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Research Article

Molecular Characterization of Enteropathogenic Escherichia coli (EPEC) among Children with Gastroenteritis in Ogun State, Nigeria

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Abstract: Enteropathogenic *Escherichia coli* is one of the most important pathogens causing severe and persistent watery diarrhoea, abdominal pain, fever, vomiting and often lead to death in humans. Diarrhoeic faecal samples were collected from 210 children (aged ≤ 5 years) in three different hospitals which are Federal Medical centre Idi-Aba Abeokuta, Otunba Tunwase Padectrics Centre, Ijebu Ode and Olabisi Onabanjo Teaching hospital Sagamu after questionaires were administered. The stool samples were cultured for bacteria on Eosin methylene blue agar, MacConkey agar, and Salmonella-Shigella agar. *Escherichia coli* were identified using biochemical characterization. Enteropathogenic *Escherichia coli* were serotyped by slide agglutination test using specific Enteropathogenic *Escherichia coli* monovalent antisera (EPEC O111, O126, O86, O55, O128 and O26). Antibiotic sensitivity was carried out using disc diffusion method. Plasmids from multi-drug resistant Enteropathogenic *Escherichia coli* were extracted by Alkali-lysis method and electrophoresed. Genomic DNA were extracted from Enteropathogenic *Escherichia coli* and subjected to multiplex Polymerase Chain Reaction for detection of effacing and attaching (*eae*), bundle-forming pili (*bfp)* and enteropathogenic *Escherichia coli* adherence factor (*eaef)* genes. The strains that exhibited multiple drug resistant were 21(18.8%) with plasmids detected in 12(57.1%) of the 21 multi-drug resistance *Escherichia coli*. Twenty-one of the Enteropathogenic *Escherichia coli* strains possessed effacing and attaching genes, 6 possessed *bfp w*hile 18 possessed *eaef.* Typical Enteropathogenic *Escherichia coli* strains were more prevalent (tEPEC) with (71.4%) than atypical (aEPEC) (28.6%). The molecular characterization of the Enteropathogenic *Escherichia coli* among children in this study, revealed typical Enteropathogenic *Escherichia coli* (tEPEC) as the dominant strain in Ogun state.

Keywords: Multidrug, Enteropathogenic *E. coli*, Prevalence, Gastroenteritis, Typical Enteropathogenic *E. coli.*

INTRODUCTION:

Gastroenteritis is an infection of the stomach that causes diarrhoea and vomiting. The illness is characterized by fever, diarrhoea that is often severe and can result in dehydration, abdominal distension and paralytic ileus (Singh, 2010). It has been demonstrated that the incidence of community acquired Enteropathogenic *Escherichia coli* infection is high in the six-month period following childbirth (Nweze, 2009) and that the infection is more severe in younger children (Cravioto *et al.*, 1998).

Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. *Antibiotic treatment of common bacterial infections plays a crucial role in reducing morbidity and mortality of diseases (Jafari et al., 2008). T*he widespread misuse of antimicrobial agents in the treatment of infections might be the reason why there is a serious problem of antimicrobial resistance (Akingbade *et al.*, 2014).

Diarrheagenic Escherichia coli is the major cause of gastroenteritis in children in the developing world and is associated with high resistance levels to antibiotics (Ochoa and Contreras, 2011). Escherichia coli strains have been associated with a number of disease syndromes, among these, often severe and fatal infections are pyelonephritis, septicemia, meningitis, endocarditis, and epidemic diarrhoea in adults and children.

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Escherichia coli is a genetically and phenotypically diverse species whose strains are identified on the basis of 'O', 'H' and sometimes 'K' antigens, which together constitute the serotype. Based on toxigenicity, *Escherichia coli* is now classified into distinct groups, viz. enterotoxigenic *Escherichia coli* (ETEC), which causes diarrhoea by producing heat-labile and/or heat-stable enterotoxins. Verocytotoxin or Shiga toxin-producing *E. coli* (VTEC or STEC) that causes severe disease in humans, such as haemorrhagic colitis and heamolytic uremic syndrome by the production of Shiga toxin (*stx1, stx2* and their variants). Those VTEC strains that are able to induce haemorrhagic colitis and heamolytic uremic syndrome are called enterohaemorrhagic *E. coli* (EHEC). Necrotoxigenic *E. coli* (NTEC) is able to elaborate two types of cytotoxic necrotizing factors (CNF1 and CNF2)

(Orden *et al.*, 1999; Staats *et al.*, 2003). Enteropathogenic *Escherichia. coli* (EPEC), a term more recently used as a synonym for attaching and effacing *E. coli* (AEEC), is an emerging cause of diarrhoea in humans and animals (Beutin *et al.*, 2003)

Enteropathogenic *Escherichia coli* belongs to a group of *Escherichia coli* collectively known as attaching and effacing pathogens based on their ability to form distinctive lesions on the surfaces of intestinal epithelial cells (Lai *et al.*, 2013). The attachment and effacing of EPEC is due to a pathogenicity island known as the locus of enterocyte effacement (LEE). Typical Enteropathogenic *Escherichia coli* isolates carry a large EPEC adherence factor plasmid that encodes for bundle-forming fimbriae (Johnson and Nolan, 2009) while atypical enteropathogenic *Escherichia coli* isolates has attaching and effacing gene alone.

World Health Organization recognized Enteropathogenic *Escherichia coli* O111 serotypes of 12 different O serogroups (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158). The aim of the present study was to identify and molecularly characterize Enteropathogenic *Escherichia coli* isolates among children within age 5years and to characterize and determine the prevalence of effacin and attaching (*eae*A), bundle forming pili *(bfp)* and Enteropathogenic *Escherichia coli* adherence plasmid (*eaf*) produced by the Enteropathogenic *Escherichia coli* in Abeokuta, Ogun State Nigeria.

MATERIALS AND METHODS

This study was carried out at Federal Medical Centre, Idi Aba, Abeokuta, Otunba Tunwase National Paediatrics Centre Ijebu Ode, and Sagamu teaching hospital Sagamu.

The study population is patients with gastroenteritis aged 6month to 5 years attending the peadiatric hospitals. Children age 5 years and minimum age with gastroenteritis were enrolled in this study. While children age above 5 years and adult with or without gastroenteritis were excluded from the study. Total of two hundred and ten diarrhoea faecal samples were collected from the patients. The samples were collected into sterile universal bottle. The name, age and sex of the patients were properly labelled on the universal bottles

Ethical approval was obtained from the management of the hospitals. Informed assent were obtained from patients mothers/guardians and clinicians involved in the management of the patients examined.

Collection of Faecal Sample

Fresh stools specimens obtained from patients were macroscopically examined for blood and mucus, colour and consistency. Stool samples were collected in wide mouth plastic containers and sterile swap and were then transported to the laboratory.

Microbiological Studies:

The diarrhoea samples were inoculated aerobically on sterile MacConkey agar and Eosin Methylene Blue and Deoxycholate Citrate Agar. The agar plates were incubated aerobically at 37^{o}C for 24 hours. Isolates showing greenish metallic sheen with dark purple centre on Eosin Methylene Blue was subculture on MacConkey agar and Eosin Methylene Blue agar to get a pure colonies

Characterization and Identification of Isolates

Isolated pure cultures of bacteria were subjected to various morphological and biochemical tests. After which they were identified using Bergey's Manual of Systematic bacteriology. The following tests were carried out: Gram stain, Motility, Oxidase test, Urease test, Indole test, Methyl red test, Vogue Proskauer test, Citrate test, Catalase test, Coagulase test, Fermentation of glucose, lactose and sucrose.

Serological Identification for Enteropathogenic *Escherichia coli*

Isolates biochemically identified as *Escherichia coli* isolates were subjected to slide agglutination test using monovalent EPEC O - specific antisera according to the instruction of the manufacturer (Oxoid, UK). The overnight pure culture of the different *Escherichia coli* isolates were emulsified in a drop of 0.85% saline and mixed to form a smooth suspension on a clean dry tile. A drop of antiserum (O111, O126, O86, O55, O128 and O26) were added to each suspension and mixed. The suspensions were spread to cover the reaction area and rocked for one minutes. The same was done with the control latex and control organisms. Agglutination indicated positive reaction while no agglutination indicated negative reaction.

Antibiotic Susceptibility Testing

Commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the one hundred and twelve *Escherichia coli* isolates. Ten different antibiotics with different disc concentration such as Gentamycin (Gen) 10 µg/disc, Erythromycin (Ery) 15 µg/disc, Ceftriaxone (Cef) 30µg/disc, Cotrimoxazole (Cot) 25 µg/disc, Tetracycline (Tet) 30 µg/disc, Streptomycin(Str) 10 µg/disc Cloxacillin 5 µg/disc (Cxc), Amoxicillin (Amx), 25 µg/disc, Cefuroxime (Cxm) 30 µg/disc, Ceftazidime (Caz) 30 µg/disc were used in this study.

The antimicrobial sensitivity test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method (Bauer *et al.,* 1966). The turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution. The standardized bacterial suspension was inoculated on to Muller Hinton Agar (Lab M Laboratories, Mumbai, India) and left to dry for 10 minutes, before placing the antimicrobial sensitivity discs. Antibiotic impregnated discs of 8mm diameter were used for the test. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter interpretative chart CLSI, (2007) to determine the sensitivity of the isolates to antibiotics. Standard strain of *Escherichia coli ATCC*25922 was used as control.

Plasmid Extraction

Plasmids were extracted from twenty one different Enteropathogenic *Escherichia coli* isolates exhibiting multiple resistance to the antibiotics tested by the alkaline lysis method using Zyppy ™ Plasmid Miniprep Kit UK (Sambrook and Russell, 2001). Pure Enteropathogenic *Escherichia coli* isolate was inoculated on Mueller Hinton agar and incubated overnight. The grown culture (600µl) was transferred into a 1.5ml micro-centrifuge tube and was centrifuged for 30 seconds before the supernant was discarded. Sterile distilled water (600 µl) was added to the bacterial cell pellet and was re-suspended completely. The lysis buffer (100 µl) was then added and mixed by inversion of the tube 4-6 times within 2 minutes, the lysis buffer changed from opaque to clear blue indicating complete lysis. The neutralizing buffer (350 µl) was added and mixed thoroughly until the sample turned yellow forming a yellowish precipitate indicating complete neutralization and was centrifuged for 3 minutes. The supernant was transferred into column provided and was placed in a collection tube and centrifuged for 15 seconds. The flow through was discarded, 200 µl of the Wash Buffer was added to the column and centrifuged for 30 seconds before 400 µl of the Wash Buffer was added. This was centrifuged for 1minute and transferred into a clean 1.5ml micro - centrifuge tube. Elution Buffer (30 µl) was added directly into the column matrix and allowed to stand for one minute at room temperature after which it was centrifuged for 30 seconds to elude the plasmid DNA.

Agarose gel electrophoresis

Powdered agarose (0.8% w/v) was boiled in tris acetic, EDTA (TAE) buffer intermittently until the solution becomes a clear gel. The agarose solution was allowed to cool to 45^{o}C before 7µl of the ethidium bromide was added. The clear gel solution was poured into the gel tray with comb in place and allowed to solidify. Thereafter, the gel tray and the comb were removed. The gel was placed into the tank containing the gel buffer. Then, 2µl of the tracking dye (Bromophenol blue) was mixed with 1µl of the marker and loaded into the first well. Thereafter, 20µl of the bromophenol blue with 20µl of the sample was mixed and loaded into other wells. The cover of the tank was carefully placed on it, and plugged to the power source to run from negative to positive direction making sure it did not run a distance far more than ¾ of the gel for approximately 30 minutes. Then, the gel was viewed via the U-V transilluminator (Maniatis *et al.,* 1982). A 1kb standard DNA molecular weight marker (Gene Mate, UK) was used in the study

Genomic analysis: DNA extraction

Bacteria genomic DNA were extracted from each of the 21 different Enteropathogenic *Escherichia coli* serogroups using genomic DNA mini kit (QIAGEN - QIAamp, USA).

Detection of effacing and attaching and bundle forming pili genes among Enteropathogenic *Escherichia coli* isolates by Multiplex Polymerase Chain Reaction

The pure DNA of each of the 21 different Enteropathogenic *Escherichia coli* serogroups were subjected to the multiplex polymerase chain (mPCR) reaction, with the *eaeA and bfp* genes being targeted. Primers used were obtained from Fermentas™, Germany and were designed based on the sequences of the *eaeA and bfp* genes (Gunzburg *et al.,* 1995).

The primer sequences for *eae A* were forward primer 5' CCCGAATTCGGCACAAGCATAAGC-3' and reverse primer 5' CCCGGATCCGTCTCGCCAGTATTCG -3' with an expected amplicon size of 400bp.

The primer sequences for *bfp* were forward primer 5' AATGGTGCTTGCGCTTGCTGC -3' and reverse primer 5' GCCGCTTTATCCAACCTGGTA -3' with an expected amplicon of 150bp. PCR was carried out in a total volume of 25 µl containing 3 µl template DNA, 0.5 µl of the forward and reverse primers (5 mM), 2.5 µl of 10 × buffer, 0.5 µl dNTPs (5 mM), 0.5 µl (2.5 units) Taq polymerase and 1.5 µl $Mgcl_2$; 16 µl of nuclease free water was also added. PCR was performed in a DNA thermal cycler (Applied Biosystems, Gene Amp PCR system 9700). After initial

denaturation step of 5 min at 95˚C, 40 cycles of amplification were performed. Each cycle consisted of the following steps; 1 minute at 95˚C (denaturation), 1 minute at 55˚C (primer annealing) and 1 minute at 72˚C (extension) and 72˚C for 10 minute for final extension. Ten micro litres of the reaction mixture was mixed with gel loading buffer and then resolved by electrophoresis on 1% agarose gels with the 1000bp DNA ladder (Fermentas™, Germany). Negative control consisted of all contents of reaction mixture excluding template DNA which was substituted with 3µl sterile water. The reaction products were visualized by staining with ethidium bromide. Image documentation was carried out with a Gene snap ultra violet transluminator machine and viewed on a computer (Jothikumar and Griffiths, 2002).

Detection of *adherence factor plasmid (eaef)* gene

The DNA template of the 21 different Enteropathogenic *Escherichia coli* serogroups were subjected to PCR for screening of *adherence factor plasmid (eaef)* virulence gene using specific primer (Franke *et al.,* (1994). PCR was performed in 25 µl reaction mixtures containing 2 µl of template DNA, 5 µl of 5 x PCR buffer, 4 µl of a 2.5 mM mixture of deoxynucleoside triphosphates, 2 µl of 25 mM $MgCl_2$, 0.25 µl of 5U of Ampli Taq Gold DNA polymerase per µl, and 2 µl of a 20 µM concentration of each primer. The thermo-cycling conditions were done in a Gene Amp PCR system 9700 (AB Applied Biosystems). PCR products (5 µl) were loaded onto 1% agarose gels and run at 120 mV for 30 min. A molecular marker (1-kb DNA ladder) was run concurrently. The DNA bands were visualized and photographed under UV light after the gel has been stained with ethidium bromide.

Statistical Analysis

Data generated were analysed using Statistical package for social science (SPSS) version 17.0 for windows (SPSS, Chicago IL and USA). The level of significance was considered at five percent (5%).

RESULT AND DISCUSSION

Out of the two hundred and ten diarrhoeal faecal samples cultured, 156(76.7%) yielded pure growth of bacterial isolates while nine of the faecal samples had mixed bacterial growth. *Escherichia coli* accounted for 112 (53.3%) of the bacteria isolated while *Klebsiella pneumonia* (24), *Enterobacter aerogenes* (14*) Shigella* sp (10), *Pseudomonas aeruginosa* (8) *Proteus mirabilis* (7), *Salmonella typhi* (3) and *Staphylococcus aureus* (1) constituted 11.4%, 6.7%, 4.8%, 3.8%, 3.3%, 1.4% and 0.5% respectively (Table1).

Table1: Percentage occurrence of bacterial isolates in gastroenteritis

ISOLATED ORGANISM	n=210	Number and Percentage of bacterial isolates1
Escherichia coli		112(53.3)
Klebsiella pnuemoniae		24(11)
Enterobacter aerogenes		14(6.6)
Shigella sp		10(4.7)
Pseudomonas aeruginosa		8(3.8)
Proteus mirabilis		7(3.0)
Salmonella typhi		3(1.4)

N = **Number of faecal samples collected**

The antibiotic susceptibility showed that the 112 different *Escherichia coli* isolates tested, were resistant to one or more antibiotics used. Sixty-nine (61.6%) of the isolates were resistant to ampicillin, 65(58.4%) cloxacillin while 61(54.5%) were resistant to cotrimoxazole and erythromycin respectively. Fifty-nine (52.7%) of the *Escherichia coli* isolates were resistant streptomycin, 50(44.4%) to tetracycline, 42(37.5 %) to gentamycin and 30(26.8%) to cefuroxime. While 28(25%) and 21(18.8%) were resistant to ceftriaxone and ceftazidime (Table2).

Table2: Antibiotic resistance profile of Enteropathogenic *Escherichia coli*

Antibiotic	Concentration	Number (%) resistant
Ampicillin	10µg	39 (73.6)
Cloxacillin	5µg	35(66.0)
Cotrimoxazole	25µg	31(58.5)
Erythromycin	15µg	31(58.5)
Streptomycin	10µg	29(54.7)
Tetracycline	30µg	20(37.7)
Cefuroxime	30µg	10(18.9)
Ceftriaxone	30µg	8(15.1)
Ceftazidime	30µg	2(3.8)
Gentamycin	10µg	12(22.6)

The reaction of the *Escherichia coli* isolates to different Enteropathogenic *Escherichia coli* antiserum is shown in plate 1. Out of the one hundred and twelve *Escherichia coli* isolates subjected to slide agglutination test, eighteen (16.1%) were reactive to Enteropathogenic *Escherichia coli* O111, 13(11.6%) to

O126 while 8(7.1%) were reactive to O86. Six (5.4%) of the isolates were reactive to enteropathogenic *Escherichia coli* O55, 5(4.5%) to O128 and 3(2.7%) to O26.

The distrubtion of the enteropathogenic *Escherichia coli* serotypes in the three location studied.

The prevalence of enteropathogenic *Escherichia coli* among the *Escherichia coli* isolated from children in the three different hospitals. The highest (17.8%) occurrence of enteropathogenic *Escherichia coli* in faecal samples was found among children tested in Sagamu (Table3).

Table 3; Prevalence of Enteropathogenic *Escherichia coli* among *Escherichia coli* Isolates

Location	**Number (%) of *Escherichia coli* isolates**	**Number (%) of Enteropathogenic *Escherichia coli***	**Number (%) of non Enteropathogenic *Escherichia coli***
Abeokuta	39(34.8)	18(16.1)	21(18.8)
Ijebu Ode	36(32.2)	15(13.4)	21(18.8)
Sagamu	37(33.0)	20(17.8)	17(15.2)
Total	112(100)	53(47.3)	59(52.7)

The agarose gel electrophoretic analysis of the Multi drug resistant Enteropathogenic *Escherichia coli* (EPEC) showed that out of the twenty-one multi drug resistant Enteropathogenic *Escherichia coli* analysed twelve possessed plasmid bands while nine had no bands. In figure 4, two of the multi-drug resistant Enteropathogenic *Escherichia coli* A13 and A23 had single plasmid band with molecular size of 962bp and 454bp respectively while *Escherichia coli* A29 had multiple bands (981bp and 454bp). *Escherichia coli* isolates A4, A7, A67, J84, J89 and J92 had no plasmid bands.

In figure 5, four of the multi-drug resistant Enteropathogenic *Escherichia coli* isolates A29, J106, S141 and S147 possessed multiple bands (962bp and 812bp), (745bp and 412bp) and (812bp and 412bp) respectively. *Escherichia coli* isolates J134, J140 and S152 had single band with weight of 875bp, 812bp and 745bp respectively while *Escherichia coli* isolates J123 and S160 had no bands.

In figure 6, three of the multi-drug resistant *Escherichia coli* isolates: S171, S180 and S189 possessed single band with molecular weight of 865bp, 952bp and 752bp respectively while *Escherichia coli* isolate S183 had no band.

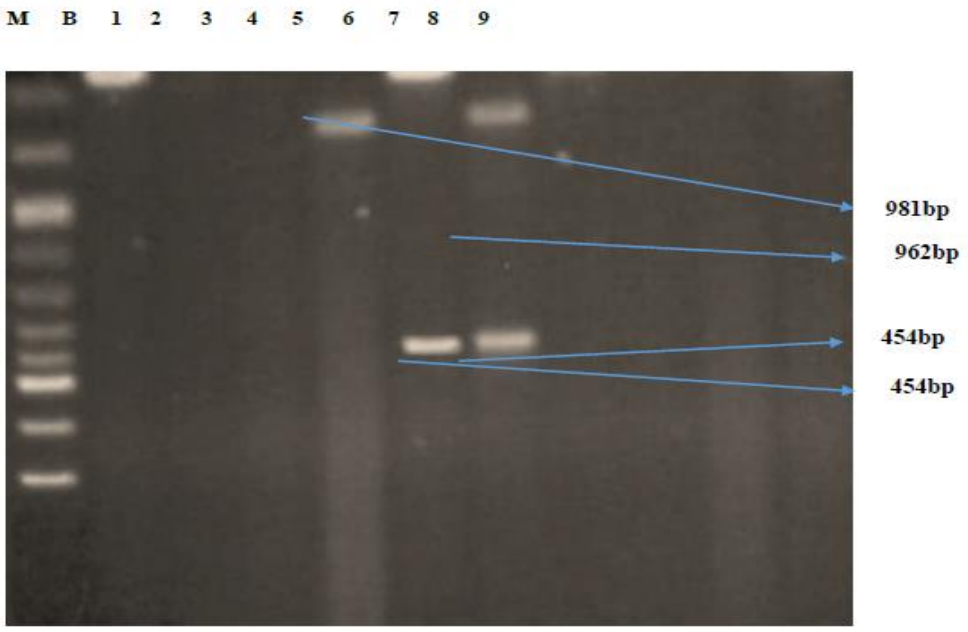

Figure 1: Plasmid profiles of the multi-drug resistant enteropathogenic *E. coli* detected from diarrhoea faecal samples of children: A13 (962bp), A23 (454bp), A29 (981bp; 454bp) no bands for A4, A7, A67, J84, J89 and J92 Isolates. *Lane* M, 1000bp Ladder.

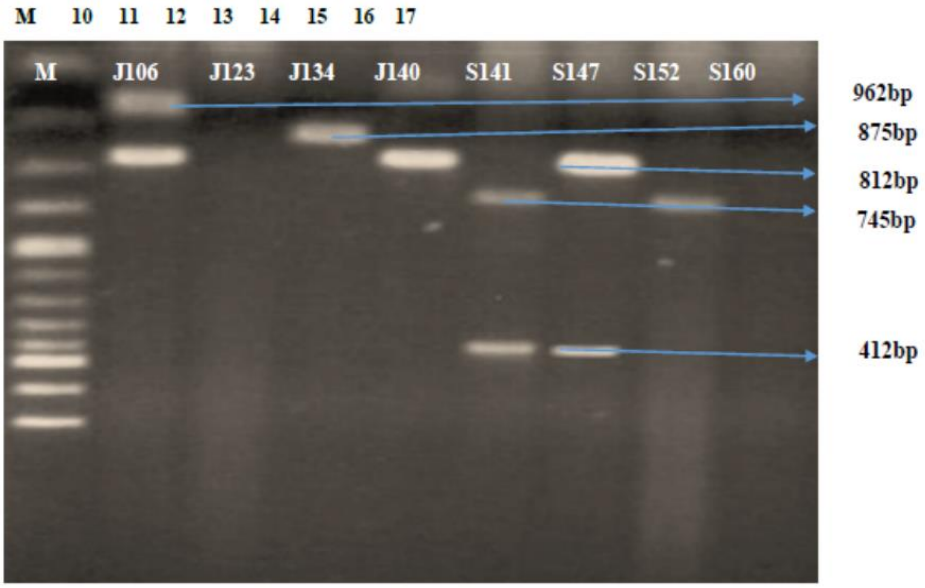

Figure 2: Plasmid profiles of the multi-drug resistant Enteropathogenic *E. coli* detected from diarrhoea faecal samples of children: J106 (962bp; 812bp), J134 (875bp), J140 (812bp), S141 (745bp; 412bp), S147 (812bp; 412bp), S152 (745bp) no bands for J123 and S160 isolates. *Lane* M, 1000bp ladder.

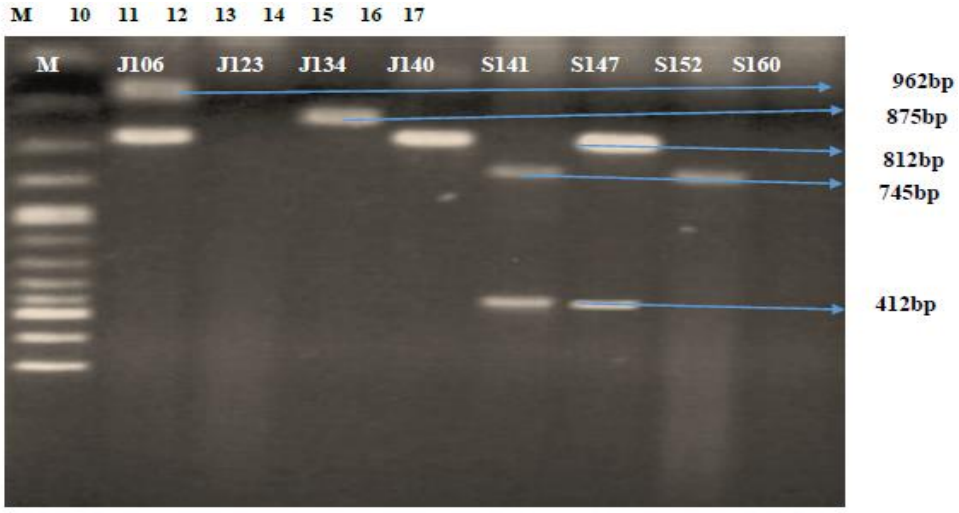

Figure 3: Plasmid profiles of the multi-drug resistant Enteropathogenic *E. coli* detected from diarrhoea faecal samples of children: S171 (865bp), S180 (952bp) and S189 (181bp) no band for S183 isolate. *Lane* M, 1000bp ladder.

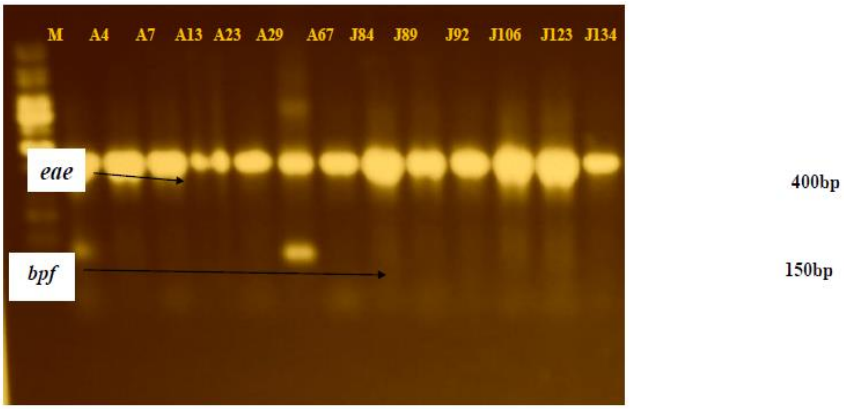

Figure 4: Agarose gel (1.0%) Electrophoretic analysis of effacing and attaching and bundle-forming pili genes detected from Enteropathogenic *Escherichia coli* isolates. *eaeA* (A4, A7, A13, A23, A67, J84, J89, J92, J106, J123, J134 and J140), *bfp* (A67). M, Lane M: Molecular weight sizes.

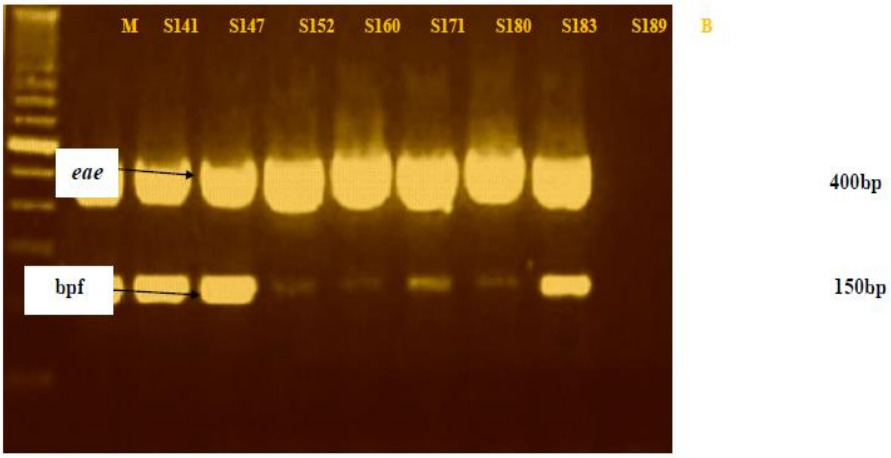

Figure 5: Agarose gel (1.0%) Electrophoretic analysis of effacing and attaching and bundle-forming pili genes detected from Enteropathogenic *Escherichia coli* Isolates. *eae A* (S141, S147, S152, S160, S171, S180, S183 and S189), *bpf* (S141, S147, S152 and S189). M, Lane M: Molecular Weight Sizes, Lane B: Blank

DISCUSSION

Out of the two hundred and ten diarrhoea faecal specimens examined, 53.3% were positive for *Escherichia coli*. This is lower than 83.1% prevalent rate reported by Ogbu *et al.*,(2008) in Abakaliki but similar to 54% reported by Akingbade *et al.*, (2014) in Abeokuta and higher than 34% prevalence rate recorded by Sule *et al.*, (2011) in Kaduna.

The rate (25.2%) of Enteropathogenic *Escherichia coli* isolation in the diarrhoea faecal samples of children in this study was similar to 28.6% reported by Motallebi *et al.*, (2011) in Iran but lower to 34.0% in Brazil, (Gomes *et al.*, 1991).

Most of the *Escherichia coli* isolates obtained in this study were resistance to ampicillin, cloxacilin, cefuroxime and gentamycin. This same trend was reported by Akingbade *et al* (2014) in Abeokuta, Kandakai-Olukemi *et al.*, (2009) in Abuja and Bii *et al.*, 2005: Brooks *et al.*, 2006 in Kenya. In this study six EPEC serogroups were identified (O111, O126, O86, O55, O128 and O26). Serotype O111 had the highest occurrence 16.1%, followed by O126 (11.6%) and O86 (7.1%).

In Aba serotype 026 had the highest prevalence 48.7%, followed by 0126 (20.5%) and 0111 (12.8%) Ome and Nonye (2015).

In Enugu, serotypes O128 and O26 were the most prevalent (Njoku-Obi and Anozie (1984) while in Venezuela serotypes O26 and O55 were most prevalent (Gonzalez *et al.*, 1997). This shows variation in occurrence of EPEC serotypes in geographical regions.

There were detectable plasmids in twelve (57.1%) of the twenty one multi - drug resistant Enteropathogenic *Escherichia coli* isolates.

The plasmids weight ranged in this study is from 412bp to 981bp. These plasmids weights were higher than 0.55 to 1.14bp plasmids reported in Rivers state, Nigeria by Clarence *et al.*, (2007).

Effacing and attaching (*eae)* genes were detected in all the twenty one multi drug resistant Enteropathogenic *Escherichia coli* in this study. Similar prevalence of *eae* gene was found in other studies (Duffy *et al.*, 2000; Effler *et al.*, 2002).

CONCLUSION AND RECOMMENDATION

Enteropathogenic *Escherichia coli* isolates was isolated from children in all the age group studied. Majority of the EPEC strains were typical EPEC.

The prevalence revealed that EPEC is one of the most important diarrheagenic agents in children within age 5 years old.

Most of the pathogenic Enteropathogenic *Escherichia coli* isolated were found to be resistant to cloxacillin, amoxicillin and ampicillin and other commonly used antibiotics in the three locations

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