18	20	0	28	30	0
Klebsiella spp.	31	18	20	18	0	12	32	16
Klebsiella spp.	26	20	26	15	30	20	24	0
Klebsiella spp.	28	25	26	20	26	0	20	23
Proteus spp.	17	15	13	0	0	0	16	17
E. coli	17	0	25	0	0	0	15	0
Proteus spp.	0	0	0	0	0	0	0	0
Proteus spp.	21	20	21	20	18	0	20	21
Proteus spp.	17	13	0	0	21	0	23	0
Proteus spp.	26	18	19	23	14	0	27	18
E. coli	0	0	25	14	16	0	32	15
Proteus spp.	0	16	0	14	0	0	0	14

Klebsiella spp.	14	18	10	0	0	0	18	0
Klebsiella spp.	17	16	19	14	20	0	21	15
Klebsiella spp.	15	18	20	0	15	0	22	16
Proteus spp.	0	0	10	13	0	0	21	13
Proteus spp.	0	0	15	0	0	0	0	0
Proteus spp.	0	0	0	0	16	0	0	0
Klebsiella spp.	0	17	0	0	0	0	0	0
Klebsiella spp.	22	25	20	21	20	0	25	21
Klebsiella spp.	0	16	15	0	8	0	17	21
Klebsiella spp.	0	0	13	0	0	0	18	0
Proteus spp.	0	16	18	20	0	0	21	15
Proteus spp.	12	14	0	0	12	0	12	0
Proteus spp.	12	10	15	12	13	0	22	15

Note: OFX–Ofloxacin; PEF–Levofloxacin; CPX–Ciprofloxacin; AU-Augmentin; CN–Gentamicin; CEP–Ceporex; SXT–Co-trimoxazole; PN–Ampicilin.

Table 3: Phenotypic confirmation of ESBL production using the Double Disc Synergy Test (DDST) Inhibition Zone Diametre (IZD).

Isolate	IZD of CR	IZD of CF+AMC	IZD of CF	IZD of CR+AMC	Remark
Klebsiella spp.	7	20	9	23	+
Klebsiella spp.	10	25	10	26	+
E. coli	9	25	11	27	+
Proteus spp.	3	0	4	0	-
Proteus spp.	0	0	0	0	-
Proteus spp.	12	26	11	21	+
E. coli	13	30	11	24	+
Proteus spp.	7	23	5	22	+
E. coli	4	0	1	0	-
E. coli	9	22	8	21	+
Proteus spp.	0	0	0	0	-

Proteus spp.	3	0	4	0	-
E. coli	12	0	11	0	-
E. coli	8	20	10	22	+
Proteus spp.	14	24	12	25	+
Klebsiella spp.	11	25	9	23	+
Proteus spp.	6	20	7	16	+
Proteus spp.	6	19	5	16	+
Proteus spp.	7	0	8	0	-
Proteus spp.	0	0	0	0	-
E. coli	12	0	0	0	-
E. coli	14	0	10	0	-
Klebsiella spp.	10	0	10	0	-
Klebsiella spp.	10	0	10	0	-
Klebsiella spp.	20	0	19	0	-
Proteus spp.	0	0	0	0	-
Klebsiella spp.	0	0	0	0	-
Proteus spp.	20	0	0	0	-
E. coli	20	0	17	0	-
Proteus spp.	19	0	14	0	-

Note: Positive (+) This means that the resistance is mediated *via* the beta-lactamse enzyme. Negative (-) This means that the resistance is not mediated *via* beta-lactamase enzyme. IZD-Inhibition Zone Diameter; CF-Cefotaxime; CR-Ceftriaxone; AMC-Augmentin (Amoxicillin-clavulanic acid)

Result of the phenotypic double disc synergy test is shown in Figure 1 below. The result showed positive for twelve (12) isolates. Of the twelve isoltes identified as ESBL producers, 5 (41.67%) were *Proteus* spp., 4 (33.33%) were *E. coli* and 3 (25%) were *Klebsiella* spp. (Figures 1 and 2).

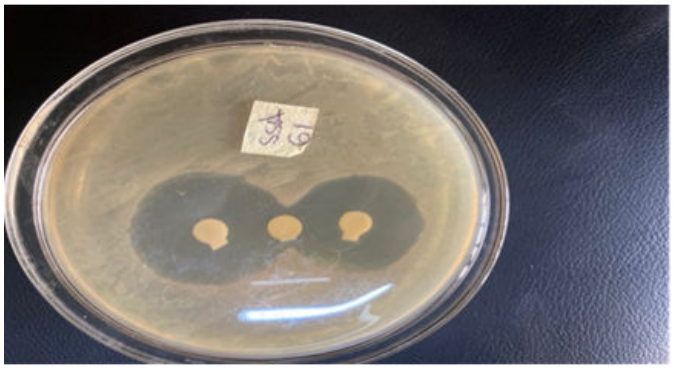

Figure 1: Phenotypic confirmation of ESBL production using double disc synergy test method.

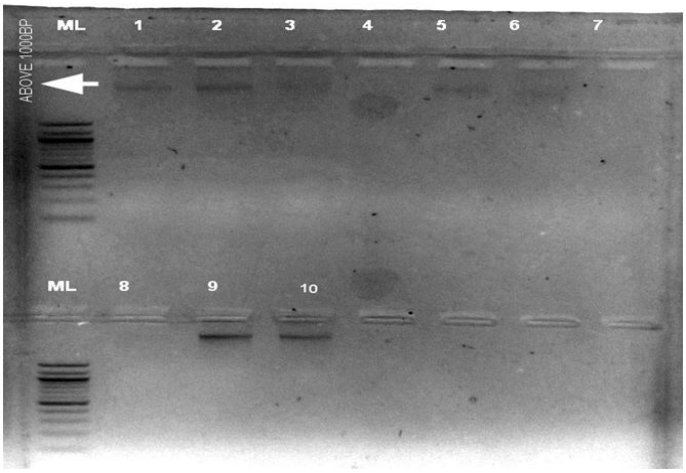

Figure 2: Gel Image showing the presence (+ve) of the *BlaSHV* gene after PCR. Gene is above 1000 bp in size.

Discussion

The amount of infections caused by Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-E) has increased exponentially around the world and this infections are associated with higher morbidity and mortality rates. Among various reasons, this problem has been linked with improper use of antibiotics during these years [11]. Enterobacteriaceae are the most common pathogens causing Urinary Tract Infections (UTIs). Increasing rates of antimicrobial resistance among Enterobacteriaceae strains decreases the option of empiric treatment of these infections [12].

Results of antimicrobial susceptibility test for the isolates respectively using the multi-antibiotic disc (gram-negative) were interpreted using the EUCAST 2021 standard breakpoints. For *E. coli*, our study indicated higher percentage resistance of isolates to floro quinolones. This is in contradiction with what was reported by Ugwu, et al., which recorded susceptibility of *E. coli* to fluoro quinolones (75.86%). This could be explained that *E. coli* around Igbariam axis is expressing higher resistance gene towards ciprofloxacin.

For *Proteus* spp., 84% of the isolates were resistant to cephalexin. Levofloxacin and ofloxacin had the same resistance pattern of 78% resistance. The isolates when tested against ciprofloxacin had 71% resistance, 16% intermediate and 13% susceptibility while ampicillin and Augmentin had 69% and 71% resistance respectively. Gentamicin had 69% resistance and 31% susceptibility while co-trimoxazole had 62% susceptibility and 38% resistance. Similar data regarding resistance against ampicillin and amoxicillin clavulanate were reported by Gonzalez, et al. and Pathak, et al. [4,13].

For *Klebsiella* spp., ofloxacin, ciprofloxacin and Augmentin showed 73% resistance while levofloxacin and cephalexin showed 67% resistance to the isolate. The isolate when tested against gentamicin 47% resistance and 53% susceptibility while ampicillin had 53% resistance and 47% susceptibility. Co-trimoxazole showed improved action against the isolate with 93% susceptibility and 7% resistance. Our data is consistent with those obtained by Pathek, et al., and Teklu, et al., showing high resistance of *Klebsiella* spp. to floro quinolones and augmentin [14].

Using the double disc synergy test as described by Rawat, et al., our findings revealed that out of 30 isolates tested, 40% were ESBL positive having a zone of inhibition of ≥ 5 mm. This is in agreement with studies performed by Tanko, et al., 50.8% and Abdelghani, et al., 46% [15]. Also, Afunwa, et al., reported that the community prevalence of ESBL in Southeastern Nigeria were placed at 4.4% which shows a wide margin from our findings. This can be attributed to the growing trend of inappropriate consumption of wide spectrum antibiotics over the years. Another interesting fact is that young people are habitual consumers of hamburgers/shawarma/pizza (pork, beef, and chicken), foods in which a high prevalence of these ESBL bacteria has been found [16]. Mandal, et al., and Nwankwo, et al., reported prevalence of 28.46% and 12.8% respectively. The variations may be due to the different sources of samples used in the studies [17].

The prevalence of ESBL in developing countries like Nigeria is higher than in developed countries because of the absence of antimicrobial stewardship program, indiscriminate use of antibiotics and lack of adequate antimicrobial resistance surveillance [18-20].

Conclusion

The findings from this study show a 40% prevalence of ESBL producing organism among tested students within the University. This can be as a result of uncontrolled use of antibiotics and poor level of enlightenment about antimicrobial stewardship programs. This study provides data to monitor the surge of ESBL producing isolates amongst the general population in order to reduce the economic impact, improve health and avoid deaths resulting from antibiotics resistance.

References

1. Adesoji AT, Ogunjobi AA (2016) Detection of extended spectrum beta-lactamases resistance genes among bacteria isolated from selected drinking water distribution channels in Southwestern Nigeria. Biomed Res Int 1-9
2. Afunwa RA, Esimone CO, Iroha RI, Odimegwu DC (2011) Antimicrobial resistance status and prevalence rates of extended spectrum beta lactamase producers isolated from a mixed human population. Bosn J Basic Med Sci 11:91-96
3. de Angelis G, del Giacomo P, Posteraro B, Sanguinetti M, Tumbarello M (2010) Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in Enterobacteriaceae. Int J Mol Sci 21:5090
4. Gonzalez D, Gallagher E, Zuniga T, Leiva J, Vitas AI (2019) Prevalence and characterization of β lactamase producing Enterobacteriaceae in healthy human carriers. Int Microbiol 23:171-177
5. Iroha IR, Mohammed ID, Moses IB, Ngwu NJ, Uzoeto HO, et al. (2022) Molecular characterization of enterobacteriacea isolated from gingivitis and periodontitis patients and the antimicrobial activity of mouth wash agents. Scientific African 15:e01106

6. Jacoby GA, Medeiros AA (1991) More extended-spectrum beta lactamases. Antimicrob Agents Chemother 35:1697-1704

7. Lagadinou M, Onisor MO, Rigas A, Musetescu DV, Gkentzi D, et al. (2020) Antimicrobial properties on non-antibiotic drugs in the era of increased bacterial resistance. Antibiotics 9:107

8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. (2012) Multidrug resistant, extensively drug resistant and drug-resistant bacteria: An international expert proposal for interim standard deffnitions for acquired resistance. Clin Microbiol Infect 18:268-281

9. Mandal DK, Sah SK, Mishra SK, Sharma S, Kattel HP, et al. (2020) Carriage of extended-spectrum-β-lactamase-and AmpC-β-lactamase-producing Enterobacteriaceae (ESBL-PE) in Healthy Community and Outpatient Department (OPD) patients in Nepal. Can J Infect Dis Med Microbiol 2020:5154217

10. Mohamed ES, Khairy RM, Abdelrahim SS (2020) Prevalence and molecular characteristics of ESBL and AmpC β-lactamase producing Enterobacteriaceae strains isolated from UTIs in Egypt. Antimicrob Resist Infect Control 9:1-9

11. Musa BM, Imam H, Lendel A, Abdulkadir I, Gumi HS, et al. The burden of extended-spectrum β-lactamase-producing Enterobacteriaceae in Nigeria: A systematic review and meta-analysis. Trans R Soc Trop Med Hyg 114:241-248

12. Nwankwo EO, Magaji NS, Tijjani J (2015) Antibiotic susceptibility pattern of Extended Spectrum Betalactamase (ESBL) producers and other bacterial pathogens in Kano, Nigeria. Trop J Pharm 14: 1273-1278

13. Pathak S, Sharma M, Srivastava P (2013) Gram negative Bacilli and further molecular *Escherichia coli* and *Klebsiella* spp. J Clin Diagnostic Res 7:21732177

14. Rosantia S, Higa T, Yagi N, Tokunaga T, Higa S, et al. (2020) Characterization of CTX-M-type-Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae isolated from Indonesian undergraduate medical students of a university in Surabaya, Indonesia. J Infect Chemother 26:575-581

15. Tanko N, Bolaji RO, Olayinka AT, Olayinka BO (2020) A systematic review on the prevalence of extended-spectrum beta lactamase-producing gram-negative bacteria in Nigeria. J Glob Antimicrob Resist 22:488-496

16. Brolund A, Sandegren L (2016) Characterization of ESBL disseminating plasmids. Infect Dis 48:18-25

17. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, et al. (2019) Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control 8:1-2

18. Ugwu MC, Shariff M, Nnajide CM, Beri K, Okezie UM, et al. (2020) Phenotypic and molecular characterization of β-lactamases among Enterobacterial uropathogens in Southeastern Nigeria. Can J Infect Dis Med Microbiol 2020:5843904

19. Adesoji AT, Ogunjobi AA (2016) Detection of extended spectrum beta-lactamases resistance genes among bacteria isolated from selected drinking water distribution channels in Southwestern Nigeria. BioMed Res Int 2016:9

20. Iroha IR, Mohammed ID, Moses IB, Ngwu NJ, Uzoeto HO, et al. (2022) Molecular characterization of Enterobacteriacea isolated from gingivitis and periodontitis patients and the antimicrobial activity of mouth wash agents. Sci Afr 15:e01106