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ANTIBIOGRAM AND ESBL GENE PROFILING OF ENTERIC ORGANISMS ISOLATED FROM DIARRHEA PATIENTS AGED 2 to17 ATTENDING COOUTH AWKA, NIGERIA

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Abstract: **This study aimed at assessing antibiotic resistance patterns and Extended Spectrum Beta-Lactmase (ESBL) genes in enteric bacteria isolated from children and adolescents in Awka. A sample size of 220 subjects drawn from four different hospitals in Awka was proposed for the study. Enteric organisms were isolated from the subjects who presented with enteritis signs that came for treatment at the selected hospitals. Bacterial isolates were characterized using standard biochemical procedures and antibiogram of the isolates was conducted using the agar disc diffusion method. Organisms that exhibited 20% resistance inclusive of resistance to penicillins and cephalosporins were picked out and identified using 16s rDNA sequencing and molecular typing. ESBL genes were assessed in those isolates and identified using the PCR and electrophoresis techniques. Data obtained from the resistance were analyzed using frequency distribution in percentages and Chi-square. P values <0.05 were considered significant. Male subjects were 122 (55.45%) while the female subjects were 98 (44.54%). Biochemical characterization of the isolates revealed the presence of the following enteric organisms: *Escherichia coli*, *Enterobacter* spp, *Klebsiella* spp, *Proteus* spp, and *Shigella* spp. The percentage occurrence of each isolate was *E. coli* (49.54%), *Enterobacter* (20.91%), *Klebsiella* (19.09%), *Proteus* (11.82%) and *Shigella* (5.45%). Antimicrobial resistance profiles of the isolated bacterial species showed lowest resistance to Ciprofloxacin (CPX) and highest resistance to perfloxacin (PEF). Eighty three (37.73%)) of the bacteria species isolated showed resistance to at least one of the tested antibiotics. 38 (17.27%) occurred in female while 45 (3.8%) occurred in men. The differences observed was statistically significant (p=0.001). Molecular typing of isolates that exhibited resistance to beta-lactam antibiotics revealed them as *Escherichia coli* and *Enterobater cloacae* strains. Their ESBL gene screening showed that two of the isolates had *bla-SHV* gene, all the isolates had *bla-TEM* gene, while three of the isolates had the *bla-OXA* gene. It is thus advised that antibiotic use on these children be controlled and should only be prescribed by designated health personnel.**

Keywords: **Extended Spectrum Beta-Lactmase (ESBL), enteric bacteria isolated, biochemical procedures, electrophoresis techniques, health personnel.**

I. INTRODUCTION

Approximately 1.6 million deaths occur each year globally due to diarrhea with the highest-burden occurring in developing countries and economically disadvantaged regions (Wolde et al., 2022). Of all child deaths from diarrhea, 78% occur in the African and Southeast Asian regions. According to WHO report in 2017, diarrhea accounts for about 8%

of all deaths among children most of who are living in South Asia and sub-Saharan Africa. Diarrhea has a lot of symptoms such as nausea, vomiting, frequent passage of stool with loose watery consistency, and abdominal pain. Important risk factors of diarrhea are age, decreased gastric acidity, abuse of antibiotics, immunosuppression, and poor sanitation. Nowadays, infectious diarrhea has become one of the main health problems worldwide. Enteric infections from multi-drug resistant pathogens are more difficult to treat as a result of antibiotic resistance, which has been declared to be an issue of global health concern (Carvalho *et al*., 2020). Factors such as self-medication, antibiotic consumption pattern and horizontal gene transfers of resistant plasmids amongst enteric organisms culminate to the high rate of antibiotic resistance expressed by these organisms. Microorganisms adopt various mechanisms for growing resistance against antimicrobial substances due to supporting factors like uncontrolled antibiotic use, under-dosage of antibiotic use, self medication and also horizontal gene transfers amongst microbial population. These factors culminate in antibiotic selection pressures amongst organisms, especially the pathogens, thus, making their therapeutic control more difficult.

Antibiotics are chemical compounds capable of killing or inhibiting the growth of microorganisms. These agents are broadly divided into four main types depending on the type of microorganism they act upon: antibacterial, antifungal, antiviral and anti-parasitic (Pandit *et al*., 2020; Adekanmbi *et al*., 2020). These agents have been developed largely over so many years and their use has been expanded from humans to animals, especially livestock. They do not only serve therapeutic reasons but are also used as growth promoters (Carvalho *et al*., 2020). However, rising antibiotic resistance cases in microorganisms are threatening the usefulness of these compounds.

Antibacterial agents are further classified into four groups based on their mechanisms of action in the microbial cells viz: cell wall synthesis inhibitors, cell membrane inhibitors, protein synthesis inhibitors and nucleic acid synthesis inhibitors. β- Lactam antibiotics belong to the cell wall synthesis inhibitors. Bacteria that are able to resist their activities possess the ability to produce β- lactamase enzyme that inactivate the β- lactam ring in the antibiotic chemical structure (Montso *et al*., 2019). β- Lactamase production by bacteria has been reported to be associated with the role of R-plasmids that encode the resistant genes in them. Plasmids are extra-chromosomal genetic elements ubiquitous in bacteria, and commonly transmissible between host cells. (Orlek et al, 2017). The most common type of β- lactamase gene coded on the R-plasmids is the extended spectrum β- lactamase (ESBL) (Pandit *et al*., 2020). Other types of β- lactamases produced by bacteria are broad spectrum β- lactamases (BSBLs), metallo β- lactamases (MBLs), AmpC β- lactamases and oxa β-lactamases (Ezeora et al., 2020). In addition to plasmid mediated antibiotic resistance, there are other known ways through which bacteria resist actions of antibiotics such as, efflux pumping and alteration of target sites or binding sites. Amani *et al*. (2015) reported that Enterobateriacae family carry more of plasmid-mediated resistant genes especially that of the ESBL.

Members of the Enterobacteriacae are usually referred to as enteric bacteria because they inhabit the intestine of man. Some of these organisms are pathogenic while some live as commensals in the gut of man. The pathogenic members elicit diarrhea of varying severity which can sometimes be febrile. Some bacteria are producing enterotoxins such as cholera toxin, the heat-labile or heat-stable enterotoxins produced by *Escherichia coli*. Others produce cytotoxins like Shiga toxins produced by *Shigella*, which damage cells. Both of them can cause diarrheal diseases. (Ghazaei, 2022). When pathogenic bacteria overcome host microbiome of normal flora, diarrhea develops (Ghazaei, 2022). This present study sought to investigate the antibiotic resistance patterns of enteric organisms, as well as profile for the presence of Extended Spectrum Beta Lactamase (ESBL) genes, so as to establish the antibiotic selection pattern of these organisms in Nigerian children and adolescents residing in Awka, Anambra State.

II. METHOD

A. Study area

Awka is the capital city of Anambra State, south east Nigeria with an area of 523.2 km² and Latitude and longitude coordinates as **6.210528, 7.072277**. The city has an estimated population of 301,657 as of the 2006 Nigerian census, and over 2.5 million as of a 2018 estimate. The population is 51.1% males and 48.9% females. 32.8% are aged 0 to 14 years, 63.2% aged 15 to 64 while the rest are 65 years and above (National Population Commission of Nigeria/National Bureau of Statistics).

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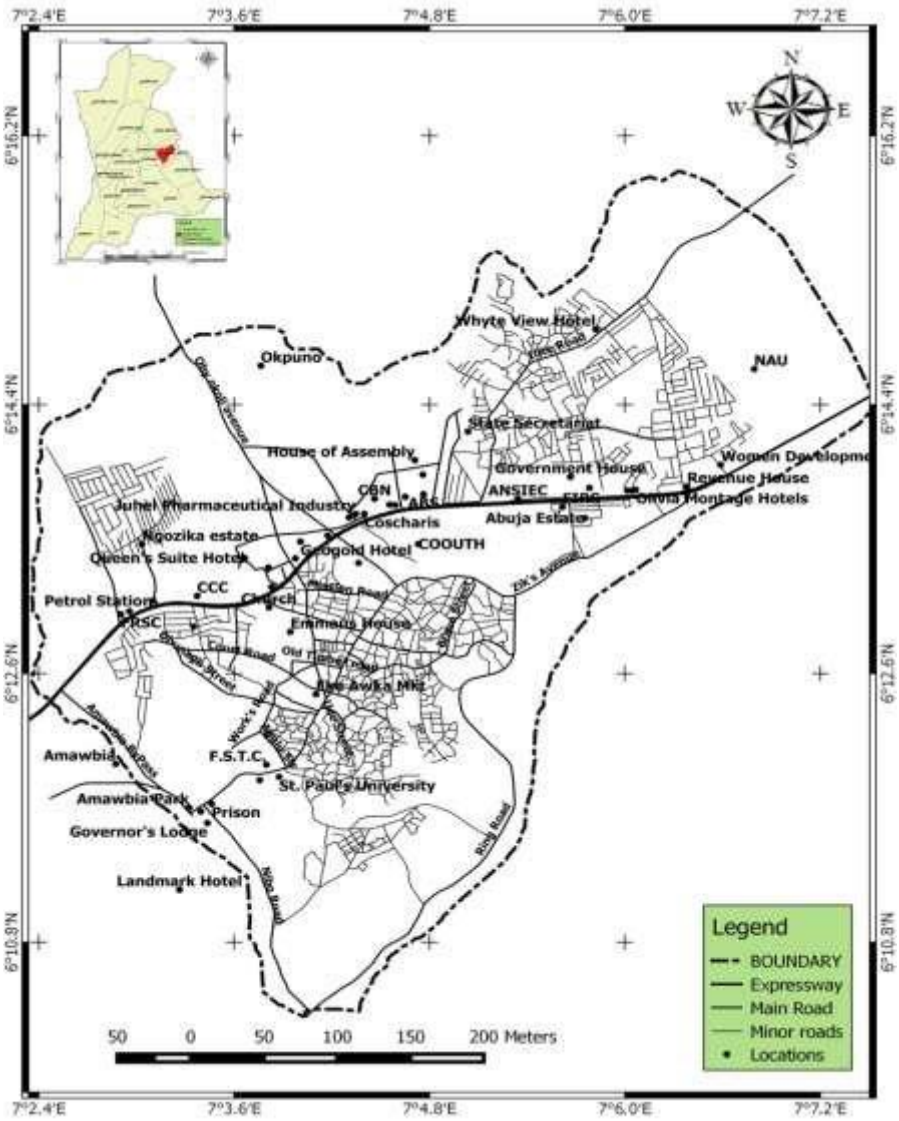

Figure 1: Map of Awka. Source: Nzoiwu *et al,* 2017

The area lies within the tropical rainforest zone of West Africa with an average humidity of 80%. Its mean daily temperature is 20^{O}C, while the mean annual rainfall is 200 cm (Ibeje, 2019) as it witnesses two distinctive climatic changes in a year. The dry season occurs between early November and March with prevailing dust-laden Northeasterly wind and rainy season occurs from April up to October with Southwesterly moisture laden air mass moving. The area is mostly inhabited by Igbo tribe and they are mainly Christians.

Strategically, Awka is located midway between two major cities in Northern Igbo land, Onitsha and Enugu as seen in figure 1. The town is known for metal works as blacksmiths. The economy of Awka city revolves primarily around government since many state and federal institutions are located there. Awka has a large university community which at times comprises around 15% of the population of the town. Awka is home to several educational institutions, including universities, colleges, and secondary schools. It hosts two primary universities of higher/tertiary education – Nnamdi Azikiwe University and Paul University. It has one tertiary health facility (Chukwuemeka Odumegwu Ojukwu teaching hospital) and several private hospitals and primary health centres.

Awka like most Nigerian cities is defined by large rudimentary informal markets where everything from basic food produce to clothes, cosmetics and household items are sold. The largest market in the town is Eke Awka. In recent years, several new businesses have sprung up and have largely changed the face of Awka city. The area is characterized by the

presence of many tree species of the rainforest interspersed by tall grasses. Vegetation therefore consists mostly of wooded shrublands and pockets of forests. Awka is reversed by rivers such as Imo Awka River, Uvunu River and Obibia River. Some of the rivers dry up in the middle of the dry season leaving sandy exposed surfaces. These river valleys support the growth of dense vegetation. The crops within the study area are grown as subsistence or market garden crops. A fallow system is practiced within the area and major crops are yam, maize, cassava, cocoyam, vegetables and fruits.

B. Study Design

This was a hospital-based cross sectional study carried out on children and adolescents who attended medical care at Chukwuemeka Odumegwu Ojukwu Teaching Hospital (COOUTH), Covenant Children's hospital Okpuno, Ifebi Hospital Awka and Divine Hospital and Maternity, Amawbia. The choice of hospitals was randomly made based on their geographical location. The study was carried out over a period of six months.

C. Ethical Consideration

Ethical approval (COOUTH/CMAC/ETHC/Vol.1/FN:04/0108) was gotten from the ethics board and research committee of Chukwuemeka Odumegwu Ojukwu Teaching Hospital Awka. Patients' consents were duly obtained orally through the parent/guardian.

D. Study population

Children and adolescents (2 to 17 years) who present with enteritis symptoms (such as frequent, loose, or watery bowel movements and abdominal discomfort) that come for treatment at the listed hospitals were used for the study.

E. Sampling Method

In this research, a two-stage sampling approach was employed to ensure the representation of the target population. Firstly, a random sampling technique was used to select the hospitals from which participants were recruited. Secondly, a stratified sampling method was applied within each selected hospital, dividing the population of diarrheic children into subgroups based on age and gender. Random sampling was then used within each stratum to randomly select participants.

F. Sample size determination

The sample size (n) was determined using Cochran's formula. The formula states as follows:

$$n=\frac{z^2pq}{e^2}$$

n= sample size

z= Standard deviation at 95% which corresponds to confidence interval of (1.96).

p= Proportion of the population having the desired characteristics.

q= (I-P), Proportion of the population without the desired characteristics

e= degree of precision; i.e. the margin of error that is acceptable (0.05)

P was set at 84.13% (0.84) according to Oli e*t al*. (2019).

This calculation gives us a sample size value of 206.52; but a total of 220 samples were used in this study.

G. Eligibility Criteria

Inclusion Criteria

- Patients between 2-17 years.
- Patients who sought medical care and attended the specified select hospitals during the study period.
- Patients that were designated to undergo stool analysis as part of their medical evaluation or diagnostic process.
- Patients **willing** to comply for their stool samples to be used.

Exclusion Criteria

- Patients that were not scheduled for stool analysis by their doctor.
- Patients below and above 2-17 years.
- Patients **unwilling** to comply for their stool samples to be used.
- Patients who did not attend the specified select hospitals during the study period.

H. Collection of Stool Samples

Stool specimens were collected in a sterile specimen bottle with the assistance of certified laboratory scientists. Loops full of the collected stool samples were transferred into 5ml normal saline. Their age and sex data were obtained from their filled laboratory request form.

I. Isolation of Bacteria

A 0.1 ml aliquot of the stool suspension was spread on sterile MacConkey, Eosin methylene blue (EMB) and Salmonella-Shigella agar plates with the aid of a sterile glass rod spreader, and incubated at 37°C for 24 hours. After incubation, the plates were carefully observed for bacterial colonies. Using a properly flamed wire loop, observed bacterial colonies were aseptically sub-cultured in order to obtain pure cultures of the bacterial colonies (Cheesbrough M., 2006). All the media that were used for the microbial isolation were prepared according to the manufacturers' instructions.

J. Characterization of Bacteria Isolates

The bacterial isolates were identified using morphological features such as colour, gram's reaction, and biochemical analysis such as Indole, Methyl red, Voges proskauer, Citrate utilization, Oxidase, Catalase, as given in Bergey's Manual of Systemic Bacteriology (Hemraj *et al*., 2013).

K. Antibiotic Susceptibility Profiling of Isolates

Disc diffusion method was used for this assay. Bacteria isolates were inoculated into nutrient broth and incubated at 30˚Cfor 24 h. Each isolate (0.1 ml) was spread-plated on Mueller Hinton agar plate using sterile swab glass spreader. OPTU-discs (10 antibiotics in a single ring namely Cefoperazone (10 µg) , Ofloxacin (10 µg), Pefloxacin (10 µg), Gentamicin (10 µg), Augmetin (30 µg), Ciprofloxacin (10 µg), Trimethoprin- sulfamethoxazole (30 µg), Streptomycin (30 µg), Penicillin (30 µg), Nafcillin (30 µg)) was placed on the top of the agar plates and was incubated at 37ºC for 24 h. Resistance was measured as the absence of a growth inhibition zone around the discs (Davidova-Gerzova *et al*., 2023).

L. Molecular Characterization of Bacterial Isolates

Isolates were characterized using 16s rDNA sequencing at Chisco Molecular laboratory, NAU Awka.

M. Determination of ESBL type using multiplex PCR

Multiplex PCR was performed in order to identify the type(s) of ESBLs present in the clinical isolates. Bacterial DNA was prepared by re-suspending 1-2 colonies of each test isolate in 200µl dH_2O and heating the solution at 95˚C for 10 mins. The presence of *bla*CTX-M, *bla*SHV, *bla*TEM and *bla*OXA genes was tested for using previously published primer sets and conditions (Sun Z. *et al.*, 2021). Each reaction tube containing 10µl of Master mix (Qiagen), 4µl of primers, 1µl of DNA and was made up to 20µl total volume with sterile H2O. The PCR reaction conditions consisting of a 15 min denaturation step at 95˚C, followed by 30 amplification cycles of 30s at 94˚C, 90s at 62˚C and 60s at 72˚C, with a final extension step of 10 mins at 72˚C was carried out. In order to visualize the PCR amplicons, samples was mixed with 4 µl of Thermo Scientific loading dye and loaded into the wells of a 1% agarose gel. The gel was prepared by melting 1g of agarose in 100ml Tris-acetate-EDTA (TAE) buffer. After the solution is cooled (but was still molten), 5 µl of Red Safe, a DNA chelating agent, was added before casting the gel in a gel tray. Gels were run at 130 volts for 60 minutes. Amplicons was visualized using an Ultra-Violet transilluminator system (DIGI DOC-IT System TM) for analysis. This process was carried out at Inqaba Biotech South Africa through Chisco laboratory NAU, Awka.

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N. Data Collation and Analyses

All the data were summarized using the frequency distribution in percentage (%). Data were analyzed using SPSS Statistical software Package version 21.0. The statistical tools used were Chi-Square, Brown-Forsythe test, Bartlett's test. P values of <0.05 were considered significant.

III. RESULTS

A. Demographic Profile of Examined Patients

A total of 220 patients were used for the study between November 2021 and April 2022. Male patients were 122 (55.45%) while the female patients were 98 (44.54%), participation based on age group was 31 patients for ages 2-10 years and 189 for ages 11-17 years, accounting for 14.09% and 85.91% respectively as shown in Table 1.

B. Isolation and Characterization of Enteric Isolates from Stool Samples

Enteric bacteria were detected in 172 (78.2%) of the 220 enrolled cases, and 235 bacterial isolates were isolated from the positive samples. This was because some of the specimens had more than one type of bacterial isolate. Biochemical characterization of the isolates revealed the presence of the following enteric organisms: *Escherichia coli* (49.54%), *Enterobacter* spp (20.91%), *Klebsiella* spp (19.09%), *Proteus* spp (11.82), and *Shigella* spp (5.45) as shown in Table 2. The presumptive characterization of isolates showed that *E. coli* had the highest occurrence as shown in Table 3 The percentage occurrence of each isolate based on gender and age distribution is shown in Table 4 and 5 respectively.

C. Antibiogram of Enteric Isolates

Out of the 220 samples, 83 (37.73%) were resistant to at least one of the tested antibiotics. Pefloxacin and oflaxacin had the most resistance while cefoperazone had the least as shown in Tables 6-7. The pattern of resistance based on gender and age is shown in Tables 8 and 9 respectively. The mean values of the observed diameter zone of inhibition for the tested antibiotics group are presented in Table 10 with ciprofloxacin having the highest zone of inhibition. Five out of these isolates that exhibited resistance to penicillin and nafcillin, and also cefoperazone (which are all known beta-lactam antibiotics) were randomly selected for molecular screening.

D. Molecular Typing of Beta-lactam Antibiotic Resistant Isolates and ESBL Gene Screening

Molecular typing of isolates that exhibited resistance to beta-lactam antibiotics revealed them as *Escherichia coli* and *Enterobater cloacae* strains. Their ESBL gene screening showed that two of the isolates had *bla-*$_{SHV}$ gene, all the isolates had *bla-*$_{TEM}$ gene, while three of the isolates had the *bla-*$_{OXA}$ gene as shown in Figures 2 and 3. None of the isolates had the *bla-*$_{CTX\text{-}M}$ gene.

Table 1: Distribution of Examined Patients Based on Gender and Age

Age	2-10 years	11-17 years	Total	Percentage (%)
Sex				
Male	20	102	122	55.45
Female	11	87	98	44.55
Total/percentage	31 (14.09%)	189 (85.91%)	220	100

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates

Biochemical Tests	Results				
Colony morphology	**Rod**	**Rod**	**Rod**	**Rod**	**Rod**
Gram Stain	-	-	-	-	-
Catalase	-	+	+	-	-
Indole	-	-	-	+	-
Methyl red	-	-	-	+	+
Voges prauskeur	+	+	+	-	-
Citrate	-	+	-	-	-

Urease	-	+	-	-	-
Motility	-	-	-	+	+
Glucose	+	+	+	+	+
Sucrose		+	+	+	+
Mannitol	+	+	+	+	+
Lactose	+	+	+	+	+
Hydrogen Sulphide	-	-	-	-	-
Presumptive Organisms	***Proteus* sp.**	***Klebsiella* sp.**	***Shigella* sp.**	***Escherichia coli***	***Enterobacter* sp.**

Table 3: Occurrence of Enteric Bacterial Isolates in Stool Samples of Examined Patients

Isolates	Total positive plates	Percentage (n=220)
Escherichia coli	109	49.54
***Enterobacter* spp.**	46	20.91
***Klebsiella* spp.**	42	19.09
***Proteus* spp.**	26	11.82
***Shigella* spp**	12	5.45

Table 4: Occurrence of Enteric Bacterial Isolates in Stool Samples of Examined Patients based on gender

Isolates	Male (%)	Female (%)	Total
Escherichia coli	61(27.72)	48(21.82)	109
***Enterobacter* spp.**	22(10)	24(10.91)	46
***Klebsiella* spp.**	18(8.18)	24(10.91)	42
***Proteus* spp.**	20(9.09)	6(2.73)	26
***Shigella* spp**	8(3.64)	4(1.81)	12
Total	129	106	235

Note: total sample examined = 220; *P* -value = 0.106

Table 5: Occurrence of Enteric Bacterial Isolates in Stool Samples of Examined Patients based on age

Isolates	2-10years (%)	11-17years (%)	Total
Escherichia coli	26(11.82)	83(37.72)	109
***Enterobacter* spp.**	8(3.64)	38(17.27)	46
***Klebsiella* spp.**	9(4.09)	33(15)	42
***Proteus* spp.**	7(3.18)	19(8.64)	26
***Shigella* spp**	5(2.27)	7(3.18)	12
Total	55	180	235

Note: total sample examined = 220

Table 6: Inhibition zones diameter of bacterial isolates to tested antibiotics

Isolated Bacteria	Antibiotics/mean diameter zones of inhibition(mm)									
	CFP	OFX	PEF	CN	AU	CPX	SXT	S	PN	NA
Escherichia coli	15.31	8.6	3.92	9.23	16.5	18.21	6.31	6.51	12.58	15.5
Enterobacter spp.	14.18	7.41	4.2	8.89	12.86	15.56	5.12	4.64	8.25	14.89
Klebsiella spp.	13.32	6.51	5.12	7.71	14.56	16.85	6.52	8.67	9.2	5.23
Proteus spp.	0	7.56	6.16	0	12.25	15.68	5.1	9.8	7.83	7.86
Shigella spp	0	4.42	5.45	0	11	17.01	4.5	7.1	7.3	6.73

P value = 0.001

CFP: Cefoperazone(10µg), OFX: Oflaxacin (10µg), PEF: Pefloxacin(10µg), CN: Gentamicin(10µg), AU:Augmetin(30µg), CPX: Ciproflaxin(10µg), SXT: Trimethoprin- sulfamethoxazole(30µg), S: Streptomycin(30µg), PN: Penicillin(30µg) NA: Nafcillin(30µg).

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Table 7: Resistance profiles of bacterial isolates to tested antibiotics

Isolated Bacteria	Resistance to antibiotics (%)									
	CFP	OFX	PEF	CN	AU	CPX	SXT	S	PN	NA
Escherichia coli n=38	12(5.45)	26(11.82)	27(12.27)	15(6.82)	10(4.55)	29(13.18)	30(13.64)	36(16.36)	38(17.27)	32(14.55)
Enterobacter spp. n=19	5(2.27)	14(6.36)	12(5.45)	9(4.09)	7(3.18)	19(8.64)	11(5.0)	17(7.73)	17(7.73)	16(7.27)
Klebsiella spp. n=14	6(2.73)	10(4.55)	10(4.55)	8(3.64)	9(4.09)	12(5.45)	12(5.45)	9(4.09)	14(6.36)	12(5.45)
Proteus spp. n=8	0(0)	0(0)	3(1.36)	0(0)	6(2.73)	6(2.73)	7(3.18)	8(3.64)	8(3.64)	5(2.27)
Shigella spp n=4	0(0)	3(1.36)	3(1.36)	0(0)	0(0)	3(0.90)	2(0.90)	4(1.82)	4(1.82)	3(1.36)
Total resistant isolates=83	23(10.45)	53(24.09)	55(25)	32(14.54)	32(14.54)	69(31.36)	62(28.18)	74(33.64)	81(36.82)	68(30.91)

Table 8: Distribution of bacterial resistance in isolates according to gender

Isolated Bacteria	Male (%)	Female (%)
Escherichia coli n=38	21(9.55)	17(7.73)
Enterobacter spp. n=19	11(5.0)	8(3.64)
Klebsiella spp. n=14	5(2.27)	9(4.09)
Proteus spp. n=8	5(2.27)	3(1.36)
Shigella spp n=4	3(1.36)	1(0.45)
Total resistant isolates=83	45(20.45)	38(17.27)

Note: total sample examined = 220; *P*-value=0.001

Table 9: Distribution of bacterial resistance in isolates according to age

Isolated Bacteria	2-10years (%)	11-17 (%)
Escherichia coli n=38	7(3.18)	31(14.09)
Enterobacter spp. n=19	4(1.82)	15(6.82)
Klebsiella spp. n=14	3(1.36)	11(5.0)
Proteus spp. n=8	1(0.45)	7(3.18)
Shigella spp n=4	2(0.91)	2(0.91)
Total resistant isolates=83	17(7.73)	66(30.0)

Note: total sample examined = 220

Table 10: Mean Susceptibility (mm) of Isolates to tested antibiotics

Antibiotics	Mean±Std. Deviation
CFP	14.2771 ± 5.04
OFX	6.9036 ± 5.56
PEF	4.9759 ± 5.18
CN	8.6024 ± 8.37
AU	13.4217 ± 5.92
CPX	16.7108 ± 4.50
SXT	5.5181 ± 6.71
S	7.3373 ± 6.65
PN	9.0241 ± 6.01
NA	10.0482 ± 8.12

CFP: Cefoperazone(10µg), OFX: Oflaxacin (10µg), PEF: Pefloxacin(10µg), CN: Gentamicin(10µg), AU:Augmetin(30µg), CPX: Ciproflaxin(10µg), SXT: Trimethoprin- sulfamethoxazole(30µg), S: Streptomycin(30µg), PN: Penicillin(30µg) NA: Nafcillin(30µg). No. of isolates = 83.

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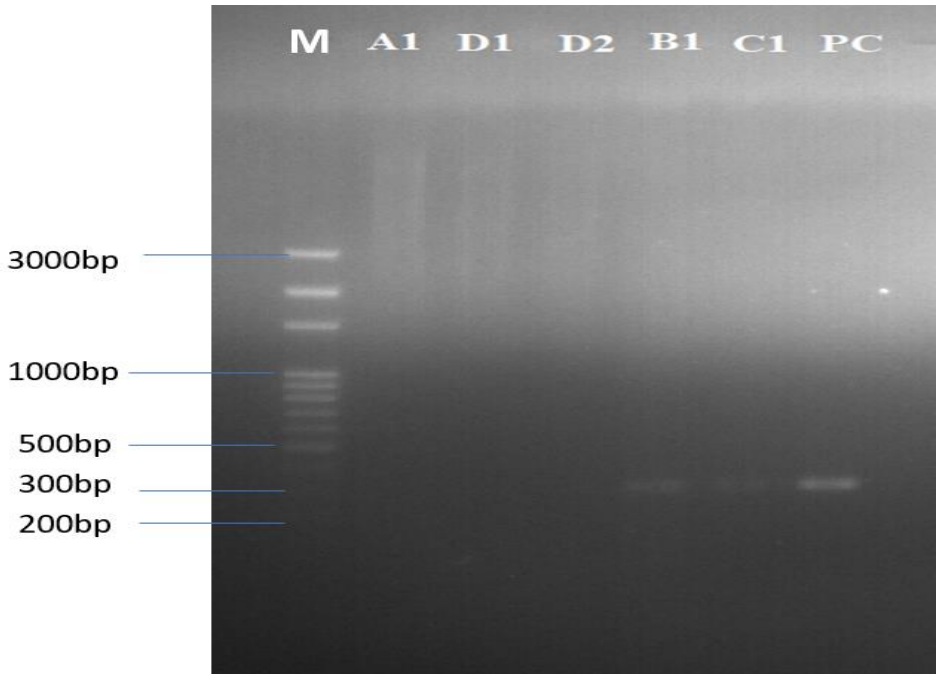

Figure 2: ESBL genes after PCR. Lane M: 3000bp DNA ladder, lane A1: negative, lane D1: negative, lane D2: negative, lane B1: positive *SHV*, Lane C1: positive *SHV*, Lane PC: positive control for *SHV* (367-401 bp)

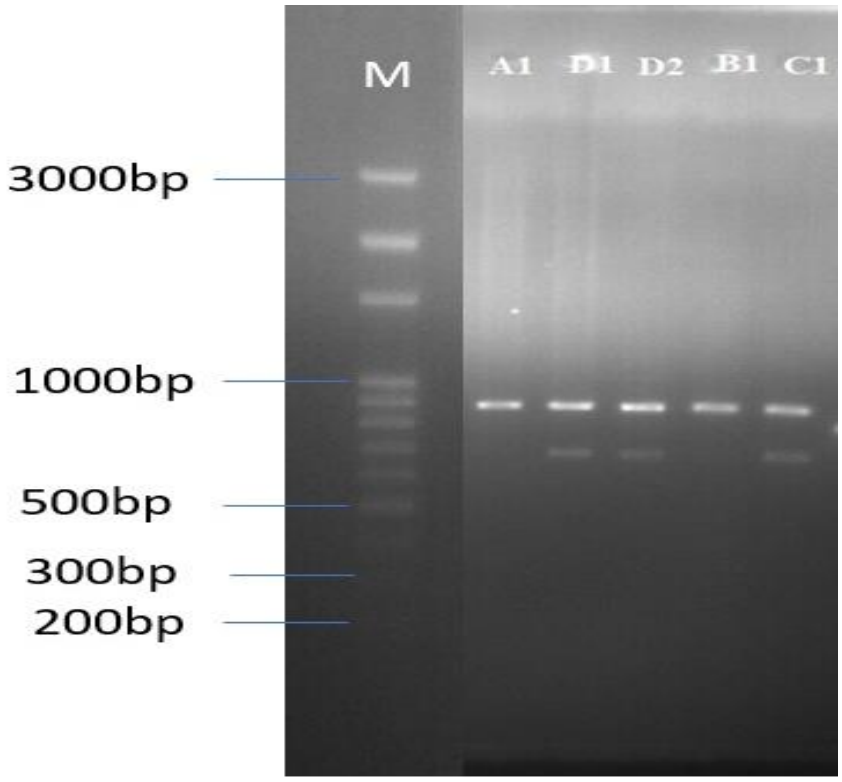

Figure 3: Multiplex PCR result. Lane M: 3000bp DNA ladder, Lane A1 –C1: positive for *TEM* (940-1060 bp), Lane D1, D2 and C1: positive for *OXA* (720-810 bp).

IV. DISCUSSION

Enteric microorganisms are known to inhabit the gut. They are known to be either invasive or pathobionts or commensals in the gut of humans. The role of some members of Enterobacteriaceae in causing diarrhea in children is well known. Likewise, the capacities of this group of organisms to harbor ESBL resistance genes have been well established.

Drug resistance by ESBL producing microbes is an emerging subject of study amongst researchers with regards to establishing antibiotic resistance patterns amongst pathogens across the world. The World Health Organization (2020) classified antimicrobial resistance as a global threat and ESBL resistance as an urgent threat to the world, in terms of disease treatment challenges.

This present study evaluated enteric microorganisms found in children and adolescents that attended four hospitals in Awka. It was observed from their stool samples that male subjects had more enteric organisms than the female subjects. The difference observed was not statistically significant at $p<0.05$. This finding varies with the report of Ogefere *et al.* (2016) who stated that female subjects had higher *E. coli* counts in children stool samples examined at Yenagoa, Nigeria. *Escherichia coli* (49.54%) were predominant in the stool samples examined in this study, followed by *Enterobacter* (20.91%) and *Klebsiella* (19.09%) whose occurrences were closely related. This finding partly corresponds with the report of Dela *et al.* (2022) who reported *E. coli* as the predominant enteric organism isolated from children's stool samples examined at Accra Ghana, followed by *Klebsiella* species and then *Proteus* species. Our finding also corresponds with that of Ezeora *et al.* (2020) who examined children stool specimens from different hospitals at Abakiliki, Nigeria.

37.73% of the examined isolates exhibited drug resistance towards at least one of the tested antibiotics, while 25% had multiple drug resistance. 35% of the resistant isolates exhibited resistance to beta-latam antibiotics. This finding partly corresponds with the report of Ezeora *et al.* (2020) who reported 33.3% antibiotic resistance frequency from enteric organisms examined in stool patients of children in Abakiliki, Nigeria. This study finding also partly vary with the report of Ogefere *et al.* (2016) who reported 10% frequency of ESBL gene bearing enteric isolates from children examined at Yenagoa, Nigeria. Dela *et al.* (2022) reported ESBL gene occurrence of 20.4% in examined stool specimen of children in Accra, Ghana, with *Escherichia coli* being the most ESBL gene bearing bacterium; while, in this study, *Enterobacter cloacae* was the most ESBL gene bearing bacterium. The isolates showed high resistance to perfloxacin, oflaxacin and gentamicin. However, they showed least resistance to augmentin and ciprofloxacin. The 100% susceptibility of the *Proteus spp* and *Shigella spp* isolates to cefoperazone and gentamicin was noted in this study. Statistical analysis of the isolates resistances to the used antibiotics showed that they were significantly resistant ($p = 0.001$). The occurrence of resistance based on gender was evaluated. Out of the eighty three (83) resistant bacteria species isolated, 38 (17.27%) occurred in female while 45 (3.8%) occurred in men. However, *Klebsiella spp* had more resistance in females while *E. coli* had higher value in males. The differences observed was statistically significant ($p=0.001$). Despite the resistances recorded, a good number of the bacteria isolates were susceptible to some of the antibacterial agents.

Molecular typing of the bacteria that phenotypically showed both multiple drug resistance and beta-lactam antibiotic resistance, revealed them to be three *Escherichia coli* strains and two *Enterobacter cloacae* strains. Evaluation of the presence of ESBL genes in these microorganisms using conventional and multiplex PCR showed that these isolates all had the *bla-*$_{TEM}$ gene, three of the isolates which were *E. coli* strain C289, *Enterobacter cloacae* strain ACD1 and *Enterobacter cloacae* strain ZG-LB-3-2, had the *Bla-*$_{OXA}$ gene; while, two of the isolates – *E. coli* strain 68 and *E. coli* strain C289 had the *bla-*$_{SHV}$ gene. None of the isolates had the *bla-*$_{CTX\text{-}M}$ gene. Prevalence of ESBL gene- positive *Enterobacter cloacae* strains in nosocomial blood and urinary tract infections in children across Africa and Europe has been reported by the following researchers: Sumbana *et al.* (2021) for Mozambique; Wendel *et al.* (2022) for Germany; Adiguzel *et al.* (2021) for Turkey. They also associated *E. cloacae* occurrence in children to septicaemia complications during rotavirus gastroenteritis. They all reported that the presence of ESBL genes in *Enterobacter cloacae* and *Enterobacter aerogenes* makes the treatment of opportunistic infections caused by these enteric bacteria with first line beta-lactam antibiotics more difficult.

Hamad *et al.* (2022) have reported on the high prevalence of ESBL genes in *E. coli* strains found in enteritis positive and also in healthy children. They however, reported the predominant presence of the *bla-*$_{CTX\text{-}M}$ gene, which was not found in any of the examined isolates in this study.

V. CONCLUSION

This study has been able to show the occurrence and antimicrobial resistance patterns of ESBLs in the predominant bacteria isolated from stool samples of children (2-17 years) with enteritis symptoms. They have high *E. coli*, *E. cloacae* and *Klebsiella* counts in their stool, which are possible aetiologic agents of nosocomial infections. Their multiple drug resistant patterns also gave insight on possible impending cases of drug resistant enteric infections that could be

experienced in the nearest future, especially those related to *Enterobacter cloacae*. It is thus advised that antibiotic use on these children be controlled.

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