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Plasmid Profiling of Bacteria Associated with Gastroenteritis among Children in Owo, Ondo State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Antibiotic resistance among the children with gastroenteritis remains a major global problem around the world. This study was therefore carried out to determine the plasmid profile of bacterial isolates from children with gastroenteritis in Owo, Ondo State. A total of 115 stool samples were collected from the children with gastroenteritis. The stool samples were cultured on MacConkey Agar using standard laboratory technique. Plasmid analysis of the some selected multiple resistant isolates was carried out using alkaline Phosphate method. Post plasmid curing was also carried out using sodium deodecyclsulphate (SDS). Out of 115 stool samples analyzed, eighty seven (75.7%) bacteria were isolated. Four different bacteria belonging to 4 genera were isolated with *Escherichiacoli* having the highest occurrence rate of 70(80.5%), followed by *Pseudomonas aeruginosa* 10 (11.5%), *Proteus mirabilismirabilis*5(5.7%) and *Salmonella typhi* 2 (2.3%). Out of 70 *Escherichiacoli* serotyped, 17 (24.3%) were *Escherichia coli* 0157 and 53 (75.7%) were Non-*Escherichia coli* 0157. The antibiotic resistant of the bacteria isolates ranges from 5.8% to 100.0% with chloramphenicol and augmentin exhibiting highest resistant. The antibiotic tested exhibited 100% resistant to *Salmonellatyphi*. Out of the six bacteria isolates tested for the present of plasmid, four had a plasmid with 3 bacteria having molecular weight of 2120bp and one bacterium having

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molecular weight of 1910bp. Plasmid mediated resistant is more than chromosomal mediated resistant. However, antimicrobial resistant pattern of the bacteria isolates in this study is worrisome. Nevertheless, Amoxicillin and sparfloxacin with least resistant pattern could be used for the treatment of gastroenteritis.

Keywords: Plasmid; bacteria; gastroenteritis; children-Nigeria.

1. INTRODUCTION

Gastroenteritis is the inflammation of the gastrointestinal tract lining, which involves both the stomach ("gastro") and the intestines ("entero") and this results in sudden onset of diarrhoea and vomiting [1]. Gastroenteritis remains a major global problem in children around the world. Children in sub-Saharan Africa are 15 times more prone to death from diarrhoeal diseases before they attain the age of 5 years than children living in countries that are developed [2]. Infection due to gastroenteritis has been known to be caused by microorganisms such as:*Salmonella species*, *Shigella species*, *Campylobacter species*, *E. coli O157:H7*, *Yersinia enterocolitica*, *Vibrio cholera*, Rotavirus, *Cryptosporidium species*, *Entamoeba histolytica*, *Listeria monocytogenes* and *Giardia lamblia* [1]. Other causes are by ingestion of some food items, chemical toxins or drugs.

"A change in bowel habit from normal with an increase in stool volume and/or fluidity resulting in an increase in stool frequency is referred to as diarrhoea. It is also defined as a form of gastrointestinal infection caused by a variety of bacterial, parasitic and viral organisms or via contaminated drinking water, , food or from person-person as a result of poor hygienic practices. If not untreated, diarrhoea can typically last several days" [3]. "World Health Organization (WHO) regards a disease to be diarrhoea if there is a passage or excretion of watery stools in about two-three times within 24 h period" [3]. "However, factors such as; stool frequency, stool consistency, and the usefulness of parental discernment in determining whether children have diarrhoea or not is clearly important to ascertain if diarrhoea has occurred or not. Acute diarrhoeal illnesses or dysentery is often easily characterized by bloody appearance of stool, irrespective of frequency or consistency" [3].

"Diarrhoeal episode is usually divided into acute, persistent and chronic. The most common of diarrhoea disorders, acute diarrhoea often begins abruptly, are as a result of infections and are subdue/resolved within 14 days. Persistent diarrhoea arises as a result of secondary infections in the presence of complications like malnutrition while chronic diarrhoea is majorly a product of congenital defects of digestion, absorption in the body system and it lasts for a minimum of 14 days" [3]. Among children below the age of 5 years old, diarrhoea- related diseases account for the second highest cause of death [4]. Although the diarrhoea mortality rate has reduced globally, morbidity rate is still high in Sub-Sahara Africa because the region is experiencing increased population growth, practices, lack of proper hygiene conditions and resources for surveillance, diagnosis, treatment and prevention of the disease is scarce in the region [4]**.**

"In sub-Saharan Africa, there are more than one billion diarrhoeal cases and an estimated 606,024 deaths of diarrhoea yearly with nearly half of the deaths occurring in children lesser than five years of age" [5]. "In Nigeria, there are an estimated 151,700 yearly child mortality as a result of diarrhea" [6] "with the prevalence rate of diarrhoea ranging between 10% and 18.8%" [6] and "80,968 deaths as a result of unsafe water and unhygienic sanitation thus making Nigeria one of the leading contributors to diarrhoeal morbidity and mortality worldwide" [6].

Bacteria associated with enteric infections are treated with the use antibiotics. The advent of Penicillin and many other antibiotics for clinical use has significantly transformed the management of clinical diseases and public perception to some threats usually pose by bacteria. These achievements are increasingly threatened with an increase in different levels of antibiotics resistance [7]. In the developing world where the incidence of diarrheal diseases is highest, antimicrobial resistance in enteric pathogens is of great value. The relative increase of antimicrobial resistance in enteric pathogens among developing countries is becoming a point of concern. The resistance to commonly prescribed antibiotics by entero-pathogenic bacteria is increasing both in developing and developed countries.

However, resistance has emerged even to newer, more effective antimicrobial agents [8]. Among the factors leading to increased risk of diarrhoea among children are: failure to adequately breast-feed a child for the first 4-6 months of life. Diarrhoea has been observed to be much greater in non-breastfed than adequately breastfed infants. Susceptibility of host to infection is assessed by the child's age, presence of protective maternal factors (trans-placental antibodies), immunological status, and nutritional status, prior exposure to foreign harmful entities, acquired immunity and genetic susceptibility [8]. Increased emergence of resistance among these bacteria pathogens is posing a significant threat. Thus, necessitating the need for the study to: isolate bacteria from children with gastroenteritis, determine the demography of children with gastroenteritis, carry out antibiogram on the bacteria isolate, determine the present of plasmid among the isolate and determine whether the antibiotic resistance is plasmid mediated

2. MATERIALS AND METHODS

2.1 Study Area / Location

This study was carried out at the Federal Medical Centre, Owo, Ondo State. The hospital is a tertiary health institution with a referral status to Primary Health Care Centre's, Maternity and Antenatal Clinics around Owo town with estimated population of 276,574 having latitude 7^0 11' 46.32^{0}N and longitude 5^0 35' 12.52''E.

2.2 Study Population

The study population for this research work were patients that visited the above named hospital within age group 0-5years with diarrhoea stool: 'Diarrhoea is defined as passing out loose or watery stools at least three times per day, or more frequently than what is normal for an individual' [10].

2.3 Sample Size Determination

The sample size of this research was calculated using the formula of Charam and Biswas [11].

Applying the formula of Charam and Biswas [11], a sample size of 114 was calculated for this study using the prevalence rate of 8.1% [12] gastroenteritis in Lagos State, South West Nigeria.

Where

B= is the prevalence

(A) = standard deviation of 1.96

(D) =level of inaccuracy at 95% confidence level is 0.05:

Map 1. Map of Ondo State Nigeria depicting the study area, Owo [9]

$\frac{A^2 BC}{D^2}$

Sample size of 114 will be used

A= 1.96^2= 3.8416
B= 8.1% = 0.081
Noting that (C=1-B)
D = 0.05^2= 0.0025
1-0.081=0.919

Thus,

$\frac{A^2 BC}{D^2} = \frac{3.8416 \times 0.081\ (0.919)}{0.0025} = \frac{0.2859}{0.0025} = 114$

2.4 Sample Collection

A total of one hundred and fifteen (115) stool specimen was collected from the patients in Paedriatric Ward (PW) Department of the above named hospital into a sterile universal bottle and then transported to the laboratory within 1hr of collection [4].

2.5 Specimen Processing

The stool sample was cultured on the Mac-Conkey agar as described by Cheesbrough [13] and incubated overnight at 37^0C.

1. **Gram Staining**: The colony was stained using gram stain technique as described by Cheesbrough [13]. A smear of the culture between 18 to 24 hours was prepared on a clean, grease free microscopic glass slide with a drop of sterile distilled water. The smear was allowed to dry and then heat fixed by passing the slide through a Bunsen flame once or twice. The fixed smear was flooded with crystal violet and allowed to stain for 60 seconds after which the stain was poured off the slide. The slide was then flooded with Lugol's iodine solution which was allowed to stand for another 60 seconds and then poured off. The smear was later decolorized with 95% ethanol for 5 seconds. The smear was then counterstained with Safranine for 60 seconds, after which the slide was gently rinsed with running tap water. It was then blotted dry with filter paper and examined under the oil immersion objectives of the light microscope.

2. **Identification of colony:** The colony on the agar plate was identified using morphological and biochemical characterization. The morphology characteristics was done using visual examination; the size, colour, odour, pigmentation, elevation will be recorded as described by Ochei and Kolhatar [14]. Mac-Conkey agar was prepared and poured into plates aseptically and allowed to solidify. Distinct colonies on the samples obtained was sub-cultured by streaking on to plates and slants. These will be incubated at 37^0 C for 24 hours.

3. **Biochemical characterization**: The colony on the Mac-Conkey agar was identified using the following biochemical test as described by Cheesbrough [13].

1. **Indole Test**

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole by the activity of tryptophanase enzymes. Tryptophanase enzyme. Tryptophan + water = indole + pyruvic acid +ammonia. Indole test was performed as described by Abdulkadir and Waliyu [15]. A tube of peptone water was inoculated with the organism under study. It was then incubated for 48 hours at 37^0C. The test tube was shaken gently and 5 drops of Kovac's reagent was added in the tube and allowed to stand for a few minutes. A deep red color develops in the presence of indole which separates out at the alcohol or upper layer indicates positive and yellow coloration indicates negative.

2. **Citrate Test**

The test was carried out on the test organisms to examine the ability of the isolates to utilize citrate as sole carbon source. The test was carried out by using Simmon citrate medium. Utilization of citrate was indicated by change of the green color of the medium from green to deep blue.

3. **Oxidase Test**

The oxidase test was used to identify bacteria that produce cytochrome C oxidase, an enzymes of the bacterial electron transport chain when the enzymes is not present, the reagent remains reduced and is colourless.

4. **Urease test**

Urea slant in the bijou bottle was stabbed with organism using a straight wire; it was then incubated at 37^0C for 24hours. The development of a bright pink or red color indicate a positive reaction [14].

2.6 Antibiotic Susceptibility Testing

The antibiogram of the isolates was determined by using the modified Kirby-Bauer disk diffusionmethod [16]. Standard bacteria suspension which is equivalent to 0.5 McFarland standards with a yielded suspension containing 10^5-10^6 cells/ml was used. The bacteria suspension was inoculated on Mueller Hilton agar (MHA) as described by [17]. The following antibiotic disc will be used;CPX= Ciprofloxacin (10µg), COT= Co-trimoxazole (25µg), AM= Amoxacillin (30µg), SP=Sparfloxacin (30µg), CH=Chloramphenicol (30µg), AU=Augmentin (30µg), CAZ=Ceftazidime(30µg)These antibiotics are commonly prescribed in the hospital. The disc was dispensed at equidistance on the plates using multi disc dispenser for 1hour before incubating the plates at 37°C for 24 h and the diameter zone of inhibition was measured using meter ruler. The isolates recovered overnight were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant which was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [18].

2.7 Plasmid Profile of Isolate

1. **Plasmid extraction and analysis: [19]**

   1. 1.5ml of overnight culture was spun for 1 minute in a micro-centrifuge to pellet cells.
   2. The supernatant was gently discarded, leaving 50 - 100µl together with cell pellet (when cells are grown on agar, the cells are harvested into 100µl of nutrient broth) and vortex at high speed to re-suspend cells completely.
   3. 300µl of TENS was added. It was mixed by inversion of the tubes 3 – 5 times until the mixture becomes sticky. After 10 minutes is before moving to the next step, samples were set on ice to prevent them from the degradation of chromosomal DNA which may be co-precipitated with plasmid DNA in steps 6 and 7.
   4. 150µl of 3.0M sodium acetate pH 5.2 was added and vortexed to mix completely.
   5. The mixture was spun 5 minutes in micro-centrifuge to pellet cell debris and chromosomal DNA.
   6. The supernatant was transferred into a fresh tube; mixed well with 900µl of ice-cold absolute ethanol.
   7. The mixture was spun for 10 minutes to pellet plasmid DNA. (White pellet was observed)
   8. Supernatant was discarded; and the pellet was rinsed twice with 1ml of 70% ethanol and dry pellet.
   9. The pellet was re-suspended in 20 - 40µl of TE buffer or distilled water for further use. (TENS composition: Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%.)

2. **Plasmid curing of isolates**

   1. The bacteria cells were grown in nutrient or Mueller Hinton broth overnight
   2. 5mls of Nutrient broth supplemented with 1mg/ml acridine orange was prepared
   3. Organisms were sub-cultured into Nutrient broth containing the acridine orange.
   4. It was incubated at 37ºC from 48 hours to about one week
   5. Cured organism was Plate out on Nutrient agar.

3. **Antimicrobial susceptibility of post-plasmid curing isolates.**

The disk diffusion method of Bauer and Kirby was used for the antimicrobial assay of post plasmid curing experiment of the isolates. Obtained colonies were selected and plated out on Muller Hilton agar containing the following antibiotic disks: CPX= Ciprofloxacin (10µg), COT= Co-trimoxazole (25µg), AM= Amoxacillin (30µg),SP=Sparfloxacin (30µg), CH=Chloramphenicol (30µg), AU=Augmentin (30µg), CAZ=Ceftazidime(30µg). All plates were incubated aerobically overnight at 37°C. The zones of inhibition was measured (mm) using a millimetre rule and the results were compared with the zones of inhibition for the initial zones of inhibition for the clinical isolates recommended standards by National Committee for Clinical Laboratory Standards for the interpretation of zone of inhibition was used for the interpretation of zones of inhibition.

2.8 *Escherichia coli* Serotyping with *Escherichia coli* O157 Antiserum [20]

1. Two separate drops of normal saline (0.85% sodium chloride) was placed on a clean glass slide.

2. An *Escherichia coli* colony from an overnight culture plate was taken and mixed thoroughly with both drops of normal saline on the slide to obtain a smooth suspension.
3. One drop of antisera was added to one of the bacterial suspension drops on the slide, to the other (control), one drop of normal saline was added.
4. The antiserum with the bacterial suspension was mixed using a sterile applicator stick. While the saline (control) was mixed with a fresh sterile applicator stick.
5. The slide was gently rocked back and forth for one minute and observed for agglutination under normal lighting conditions and with low power objective to ascertain agglutination.

2.9 Statistical Analysis

The data was analyzed using statistical packages for social sciences (SPSS, Version 20.0). Data were expressed as percentage frequencies.

3. RESULTS AND DISCUSSION

Table 1 shows the demographic data of the patients with gastroenteritis in Federal Medical centre Owo, Ondo State. It showed that the age distribution of the patients were 22(19.1%) and 93(80.0%) within the age group 0-2years and 3-5years respectively. Age group 0-2years had 4(4.6%) isolation rate while age group 3-5years had 93(80.9%) isolation rate.

The sex distribution of the patient showed that there were more of female than male in ratio 53:62. Male subject with gastroenteritis were 3 (35.7%) while female subjects were 56 (64.4%).

Table 2 shows the cultural characteristic and morphological appearance of bacterial isolates. There were 70 (Seventy) isolates with about 1.5mm in size, pinkish in colour with serrated edges, opaque, soft, odourless and not pigmented. Another 5 (Five) isolates were about 1.8mm in size, creamy in colour, raised elevation with circular edges, opaque, soft in consistency, with odour and not pigmented. 10 (Ten) isolates were also seen to be about 2.0mm in size, greyish, flat with circular edges, smooth with odour and pigmented with greenish discolouration. Lastly, 2 (two) isolates were recovered from the sample with 1.3mm in size, creamy, raised with circular edges, soft with odour and not pigmented.

The table in Table 3. shows that isolates that are catalase +veindole +ve, citrate–ve, urease –ve, Oxidase–ve, motile and slant (acidic), butt (acidic), (+) and H_2S (-) were identified as *Escherichia coli.* Those isolates that were catalase +veindole -ve, citrate+ve, urease +ve, Oxidase–ve, motile and slant (alkaline), butt (acidic), Gas (+) and H_2S (+) were identified as *Proteus mirabilis.* Isolates that shows catalase +ve, indole–ve,citrate +ve, urease –ve, Oxidase +ve, slant (alkaline), butt (alkaline), Gas (-) and H_2S (-) were identified as*Pseudomonas aeruginosa.* Lastly those that are catalase +ve, indole–ve,citrate-ve, urease –ve, Oxidase–ve, slant (alkaline), butt (acidic), Gas (+) and H_2S (+) were identified as *Salmonella typhi.*

The table in Table 4 shows that *Escherichia coli* predominates the bacteria isolated with occurrence rate of 70(80.5%), followed by *Pseudomonas aeruginosa* with 10 (11.5%), *Proteus mirabilis* and *Salmonella typhi* has low isolation rate with 5 (5.7%) and 2 (2.3%) respectively.

The table in Table 5 revealed that 70 (seventy) *Escherichia coli*were isolated out of which 17(24.28%) were *Escherichia coli*0157 and 53 (75.71%) were Non-*Escherichia coli*0157.

Table 6. shows the antibiotic resistance pattern of the bacteria isolates to commonly used antibiotics and it reviewed that *Escherichia coli* 0157was resistant in the following sequence7(41.2%), 3(17.6%), 0(0.00%), 0(0.00%), 4(23.5%), 13(76.5%), 1(5.9%) to CPX (10ug), COT (25ug), AM (30ug), SP (30ug), CH (30ug), AU(30ug), CAZ(ug) respectively. Non-*Escherichia coli* 0157was resistance by19 (35.8%), 11(20.8%), 23(43.4%), 6(11.3%), 4(7.5%), 33(62.3%), 13(24.52) resistance. *Proteus mirabilis* was3 (60.0), 1(20.0), 3(60.0), 0(0.00), 0(0.00), 4(80.0), 5(100.0) to CPX (10ug), COT (25ug), AM (30ug), SP (30ug), CH (30ug), AU (30ug), CAZ (ug) respectively. *Pseudomonas aeruginosa*was5(50.0%), 6(60.0%), 3(30.0%), 3(3.0%), 3(30.0%), 10(100.0%), 4(40.0%) to CPX (10ug), COT (25ug), AM (30ug), SP (30ug), CH (30ug), AU(30ug), CAZ(ug) respectively. *Salmonella typhi* was 2(100.0%), 2(100.0%), 2(100.0%), 2(100.0%), 2(100.0%), 2(100.0%) and 2(100.00%) to CPX (10ug), COT (25ug), AM (30ug), SP (30ug), CH (30ug), AU(30ug), CAZ(ug) respectively.

Table 1. Demographic data of patients with gastroenteritis

Age (Years)	Number ofstool examined (%)	Numberyielded bacterial growth (%)
0-2	22 (19.1)	4 (4.6)
3-5	93 (80.9)	83 (95.4)
Total	115 (100)	87 (100)
Sex		
Male	53 (46.1)	31 (35.7)
Female	62 (53.9)	56 (64.4)
Total	115 (100)	87 (100)

Table 2. Colonial morphology of the bacterial isolates from cultured stool sample of children with gastroenteritis

Bacterial Growth	Size	Colour	Elevation	Edges	Opacity	Consistency	Odour	Pigmentation (mm)
I_1(n=70)	1.5	Pinkish	Raised	Serrated	Opaque	Soft	Odourless	Not Pigmented
I_2 (n=5)	1.8	Creamy	Raised	Circular	Opaque	Soft	Odour	Not Pigmented
I_3 (n=10)	2.0	Greyish	Flat	Circular	Opaque	Smooth	Odour	Pigmented
I_4(n=2)	1.3	Creamy	Raised	Circular	Opaque	Soft	Odour	Not pigmented

Table 3. Biochemical characteristics and Gram reaction of the isolates recovered from children with gastroenteritis

Isolates	Gram Reaction	Catalase	Indole	Citrate	Urease	Oxidase	Motility	TSI (Reaction)	Presumptive Organism
I_1 (n=70) Butt-Acid	GNB	+ve	+ve	-ve	-ve	-ve	Motile	Slant-Acid Gas- (+) H_2S- (-)	*Escherichia coli*
I_2 (n=5) Butt Acid	GNB	+ve	-ve	+ve	+ve	-ve	Motile	Slant-Alkaline Gas- (+) H_2S- (+)	*Proteus mirabilis*
I_3 (n=10) Butt Alkaline	GNB	+ve	-ve	+ve	-ve	+ve	Motile	Slant-Alkaline Gas- (-) H_2S- (-)	*Pseudomonas aeruginosa*

Isolates	Gram Reaction	Catalase	Indole	Citrate	Urease	Oxidase	Motility	TSI (Reaction)	Presumptive Organism
I_4 (n=2) Butt- Acid	GNB	+ve	-ve	-ve	-ve	-ve	Motile	Slant-Alkaline Gas- (+) H_2S- (+)	*Salmonella typhi*

Key: + (Positive), - (Negative), GNB (Gram Negative Bacilli), Alkaline (Red colour), Acid (Yellow colour).

Table 4. Isolation reaction rate of bacterial with growth

Bacteria	Number of isolates (%)
Escherichia coli	70 (80.5)
Proteus mirabilis	5 (5.8)
Pseudomonas aeruginosa	10 (11.5)
Salmonella typhi	2 (2.3)
Total	87 (100)

Table 5. Serotyping of *Escherichia coli* isolated from children with gastroenteritis

	Number of positive (%)
Escherichia coli 0157	17 (24.3)
Non-Escherichia coli0157	53 (75.7)
Total	70 (100)

Table 6. Antibiotic resistance pattern of bacteria isolated from patients with gastroenteritis

Bacteria	Antibiotics (%)						
	CPX (10ug)	COT (25ug)	AM (30ug)	SP (30ug)	CH (30ug)	AU (30ug)	CAZ (30 ug)
Escherichia coli0157 (n=17)	7(41.2)	3(17.6)	0(0.0)	0(0.0)	4(23.5)	13(76.5)	1(5.8)
Non-*Escherichia coli (n=53)*	19(35.8)	11(20.8)	23(43.4)	6(11.3)	4(7.5)	33(62.3)	13(24.5)
Proteus mirabilis (n=5)	3(60.0)	1(20.0)	3(60.0)	0(0.0)	0(0.0)	4(80.0)	5(100.0)

Bacteria	Antibiotics (%)						
	CPX (10ug)	COT (25ug)	AM (30ug)	SP (30ug)	CH (30ug)	AU (30ug)	CAZ (30 ug)
Pseudomonas aeruginosa (n=10)	5(50.0)	6(60.0)	3(30.0)	3(30.0)	3(30.0)	10(100.0)	4(40.0)
Salmonella typhi (n=2)	2(100.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)

Key : CPX= Ciprofloxacin, COT= Co-trimoxazole, AM= Amoxacillin, SP=Sparfloxacin, CH=Chloranphenicol, AU=Augmentin, CAZ=Ceftazidime

Table 7. Pre and Post plasmid sensitivity of the bacteria isolated from gastroenteritis

Isolate	CPX(10ug)		COT(25ug)		AM(30ug)		SP(30ug)		CH(30ug)		AU(30ug)		CAZ(30ug)		Interpretation
	(pre)	(post)	(pre)	(post)	(pre)	(post)	(pre)	(post)	(pre)	(post)	(pre)	(post)	(pre)	(post)	
Escherichia coli	R	S	R	S	R	S	R	S	R	S	R	S	R	S	Plamid mediated
Escherichia coli	R	S	R	S	R	S	R	S	R	S	R	S	R	S	Plamid mediated
Pseudomoas aeruginosa	R	S	R	S	R	S	R	S	R	S	R	S	R	S	Plamid mediated
Proteus mirabilis	R	S	R	S	R	S	R	S	R	S	R	S	R	S	Plamid mediated

Key: Pre (Pre plasmid sensitivity pattern), Post (Post plasmid curing sensitivity pattern), R (Resistant), S (sensitive)

Table 7 shows the Pre and Post plasmid sensitivity and resistance pattern of the bacteria isolated from gastroenteritis in which *Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* were resistant to amoxicillin, chloramphenicol and co-trimoxazone respectively and shows plasmid mediated antibiotic resistance pattern while *Salmonella typhi*was not plasmid mediated.

4. DISCUSSION

Acute gastroenteritis (AGE) is a foremost reason of disease and death in children aged less than 5 years in developing countries where limited access to poor sanitation, potable water, and food product contamination and deficient hygiene are prevalent [21].

In this study the age and sex distribution of patients with gastroenteritis were revealed in Table 1.0 showing their age group as 0-2years and 3-5years. 53 (46.1%) were male while 62(53.9%)were female respectively. A total number of 115 stool sample were examined and number of bacteria growth yielded were 4(4.6%) and 83(95.4%) among age group 0-2years and 3-5years respectively. The high occurrence of gastroenteritis recorded among age group 3-5years was due to their exposure while playing. This was in agreement with study conducted by Moharana et al*.* [22] who attributed this to the playful and crawling attitude of children of that age group as compared to age group 0-2year. Also, there were more of females than male children that were presented with gastroenteritis with 56(64.4%) and 31(35.7%) bacteria isolates respectively which was in agreement with study conducted by Lingfei et al*.*[23] and this is attributed to the high increased female children participation in the study.

The cultural characteristics of the bacterial growth on Mac-conkey agar were based on their size in (mm), colour, odour, pigmentation, elevation, edges, consistency and opacity which was similar to the identification by Ochei and Kolhatkar [14] as shown in table 2. Also, the bacteria biochemical characterization and Gram reaction of the bacteria isolates were similar to the one stated by Cheesbrough [13] as seen in Table 3.0.

In this study a total of eighty seven (87) bacteria were isolated from one hundred and fifteen (115) patient with gastroenteritis, isolates belonging to four different genera were identified with *Escherichia coli* dominating the occurrence rate 70 (80.5%),*Pseudomonas aeruginosa*10(11.5%), *Proteus mirabilismirabilis*5(5.8)and *Salmonella typhi* was the least dominating 2(2.3%). The specie of bacteria isolate was similar to the one isolated by Rathaur et al*.*[24], the high rate of antibiotic use could have suppressed the growth of other bacteria giving room for *Escherichia coli* to thrive well. Incidence of other bacteria (*Pseudomonas aeruginosa* and *Proteus mirabilis*) among the study participants strongly suggests that individuals studied have either just acquired the organisms at an early stage of infections or suffered from previous infections due to the organisms and hence are carriers of the organisms. The wide array of organisms agrees with previous work by Rathaur et al.[24] that simultaneous infection by some pathogens may lead to a synergistic effect on the severity of the disease. *Escherichia coli* being the most predominant in this study is similar to the findings of Ifeanyi et al*.* [25] and the low incidence of *Salmonella typhi*is in conformity with the findings from Abakaliki, south –eastern Nigeria [26] and Akinnibosun and Nwafor [27]. The isolation of *proteus mirabilis*and *Pseudomonas aeruginosa* were also in accordance to study carried out by Adejo et al*.*[1] been the fact that these bacteria are usually normal flora of human intestinal tract but could become opportunistic pathogens responsible for a wide range of infections [28].

However, *Escherichia coli* remains a normal intestinal flora and as such, *Escherichia coli* isolated in this study were serotyped with *Escherichia coli*O157 antisera out of which 17(24.28%) were *Escherichia coli* 0157 and 53 (75.7%) were Non-*Escherichia coli* 0157.*Escherichia coli* O157 are recognized as important aetiological agents of diarrhoea in children that causes mild diarrhoea and leads to haemolytic uremic syndrome or death, particularly in developed countries [29]. The prevalence of *E.coli*O157 related gastroenteritis was significantly associated with rural residence, under-cocked meat consumption, raw vegetable consumption, dysentery, type of diarrhea, number of under-five children contained in the household, educational status of mothers, livestock ownership, and households with a history of diarrhea and was in accordance with study conducted by Getaneh et al*.* [30] that identify *E.coli*O157 strain in 15.3%. Also, majority of studies had shown that *Escherichia coli* remains the most important etiological agents of childhood diarrhoeal and represents a major

public health problem in developing countries like Nigeria [31].

Antimicrobial resistant pattern of the bacteria isolates in this study is frighten because it ranges from 5.8% to 100.0%,*Salmonella typhi* were resistant to all antibiotic used. Non-*Escherichia coli*O157 has high rate resistance pattern which may be as a result of large number of the isolate of non-*Escherichia coli*O157 in the study. *Pseudomonas aeruginosa* were resistant to the commonly used antibiotics in this study which was similar to the study conducted by Yayan et al*.* [32]. All the isolates in this study were resistant to augmentin and is in accordance to study conducted by Mzungu et al*.* [33].Most of the bacterial pathogen isolated were multidrug resistant to the antibiotic disc used in the study which was similar to the work by Moharana et al*.* [22]. However, such antibiotic resistance pattern might be because of early administration of antimicrobial therapy leading to prevalence of resistant strains [34]. Above all, antimicrobial agent with the least resistant pattern (Amoxacillin and sparfloxacin) could be used as agent of treatment in gastroenteritis.

Plasmids are extra-chromosomal materials important in horizontal gene transfer and are critical in facilitating genome restructuring by providing a mechanism for distributing genes that provide a selective advantage to their host [35]. In this study, the resistance plasmids to the various isolates were very diverse and distributive among the isolates was shown in Fig. 1. Plasmid profile analysis of 6 (six) bacteria isolates by agarose gel electrophoresis showed a total of 7 (seven) different plasmid bands occurring in various combinations. The size of these bands ranged from 2120 to 1910bp (Fig. 1). The distribution of different plasmid bands of these isolates appeared to have been discriminated at random. The plasmid profiles were compared with reference molecular marker “Supermix DNA ladder” (M). After electrophoresis the bands size were estimated comparing with DNA maker by careful eye estimation. In the plasmid profile, it was observed that out of 6 (six) bacteria isolates 4 (four) (*Escheria coli* O157, Non-*Escheria coli, Pseudomonas aeruginos a*and *Proteus mirabilis*) showed plasmid band which implies that the antibiotic resistance pattern was plasmid mediated while the other 2 (two) isolates without band (*Salmonella typhi*) were resistant to all antibiotics but they did not possess any plasmid band which was similar to the study conducted by Alam et al*.* [36]. Those without plasmid could carry other antibiotic resistance genes not tested in this study or be associated with virulence, conjugation, metal resistance, or other significant bacterial traits that have been associated with megaplasmids [37-39].

5. CONCLUSION

Gastroenteritis remain a major problem among children in developing country including Nigeria. *Escherichia coli* and *Escherichia coli* O157 were identify in this study which suggested poor hygenicity. Age also play a significant role in the prevalence of gastroenteritis among the study participants. Multi-drug antibiotic resistance pattern was observed with plasmids found randomly distributed among bacteria isolates. Above all, antimicrobial agent with the least resistant pattern (Amoxacillin and sparfloxacin) could be used as agent of treatment in gastroenteritis.

CONSENT

As per international standard, parental written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical clearance of this work was obtained from the ethical committee of the Federal Medical Center, Owo after due process was followed. Study participation is at free-will and individual whom are not willing to participate in the study were given the autonomy withdraw from the study at any time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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