Original Research Article

Molecular Characterization of Extended-spectrum β-Lactamase Producing *Escherichia coli* from Suspected Cases of Diarrhea in Nasarawa South Senatorial Zone, Nigeria

ABSTRACT

Aims: To investigate the prevalence and molecular diversity of ESBL producing *E.coli* isolates from children with suspected cases of diarrhea, in Nasarawa South, senatorial zone, Nigeria
Study design: Investigative study.
Place and Duration of Study: Nasarawa South Senatorial District, Nigeria, in 2023
Methodology: A total of eight (8) phenotypic ESBL producing isolates that form suspected cases of diarrhea in Nasarawa south, Nigeria, from previous study were used for polymerase chain reaction detection of ESBL genes and molecular diversity of ESBL isolates were determined using restriction fragment length polymorphism.
Results: The prevalence of $bla_{CTX\text{-}M}$ gene (4, 50%) was found to more prevalent among ESBL producing isolates than bla_{sHV} and *blaTEM* (3, 37.5%). The strains of ESBL isolates that form the suspected cases of diarrhea among children in Nasarawa South were A and B and the proportion was 50%. The $bla_{CTX\text{-}M}$ gene was frequently detected in the ESBL producers and strain A and B were the only strain of ESBL producing isolates that form suspected cases of diarrhoea in the study location.

Conclusion: $bla_{CTX\text{-}M}$ gene was more predominant in ESBL producing isolates in the study location and the strains A and B ESBL producing isolates were prevalent among children with suspected cases of diarrheic in the study location.

Keywords: Escherichia coli; diarrhea; antimicrobial; Nasarawa; extended-spectrum beta-lactamase; Nigeria.

1. INTRODUCTION

Diarrhoea disease cause by *Escherichia coli* (*E. coli*) is serious global health problem in children and adults worldwide (Dela *et al*., 2022) with highest mortality rate in Sub-Sahara Africa (Ogunbiyi *et al*., 2023; Zelelie *et al*., 2023). This disease account 1.7 billion annual global cases with estimated 1.3 million deaths of which 25% of this were mainly occurring in Low- and Middle-Income Countries (LMICs) (Mokomane *et al*., 2018, Ogunbiyi *et al*., 2023).

Worldwide, reports have shown that *E. coli* causing diarrhoea are classified into six different pathotypes nemaly; enteroaggregative Escherichia coli (EAEC), Enteroinvasive *Escherichia coli* (EIEC) Enterohemorrhagic *Escherichia coli* (EHEC)/Shiga-toxin producing *Escherichia coli* (STEC), enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC) and diffusely adherent *Escherichia coli* (DAEC) (Sani et al., 2015; Abimiku et al., 2016; Dela et al., 2022). Among the diarrheagenic *E. coli* pathotypes, EAEC along with the well-established ETEC and EPEC cause a substantial health burden of infant diarrheal cases and a variety of animal's species (Abimiku *et al*., 2019).

The antimicrobials such as beta-lactams and fluoroquinolones are commonly as therapeutic options of infections caused by Gram-negative Enterobacteriaceae in both human and veterinary medicine (Egwu et al., 2023).

Antimicrobial resistance in enteric bacteria is a serious global problem that have associated with increased prolonged hospitalization, increased in morbidity and mortality; and high cost of treatment (Chellapandi *et al*., 2017, Zelelie *et al*., 2023).

The emergence and spread of ESBL strains of diarrheagenic *E. coli* is a serious challenge, and has led to increase severity of infection, cost of treatment, and duration of diarrhoea episodes (Ugwu et al. 2019; Heredia and García 2018; Odetoyin et al. 2022). Therefore, the focus of this study is on the prevalence and molecular diversity of ESBL producing *E. coli* isolated from stool of suspected cases of diarrhoea among children of <5 years in Nasarawa South Senatorial zone, Nasarawa State, Nigeria.

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Bacteria Isolates

A total of eight (8) ESBL producing *E. coli* from stool of children with suspected cases of diarrheic from our previous study (Zakou *et al*., 2024) maintained at -80 °C were used for this study.

2.2 DNA extraction

The DNA of phenotypic ESBL producing isolates was extracted using boiling method with minor modification (Nkene *et al*., 2020). One thousand microlitre (1000 µL) of an overnight broth culture of the isolates were centrifuge at 14000 rpm for 3 min. The supernatant was discarded, and the harvested cell pellet was resuspended in 1000 µL sterile distilled water and centrifuged at 14000 rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 µL of sterile distilled water by vertexing. The tube was centrifuged again at 14000 for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500µl of normal saline and heated at 95 °C for 20 min. The heated bacterial suspension was cooled on ice for 10 mins and spun for 3 min at 14000 rpm. The supernatant containing the DNA 52 was transferred to a 1.5ml microcentrifuge tube and stored at -20 °C for other downstream reactions. The purity and concentration of extracted DNA was quantified using the Nanodrop 1000 spectrophotometer.

2.3. Amplification and Separation of Extended-Spectrum Beta-Lactamase Genes

The single plex Polymerase Chain Reaction (PCR: ABI 9700 Applied Biosystems thermal cycler) were performed to amplify the presence of ESBL genes such as $bla_{CTX\text{-M}}$, bla_{SHV} and bla_{TEM} in the phenotypic ESBL producing isolates using the following forward (F) and reverse (R) primers as shown in Table 1 under the following condition with minor modification (Nkene *et al*., 2020); initial denaturation step at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 3 sec, annealing at 58 °C for 30 sec, initial extension at 55 °C for 30 sec, final extension at 68 °C for 7 minutes and hold at 4 °C indefinitely for cooling. The amplified ESBL genes were separated on 1% agarose geI at 120 V for 15 minutes and visualized on a UV trans illuminator.

2.4 16s rRNA Amplification

The amplification of 16s rRNA of the ESBL producing isolates was carried out using the F: AGAGTTTGATCMTGGCTCAG and R: CGGTTACCTTGTTACGACTT and the PCR conditions were as follows: Initial denaturation, 96 °C for 5 min; denaturation, 95 °C for 30 sec; annealing, 52 °C for 30 sec; extension, 72 °C for 30 sec for 25 cycles and final extension, 72 °C for 5 minutes (Nkene *et al*., 2020). The amplified 16s rRNA of the phenotypic ESBL isolates were separated on 1% agarose gel at 120 V for 15 minutes and visualized on a UV trans illuminator.

2.5 Restriction Fragment Length Polymorphism

The Restriction Fragment Length Polymorphism (RFLP) assay of the ESBL producing isolates by digesting the amplified 16s rRNA of the ESBL isolates with restriction enzymes ECOR1 following a method described by Nkene et al. (2020). The restriction fragments were separated on 1% agarose gel and visualized on a UV trans illuminator.

Table 1: Primers and amplicon sizes for Extended spectrum β-lactamase genes

Target genes	Gene sequence	Amplicon size	References
blaTEM	F 5´-TCGGGGAAATGTGCGCG-3´ R 3´-TGCTTAATCAGTGAGGCACC-5´	972	Abrar *et al*., 2019
blaSHV	F 5´-GGGTTATTCTTATTTGTCGC-3´ R 3´-TTAGCGTTGCCAGTGCTC-5’	615	Abrar *et al*., 2019
blaCTX-M	F 5´-ACGCTGTTGTTAGGAAGTG-3´ R 3´-TTGAGGCTGGGTGAAGT-5´	757	Abrar *et al*., 2019

3. RESULTS AND DISCUSSION

3.1 Prevalence of Extended Spectrum β-Lactamase Genes

The prevalence of ESBL genes in phenotypic ESBL producing isolates was determined using descriptive statistics and the overall prevalence $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} were 4(50.0%) and 3(37.5%) respectively as shown in Figure 1. Similarly, isolates with co-carriage of bla_{SHV}/bla_{TEM} (1, 12.5%) were found to be more prevalent than the co-carriage of but none of the isolates were co-carriage of $bla_{TEM}/bla_{CTX\text{-}M}$, $bla_{SHV}/bla_{CTX\text{-}M}$ and $bla_{CTX\text{-}M}/bla_{SHV}/bla_{TEM}$.

3.2 Molecular Diversity of Extended- Spectrum β-Lactamase Genes producing *Escherichia coli*

To determine the diversity of ESBL producers from children with suspected carrier of diarrheic in Nasarawa South, Nigeria RFLP approach was used to digest the amplified 16rRNA sequence with ECOR1 and the different strains of isolates based on the size of fragments generated were assigned as A (200bp, 300bp, 500bp, 700bp) and B (100bp, 200bp,300bp, 600bp) of which the proportion of both A and B were 4(50.0%) as shown in Figure 2 and present both in General Hospital Doma (GHD) and Dalhatu Araf Specialist Hospital Lafia (DASHL) as shown in Plate 4.

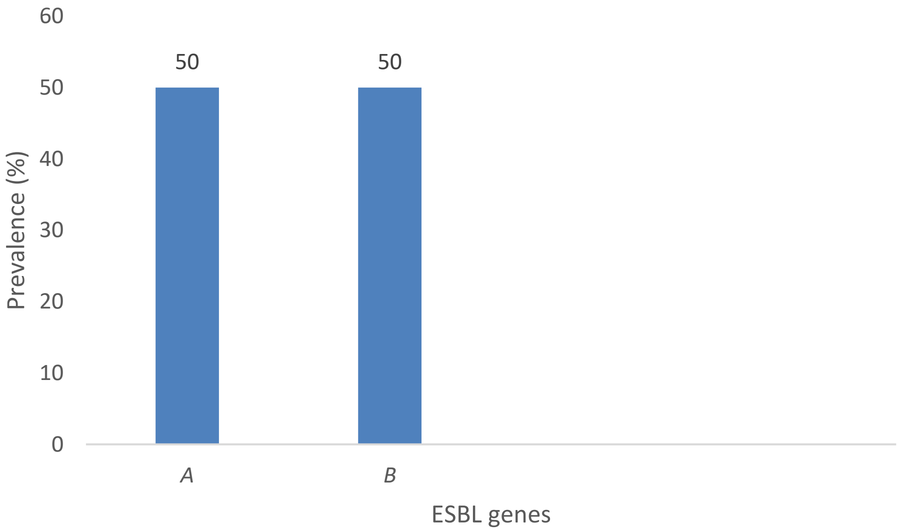

Figure 1: Prevalence of Extended spectrum β-lactamase genes in *Escherichia coli* from children with suspected cases of diarrheic in Nasarawa South, Senatorial Zone, Nigeria

ESBL= Extended spectrum β-lactamase

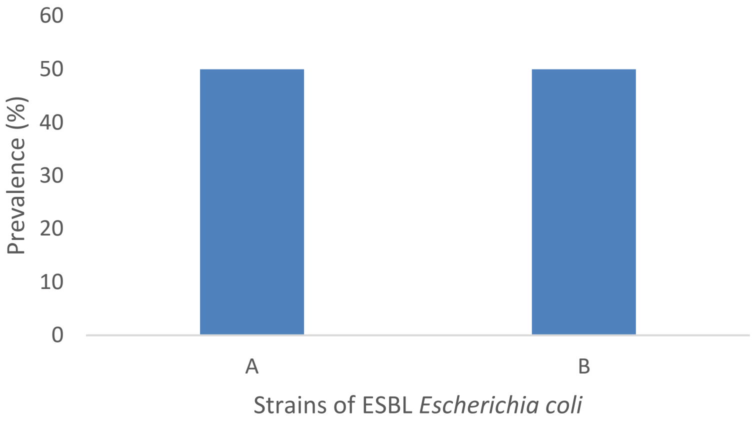

Figure 2: Prevalence of strains of Extended spectrum β-lactamase producing *Escherichia coli* from children with suspected cases of diarrheic in Nasarawa South, Senatorial Zone, Nigeria

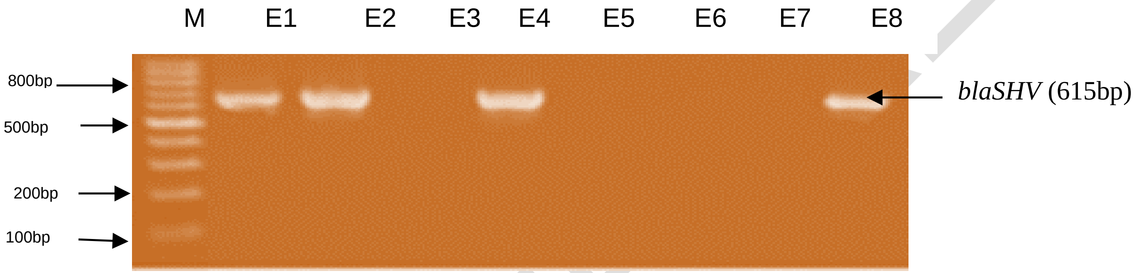

Plate 1: Agarose gel electrophoresis of the amplified ESBL gene bacteria isolates. Lane M represents 1500bp DNA molecular ladder. Lane E1, E2 E5 and E64 represent the expression of the *blaSHV (615bp)* gene for *Escherichia coli.* While other Lanes did not show the expression of any ESBL genes.
Key: E1-E4=DASHL; E5-E7=GHD, E8=GHO; DASHL=Dalhatu Araf Specialist Hospital Lafia, GHD=General Hospital Doma, GHO=General Hospital Obi

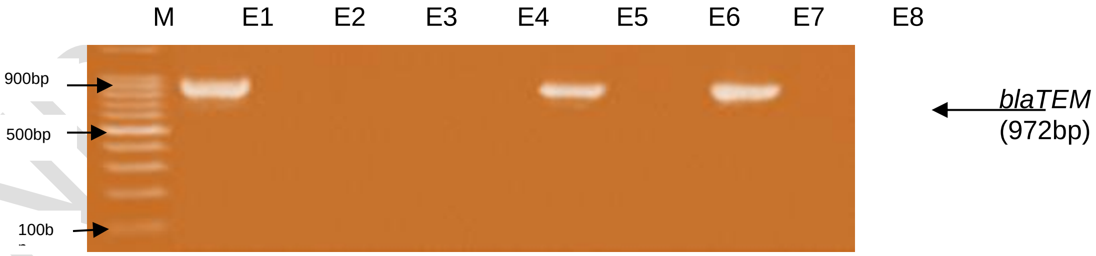

Plate 2: Agarose gel electrophoresis of the amplified ESBL gene in *Escherichia coli* isolates. Lane M represents 1500bp DNA molecular ladder. Lane E1, E7 and E35 represent the expression of the *blaTEM* (972bp) gene for *Escherichia coli.* While other Lanes did not show the expression of any ESBL genes.
Key: E1-E4=DASHL; E5-E7=GHD, E8=GHO; DASHL=Dalhatu Araf Specialist Hospital Lafia, GHD=General Hospital Doma, GHO=General Hospital Obi

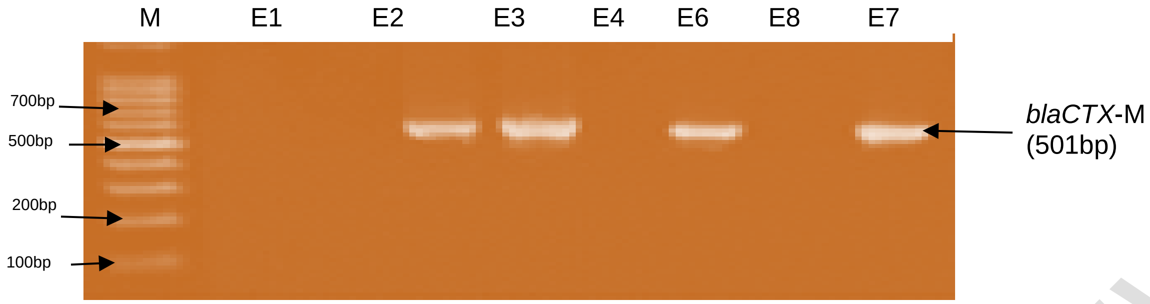

Plate 3: Agarose gel electrophoresis of the amplified ESBL gene in *Escherichia coli* isolates. Lane M represents 1500bp DNA molecular ladder. Lane E4, E5, E8 and E64 represent the expression of the *blaCTX*-M-4 (501bp) gene for *Escherichia coli.* While other Lanes did not show the expression of any ESBL genes.
Key: E1-E4=DASHL; E5-E7=GHD, E8=GHO; DASHL=Dalhatu Araf Specialist Hospital Lafia, GHD=General Hospital Doma, GHO=General Hospital Obi

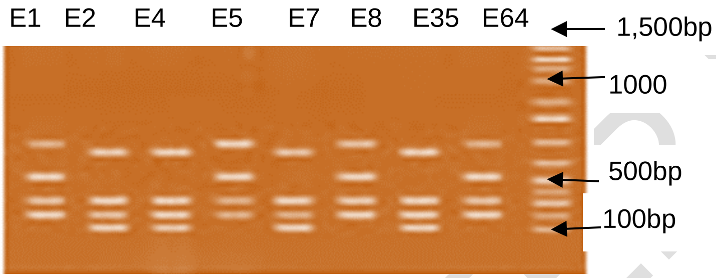

Plate 4: Restriction Fragment Length Polymorphism (RFLP) analysis of *Escherichia coli* isolates cluster. Lane M represents 1500bp DNA molecular ladder. Each lane corresponds to an isolate' DNA. Identical and unidentical banding patterns were evident in all samples from Lane E1 to Lane E64
Key: E1-E4=DASHL; E5-E7=GHD, E8=GHO; DASHL=Dalhatu Araf Specialist Hospital Lafia, GHD=General Hospital Doma, GHO=General Hospital Obi

3.3 Discussion

This study investigates the prevalence and molecular diversity of ESBL producing *E. coli* isolates from children with suspected cases of diarrheic. The results of our study on detection of ESBL genes in ESBL producing *E. coli* with suspected cases of diarrheic shows that bla_{CTX-M}, bla_{SHV} and bla_{TEM} were detected and this suggest that the genes may be responsible for extended-spectrum cephalosporin resistance in *E. coli* with suspected cases of diarrheic in the study location.
The result of our study also shows that bla_{CTX-M} gene was found to be more prevalent than other ESBL gene and this however contradict the previous study conducted by Abimiku *et al*. (2019) who reported high prevalence of bla_{SHV} in *E. coli* with suspected cases of diarrheic. The results of our study also agree with previous study that reported bla_{CTX-M} as most prevalent ESBL gene in intestinal *E. coli* causing diarrheic, blood stream pathogenic *E. coli* (Kuenzli, 2016, DeFrancesco, 2017, Hamad, 2022).
The results of our study on the RFLP of the ESBL producing isolates shows that the isolates were distributed into two different strains namely A and B with equal proportion and these findings contradict with the with the study conducted by Abimiku *et al*. (2019) who reported the distribution of the ESBL strain A-J. The presence of the above strains suggest that they are likely response for suspected cases of diarrheic in the study location.

4. CONCLUSION

The results of this study shows that $bla_{CTX\text{-}M}$ gene was more predominant in ESBL producing isolates in the study location and the strains A and B ESBL producing isolates were prevalent among children with suspected cases of diarrheic in the study location.

ETHICAL APPROVAL

Appropriate ethical committee approval was obtained prior to start of the research and is available for review (Attached to this publication draft)

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