PREVALENCE OF DIARRHEAGENIC BACTERIA IN STOOL SAMPLES OF ADULT PATIENTS ATTENDING DUTSIN-MA GENERAL HOSPITAL, KATSINA STATE, NORTHWESTERN NIGERIA

Abstract

Enteric infections and diarrheal diseases (EIDD) constitute a pervasive health burden throughout the world, closely associated with poor water supply, poor environmental sanitation and unhygienic status of individuals, which are common in developing countries. Diarrhea can be caused by a wide range of microbial agents including viruses, bacteria and parasites. Among the bacterial agents, *Escherichia coli, Salmonella*, and *Shigella* species are one of the most common causes of diarrhea. This study was carried out to investigate the prevalence of *Escherichia coli* and *Salmonella* species among adults attending Dutsin-Ma General Hospital, Katsina State, and also to determine the antimicrobial susceptibility profile of the bacterial isolates to commonly prescribed antibiotics against them. A total of 30 fresh diarrheic samples were collected from the study participants, 17 samples from male and 13 from female participants in sterile sample containers between May and July, 2021. The samples were put in ice packed containers and transported to the laboratory of the Department of Microbiology, Federal University, Dutsin-Ma for processing and analyses. Out of the 30 samples examined, 29 (96.67%) were found to be positive, 16(53.33%) *E. coli* and 13(43.33%) *Salmonella* species, respectively. The result of antibiotic susceptibility test showed that both *E. coli* and *Salmonella* species had highest resistance to ampicillin but were found to be sensitive to chloramphenicol, hence, it could be used as a best drug of choice for the treatment of diarrhoea caused by the pathogens.

Key Words: *Escherichia coli, Salmonella,* diarrhoea, dehydration, adults, antibiotics

1.0 Introduction

Enteric infections and diarrhoeal diseases (EIDD) constitute a pervasive health burden throughout the world (*Hien et al., 2017*), closely associated with poor water supply, sanitation and hygiene status, which are common in developing countries (*Hien et al., 2017*). EIDD affect a large number of people globally and constitute a leading cause of morbidity and mortality in developing countries, with higher rates among children and aged individuals (Nataro *et al.*, 2006). Diarrhoea is characterized by stools of decreased consistency and increased volume due to imbalance of secretion and absorption of water and salts in the intestine (*WHO, 2017*). It is a major source of malnutrition and life threatening diseases which may be fatal (Okeke *et al.*, 2009).

Diarrhoea can be caused by a wide range of microbial agents including viruses, bacteria and parasites (*Shamki et al., 2012*). Among the bacterial agents, *Escherichia coli, Salmonella* and *Shigella* are one of the most common causes of diarrhoea. *E coli* is Gram-negative, oxidase-negative, rod-shaped bacterium from the family Enterobacteriaceae (*Croxen et al., 2013*) that is encountered as a normal inhabitant of human and other mammalian intestine (Bekal *et al.*, 2018). A total of six *E. coli* pathotypes have been found to have been implicated in diarrhoea, and hence, they area called diarrhoeagenic *E. coli*, including enteropathogenic *Escherichia coli*, Shiga like toxin producing *E. coli* (STEC), enteroinvasive E. *coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli* and adherent invasive *E. coli* (Nataro and Kaper, 1998). These are characterised by expression of different group specific virulence factors. The most commonly

identified pathotype is STEC which was previously called as enterohemorrhagic and verocytotoxin producing *E. coli*.

Salmonella, a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae, causes a wide range of human diseases, such as enteric fever, gastroenteritis, endocarditis, and bacteraemia. It is one of the most common and widely distributed food borne diseases. It constitutes a major public health burden and represents a significant health cost in many countries. Salmonella serovars such as *Salmonella* serovar *Typhimurium*, *Salmonella* serovar *Paratyphi*, and *Salmonella* serovar *Choleraesuis* among others are grouped as Non-tyhoidal Salmonella (NTS) which also known to be implicated in the cause of diarrhea (*Gal-mor et al., 2014*). The genus *Salmonella* is divided into two species, *Salmonella enterica* (type species) and Salmonella bongori, based on differences in their 16S rRNA sequence analysis. The species, *S. enterica*, is classified into six subspecies based on the genomic relatedness and biochemical properties. The subspecies are denoted with roman numerals: I, *S. enterica* subsp. enterica; II, *S. enterica* subsp. salamae; IIIa, *S. enterica* subsp. arizonae; IIIb, *S. enterica* subsp. diarizonae; IV, *S. enterica* subsp. houtenae; and VI, *S. enterica* subsp. indica. *S. enterica* subsp. enterica (I) is responsible for approximately 99% of Salmonella infections in humans and warm-blooded animals. In contrast, the other five Salmonella subspecies and *S. bongori* are rare in humans and are found mainly in the environment and in cold-blooded animals (*DuPont*, 2009).

Based on the clinical disease in humans, *Salmonella* strains can be grouped into typhoidal Salmonella and non-typhoidal Salmonella (NTS). The NTS strains are found in animals and cause gastroenteritis in humans. The disease occurs worldwide and is characterized by vomiting, abdominal pain and cramps, myalgias and non-bloody diarrhea. The disease is

limited to the lamina propria of the small intestine and usually antimicrobial therapy is not given. Extraintestinal features include bacteremia, septic arthritis, urinary tract infections, and osteomyelitis which may be seen in 5% of cases (Que, 2009). Some individuals may become asymptomatic carriers and shedding may last for several weeks to a few months.

Moreover, Antimicrobial drug resistance is a global health problem that continues to expand as microorganisms continue to adapt to antibiotics used to treat them (Ochoa *et al.*, 2009), especially in settings where few treatment options are available in the treatment of infective bacterial diarrhoea (Binsztein et al., 1999). The emergence of antimicrobial resistance, including resistance to the new and potent antimicrobial agents, is a major public health concern especially developing countries, like Nigeria , where bacterial infections are still among the major causes of death (Estrada *et al., 2005)* especially among young children, is of particular concern (GebreSilasie *et al.,* 2018). Antibiotic resistant diarrhoeagenic *E. coli* and *Salmonella* species cause severe diarrhoeal disease, and could be associated with treatment failures among diarrhoeal patients (Adesoji and Liadi, 2020).

2.0 Materials and Methods

2.1 Study Area

The study was conducted in Dutsin-Ma town, Dutsin-Ma Local Government Area of Katsina State. Dutsin-Ma Local Government lies on the latitude 12^{o}26N and longitude 07^{o}29E. It shares boundary with Kankia Local Government Area to the East, Safana and Danmumsa Local Government Areas to the West, Kurfi and Charanchi Local Government Areas to the North and12qWSE34DZWSA Matazu Local Government Area to the Southeast (Abaje *et al.*, 2012).

Dutsin-Ma Local Government has a size of about 552, 323 Km^2 with a total population of 169, 829 people as at 2006 National Census (National Population and Housing Census, 2006). The people of the area are predominantly farmers, cattle rearers and traders. The climate of Katsina State is a tropical wet and dry type. Rainfall is between May and September with a peak in August and the average annual rainfall is about 700 mm. Due to severe and widespread drought, the pattern of rainfall in the area is variable which poses serious socio-economic constraints (Abaje, 2012). The mean annual temperature ranges between 29^oC to 31^oC. The vegetation of the area is the Sudan Savannah type which comprises the characteristics and speciesmof Guinea and Sahel Savannah (Abaje *et al.,* 2014).

2.2 Study Population

A total of thirty (30) diarrheic patients, 17 males and 68 females attending Dutsin-Ma General Hospital participated in the study. Ethical approval was obtained from Katsina State ministry of health for permission to obtain diarrhoea samples from patients at the selected Hospital. Written informed consent was also obtained from the study participants.

2.3 Sample Collection

A total of thirty (30) diarrheic stool samples were collected from the patients. The samples were collected into sterile, transparent, wide mouthed bottles. The name, age, sex and class of the participants were properly labelled on the universal bottles after which they were placed into an ice parked container and transported to the laboratory of the Department of Microbiology for processing and microbiological analyses.

2.4 Sample Processing and Analysis

2.4.1 Isolation and identification of *Escherichia coli:* sterile swab stick was used in streaking diarrheic stool samples collected from diarrheal patients on sterile Eosin Methylene Blue Agar and MacConkey Agar for isolation of *E. coli.* Subsequently the plates were incubated at 37°C for 24 hours in an incubator. Afterwards, the plates were observed for colony formation after 24-48 hours of incubation. In order to obtain discrete colonies, the bacterial colonies on the primary plates were subcultured on EMB and MacConkey agar. After 24hrs of incubation at 37^{o}C, colonies with characteristic green metallic sheen and pink colour were aseptically picked and streaked on nutrient agar slant and stored for further biochemical characterization of the isolates.

2.4.2 Isolation of *Salmonella* species: About one gram (1g) of diarrheic stool sample from diarrheal patients was enriched in 5ml of Rappaport Vassiliadis R10 broth at 37°C for 24 hours followed by subculturing on both MacConkey Agar and *Salmonella-Shigella*(SS) agar and the plates were incubated at 37°C for 24 hours. Afterwards, the plates were observed for colony formation after 24-48 hours of incubation. Pure cultures were prepared from *Salmonella* like colonies, i.e. colorless colonies on MacConkey Agar, and colorless colonies with black spot at the center on SS Agar, were aseptically picked and stored on Nutrient agar slant for further biochemical characterization.

2.5 Gram Staining

Gram staining of the isolated colonies was carried out to identify the Gram reaction of the isolates. Using a sterile wire loop, clean glass slides, and a dropper, a drop of sterile normal saline was placed on a clean slide and thin smear of the isolate was made and allowed to air dry. The smear was then heat fixed, flooded with crystal violet (primary stain) for 30secs and rinsed with a tap water for 5secs, and then flooded with iodine (mordant) for about 1min and

decolorized with 90% alcohol. It was then stained with safranin (counter stain) for about 80secs and rinsed with water. The preparation was inverted, air-dried and observed using an oil immersion objective lens (X100) (Cheesbrough, 2010).

2.6 Biochemical Test and Characterization of the Isolates

Escherichia coli and *Salmonella* species isolated were subjected to various biochemical tests such as: motility test, urease test, indole test, methyl-red, vogues proskauer test, citrate test, oxidative fermentation test, triple sugar iron agar test for biochemical characterization, and identified using standard bacteriological procedures (Cheesbrough, 2010).

2.7 Antibiotic Susceptibility Testing: *Escherichia coli* and *Salmonella* species isolates that tested positive for the battery of biochemical tests used were subjected to antimicrobial susceptibility testing using disk diffusion method. A bacterial lawn was prepared by transferring overnight grown bacterial colonies to a glass tube containing 5 ml sterile normal saline water with a sterile inoculating loop. The suspension was vortexed and visually matched with 0.5 MacFarland standard for turbidity. Sterile cotton tipped swab was immersed in the suspension and spread onto Mueller Hinton agar (Oxoid, UK) to obtain a semi-confluent growth. Discs impregnated with predetermined amounts of antibiotics were placed onto the bacterial lawn and the plates were inverted and incubated for 18-24 hours at 37°C. After the incubation, the diameter of the inhibition zones was measured in millimeters using ruler and interpreted as sensitive, intermediate, and resistant using the criteria described by the Clinical and Laboratory Standards Institute. The antibiotics discs (Oxoid, UK) used included: amoxicillin (10µg), ampicillin (10µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (10µg), nalidixic acid (30µg) and imipenem (10µg), respectively.

3.0 Results

Analysis of 30 fresh faecal samples revealed positive growth of diarrhoea causing *E. coli* and *Salmonella* species in 29(96.67%) samples out of the total samples analyzed. *Escherichia coli* (53.33%) had higher prevalence than *Salmonella* species (43.33%) as presented in table 1. Patients of age group 25– 36 years had the highest prevalence of 37.50% of diarrhoea causing *E. coli* and *Salmonella* species, while the lowest prevalence (18.75%) of *E. coli* was in the age groups of 18-24 and 49-69 years, respectively, it was more prevalent in males then in female patients (Table 2). Table 3 shows age and gender distribution of *salmonella* isolates among the study participants. Table 4 shows the antibiotic profile of both *E. coli I* and *Salmonella* species. Highest resistance was observed in ampicillin antibiotic while chloramphenicol was found to be most effective drug of all the tested drugs, hence, considered the best drug for treatment of diarrhoea caused by the bacterial agents.

Table 1: Percentage Distribution of Bacterial Isolates among the Study Population

Bacteria	No. of positive sample (%)	No. of negative sample (%)	Total sample (%)
Eschericia coli	16(53.5)	14(45.7)	30(100)
Salmonella	13(43.3)	17(56.7)	30(100)
Total	29(96.6)	31(103.4)	30(100)

Table 2: Distribution of *Escherichia coli* among the study population according to age and gender

Age group (years)	male	female	total (%)
18-24	2	1	3(18.75)
25-36	4	2	6(37.5)
37-48	1	3	4(25)
49-60	2	1	3(18.75)
Total	9	7	16

Table 3: Distribution of *Salmonella* species among the study population according to age and gender

Age group (years)	male	female	total (%)
18-24	2	1	3(23.07)
25-36	2	1	3(23.07)
37-48	2	2	4(25)
49-60	2	1	3(23.07)
Total	8	6	13

Table 4: Antibiotic susceptibility pattern of *Escherichia coli* and *Salmonella spp.* in diarrheic stools from the study population

Antibiotic	Resistance	Intermediate	sensitive
Amoxicillin	10(55.6) 7(100)	5(27.8) 0(0)	3(16.6) 0(0)
Ampicillin	17(94.4) 5(71.4)	1(5.6) 2(28.6)	0(0) 0(0)
Cefrioxone	8(44.4) 2(28.6)	5(27.8) 2(28.8)	5(27.8) 3(42.9)
Chloramphericol	0(0) 0(0)	0(0) 0(0)	18(100 17(100)
Ciproflocacin	3(16.6) 0(0)	5(27.89) 0(0)	7(100)
Imipenem	10(55.6) 3(42.9)	5(27.8) 4(57.1)	3(16.7) 7(100)
Nalidixic acid	2(11.1) 2(28.6)	2(11.1) 0(0)	14(77.8) 5(71.41)

4.0 Discussion, Conclusion and Recommendation

4.1 Discussion

In this study, 53.3% occurrence of *Escherichia coli* was observed from the stool samples. (Table 2), which is higher compared to 41.4% occurrence of *E. coli* reported by *Korie et al.* (2011), and 59% occurrence of *E. coli* from diarrheic stools reported by *Dormanesh et al.* (2015) in a study conducted in Enugu, Nigeria and Iran respectively. *Nan-Ok et al.* (2015) also reported 22.0% of *E. coli* from diarrheic stool specimen in a study conducted in Korea. However, 43.3% prevalence of *Salmonella* spp. was observed in this present study, which is higher than 1.2% and 5% occurrence of *Salmonella* spp. reported by *Nair et al.* (2015) and *Mzungu et al.* (2016) in a study conducted in India and Nigeria, respectively. *Tesfahun et al.* reported 10.8% occurrence of *Salmonella* spp. from diarrheic stools in a study conducted in Ethiopia while *Kabir et al.* reported 17% occurrence of *Salmonella* spp. from stool specimen of patients with gastroenteritis in a study conducted in Lagos, Nigeria. This disparity may be attributable to differences in the study designs, patients´ selection, differing environmental condition and behavioral pattern in those regions. The study reported highest resistance (82.90%) of the organisms to ampicillin. This is similar to the study conducted by Ochoa *et al. (2009) who reported 85% resistance to ampicillin by diarrhoeagenic E. coli among children in Peru. It also agrees with other studies such as those carried out by Binsztein et al. (1999) (75%) in Argentina and Estrada et al. (2005) who recorded 85% resistance to ampicillin antibiotic in Tanzania among young children.* The highest resistance to ampicillin showed by the bacteria in the study could be attributed to continuous use of such drugs which might most likely be associated with treatment failure and serious antimicrobial resistance. It might also be because of the fact that these drugs are relatively cheap and available. This could have rendered them readily accessible to the patients, thereby increasing their misuse and overuse, thus enabling the bacteria to acquire resistance

genes, for examples, possession of penicillinases producing genes. The enzymes hydrolyze the beta lactam ring in the antibiotics, and as such render them ineffective, hence, the resistance.

5.2 CONCLUSION

In conclusion, from this present study, *Escherichia coli* and *Salmonella* spp. were frequently isolated among age groups (18-60years) with prevalence rate of 53.3% and 43.3% respectively. Diarrheal diseases caused by enteric infections remain a leading global health problem. Two to four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting to death of large number of individuals across age groups.

5.3 RECOMMENDATION

1. Potential source of infection as well as food and meat inspection must be followed up.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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