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Prevalence of Extended Spectrum β -Lactamase among Diarrheogenic Strains of *Escherichia Coli* among Children in Yenagoa, Nigeria.

Ogefere, H.O.^{*1}, Ibadin, E.E.², Omoregie, R.^{2,3} and Ierhunwa, I.⁴

Department Of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University Of Benin, Benin City, Nigeria ¹, Medical Microbiology Unit, Medical Laboratory Services University of Benin Teaching Hospital, Benin City, Nigeria ², School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Nigeria ³, Department of Medical Microbiology, Medical Laboratory Services, Federal Medical Centre, Yenagoa, Nigeria ⁴.
Corresponding Author: helenogefere@yahoo.com/ +234-802-350-9447

Abstract

Diarrheogenic *Escherichia coli* (DEC) have increasingly shown to harbor resistance to antimicrobial agents including genes for extended spectrum beta-lactamases (ESBL). The aim of this study was to evaluate the prevalence of ESBL-production among DEC children less than five years. A total of 139 children aged 0 to 5 years with symptoms of diarrhea attending public and private healthcare institutions in Yenagoa were recruited for this study. DEC were recovered from these stool specimens and screened for ESBL production. A total of 57 (41.0%) DEC strains were isolated from 139 stool specimens. The isolation rate of DEC was significantly higher ($p=0.0148$) in private healthcare facilities (60%) than in public healthcare institutions (32.5%). Of the 57 DEC strains, 12 (21.1%) strains were positive for ESBL enzyme, but its distribution did not differ significantly between DEC isolates recovered from public and private health institution ($p=0.5191$). Age and gender did not significantly ($p>0.05$) affect the distribution of ESBL-producing isolates among the recovered DEC. Similarly, there was no significant difference ($p>0.05$) in the susceptibility profiles of ESBL-producing and non-ESBL producing DEC isolates. Screening for ESBL-production for DEC should be done routinely. Prudent use of antibiotics and practice of good hygiene is advocated to stem the tide of ESBL-producing DEC.

Keywords: Diarrhea, *Escherichia coli*, Extended spectrum beta-lactamase (ESBL), Children, Yenagoa, Nigeria.

Introduction

Diarrheal disease - a scourge plaguing developing countries is caused by a range of enteric pathogens such as bacteria, viruses and parasites (Hien *et al.*, 2008; Walker *et al.*, 2012). Infectious diarrhea is the second most common cause of death in children under 5 years in developing countries, with Nigeria bearing a huge chunk of the global burden (Walker *et al.*, 2012). Previous estimates have placed the number of deaths from the clinical illness at 760,000 on a yearly basis (Walker *et al.*, 2012). Diarrheogenic *Escherichia coli* (DEC) and *Shigella* species are the most popular bacterial pathogens causing diarrhea (Hien *et al.*, 2008). While antibiotics have played key roles in combating the disease when the causative agent is bacterial, resistance to antibacterial drugs have been on the increase worldwide (Hien *et al.*, 2008).

E. coli has been shown to be efficiently capable of accepting and transferring genetic materials and under stress, readily transfers those genetic materials to enteric pathogens and gut flora including *Salmonella*, *Shigella*, *Yersinia* and *Vibrio* species, therefore making it an important reservoir of transferable resistance (Aibinu *et al.*, 2007). Production of extended-spectrum β -lactamases (ESBLs) is the most common mechanism of resistance to penicillins, cephalosporins and aztreonam in the treatment of serious infections caused by the Enterobacteriaceae, notably *E. coli* (Bradford. 2001; Paterson and Bonomo, 2005). Its genetic determinants are largely plasmid borne,

being of SHV, TEM, OXA and CTX-M genes predominantly (Lautenbach *et al.*, 2001; Ogbolu *et al.*, 2013). Strikingly, multidrug resistance in ESBL producers tends to limit therapeutic options and subsequently facilitate the dissemination of these bacteria strains (Paterson and Bonomo, 2005)..

Epidemiological studies have suggested that the increasingly widespread use of third-generation cephalosporins is a major risk factor that has contributed to the emergence of ESBL-producing bacteria (Gupta *et al.*, 2003). Poor hygiene and gut colonization of ESBL-producing bacteria have been associated with subsequent infections (Meyer *et al.*, 1993). A study in Nigeria demonstrated the presence of ESBL-producing genes in *E. coli* isolated from the faeces of non-diarrhoeic out-patients (Ogbolu *et al.*, 2013). The aim of this present study was to evaluate ESBL production among DEC among children less than five years by using a phenotypic method.

Materials and Methods

Study area

Yenagoa is the capital of Bayelsa State, Nigeria with a population of 352,285 and a land area of 1,698 km² (National Population Commission, 2006). It is located in the South-South geo-political zone of the country. It is located within the tropical rain forest region of the country and more than three-quarter of it is covered by water. The residents are mainly fishermen.

Study population

A total of 139 children aged 0 to 5 years with symptoms of diarrhea attending public and private healthcare institutions in Yenagoa were recruited for this study. Informed consent was obtained from the parents of each child prior to specimen collection. The protocol for this study was approved by Bayelsa State Ministry of Health.

Specimen collection and processing

Stool specimens were collected from each child into sterile wide-mouth container, labeled and transported to the laboratory for analysis. The stool specimens were inoculated on MacConkey and Deoxycholate citrate agar and incubated aerobically at 37°C overnight. The emergent isolates were identified using standard techniques (Barrow and Feltham, 2003). Only the *Escherichia coli* isolates were used for further analysis.

Detection of extended spectrum β -lactamase

The presence of ESBL was detected in only *Escherichia coli* isolates using the double-disc

synergy test (Livermore and Brown, 2001). Briefly, test organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standards. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. A disc containing 30 μ g amoxicillin–clavulanate (Oxoid, England) was placed at the centre of the agar plate. A 30 μ g ceftazidime disc (Oxoid, England) was placed 25 mm from the amoxicillin–clavulanate disc and another disc containing 30 μ g cefotaxime (Oxoid, England) was placed on the opposite side of the amoxicillin–clavulanate disc (25 mm apart). The plates were incubated at 37°C overnight and ESBL production was inferred as positive if there was an expansion of the zone of inhibition between the ceftazidime and amoxicillin–clavulanate disc, cefotaxime and amoxicillin–clavulanate disc or both.

Disc susceptibility test

Disc susceptibility tests were performed on all bacterial isolates using the British Society for Antimicrobial Chemotherapy (BSAC) method (Andrews, 2009).

Data analysis

The data obtained were analyzed with Fisher's Exact test using the statistical software INSTAT® (Graph Pad Software Inc, La Jolla, CA, USA). A p-value of ≤ 0.05 was considered significant in all statistical comparisons.

Results

A total of 57 (41.0%) diarrheagenic *Escherichia coli* strains were isolated from stool of 139 infants with symptoms of diarrhea. The prevalence of DEC was significantly higher among children attending private health facility compared with those receiving care in public health institutions ($p = 0.0148$). Of the 57 *E. coli* strains, 12 (21.1%) *E. coli* isolates were positive for ESBL enzyme, but its distribution did not differ significantly between DEC isolates recovered from public and private health institution ($p = 0.5191$) (Table 1).

Age and gender did not significantly ($p > 0.05$) affect the distribution of ESBL-producing isolates among the recovered DEC (Table 2). Similarly, there was no significant difference ($p > 0.05$) in the susceptibility profiles of ESBL-producing and non-ESBL producing DEC isolates (Table 3)

Table 1: Distribution of ESBL-producing *E. coli* according to hospital

Health Facility	Number of infants tested	Number (%) of infants infected with <i>E. coli</i> strains.	Number (%) of ESBL positive <i>E. coli</i> strains.
Public*†	83	27 (32.5)	7 (25.9)
Private	56	30 (60.0)	5 (16.6)
Total	139	57 (41.0)	12 (21.1)

Legend

*DEC (Public vs Private): p=0.0148

†ESBL (Public vs Private): p=0.5191

Figures in parenthesis are in percentages

Table 2: Distribution of ESBL-producing *E. coli* according to gender and age

Socio-demographic factors	No (%) of infants infected with <i>E. coli</i>	No (%) of ESBL positive <i>E. coli</i> strains	p-value
Gender			0.0849
Male	24	2 (8.3)	
Female	33	10 (30.3)	
Age (years)			0.9361
≤ 1	26	6 (23.1)	
2-3	20	4 (20.0)	
4-5	11	2 (18.2)	

Legend

Number in brackets = Value in percentage

ESBL- Extended spectrum beta-lactamase

Table 3: Antimicrobial susceptibility profile of *E. coli* from stool of diarrhoeic infants.

Antibiotic	Number (%) Non ESBL-Producing <i>E. coli</i> (n= 45)	Number (%) ESBL-Producing <i>E. coli</i> (n=12)	p-value
Ceftazidime	33 (73.3)	9 (75.0)	1.0000
Cefuroxime	12 (26.7)	3 (25.0)	1.0000
Gentamicin	1 (2.2)	2 (16.6)	0.7441
Cefixime	26 (57.8)	8 (66.7)	0.3321
Ofloxacin	27 (60.0)	5 (41.7)	0.1090
Amoxicillin-clavulanate	7 (15.6)	2 (16.6)	1.0000
Ciprofloxacin	23 (51.1)	5 (41.7)	0.7470

Legend

Number in brackets = value in percentage.

ESBL- Extended spectrum beta-lactamase.

Discussion

DEC of different pathotypes has been largely implicated in infectious diarrhea among infants in various regions in Nigeria (Okeke *et al.*, 2000; Nweze, 2009; Ifeanyi *et al.*, 2015). The isolation rate of DEC in this study was 41%. This observation is consistent with findings from a previous report in South-Eastern Nigeria where 44.74% of microbial isolates were DEC (Nweze, 2009). A prevalence rate of 18.4% and 60.3% has equally been shown in Northern and South-western Nigeria respectively (Okeke *et al.*, 2000; Ifeanyi *et al.*, 2015). Among children with diarrhea, prevalence of the infection varied with geographical locations, regions within the same country, and even over time in the same location and population (Petri *et al.*, 2008). DEC isolates were recovered more from private health facilities than public health facilities (p=0.0148). Parents may patronize private health care facilities more than public due to its closeness to the people than public health facilities. This may explain the findings in this study.

ESBL-producing bacteria are of public health significance because of the limited therapeutic options they provide clinicians when they are implicated as causing infection, as well as the high cost of treating patients (Paterson and Bonomo, 2005). Failure of antibiotics could also lead to exacerbated clinical symptoms especially among infants. This study observed a 21.1% positivity rate for the ESBL among DEC isolates. Several studies from developing nations have noted different prevalence rates of ESBL-producing DEC in infants; 31.1% in Egypt, 48.7% in Iraq and 64.3% in India (Aggarwal *et al.*, 2013; Al-Dahmoshi *et al.*, 2015 and Maysaa *et al.*, 2015 respectively). This difference may be due to geographical location. At

over 350,000 deaths yearly and towering just above Nigeria, India has the highest figure for mortality due to diarrhea in children below 5 years (UNICEF, 2009). ESBL-producing strains may therefore play a contributory role in severity of symptoms and mortality rates among diarrhoeic infants.

In our study, there was no significant difference between Private and Public Hospitals in the distribution of ESBL-producing *E. coli*. Studies have shown an association between abuse of third generation cephalosporins and the emergence of ESBL-producing bacteria (Meyer *et al.*, 1993). In Nigeria, antibiotic use is not regulated and over the counter sales of antibiotics without prescription is rife (Okeke *et al.*, 1999; Omoregie and Eghafona, 2009; Ogbolu *et al.*, 2013). Similarly, the metropolis where the study was carried out is surrounded by rivers, with fishing and trading been a major occupation of residents. ESBL-producing *E. coli* has been isolated from rivers in various regions of the world such as in Netherlands (17.1%) and in South Korea (60%) (Jang, *et al.*, 2013; Franz *et al.*, 2015). In our study area, where poor hygiene is commonplace, and sewage and waste management is practically non-existent, there is a high possibility of having a higher percentage of ESBL-producing *E. coli* in surrounding rivers. Residents in our study area have similar source of water; shallow wells and rivers and may therefore have higher likelihood of been infected with ESBL-harboring DEC. Further investigations are needed to verify this.

Consumption of sea food has been associated with a reduced risk for been infected with ESBL-producing *E. coli* (Soraas *et al.*, 2013). This study showed a prevalence of 21.1% of ESBL-producing *E. coli* with a reduced positivity rate with increasing age. While we may infer a change in diet from milk-based to

fish-based with increasing age, there was no statistical significance observed with respect to age and ESBL-production. More research is therefore required in this regard, particularly, among older individuals.

In our study, ceftazidime and cefixime appeared to be the most active antibiotics against ESBL-producing *E. coli* strains. However, ESBL producers have intrinsic resistance to third generation cephalosporins (which include ceftazidime and cefixime) which could lead to treatment failure if they are administered (Paterson and Bonomo, 2005). It has been reported that ESBL-mediated resistance is not obvious in disc or dilution susceptibility testing but is associated with clinical failure (Brun-Buisson *et al.*, 1987; Rice *et al.*, 1991; Livermore and Brown, 2001). This may explain the *in-vitro* susceptibility of β -lactam drugs against ESBL-producing DEC. Most diagnostic laboratories in our country usually perform antimicrobial susceptibility testing for gram negative rods without screening for ESBL production. This may therefore be misleading to clinicians and may exacerbate the condition of these diarrhoeic infants. Several recommendations from previous studies around the world have justified screening for ESBL production as an adjunct to susceptibility testing when gram negative rods are incriminated in clinical infections (Lautenbach *et al.*, 2001; Paterson and Bonomo, 2005).

The fluoroquinolones showed moderate antibacterial activity against ESBL-producing DEC. A strong association has also been shown between quinolone resistance and ESBL production (Lautenbach *et al.*, 2001). This may explain the finding in this study. However, fluoroquinolones are contraindicated in children (Egbe *et al.*, 2011). Considering the above explanation in terms of β -lactam antibiotics and fluoroquinolones, and the finding that there was no significant difference in the susceptibility profiles of ESBL-producing and non-ESBL-producing DEC, ESBL-producing DEC were more resistant to the tested antibiotics than non-ESBL-producing DEC. Thereby, resulting in limitation of therapeutic options. This finding may not be too surprising as ESBL-producing *E. coli* harboring plasmid genes encoding ESBLs frequently also carry genes encoding resistance to other classes of antibiotics like aminoglycosides and trimethoprim/sulfamethoxazole (Paterson and Bonomo, 2005).

Summarily, an overall prevalence rate of 21.1% of ESBL-producing *E. coli* was observed for DEC

isolated from the stool of diarrhoeic infants in public and private hospitals in Yenogoa metropolis. Prudent use of antibiotics and practice of good hygiene is advocated to stem the tide of ESBL-producing DEC.

Conflict Of Interest

The authors declare no conflict of interest

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