

Isolation and characterization of some enteric bacteria associated with acute diarrhea among children in Kano, Northern Nigeria

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ABSTRACT: Diarrhea is a common cause of illness and death among children in developing countries including Nigeria. The study aimed to isolate and characterize the enteric bacteria associated with acute diarrhea among children in Kano, Northern Nigeria. This study involved 250 patients attending 3 major health centres within Kano State (Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital and Wudil General Hospital) for treatment of diarrhea. Fecal samples from the patients were streaked onto the surface of MacConkey agar and *Salmonella-Shigella* agar plates and incubated aerobically at 37°C for 24 hours. After incubation, bacterial growth was observed for colony appearance and morphology. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained. For identification, each pure colony was Gram stained and subjected to further biochemical tests such as indole, methyl-red, Voges Proskauer, citrate utilization and motility test. A total of 523 bacteria were isolated comprising 8 genera. They include *E. coli*, *Salmonella* spp, *Shigella* spp, *Klebsiella* spp, *Proteus* spp, *Enterobacter* spp, *Citrobacter* spp and *Serratia* spp. Highest number of isolates was recovered from patients aged 1 – 2 years with 174 bacterial isolates accounting for 32%, followed by patients less than a year with 141 bacterial isolates (27%). Patients aged 4 – 5 years had the least number of isolates (50) which accounted for 9.6% of the total isolates. The result further showed that *E. coli* has the highest occurrence of 193 appearances (36.9%), *Salmonella* spp had occurrence of 92 (17.6%) while *Shigella* spp had occurrences of 63 (12%). The occurrences of *Citrobacter* spp and *Serratia* spp in this study were 28 (5.4%) and 21 (4%) respectively. It is concluded that enteric bacteria are one of the major causative agents of diarrhea among children.

Keywords: Characterization, children, diarrhea, enteric bacteri, Isolation.

INTRODUCTION

World Health Organization defines diarrhea as “the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual” (WHO, 2017). Diarrhea is a form of gastrointestinal infection caused by a variety of bacterial, viral and parasitic organisms or through contaminated food or drinking water, or from person to person as a result of poor hygienic practices. It remains a major cause of mortality among under-age children (mostly under 5 years) around the

world, especially in developing world (Black *et al.*, 2010). Diarrheal disease is the third leading cause of infant and child mortality in developing countries (Black *et al.*, 2010) and about 1.8 million children die per annum from this disease (WHO, 2013). The number of diarrheal deaths is ridiculously on the high side despite a fall in childhood diarrheal diseases from 4.6 million to 0.8 million over the last three decades (Fontaine *et al.*, 2009).

An estimated half of deaths from diarrhea among young

children occur in Africa, where diarrhea is the largest cause of death among children under five years of age and also a major cause of childhood illness (Fischer-Walker *et al.*, 2012). The prevalence of childhood diarrhea in Nigeria is 18.8% (WHO, 2009). Amongst children below five years old, diarrhea accounts for over 16% of deaths and estimated at 150,000 annually (UNICEF, 2013). Exposure to diarrhea-causing pathogens is frequently related to the consumption of contaminated water and to unhygienic practices in food preparation and disposal of excreta. The combination of high cause-specific mortality and the existence of an effective remedy make diarrhea and its treatment a priority concern for health services (UNICEF, 2013). Diarrhea is a health problem that is connected to water and sanitation, therefore can be both 'water borne' and 'water washed'. Transmission of agents causing diarrhea are usually by the faecal oral route, which include the ingestion of faecal contaminated water or food, person to person contact and direct contact with infected faeces (Andu *et al.*, 2002).

Severe acute bacterial gastroenteritis is caused majorly by *Shigella* spp, but *Salmonella* spp., *E. coli*, *Campylobacter* spp and *Vibrio* spp. play an important role in the epidemiology of diarrhea, especially in certain areas of the globe (Humphries and Linscott, 2015). Among pathogenic bacteria, Gram-negative *Shigella* spp. can cause serious intestinal diseases, including bacillary dysentery. Many cases of the *Shigella* infections occur in children under 10 years of age. Ingestion of fewer microorganisms may lead to infection, which is substantially lower than the infective dose of other enteric bacteria. In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process with bloody diarrhoea and high concentration of neutrophils in the stool (Oloruntoba *et al.*, 2014). *E. coli* are known to be multi-resistant bacteria and present in large number in the normal intestinal flora of humans and animals where it generally cause no harm. However, a number of enteropathogenic strains can cause acute diarrhea that ranges from mild and non-bloody to highly bloody including mild watery diarrhoea, abdominal cramps, nausea and headache. Others have been associated with severe, chronic, non-bloody diarrhoea, vomiting and fever in infants, causing watery and occasionally bloody diarrhea where strains invade colon cells by a pathogenic mechanism similar to that of *Shigella* (O'Connor, 2002). *Salmonella* infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia, typhoid fever/enteric fever (sustained fever with or without diarrhea) and a carrier state in persons with previous infections. *K. pneumoniae* is approximately 60–80% of all *Klebsiella* spp. isolated from faeces and clinical specimens.

The most frequently studied diarrhoea pathogens in Nigeria are however bacteria. At various times, Okeke *et*

al. (2003), Akinkunmi and Lamikanra (2010) and Oloruntoba *et al.* (2014) have identified various species of bacteria as being involved in cases of diarrhea in Nigeria. Over the years, the bacterial agents associated with childhood diarrhea in Nigeria where the present study was carried out include various diarrheogenic strains of *Escherichia coli* by Okeke *et al.* (2003) as well as Igbeneghu (2009); *Salmonella* species by Omololu-Aso *et al.* (2009); *Shigella* species by Abdu *et al.* (2014). In view of this, the study was aimed to isolate and characterize enteric bacteria associated with acute diarrhea among children in Kano, Northern Nigeria.

MATERIALS AND METHODS

Sampling sites

The study was conducted partly at Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital, Wudil General Hospital and largely at Laboratory of Microbiology Department of Kano University of Science and Technology Wudil all in Kano. Kano State is located in the North-western Nigeria, it is coordinated at latitude 11°30'N and longitude 8°30'E (Wikipedia, 2021). It shares borders with Kaduna State to the South-West, Bauchi State to the South-East, Jigawa State to the East and Katsina State to the North. It has a total area of 20,131 km² (7,777sqm) and estimated population of 13.4 million (NPC, 2014).

Ethical consideration

An approval for the study (Reference Number: NHREC/17/03/2030) was obtained from Research and Ethic committee Kano State Ministry of Health through Health Service Management Board Kano in conjunction with the approval from Ethical Committee of Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital and Wudil General Hospital. The aim of the study was explained clearly to the patients and informed consent obtained before commencing the study (Salisu *et al.*, 2018).

Determination of sample size

Sample size for the study was determined using the formula; $N = (Z_{1-\alpha})^2 \times p \times (1-p) / d^2$ as described by Omole *et al.* (2019) where N = minimum sample size, $Z_{1-\alpha}$ = Value of standard normal deviate which at 95% confidence interval was found to be 1.96. p = the best estimate of prevalence obtained from literature review (18.8%) (WHO, 2009), and d = difference between the true population rate and sample that can be tolerated, this is the absolute

precision (in percentage) on either side of the population = 0.05.

$N = (1.96)^2 \times (0.188) \times (1-0.188) / (0.05)^2 = 0.5864/0.0025 = 234.56$ which is approximately equals to 235, and the minimum number of samples for the study. Therefore, a total of 12 subjects accounted for 5% (Bankhead *et al.*, 2017) of the minimum number of subjects were added to the research for attrition, making a total of 250 samples.

Samples collection

This study involved 250 patients attending 3 major health centres namely: Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital and Wudil General Hospital all in Kano State for treatment of diarrhea. A sterile universal container was used for sample collection. Diarrhea samples were obtained from the patients and were processed for isolation and identification of bacteria according to the standard microbiological techniques (Cheesbrough, 2010). All the samples collected were immediately transferred under aseptic conditions to Microbiology Laboratory of Murtala Muhammad Specialist Hospital for isolation and identification.

Isolation and Identification of Bacteria

Isolation of enteric bacteria was conducted according to the method describe by Cheesbrough (2010). During the process, a sterile wire loop was dipped into the fecal sample of the patients and streaked onto the surface of MacConkey agar (Life save Biotech, USA) and *Salmonella-Shigella* agar (Biomark, India) plates and incubated aerobically at 37°C for 24 hours. After incubation, bacterial growth was observed for colony appearance and morphology. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained. For identification, each pure colony was Gram stained and subjected to further biochemical tests such as indole, methyl-red, Voges Proskauer, citrate utilization and motility test as described by Cheesbrough (2010).

RESULTS

Age and sex distribution of the patients

The age and sex distribution of the patients in the present study is presented in Table 1. A total of 250 under-5-years children diagnosed with diarrhea were involved. One hundred and thirty (132) of the patients were male (52.8%) while one hundred and eighteen (118) were female (47.2%). Based on age categories, patients aged 1 – 2

years has the highest frequency with 91 subjects accounting for 36.4%, followed by patients aged between 0 – 1 year with 73 subjects (29.2%), then followed by those aged between 2 – 3 years with 36 subjects (14.4%). Least number of subjects was recorded by those aged between 4 – 5 years with 21 occurrences which accounted for 8.4%.

Identification of the Isolates

Table 2 represents the morphological and biochemical characterization of the bacteria isolated from stool samples of the patients. Isolates were characterized based on gram staining, biochemical test, motility, hydrogen sulphide production and gas from carbohydrate as well as lactose fermentation. A total of eight genera were identified. They include *Salmonella* spp, *Shigella* spp, *Klebsiella* spp, *Proteus* spp, *Enterobacter* spp, *Citrobacter* spp and *Serratia* spp

Number of Isolates based on age distribution of the patients

The total number of bacteria isolated from the stool samples examined is presented in Table 3. The result showed that a total of 523 isolates were recorded. Highest number of isolates of 174 (32%) was recovered from patients aged 1 – 2 years, followed by subjects less than a year with 141 isolates (27%). Subjects aged 4 – 5 years had the least number of isolates (50) accounting for 9.6% of the total isolates.

Percentage frequency of bacteria isolates

The percentage frequency of the bacteria isolated from the stool samples of the subjects is presented in Table 4. The result showed that *E. coli* has the highest frequency with total of 193 occurrences (36.9%), followed by *Salmonella* spp with total frequency of 92 (17.6%), then *Shigella* spp with frequency of 63 (12%). The number of isolates recorded by *Citrobacter* spp and *Serratia* spp in this study were 28 (5.4%) and 21 (4%) respectively.

DISCUSSION

Diarrhea is a common cause of illness and death among children in developing countries including Nigeria (CDC, 2019). Various studies have implicated various bacteria in diarrhea diseases but all these studies were focused on individual organisms as etiologic components of diarrhea (Igbeneghu, 2009; Akinkunmi and Lamikanra, 2012). In the present study, more males (52.8%) were diagnosed

Table 1. Age and sex distribution of the patients

Age (Years)	Male	Female	Frequency (n)	Percentage (%)
0 – 1	38	35	73	29.2
1 – 2	48	43	91	36.4
2 – 3	20	16	36	14.4
3 – 4	15	14	29	11.6
4 – 5	11	10	21	8.4
Total	132	118	250	100

Table 2. Identification of the isolates from stool samples of patients attending 3 health centres in Kano

Code	GS	IN	MR	VP	CU	H ₂ S	GP	LF	MO	Expected isolate
IS ₁	-	+	+	-	-	-	+	+	+	<i>E. coli</i>
IS ₂	-	-	+	-	-	+	-	-	+	<i>Salmonella</i>
IS ₃	-	-	-	+	+	-	+	+	-	<i>Klebsiella</i>
IS ₄	-	+	+	-	-	-	+	-	-	<i>Shigella</i>
IS ₅	-	+	-	+	+	-	+	-	+	<i>Proteus</i>
IS ₆	-	-	-	+	+	-	+	-	+	<i>Enterobacter</i>
IS ₇	-	+	+	-	+	+	+	+	+	<i>Citrobacter</i>
IS ₈	-	+	+	+	+	-	-	-	+	<i>Serratia</i>

Key: GS = Gram staining, IN = Indole, MR = Methyl-red, VP = Voges Proskauer, CU = Citrate utilization, H₂S = Hydrogen sulphide, GP = Gas production, LF = Lactose fermentation, MO = Motility.

Table 3. Number of isolates based on age distribution of the patients.

Age (Years)	Samples (n)	No. of isolates	Percentage (%)
0 – 1	73	141	27.0
1 – 2	91	174	33.2
2 – 3	36	81	15.5
3 – 4	29	77	14.7
4 – 5	21	50	9.6
Total	250	523	100

Table 4. Percentage of bacteria isolates from stool samples.

S/N	Isolates	No. of isolates	Percentage (%)
1	<i>E. coli</i>	193	36.9
2	<i>Salmonella</i> spp	92	17.6
3	<i>Shigella</i> spp	63	12.0
4	<i>Klebsiella</i> spp	44	8.4
5	<i>Proteus</i> spp	39	7.5
6	<i>Enterobacter</i> spp	43	8.2
7	<i>Citrobacter</i> spp	28	5.4
8	<i>Serratia</i> spp	21	4.0
Total		523	100

with the infection than female (47.2%). However, the difference was found to be statistically non-significant at $p < 0.05$. This finding was in conformity with that of Rathaur

et al. (2014) who reported that there was a no statistically significant difference between the number of male (54.6%) and female (45.4%) children who presented with diarrhea

in India. The differences in the number of male and female may be attributed to the fact that male children are more prone to infections than their female counterpart. Males have also been observed to be more socially active than female at such ages; hence, they could be more exposed to more sources of infections than the female counterparts (Abdullahi *et al.*, 2009).

From the study, it was found that children aged 1 – 2 years (12 – 23 month) were more affected by the infection (36.4%) than their counterpart and the differences among the age categories in this study is significant ($p < 0.05$). This can be attributed to negligent attitude of parents toward children of this age group and increased mobility of children due to their recently acquired walking ability. In addition to that, teething also begins at this age which means that they are able to grasp and put contaminated items into their mouths (Akinwumi *et al.*, 2021). There is also the issue of weaning- these children are very likely to have just been introduced to family diet after they are no longer breast-fed (Akinwumi *et al.*, 2021).

A total of 523 enteric bacteria were isolated from 250 stool samples of the subjects collected in the present study, however, there was significant difference in the types of bacterial isolated from the samples. The result shows eight bacterial genera belonging to Enterobacteriaceae family were identified. They include *E. coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter* and *Serratia*. In the present study, *E. coli* was found as the predominant isolate with 193 (36.9%) followed by *Salmonella* with total frequency of 92 (17.6%), then *Shigella* with frequency of 63 (12%). The number of isolates recorded by *Citrobacter* and *Serratia* in this study were 28 (5.4%) and 21 (4%) respectively. Finding of this study was in conformity with that of Sang *et al.* (2012), Amukoshi (2017) and Adeh *et al.* (2019) who all found *E. coli*, *Salmonella* and *Shigella* as major enteric bacteria causing diarrhea in children. The present study also justifies the finding of Abdullahi *et al.* (2009) in Kano, Nigeria.

In the present study, 193 samples out of 250 were positive for *E. coli* and this accounted for 77.2%. This is lower than 83.1% prevalent rate reported from similar study in Abakaliki, South eastern Nigeria (Ogbu *et al.*, 2008) and higher than 34% prevalent rate recorded by Sule *et al.* (2011) in Kaduna. *Escherichia coli* recorded highest prevalence among the other enteric bacterial species identified in this study. This is in agreement with the report of Akingbade *et al.* (2013) and Clarke (2001) that stated that the bacterial pathogen most commonly associated with endemic forms of childhood diarrhea is *Escherichia coli*. According to Bahl *et al.* (2001), the most important cause of acute watery diarrhea in young children in Nigeria include *rotavirus*, enterotoxigenic *Escherichia coli*, *Shigella*, *Campylobacter jejuni*, *Salmonella* and enteropathogenic *Escherichia coli*.

Conclusion

In the present study, more males were infected with diarrhea than female counterparts and the frequency of the infection was more among children aged between 12 - 23 months. Findings of the study showed that eight bacterial genera belonging to Enterobacteriaceae family were identified. They include *E. coli* spp, *Salmonella* spp, *Shigella* spp, *Klebsiella* spp, *Proteus* spp, *Enterobacter* spp, *Citrobacter* spp and *Serratia* spp. *E. coli* was found as the predominant isolate followed by *Salmonella* spp, then *Shigella* spp. A total 193 samples out of 250 were positive for *E. coli* and this is accounted for 77.2% of the total isolates. It is recommended that mothers and care givers should be given health education to ensure their under-five aged children are fully immunized and see good personal hygiene as vital. This will remarkably reduce the possibility of infection in them.

CONFLICT OF INTEREST

The authors declare no conflicts of interest exist.

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