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Molecular Detection of Extended-Spectrum Beta-Lactamase (ESβL)-Encoding Genes among Clinical Isolates of Enterobacteriaceae from Federal Teaching Hospital Gombe State Nigeria

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Abstract

Beta-lactamase inhibitors, like clavulanic acid, inhibit extended-spectrum beta-lactamases (ESβLs), which are enzymes that impart resistance to penicillins, first-, second-, and third-generation cephalosporins, and aztreonam. The ESβLs are commonly found in Gram-negative bacteria particularly members of *Enterobacteriaceae*. The wide distribution of ESβL among *Enterobacteriaceae* has been attributed to the continuous misuse of these antibiotics. Global outbreaks have been linked to the genes producing the ESβLs enzymes that provide resistance to third-generation cephalosporins. This study was conducted to determine the prevalence and detect ESβLs genes among *Enterobacteriaceae* isolates from Federal Teaching Hospital, Gombe. A total of 420 clinical isolates of *Enterobacteriaceae* were collected and identified by using Gram staining and biochemical tests. As advised by CLSI, the isolates were screened using the disk diffusion method to check for the formation of ESβLs. Suspected ESβL producers based on the screening test were subjected to confirmatory test using double-disk-synergy test (DDST), while the presence of genes coding for the ESβL production was determined by Polymerase Chain Reaction (PCR). The organisms displayed high resistance to Ceftazidime, almost 90%, followed by Cefpodoxime with 75% and Cefotaxime with 74%. Among the 377 resistant isolates, 95(25.2%) were confirmed positive for ESβLs production. *E. coli* was the most common ESβL producer with 35.7%, followed by *K. pneumoniae* with 24.2%, *K. oxytoca* with 14.7% and *Citrobacter* spp. with 10.5%. Based on PCR analysis, 4 (20%) isolates had one type of ESβL gene, 11(55%) had two types and 5 (25%) isolates had three types of the genes. The most detected ESβL genes were blaCTX-M and blaSHV with equal occurrence of 63.7%, followed by blaTEM with 59.1%. The increased ESβL occurrence in clinical isolates of *Enterobacteriaceae* in our locality creates significant therapeutic problems prompting an immediate need for the establishment of local guidelines for ESβL screening and strengthening Infection Prevention and Control (IPC) and Antimicrobial Stewardship (AMS) practices in FTH Gombe, as well as other laboratories in the State.

Keywords: PCR, antibiotics, Enterobacteriaceae, ESβL, and Gram-negative bacteria
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Introduction

All antibacterial drugs having a beta-lactam ring in their molecular structures are classified as beta-lactam antibiotics, a family of broad-spectrum antibiotics. Worldwide, these antibiotics are routinely administered to treat illnesses brought on by both Gram-positive and Gram-negative bacteria (Yarima et al., 2020). Based on the beta-lactam ring's chemical structure, they have been divided into six (6) major groups: beta-lactamase inhibitors, cephalosporins,

carbapenems, monobactams, and penicillins (Gharavi *et al.,* 2021). The beta-lactam antibiotics block the bacterial cell wall synthesis by interfering with the function of penicillin-binding-protein (PBP) (Gharavi et al*.,* 2021).

The beta-lactam antibiotics have been the main antibiotics for the treatment of infections caused by *Enterobacteriaceae*. But the advent of extended-spectrum beta-lactamases has essentially rendered them ineffective (Eltai et al*.,* 2018). One of the most frequent pathways leading to beta-lactam antibiotic resistance in Gram-negative bacteria is the synthesis of beta-lactamase (Lina et al*.,* 2014; Garba et al*.,* 2020). The Extended-spectrum beta-lactamases (ESβLs) are plasmid-encoded forms of beta-lactamases that confer resistance to Penicillins, narrow and extended-spectrum Cephalosprins and Aztreonam (Ghautam et al*.,* 2019), however, they are generally susceptible to beta-lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactam (Ur Rahman et al*.,* 2018). The ESβLs are commonly found in Gram-negative bacteria particularly members of *Enterobacteriaceae* (Rameshkumar et al*.,* 2016; Garba et al*.,* 2021). ESβL-producing *Enterobacteriaceae* are Gram-negative bacteria that have complex epidemiology. The two most common of these bacteria, *Escherichia coli* and *Klebsiella pneumoniae*, have the environment as one of their reservoirs (Chishimba et al*.,* 2016). The wide distribution of ESβLs among *Enterobacteriaceae* has been attributed to persistent exposure of the bacteria to a maltitude of beta-lactam antibiotics which leads to continuous production and mutation of the beta-lactamases and extending their resistance to newer produced beta-lactam antibiotics, thus producing the Extended-spectrum beta-lactamases (Shaik et al*.,* 2015).

ESβLs were first described in Germany in 1983 by Knothe, Shah, Krcmery, Antal, and Mitsuhashi (Knothe et al*.,* 1983; Ali et al*.,* 2019). The most common types of these enzymes are the Temoniera (*bla*TEM) and Cefotaxime-hydrolysing-Beta-lactamase (*bla*CTX-M) types (Bhasin et al*.,* 2020). However, most ESβLs are derived from sulfhydryl variable (SHV) and Temoniera (TEM)-1 through mutations (Ali et al*.,* 2019). Numerous research have examined the existence of TEM, SHV, Oxacillinase (OXA), and Cefotaximase Munich (CTX-M)-type enzymes (Lina et al*.,* 2014), and as at 2020, more than 400 distinct ESβLs have been identified (Yarima et al*.,* 2020). Numerous investigations have demonstrated that the most common ESβLs are CTX-M-type enzymes (Ramatla et al*.,* 2023).

It is predicted that diseases brought on by bacteria resistant to drugs cost the economy and healthcare system at least ₦1.29 trillion (€1.5 billion) annually (Yarima et al*.,* 2020). In Nigeria, extended spectrum Cephalosporins and Flouroquinolones are extensively used as broad-spectrum antibiotics and remain the drugs of choice for treatment of infections caused by various Gram-negative pathogens (Ogefere et al*.,* 2015). The problem of antibiotic-resistant bacteria is one that has spread throughout the world. This is widespread in hospitals mainly because of the massive and often inappropriate use of antimicrobials (Delgado et al*.,* 2016; Michael et al*.,* 2023a; Michael et al*.,* 2023b).

The genes encoding ESβL enzymes confer resistance to third generation cephalosporins such as ceftriaxone, cefotaxime and ceftazidime and have been noted to be the cause of outbreaks throughout the world (Livermore, 1995). So far, in Gombe State, Yarima et al*.,* (2020) conducted a research on the occurrence of ESβL encoding genes among *E. coli* and *K. pneumoniae* only where they reported the prevalence of 40% and 54.13% respectively. However, there were no reports on the molecular detection of ESβLs among other *Enterobacteriaceae* generally in Gombe State. Therefore, this study may provide more information on the epidemiology of ESBL-producing organisms for effective measures to tackle the risk of multidrug resistance.

Materials and Methods

Study area, Isolates Gathering and their Confirmation

The Federal Teaching Hospital in Gombe was the site of this investigation. A total of 420 clinical isolates of Enterobacteriaceae from diverse clinical specimens comprising Urine (n=221), stool (n=42) wound swab (n=79), high vaginal swab (n=28),

endocervical swab (n=15), sputum (n=13), blood (n=6), cerebrospinal fluid (n=4), semen (n=4), and pleural fluid (n=2) were collected and identified based on Cultural Characteristics, Gram staining and biochemical tests which include motility, Indole, citrate, urease and Kligler iron agar tests (Cheesbrough, 2006).

Standardization of Inoculum

A 0.5 McFarland standard was prepared according to Cheesbrough, (2006). Isolated colonies of the test organisms from overnight nutrient agar plates were transferred into test tubes containing 2 ml of sterile physiological saline and vortexed until turbidity of the suspension matched that of the 0.5 McFarland standard.

Screening Test for ESβL Production

Using the Kirby Bauer disk diffusion method and the Clinical Laboratory Standard Institute (CLSI) performance guideline for antibiotic susceptibility testing, the detected Enterobacteriaceae isolates were screened for the generation of ESβLs. Each standardized inoculum was streaked on Mueller Hinton agar plate using a sterile swab, by rotating the plate approximately 60° to ensure even distribution. The inoculated plates were allowed to stand for three to five minutes after which commercial antibiotic discs, Ceftazidime (30 µg), Cefotaxime (30 µg) and Cefpodoxime (10 µg) were placed on the surface of the agar plates, within a distance of about 10-15 mm away from the edge of the plates and 25 mm from disc to disc. The plates were incubated for 16–18 hours at 35°C. Zones of growth inhibition around the antibiotic discs were measured in mm. Those that were resistant to the indicator cephalosporins (cefotaxime, ceftazidime, cefpodoxime) were recorded as potential ESβL producers. In other words, those isolates that showed inhibition zone sizes of ≤ 27 mm for cefotaxime, ≤ 22 mm for ceftazidime and/or ≤ 17 mm for cefpodoxime, were considered as potential ESβL producers and used for confirmation test (CLSI, 2022).

Confirmation of ESβL Production

Isolates that were found positive for screening test were subjected to the Phenotypic Confirmatory Test (PCT) using Double Disc Synergy Test (DDST). Each isolate's standardized inoculum was swabbed onto the Mueller Hinton agar plate surface. A disc containing 20/10 µg of amoxicillin-clavulanate (AMC) was positioned in the middle of the plate. A disc containing 30 µg of ceftazidime and 30 µg of cefotaxime was positioned between 15 and 20 mm from the center of the amoxicillin-clavulanate. The plates were incubated for 16–18 hours at 35°C. Increase in the inhibition zone of each disc towards the disc of amoxicillin-clavulanate was considered positive for ESβL production. The characteristics dumb-bell shape, keynote appearance and distortion of inhibition zone specific to ESβL producers were also noted.

Preservation of Cultures

The bacterial cultures were preserved on nutrient agar slants at 4°C in a refrigerator and were used within two weeks. However, for long term preservation, cultures were stored in Muellar hinton broth with 20% glycerol at -80°C prior to molecular analysis.

Molecular Detection of Extended-Spectrum Beta-Lactamase-encoding Genes

Genomic DNA Extraction

The QIAamp extraction kit was used to extract the genomic DNA in accordance with the manufacturer's instructions. First, a 10-milliliter broth culture of the test organism was made over night. A 560 µl of prepared lysis buffer (AVL + RNA) was pipetted into 1.5 ml microcentrifuge tube containing 140 µl of the sample and vortexed for 15 seconds. After that, the mixture was incubated for ten minutes at room temperature. A 560 µl of absolute ethanol was then added to the sample and mixed by vortexing for 15 seconds and centrifuged briefly. After that, 630 µl of the solution was placed in a spin column and spun for one minute at 8000 rpm. A new collection tube was installed in lieu of the old one, together with the flow-through.This previous step was repeated using the remaining solution and the same spin column. Next, the column was put in a fresh collection tube and buffer AW1 (500 ml) was added. The mixture was then centrifuged at 8000 rpm for one minute. After adding 500 ml of buffer AW2, the mixture was centrifuged for

three minutes at 14000 rpm. Centrifugation was used to dry the spin column for one minute at 14000 rpm. After that, the spin column was put in a fresh 1.5 ml microcentrifuge tube, 60 µl of Buffer AVE was added, and it was left to incubate for a minute at room temperature. After centrifuging it for a minute at 8000 rpm, the column was disposed of. Until it was needed for PCR, the DNA was kept in a Freezer at -20^{0}C.

PCR Detection of Extended-spectrum Beta-lactamases Genes

PCR was used to detect β-lactamase genes (blaTEM, blaCTX-M and blaSHV) in a 12.5 ml reaction mixture made up of 1 µl genomic DNA, 1 µl of each primer (Table 1), 6.25 µl of Thermoscientific DreamTaq Green PCR master mix (2X), and 3.25 µl sterile distilled water. The conditions for amplification were as follows: five minutes of initial denaturation at 95°C, thirty seconds of denaturation at 95°C, thirty seconds of annealing at 60°C, two minutes of extension at 72°C, and ten minutes of final elongation at 72°C. All the three (3) genes were amplified using the same conditions except the annealing temperature of 55°C for blaTEM.

Table 1: Primers Sequences used for Detection of ESβL Genes

Gene	Primer sequence (5'-3')	Amplicon size	Reference
BlaTEM –F	5'(GAGACAATAACCCTGGTAAAT)3'	459bp	Hassan and Ibrahim, 2022
BlaTEM –R	5'(AGAAGTAAGTTGGCAGCAGTG)3'		
BlaSHV –F	5'(GTCAGCGAAAAACACCTTGCC)3'	383bp	,,
BlaSHV –R	5'(GTCTTATCGGCGATAAACCAG) 3'		
BlaCTX-M -F	5'(GAAGGTCATCAAGAAGGTGCG)3'	560bp	,,
BlaCTX-M –R	5'(GCATTGCCACGCTTTTCATAG) 3'		

Agarose Gel Electrophoresis

Agarose gel (1%, w/v) was prepared by dissolving 1g of agarose powder in 99 ml of 1X TAE buffer. The powder was fully dissolved after five minutes of microwave heating of the mixture. The solution was allowed to cool down to about 60^o C and 5 µl (0.5 µg/ml) ethidium bromide was added. The mixture was then poured into a gel casting tray to solidify with a gel comb inserted into the agarose gel to make wells into which the PCR products were loaded. Lastly, the gel-containing casting tray was put into a gel electrophoresis tank with the same TAE buffer concentration. An aliquot of 5 µl of the PCR products containing each of the detected ESBLs genes was loaded into the wells alongside 1 kb DNA ladder or molecular marker (BioLabs Quick-Load Purple DNA ladder). The electrophoresis was ran by using a voltage of 80 V and a current of 200 mA for 30 minutes and viewed using Flou-link UV transilluminator.

Statistical Analysis

The data was analyzed into frequencies and percentages using the IBM Statistical Package for Social Science (SPSS) version 25.0 software. Results were presented in form of Tables and Charts where appropriate.

Results

Distribution of *Enterobacteriaceae* According to Clinical Specimens

Table 2 shows distribution of *Enterobacteriaceae* amongst various clinical samples as reported in the preliminary work of this study (Saleh et al., 2024). It was observed that urine samples had the highest percentage of organisms with 53.4%. This was followed by wound swab with 19.1%, stool with 10.1%, and then high vaginal swab with 6.8%. However, cerebrospinal fluid (CSF), semen and pleural fluid had the least percentage of occurrence with 1.0%, 1.0% and 0.5%, respectively.

Table 2. Percentage Occurrence of *Enterobacteriaceae* Based on Clinical Specimen

	Clinical specimen										
Bacterial isolates	**U**	**St**	**WS**	**HVS**	**B**	**CS**	**ECS**	**Sp**	**Sn**	**PF**	**Total (%)**
E. coli	94	19	23	12	3	0	8	1	1	0	161 (38.9)
K. pneumoniae	26	1	17	6	1	3	1	8	3	1	67 (16.2)
K. oxytoca	37	2	12	3	1	0	3	3	0	0	61 (14.7)
Proteus spp.	11	2	10	4	0	0	1	0	0	0	28 (6.8)
Citrobacter spp.	14	5	6	2	0	0	0	0	0	0	27 (6.5)
Enterobacter spp.	14	1	3	1	1	0	0	1	0	0	21 (5.1)
S. marcescens	9	2	1	0	0	0	1	0	0	0	13 (3.1)
Providencia spp.	5	4	3	0	0	0	0	0	0	0	12 (2.9)
Y. enterocolitica	7	1	1	0	0	1	0	0	0	1	11 (2.7)
Shigella spp.	4	2	2	0	0	0	1	0	0	0	9 (2.2)
M. morganii	0	3	0	0	0	0	0	0	0	0	3 (0.7)
Salmonella spp.	0	0	1	0	0	0	0	0	0	0	1 (0.2)
Total (%)	**221 (53.4)**	**42 (10.1)**	**79 (1**	**28 (**	**6 (**	**4 (**	**15 (**	**13 (**	**4 (**	**2 (**	**414 (100.0)**

Keys: U=urine, St=stool, WS=wound swab, HVS=high vaginal swab, B=blood, CSF=cerebrospinal fluid, ECS=endocervical swab, SP=sputum, Sm=semen, PF=pleural fluid

***Enterobacteriaceae* Screened for Extended-spectrum Beta-lactamases Production**

The percentage of *Enterobacteriaceae* suspected to be ESβL producers based on screening results using Cefotaxime (30µg), Ceftazidime (30µg) and Cefpodoxime (10µg) antibiotics are shown in Table 3. Three hundred and seventy seven (377) isolates in all displayed resistance to at least one of these cephalosporins. The organisms displayed high resistance level to Ceftazidime, almost 90%, followed by Cefpodoxime with 75% and then Cefotaxime being the least with 74%. Plate I demonstrates a representative picture of the screening tests on suspected ESβL-producing isolates.

Table 3. Percentage of *Enterobacteriaceae* Screened for ESβLs Production

Bacteria	No. Screened	No. Positive (%)		
		Cefotaxime	**Ceftazidime**	**Cefpodoxime**
E. coli	163	122 (74.8)	141 (86.5)	122 (74.8)
K. pneumoniae	69	50 (72.5)	63 (91.3)	50 (72.5)
K. oxytoca	61	50 (82.0)	59 (96.7)	52 (85.2)
Proteus spp.	28	19 (67.9)	26 (92.9)	18 (64.3)
Salmonella spp.	2	2 (100)	2 (100)	1 (50.0)
Enterobacter spp.	22	14 (63.6)	19 (86.4)	14 (63.6)
Shigella spp.	9	5 (55.6)	7 (77.8)	6 (66.7)
S. marcescens	13	9 (69.2)	11 (84.6)	8 (61.5)
Providencia spp.	12	10 (83.3)	11 (91.7)	11 (91.7)
Citrobacter spp.	27	23 (85.2)	26 (96.3)	24 (88.9)
Y. enterocolitica	11	7 (63.6)	9 (81.8)	8 (72.7)
M. moganii	3	1 (33.3)	3 (100)	1 (33.3)
Total	**420**	**312 (74.3)**	**377 (89.8)**	**315 (75.0)**

Plate 1: ESβL**s** Screening test (disk diffusion method). The isolates showed resistance to third generation Cephalosporins (Cefotaxime, Ceftazidime and Cefpodoxime).

Phenotypically Confirmed *Enterobacteriaceae* for Extended-Spectrum Beta-Lactamases Production

The result of *Enterobacteriaceae* confirmed for ESβLs production is presented in Table 4. Ou t of 377 resistant isolates tested, 95 (25.2%) were phenotypically confirmed to be ESβL-producing *Enterobacteriaceae*. The most frequent ESβL-producing organism was *E. coli* with 35.7% followed by *K. pneumoniae* with 24.2%, *K. oxytoca* with 14.7% and then *Citrobacter* spp. with 10.5%. *Salmonella* spp., *Yersinia enterocolitica*, and *Morganella morganii* had the least percentage each having 1.1%, while there was no ESβL phenotypically detected in *Proteus* spp. and *Providencia* spp. Plate II shows a representative picture of the confirmatory tests using DDST.

Table 4. Percentage of *Enterobacteriaceae* Confirmed for ESβL Production

Bacterial isolates	Number tested	Number positive	Percentage (%)
E. coli	141	34	35.7
K. pneumoniae	63	23	24.2
K. oxytoca	59	14	14.7
Proteus spp.	28	0	0.0
Salmonella spp.	2	1	1.1
Enterobacter spp.	19	7	7.4
Shigella spp.	7	2	2.1
Serratia marcescens	11	2	2.1
Providencia spp.	11	0	0.0
Citrobacter spp.	26	10	10.5
Y. enterocolatica	9	1	1.1
Morganella moganii	3	1	1.1
Total	**377**	**95**	**100.0**

Plate 2: Double disk synergy test. Enhanced zone of inhibition towards Amoxicillin-clavulanate disc in the center confirmed the ESβL production.

Percentage of Extended-Spectrum Beta-lactamase Producers among *Enterobacteriaceae* in Clinical Specimens

Based on clinical specimen, urine had the highest ESβL producers with 50.0% as shown in Table 5. This was followed by wound swab with 23.3% and then stool with 11.1%. Blood, CSF and sputum had the least percentage with 1.1% while there was no ESβL-producing organism in Pleural fluid

Table 5: Occurrence of ESβL-producing *Enterobacteriaceae* in Clinical Specimen

	Clinical specimen / Occurrence									
Bacterial isolates	**U**	**St**	**WS**	**HVS**	**B**	**CSF**	**ECS**	**Sp**	**Sm**	**PF**
E. coli	17	6	3	2	1	0	3	0	1	0
K. pneumoniae	6	0	9	1	0	1	0	1	3	0
K. oxytoca	9	1	4	0	0	0	0	0	0	0
Proteus spp.	0	0	0	0	0	0	0	0	0	0
Salmonella spp.	0	0	0	0	0	0	0	0	0	0
Enterobacter spp.	3	1	1	1	0	0	1	0	0	0
Shigella spp.	1	0	1	0	0	1	0	0	0	0
S. marcescens	2	0	0	0	0	0	0	0	0	0
Providencia spp.	0	0	0	0	0	0	0	0	0	0
Citrobacter spp.	6	1	3	0	0	0	0	0	0	0
Y. enterocolitica	1	0	0	0	0	0	0	0	0	0
*M. mo*rganii	0	1	0	0	0	0	0	0	0	0
Total n (%)	**45 (50.0)**	**10 (11.1)**	**21 (23.3)**	**4 (4.4)**	**1 (1.1)**	**1 (1.1)**	**3 (3.3)**	**1 (1.1)**	**4 (4.4)**	**0 (0.0)**

Keys: **U**=urine, **St**=stool, **WS**=wound swab, **HVS**=high vaginal swab, **B**=blood, **CSF**=cerebrospinal fluid, **ECS**=endocervical swab, **SP**=sputum, **Sm**=semen, **PF**=pleural fluid

Molecular Detection of Extended-Spectrum Beta-lactamase-Encoding Genes

About 91% of the phenotypically detected ESβL-producing isolates were found to harbour one or more genes as observed following successful PCR amplification (Table 6). Out of 22 isolates of *Enterobacteriaceae*, 4(20%) isolates had only one (1) type of ESβLs encoding gene, 11(55%) had two (2) different types of genes and 5(25%) had three (3) types of genes. Specifically, among *E. coli* isolates, only two (2) had the genes in which one had the combination of CTX-M + TEM and the other one had SHV + TEM. In *K. pneumoniae* isolates, one (1) harboured single gene (blaSHV) and the others harboured multiple genes (SHV+TEM and CTX-M+SHV+TEM). Among *K. oxytoca*, all the three (3) isolates harboured double genes; two (2) had CTX-M +TEM, and one (1) had SHV+TEM. In *Enterobacter* spp., two (2) of the isolates had single genes (blaTEM or blaSHV) while the other one had all the three (3) genes. In *Citrobacter* spp. two (2) isolates had all the three (3) genes and the other one (1) had CTX-M + SHV, likewise CTX-M + SHV was detected from each of the two (2) isolates of *Serratia marcenscens*. CTX-M alone was harboured by one (1) of the *Shigella* spp. and the other one (1) had all the three (3) genes. *Salmonella* spp. had CTX-M + TEM while *Morganella morganii* had CTX-M + SHV. The different gel electrophoresis of genes detected are shown in Figures 1, 2 and 3.

Table 6: ESβL-Encoding Genes amongst *Enterobacteriaceae*

Bacteria	No. Tested	Detected genes						
		CTX-M	**TEM**	**SHV**	**C+ S**	**C+T**	**S+T**	**C+S+T**
E. coli	3	-	-	-	-	1	1	-
K. pneumoniae	3	-	-	1	-	-	1	1
Klebsiella oxytoca	3	-	-	-	-	2	1	-
Salmonella spp.	1	-	-	-	-	1	-	-
Enterobacter spp.	3	-	1	1	-	-	-	1
Shigella spp.	2	1	-	-	-	-	-	1
S. marcescens	2	-	-	-	2	-	-	-
Citrobacter spp.	3	-	-	-	1	-	-	2
Y. enterocolitica	1	-	-	-	-	-	-	-
M. morganii	1	-	-	-	1	-	-	-
Total (%)	**22**	**1 (5)**	**1 (5)**	**2 (10)**	**4 (20)**	**4 (20)**	**3 (15)**	**5 (25)**

Key: C=CTX-M, S=SHV, T=TEM

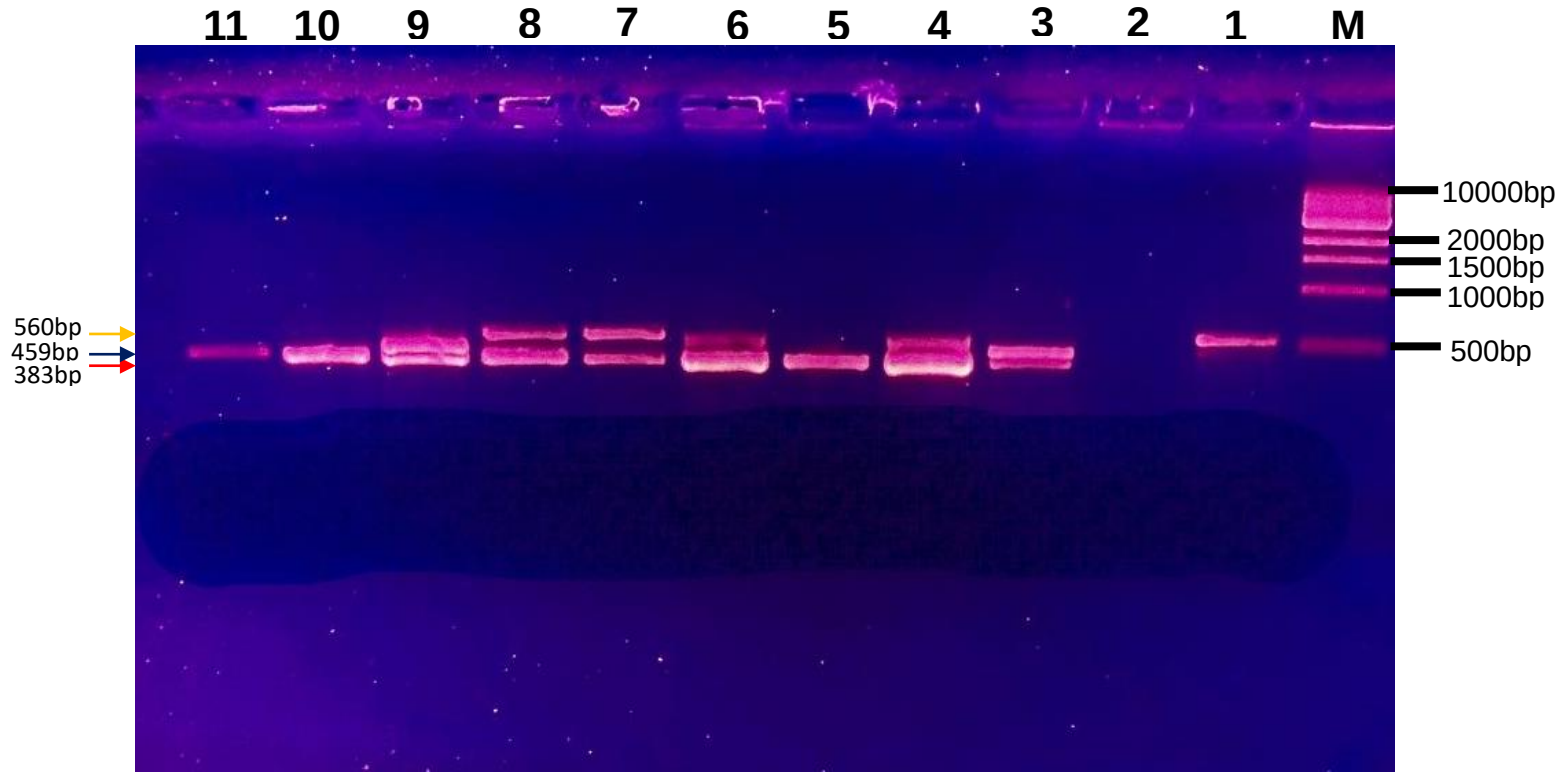

Figure 1: PCR products of blaCTX-M, blaSHV and blaTEM genes amplified from *Enterobactericeae*. M: 1Kb DNA Marker, Lanes 1,2 and 3; *E. coli*, Lanes 4, 5 and 6; *K. pneumoniae*, Lanes 7, 8 and 9; *K. oxytoca*, Lanes 10 and 11; *Enterobacter* spp. The blaCTX-M gene (560 bp), blaTEM gene (459 bp), and blaSHV gene (383 bp) are indicated by arrow heads.

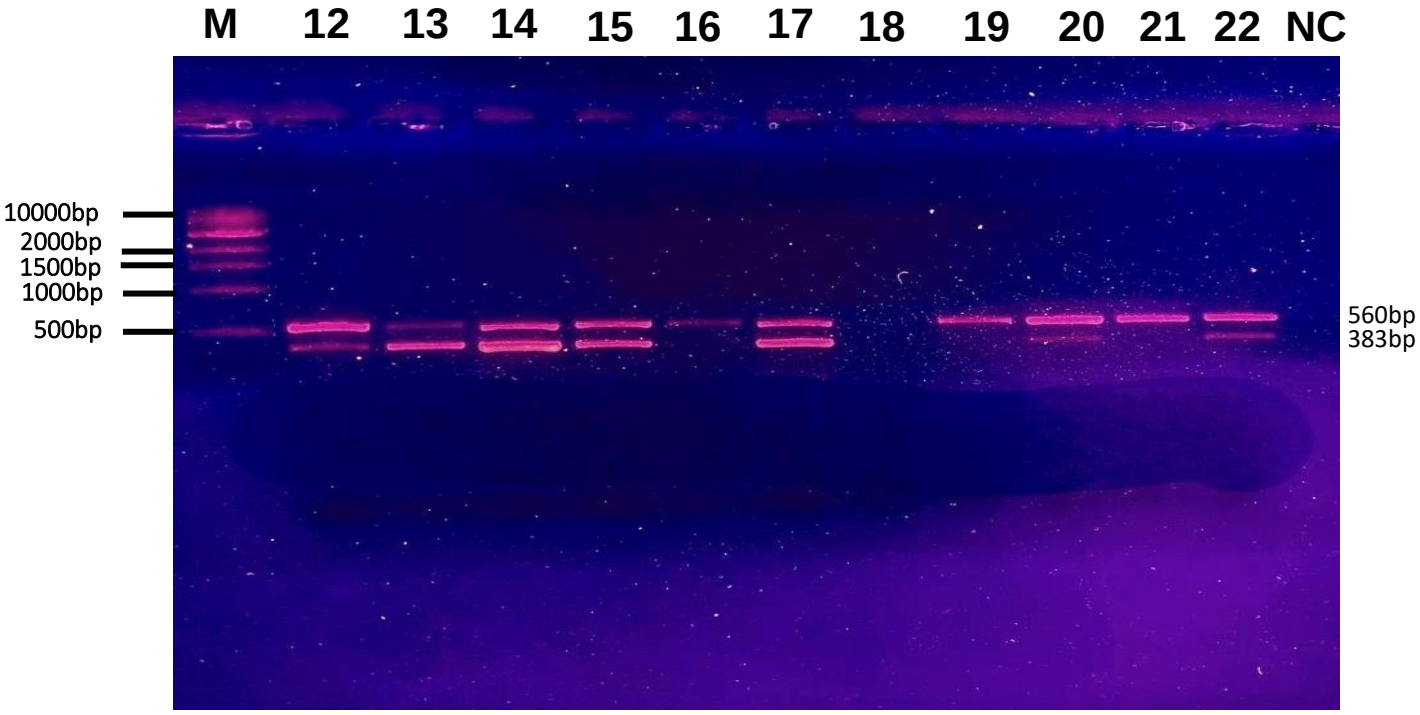

Figure 2: PCR products of blaCTX-M and blaSHV genes amplified from *Enterobactericeae*. M: 1Kb DNA Marker, Lane 12; *Enterobacter* spp., Lanes 13, 14 and 15; *Citrobacter* spp., Lanes 16 and 17; *Serratia marcescens*, Lane 18; *Yersinia enterocolitica*, Lanes 19 and 20 *Shigella* spp., Lane 21; *Salmonella* spp., Lane 22; *Morganella morganii* NC; Negative control. The CTX-M gene (560 bp), and SHV gene (383 bp) are indicated on the right

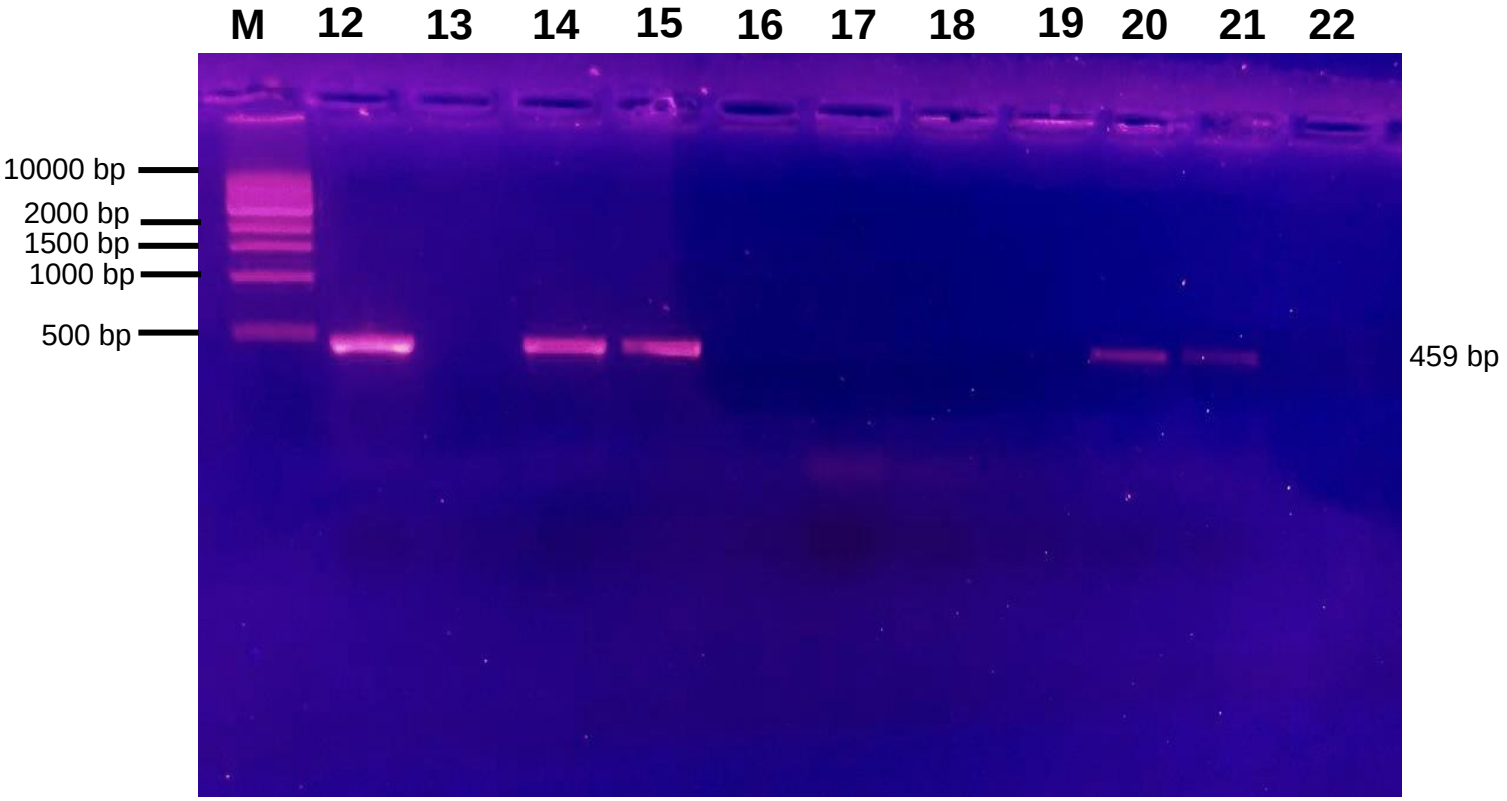

Figure 3*:* PCR products of blaTEM gene (459 bp) amplified from *Enterobactericeae*. M: 1Kb DNA Marker, Lane 12; *Enterobacter* spp., Lanes 13, 14 and 15; *Citrobacter* spp., Lanes 16 and 17; *Serratia marscescens*, Lane 18; *Yersinia enterocolitica*, Lanes 19 and 20; *Shigella* spp., Lane 21; *Salmonella* spp., Lane 22; *Morganella morganii*.

Percentage Occurrence of Extended-spectrum Beta-lactamase Genes Detected from *Enterobacteriaceae*

The percentage occurrence of ESβL genes from *Enterobacteriaceae* is shown on Figure 4. The combination of all the three (3) genes (CTX-M + SHV + TEM) was the most prevalent (25%), followed by combination of two genes (CTX-M + TEM) and (CTX-M + SHV) each corresponding to 20% and then (SHV + TEM) with only 15%. The least percentage occurrence was seen with CTX-M and TEM each having 5% prevalence rate.

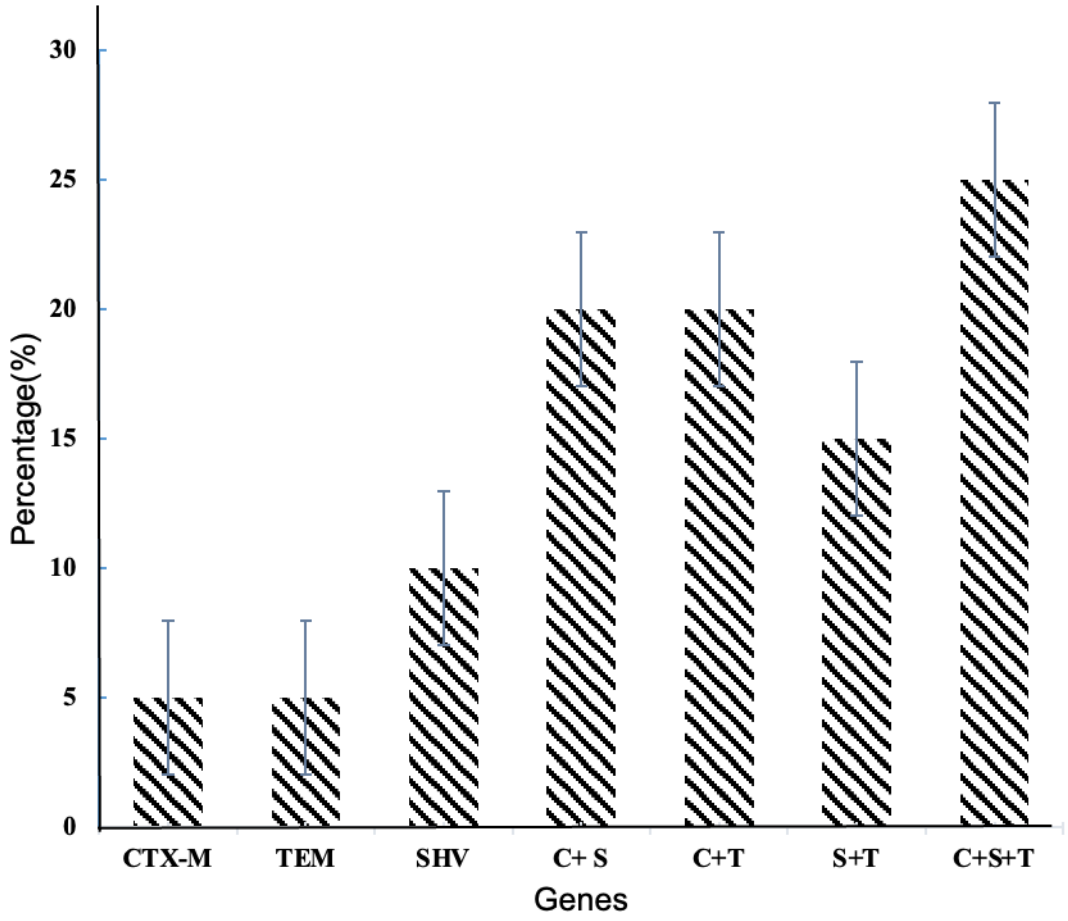

Figure 4: Percentage occurrence of ESβLs genes detected from *Enterobacteriaceae*.
Keys: C=CTX-M, S=SHV, T=TEM

Discussion

ESβLs are widespread and pose serious threat to public health worldwide due largely to their increased multiple antibiotic resistance in bacteria (Ohalete et al., 2016). Awosile et al., (2020) showed that most of the beta lactamases reported in Nigeria are associated with Gram-negative bacteria, particularly *Enterobacteriaceae*. The resistance of *Enterobacteriaceae* isolates to the third generation cephalosporins (Cefotaxime, Ceftazidime and Cefpodoxime) used for the screening of ESβLs-producing organisms in this study was observed to be high (Table 2). The results showed Ceftazidime to be the most resisted antibiotic (89.9%) over Cefotaxime (74.3%). A similar pattern of resistance was reported by Kateregga et al., (2015) who showed 73.8% Ceftazidime resistance by *Enterobacteriaceae* and that of Cefotaxime as 57.5%. However, Gautam et al., (2019), Hamid et al., (2019), and Tolulope et al., (2020) reported higher percentage of *Enterobacteriaceae* resistance to Cefotaxime more than ceftazidime. This high percentage of resistance pattern could be attributed to the extensive use of the antibiotics, sales of substandard antibiotics, incomplete antibiotic dosage, poor infection and disease prevention and control in health-care facilities and lack of awareness and knowledge (WHO, 2023).

The prevalence of ESβL varies widely within geographical locations, group of patients and types of infection (Garba et al., 2018). In the current investigation, 25.2% of Enterobacteriaceae strains produced ESβL (Table 3). Previous investigations

have reported comparable rates of prevalence, for instance, a prevalence of 26.2% was reported by Tolulope et al*.,* (2020) in the Southwestern Nigeria, 20.0% by Ungo-Kore et al*.,* (2019) in the Northwestern Nigeria, 23.1% by Hamid, (2019) in Sudan, and 20.5% by Bhasin et al*.,* (2020) in India. However, higher prevalence rates of 43.5% were reported in Adamawa Nigeria by Tula and Iyoha, (2020), 30.3% in northern Nigeria by Medugu et al*.,* (2023), and 63.9% in Ethiopia by Bitew and Tsige, (2020). This study had also shown that *E. coli* has the highest number of ESβL-producing isolates with 35.7%, followed by *K. pneumoniae* with 24.2%. This is consistent with a study conducted by Gharavi et al*.,* (2021) who reported ESβL-producing *E. coli* with 35.7% and *K. pneumoniae* with 22.7% prevalence. Other studies have confirmed *E. coli* as the most ESβL-producer and not *K. pneumoniae* (Garba and Yusha'u, 2012; Gautam et al*.,* 2019; Ouchar et al*.,* 2019; Dikoumba et al*.,* 2021; Hasan et al*.,* 2022). However, a study by Yarima et al*.,* (2020) conducted at the same Teaching Hospital reported higher ESβL prevalence in *K. pneumoniae* and *E. coli* to be 54.1% and 40.0%, respectively. The higher prevalence could be due to the fact that their work was limited to *E. coli* and *K. pneumoniae* only and the source of the isolates was restricted to only urine samples. Several other studies published in Nigeria (Mohammed et al*.,* 2016), Sudan (Hamid et al*.,* 2019) and Egypt (Mohamed et al*.,* 2020) have also revealed ESβL prevalence to be higher in *K. pneumoniae* than *E. coli*.

Although *E. coli* and *K. pneumoniae* were the most ESβL-producing organisms according to most researches (Shu'aibu et al*.,* 2018), this study has also shown ESβL-production in other species of *Enterobacteriaceae* namely; *K. oxytoca*, *Citrobacter* spp. and *Enterobacter* spp., *Serratia marcescens*, *Shigella* spp., *Salmonella* spp. and *Morganella morganii*. This investigation however, showed that other Enterobacteriaceae species have a low prevalence of ESβL production (Table 3). The prevalence of *K. oxytoca* is the highest with 14.7%, which is higher than the 5.8% reported by Hamid et al. (2019). *Citrobacter* spp. and *Enterobacter* spp. had prevalence of 10.5% and 7.4%, respectively. Contrary to this result, Moosavian & Deiham (2012) (2012) reported higher prevalence of *Citrobacter* spp. and *Enterobacter* spp. to be 16.6% and 15.8% respectively. Prevalence of 2.1% was recorded for both *Shigella* spp. and *Serratia marsescens* in this study. Similar prevalence of 2.44% was reported by Garba and Yusha'u (2012) for *S. marsescens*. However, Zahedi et al. (2016) reported higher prevalence of 5.8% for *Shigella* spp. while Hamid *at al.* (2019), reported prevalence of 5.8% for *S. marcescens*. Only 1 isolate each of *Salmonella* spp. and *Morganella morganii* was found to produce ESβL, and this agrees with studies published by Ugwu et al. (2020) and Dikoumba et al. (2021) respectively. The results of the occurrence of ESβL-producing *Enterobacteriaceae* in clinical specimens showed 50% of the ESβL-producers were from urine sample and 23.3% from Wound swab, with *E. coli* being the most predominant in the urine and *K. pneumoniae* the most common in the wound swab (Table 4). This is expected as the isolates were mostly from urine and Wound swab and also *E. coli* is the most common cause of Urinary tract infections (UTI) (Vila et al., 2016), while *K. pneumoniae* is the most common cause of nosocomial infection (Kayser et al*.,* 2005). Similarly, several other studies have reported urine as the main source of ESβL-producing isolates (Tololupe et al*.,* 2020).

Conclusion

Following screening and confirmatory testing using double disc synergy test, this study highlighted high prevalence of ESβL in clinical isolates of *Enterobacteriaceae* from Federal Teaching Hospital Gombe. The highest frequency for ESβLs production was seen in *E. coli*, followed by *K. pneumoniae*, *K. oxytoca*, *Citrobacter* spp. and some other members of *Enterobacteriaceaea*. This increased ESβL occurrence in clinical isolates is creating significant therapeutic problems prompting an immediate need for the employment of rapid method for ESβL detection in FTH Gombe and the establishment of local guidelines for ESβL screening of *Enterobacteriaceae* in hospitals.

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