

Antibiotic Susceptibility Profile of *Escherichia coli* and *Salmonella* Causing Childhood Diarrhoea in Awka Municipality, South-eastern Nigeria

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Abstract

Background: In developing countries diarrheal diseases in children are a major public health concern.

Objectives: This study investigated the incidence and antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* causing Childhood Diarrhea in Awka.

Methods: Twenty six (26) diarrheic stool samples were collected from children (<5) years and cultured. The isolated bacteria were subjected to various identification and biochemical tests. The 44 isolated bacteria (*E. coli* and *Salmonella* isolates) were subjected antibiotic susceptibility studies and ESBL-producing screening.

Results: *E. coli* and *Salmonella* were detected in 23 (88%) and 21 (80%) fecal specimen associated with diarrheal episodes respectively. *E. coli* showed 91% resistance to ceftazidime, 100% resistance to cefuroxime, 78% resistance to gentamicin, 91% resistance to ceftriaxone, 78% resistance to ofloxacin and 100% resistance to augmentin. *Salmonella* showed 100% resistance to ceftazidime, 100% resistance to cefuroxime, 100% resistance to gentamicin, 100% resistance to ceftriaxone, 69% resistance to ofloxacin and 82% resistance to augmentin®. Fifteen (65.2%) *E. coli* isolates were ESBL producers and 8 (34.7%) isolates were non-ESBL producers.

Conclusion: Overall prevalence of 88.5% of *E. coli* and 80.8% of *Salmonella* spp. were associated with childhood diarrhea in the studied locality. The *E. coli* and *Salmonella* spp. were multidrug resistant. Majority (65.2%) of the *E. coli* were ESBL producers thus the colonized children may be potential sources of multidrug ESBL-producing *E. coli* strains in the hospital and/or community.

Keywords: Antibiotic resistance; Childhood diarrhea; Enterobacteriaceae, *Escherichia coli*, *Salmonella*; ESBL

Introduction

Globally, Diarrheal diseases remain one of the leading causes of morbidity and mortality among children <5 years of age [1]. About 21% of all deaths in children under the age of five (years) are estimated to be due to diarrhoea and related infections. In Sub-Saharan Africa, it is the second leading cause of death in children under 5 years of age [2-4]. About 37% of all paediatric/childhood deaths in Nigeria are caused by acute diarrhoea, with most of the deaths occurring during the first year of life [5].

Diarrhoea is defined as three or more episodes of watery loose stools in the last 24 hours [5,6]. Every episode of diarrhoea in children could cause malnutrition, reduced resistance to infections with potential consequences of impaired physical and cognitive growth/development [7]. The high prevalence of diarrheal infections in low-income countries is due to lack of portable drinking water, poor food hygiene standards and inadequate sanitation [7-9].

Diarrhoea causing-microbes are transmitted through contaminated food, water, or direct contact with the improperly sanitized hands of people carrying the pathogens [10,11]. Bacteria, viruses, as well as parasites are all causative organisms for diarrhoea. The most commonly isolated diarrheagenic bacterial pathogens are the Enterobacteriaceae [4,8].

World Health Organization has underlined the importance of epidemiological surveys of childhood diarrhoea in all geographical areas [12]. Information on the etiology of diarrhoea is useful in planning and implementing control strategies to reduce diarrhoea-caused childhood morbidity and mortality in a country. This study is therefore designed to investigate the incidence of enterobacteriaceae as the main etiologic infectious agents of childhood diarrhoea and their antimicrobial susceptibility in the paediatrics units of a hospital in Awka, south-eastern, Nigeria. This we hope will help in infection control measures and equally guide paediatricians in choosing appropriate antibiotics for (empirical) treatment of diarrheal infections.

Methods

Ethical considerations

The study design/objective was explained to the parents/guardian of the children and a verbal consent was obtained from them. The study protocol was approved by the ethical committee of Chukwuemeke Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka (Ref: COOUTH/AA/VOI.I.008).

Study design

The inclusion criteria for the study participants were: (a) Being a child less than five years of age; (b) Having diarrhoea that fulfil the WHO criteria for ADD [13,14]. Those having a history of antibiotics use two weeks before enrolment were excluded from the study. A questionnaire was used to collect participants' information: age, sex, area of residence, socio-economic stratum, days of diarrhoea, date of collection etc. The mother or guardian of any child that met the inclusion criteria for sample collection was given a stool container and instructions for sample collection.

Bacterial isolation and identification

Stool samples collected in universal container, were inoculated using an inoculating loop of 10 µL volume calibration, into sterile nutrient broth contained in test tubes. All inoculated nutrient broths were incubated aerobically overnight at 37°C. After incubation, the broth cultures were examined for significant growth. A loop full from the culture in each broth, were streaked directly on well labelled MacConkey and Salmonella-Shigella agar, incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were prepared into plates of nutrient agar, and incubated for another 24 h. The isolates were stored at 4°C for further biochemical/identification studies: Gram staining, colony morphology and biochemical test (Indole, mannitol, TSI test, etc). The organisms were identified as *E. coli* and *Salmonella* spp. on the basis of colony morphology, Gram staining, motility, and biochemical reactions.

Antibiotic susceptibility test

Antibiotic susceptibility profiles of the bacterial isolates were evaluated using disk diffusion assay as described by Ekwealor et al. [15]. The antibiotic disc (ABTEK, India) containing the following antibiotics (disc content) was used: Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg) and Augmentin (30 µg). Standardized overnight culture of each isolate was seeded in melted Mueller-Hinton agar (MHA) at 45°C and aseptically poured into sterilized plates (in triplicate). The plates were allowed to solidify and the antibiotic disks were aseptically placed on the surface of the culture media. The MHA plates were thereafter incubated at 37°C for 18-24 h. After incubation, the inhibition zones were measured and interpreted by the recommendations of the Clinical and Laboratory Standards Institute [16,17].

ESBL detection test

The *E. coli* were first screened by phenotypic method for ESBL production and then phenotypically confirmed as per CLSI guidelines as described by Ejikeugwu et al. [16]. ESBL production was confirmed phenotypically in the *E. coli* isolates that showed reduced susceptibility

to any of the screening antibiotic disks of ceftazidime, cefotaxime, and ceftriaxone used. These agents were used for screening to improve the sensitivity of ESBL detection as recommended by CLSI [17]. ESBL production was confirmed if there is an increase of ≥ 5 mm in inhibition zone diameter for either of the cephalosporins (ceftazidime and cefotaxime) tested in combination with amoxicillin-clavulanic acid compared to its zone when each antibiotic is tested alone.

Results

Characteristics of the study cohort

Majority of the Diarrhoea cases were reported from the out-patient department, very few were admitted into the in-patient department. Many of the houses where the patients came from had modern features: Table 1 shows the characteristics of the study participants. Among the study participants 100% had electricity and refrigerator, 50% had washing machine, and 50% had cooking gas. Though no municipal water system, 70% of water is gotten from private provider and 30% from roof, jerry can for 50% of the patients, the other 50% get water from private providers only. The presence of sanitary water closet is reported in 50% and 50% sanitary latrine. Refuse containers were found in 100% of houses either inside or outside the dwelling. Fifty per cent (50%) boil water before drinking, 50% drink tap water while 50% had domestic animals like dog and chicken in the house.

| Characteristics | Percentage |
|-------------------------------|------------|
| Electricity and refrigerator | 100% |
| Washing machine | 50% |
| Cooking gas | 50% |
| Water from private provider | 70% |
| Water from roof jerry can | 30% |
| Sanitary water closet | 50% |
| Sanitary latrine | 50% |
| Refuse container in the house | 100% |
| Boil water for drinking | 50% |
| Tap water for drinking | 50% |
| Domestic animals | 50% |

Table 1: Percentage characteristics of the study cohort.

| Isolates | Number | Percentage |
|-------------------------|--------|------------|
| <i>Escherichia coli</i> | 23 | 88.5% |
| <i>Salmonella</i> spp. | 21 | 80.8% |
| Total | 44 | |

Table 2: Percentage incidence of the bacterial isolates.

Incidence of diarrhoea

A total of 26 diarrhoea stool samples were collected from the paediatric hospital in Awka Metropolis. Of the 26 diarrhoea stool

samples collected, *E. coli* was detected in 23 (88%) faecal specimen associated with diarrheal episodes. *Salmonella* spp. were also detected in 21 (80%) faecal specimen of diarrheal episodes (Table 2).

Antibiotic susceptibility and MARI

Figure 1 shows the resistance pattern of the isolated bacteria. Erythromycin and cloxacillin did not inhibit the growth of either of the organisms. *E. coli* showed 91% resistance to ceftazidime, 100% resistance to cefuroxime, 78% resistance to gentamicin, 91% resistance to ceftriaxone, 78% resistance to ofloxacin and 100% resistance to augumentin (Table 3). *Salmonella* spp. showed 100% resistance to ceftazidime, 100% resistance to cefuroxime, 100% resistance to gentamicin, 100% resistance to ceftriaxone, 69% resistance to ofloxacin and 82% resistance to augumentin. The two bacterial species recorded a high MAR indices of 50% (*E. coli*) and 75% (*Salmonella*).

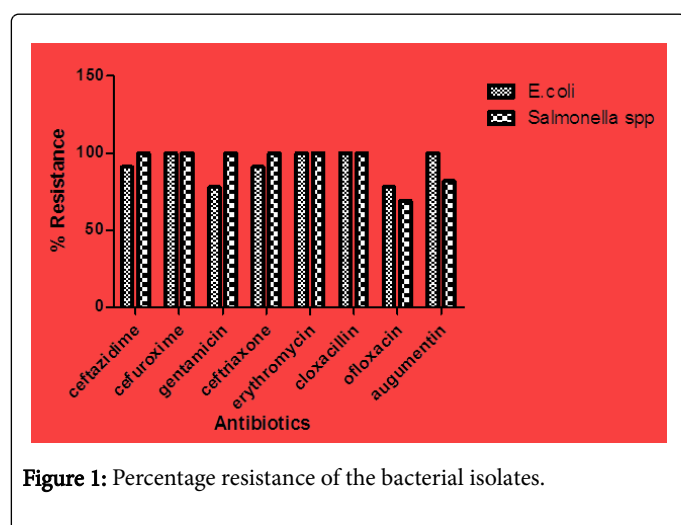


Figure 1: Percentage resistance of the bacterial isolates.

| Organism | No. of antibiotics resisted by Isolates | No. of antibiotics tested | MARI | Percentage |
|-------------------|---|---------------------------|------|------------|
| <i>E. coli</i> | 4 | 8 | 0.5 | 50% |
| <i>Salmonella</i> | 6 | 8 | 0.75 | 75% |

Table 3: Multiple antibiotic resistance index of bacterial isolates.

ESBL detection

Of the total *E. coli* isolates, 15 (65.2%) isolates were ESBL producers and 8 (34.7%) isolates were non-ESBL producers (Table 4). Figure 2 shows a pictorial effect produced by *E. coli* isolate expressing ESBL. This is characteristic of ESBL-producing bacteria because of the synergistic effect produced between a beta-lactamase inhibitor, amoxicillin-clavulanic acid, and any of the third generation cephalosporins (ceftazidime and cefotaxime).

| Isolates | Number | Percentage |
|--------------------|--------|------------|
| ESBL Producers | 15 | 65.20% |
| Non ESBL Producers | 8 | 34.70% |

Table 4: ESBL detection.



Figure 2: Mueller-Hinton agar plate showing phenotypic detection of ESBL production using the double disk synergy test (DDST) method. The bacterium is *P. aeruginosa*; the antibiotic disk on the left is ceftazidime (30 µg) while the disk on the right is cefotaxime (30 µg). The central disk is amoxicillin-clavulanic acid, a beta-lactamase inhibitor.

Discussion

Diarrheal diseases are the second leading cause of death in children less than 5 years of age. Information about household characteristics in a locality and their interrelation with diarrhoea prevalence among the children aged five years is very crucial in the infection control measures, for effective reduction of childhood morbidity and are required to make important policy decisions [6,12]. Similarly, antibiotic susceptibility testing plays a useful role in the outbreak setting and for surveillance of local trends in resistance patterns and mechanisms [6]. In this study, we highlight the importance of environmental influence on the risk of diarrhoea associated with Enterobacteriaceae during childhood. Children living in poor households are more vulnerable to diarrhoea than their wealthy counterparts [18]. The presence of a refuse container in the homes will likely increase overall risk of diarrhoea associated with *E. coli*. This association of refuse containers in the home was expected as refuse containers mostly uncovered in the study households, providing children with easy access to debris and could attract flies carrying the pathogens. Improper faecal disposal as well as lack of clean water leads to contamination of groundwater especially in areas where water filtration or purification processes are not practiced. This can help in the widespread of some bacterial pathogens among children [18,19]. Also the use of water sourced from Jerri can roof in cleaning household utensils and the presence of domestic animals in the house is also risk factors of *E. coli* associated in childhood diarrhoea. The associations strongly suggest that behavioral-hygienic and cleanliness in the household affect disease incidence. Contaminated food or drinking-water, or from person-to-person as a result of poor hygiene are known to be common means of spreading Diarrheal infection [19]. Studies have reported that variables like age of the child, quality and quantity of water, availability of toilet facilities, housing conditions, level of maternal education, household economic status etc., affect the exposure to diarrhoea pathogens and infections [18] We reported that an 88% and 80% faecal specimen yielded *E. coli* and *Salmonella* spp. respectively of diarrheal episodes This implies that there is co-infection among the study participants. Co-infection by multiple groups of enteric pathogens has been demonstrated to be the norm in diarrhoea cases [20]. The possible reasons are: (i) that when one intestinal

pathogen infects the body, the infection rate of other pathogens increases [21]; (ii), these are foodborne pathogens and can be found in the same contaminated foods increasing the likelihood of mixed infections in risk populations. It has been reported that co-infections with multiple enteric pathogens occur mainly in zones with poor quality of food, drinking water and poor sanitary conditions in the environment [20,22]. Co-infection among children with diarrhoea may cause more severe diarrhoea than infection with a single pathogen and can also increase treatment costs [20,23]. Thus we suggest that it is important to consider enteric pathogens rather than just single pathogen in every epidemiological study evaluating diarrhoea-causing agents in poor countries.

A similar percentage yield of 64% bacterial isolates of the stool samples has been reported in Kaduna, Northwestern Nigeria [24]. However, our prevalence differed from 25.1% reported in 2009 by Nweze [25].

A low susceptibility of the diarrheic organisms was observed with third generation cephalosporin (ceftazidime, cefuroxime, and ceftriaxone). The *E. coli* isolates showed moderate susceptibility to ofloxacin and gentamicin, while *Salmonella* responded better to ofloxacin and augmentin[®]. In line with our results, similar sensitive response to the fluoroquinolones was equally reported by Esegbe et al. [24]. Contrary to our results, in Niger Langendorf et al., [26] reported that most of the diarrheic *E. coli* and *Salmonella* were sensitive to antibiotics tested except amoxicillin and co-trimoxazole. Guerra et al. [27] also reported no resistance to ceftriaxone, ceftazidime and cefepime by Enterotoxigenic *Escherichia coli* clinical isolates from Northern Colombia. The MAR Index for each isolate was calculated using the relationship between the number of antibiotics to which the isolate was resistant divided by the number of antibiotics tested. Value lower than 0.2, is considered low risk, while higher than 0.2 indicates high risk [28,29]. The high MARI value observed in the study may be due to the widespread use of antibiotics in the locality [28,30]. In the present study, out of 23 *E. coli*, 65.2% (15/23) were ESBL producers by phenotypic confirmatory methods. In line with our finding, a similar high ESBL-producing was recorded of *Escherichia coli* isolated from Children with Acute Diarrhoea in Wrocław, Poland [31]. The high prevalence of ESBL-producing among the *E. coli* isolates is a big concern as they might show a false sensitive zone of inhibition in the Kirby-Bauer disk diffusion method and often remain undetected by routine susceptibility tests [26,32]. The prevalence of ESBL producing *E. coli* varies from country to country and from center to center. In the United States, ESBL producing *E. coli* ranges from 0-25% with the average being around 3% [33]. ESBL producers may have spared through communities, especially those with poor hygienic and sanitation conditions, through faecal contamination of soil and water, since most patients with ESBL producers may have had their gastrointestinal tracts colonized by these organisms [34]. β -lactamase production is perhaps the most reported important mechanism of resistance to penicillin and cephalosporins and *E. coli* is known to possess a naturally occurring chromosomally mediated and/or plasmid-mediated β -lactamases [17,31].

Conclusion

Our findings show that a high percentage of childhood diarrhoea are caused by antibiotic-resistant bacteria as most *E. coli* and *Salmonella* isolates in our study demonstrated multidrug resistance to conventional antibiotics. Majority (65.2%) of the *E. coli* were ESBL producers thus the colonized children may be potential sources of

multidrug ESBL-producing *E. coli* strains in the hospital and/or community. However, because of the safety issues with the use of fluoroquinolones in paediatrics we recommend the use of Augmentin[®] in the management of childhood diarrhoea in the studied locality with adequate fluid replacement and zinc supplement, while high hygiene practices should be encouraged.

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