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Research Paper

Characterization, Prevalence and Antibiotic Resistance Patterns of Enteric Bacterial Pathogens Associated with Infantile Diarrhea

Dauphin Dighitoghi MORO[1] *Ph.D., Department of Microbiology, Lagos State University, P.M.B. 0001, Ojo, Lagos, Nigeria.*
Ayodele Oluwaseun AJAYI[2,] *Ph.D., Department of Microbiology, Federal University of Oye Ekiti, P.M.B. 373, Oye Ekiti, Ekiti State, Nigeria.*
Jyostina CHANDWANI[3,] *MBBS., MD, Jln Medical College Ajmer, Kala bagh, Ajmer Rajasthan, 305001, India.*
Correspondence: Dauphin Dighitoghi MORO, Ph.D., Department of Microbiology, Lagos State University, P. M. B. 0001, Ojo, Lagos, Nigeria.

ABSTRACT
Objectives: *Infantile diarrhea is a very common clinical entity with several bacterial etiologies especially in developing countries. This study investigates the incidence of infantile diarrhea, the prevalence of bacterial causes and antibiotic resistance pattern of bacterial enteropathogens in some hospitals in Lagos, Nigeria.*
Setting; *Lagos University Teaching Hospital and 3 General Hospitals in Lagos, Nigeria*
Methods: *Two hundred and eighty stool samples collected from infants 1-5 months clinically diagnosed of diarrhea were enriched in selenite F broth and buffered peptone water(Oxoid, Basingstoke, UK) at 37^{0}C for 18-24 hours and were plated on MacConkey agar (Oxoid, Basingstoke, UK), Salmonella-Shigella agar (LAB M, Lancashire, UK), Eosin Methylene blue agar (Merck, Germany), Deoxycholate Citrate agar (Oxoid, Basingstoke, UK) and xylose lysine deoxycholate agar (LAB, M, Lancasure,UK). The bacterial isolates were identified on the basis of cultural, morphological and biochemical characteristics. Antibiotic susceptibility pattern was determined using the disc diffusion technique with 8 different antibiotics.*
Results*: Two hundred and fifty eight (92.1%) had one or more bacterial enteropathogens which belong to five genera which included Shigella, Salmonella, Escherichia, Vibrio and Proteus. Ninety six samples were monomicrobial while 164 were polymicrobial with 32 having two bacterial species respectively. A total of 556 bacterial isolates were recovered from the subjects constituting 168(30.2) Shigella spp., 140 (25.1%) of Salmonella typhi, with 92 (16.6%) and 64(11.5%) of Escherichia coli and Vibrio cholerae respectively. The age distribution of the pathogens (0-1, 2-3 and 4-5 months) was in a ratio of 22: 15:1.0. All isolates were resistant to at least three of the eight antibiotics tested with resistance ranging between 16.7% and 100% to modern and uncommonly used antibiotics.*
Conclusion*: Infantile diarrhea of multiple etiology is common in Nigeria with about 438(79%) of the cases in the 0-6 months age range occurring in infants between 0 and 3 months. A very high multiple resistance to the third generation cephalosporins which the infantile diarrhea thus requires prompt diagnosis, oral rehydration and treatment. Concerted efforts geared towards improved primary healthcare in developing countries will reduce the mortality rate of infantile diarrhea.*
KEYWORDS: ***Infantile diarrhea, enteric bacterial pathogens, Salmonella, Shigella, Escherichia coli, Susceptibility, Resistance.***

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I. INTRODUCTION

Diarrhea is having loose or watery stools at least three times per day or more frequently than normal for a normal individual. Mortality. Despite the efforts in controlling mortality, 9% of all deaths among children

*Corresponding Author: Dauphin Dighitoghi Moro

below 5 years globally in 2015 were due to diarrhea **[1].** World-wide, diarrheal diseases are the leading cause of death during childhood. Diarrheal diseases are the second leading cause of death for all ages behind cardiovascular diseases and probably responsible for more years of life lost than all other diseases combined [2]. Diarrheal disease accounted for 8% of all deaths in children under five years of age in 2016, and this translates to over 1300 young children dying each year or approximately 480,000 children a year [3].

While age-specific mortality due to diarrhea is highest among the elderly in the USA, most hospital admissions and death occur worldwide especially in developing countries due to diarrhea in the first year of life [2, 4]. According to Oyejide et al. [5], the rate of occurrence of diarrheal episodes remains largely unchanged with diarrhea still occurring at rates within the range of 3–10 per child per year.

Diarrhea poses very serious problem in developing countries as a leading cause of morbidity and mortality especially among children between the range of 0-10 years, with over 13% of the children born in certain parts of Latin America dieing before the 5th birthday [6].

Knowledge of etiology of diarrheal illness is essential for the development and implementation of public health measures to prevent and control this disease syndrome [7]. After enteric viruses, bacterial causes are ranked as second most common cause of diarrhea in developing countries [7, 8].

Etiological agents of diarrhea are quite many and they include bacteria like *Yersinia, Salmonella, Campylobacter, Vibrio cholerae*, viruses like rotaviruses and Adenovirus and parasites like *Giardia lamblia, Entamoeba histolytica* [6, 8]. Food-borne diarrheal diseases have been found to constitute a large proportion of all diarrheal diseases in children, majority of which occur at home [9].

After enteric viruses, bacterial causes are ranked as second most common causes of diarrhea in developing countries. Campylobacter is a potential etiological agent of enteritis both in children and adults and, it is second to *Salmonella* and *Shigella* in many countries [10]. Poor environmental sanitation, inadequate safe water supply, low level of literacy, poverty and poor personal hygiene are among the predisposing factors [11].

Various lines of treatment of diarrhea in children and adults are employed. The commonest and most widely promoted method of treatment nowadays is the concept of oral rehydration therapy (ORT), where a mother prepares the simple salt and sugar solution which is given to the child in a bid to replace as much a possible the salt and sugar lost during diarrhea [12].

However, when diarrhea is due to infection, it is better to allow the child to stool out the disease while the baby is simply rehydrated and the infections treated preferably by doctors [12]. However, infusion is sometimes needed to rehydrate the child in cases of severe diarrhea and the child admitted in a hospital for some days and some laboratory tests done for successful therapy [13].

In Nigeria, data from various studies have implicated several enteric bacterial pathogens with scanty data on the relative occurrence of these pathogens and their antibiotic susceptibility pattern in defined age groups [6, 14].

This study therefore examines the incidence of infantile diarrhea in some hospitals in Lagos, the prevalence of enteric bacterial pathogens and susceptibility pattern of the bacterial isolates to some uncommonly used antibiotics.

II. METHODOLOGY

Sample collection and Analysis

A total of 560 fecal samples were collected from acute diarrheal neonatal patients (1-16 months through the assistance of their mother) attending the Lagos University Teaching Hospital and three General Hospitals in the Lagos metropolis over a period of 2 years (October 2018 to October, 2019). Information on age and sex were obtained from the case notes and mothers after obtaining the informed consents of the parents.

The samples were examined bacteriologically using selenite F broth (SFB) and buffered peptone water (BPW) in a ratio 1:10 (w/v) as enrichment media incubated at 37°C of 24 hours. A loopful of each stool sample was inoculated onto MacConkey, Salmonella Shigella agar (SS), deoxycholate citrate agar, thiocitrate bile salts (TBS) and eosin methylene blue agar and incubated at 37° for 24 hours.

Colonies showing typical characteristics on the basis of cultural, morphological and biochemical characteristics such as Gram's stain, indole methyl-red, Voges-Proskauer and citrate (IMViC) test series, reaction on KIA, motility, urease production and sugar fermentation. Identification was according to Cheesbrough, Cowan, NCCLS [15, 16, 17]. The pure isolates were sub-cultured on nutrient agar slopes as stocks.

Antibiotic Susceptibility Test

Susceptibility to antimicrobial agents was tested by the standard disc diffusion method using Mueller Hinton Agar, the zone of inhibition were interpreted according to Cheesbrough, Cowan, and NCCLS [15, 16, 17]. The following concentrations of the antibiotics in micrograms (µg) were used; Cephalexin 30, ceftriaxone

*Corresponding Author: Dauphin Dighitoghi Moro

30, augmentin 30, ceftazidime 30, cefotaxime sodium 30, cefuroxime sodium 30, peflacin 10 and ofloxacin 10. A total of 268 randomly selected representative enteric bacterial pathogens were tested. *Escherichia coli* (NCTC 10418) and *E. coli* K12 served as control and results obtained were classified as either resistance or sensitive.

Ethical Consideration.

The purpose of the study was made known to the mothers of our prospective subjects, some accepted to allow their children to partake in the study while some mothers declined and were excluded from the study. Consequently, only children whose mothers gave informed consent were recruited for the study.

Statistical Analysis

Descriptive statistics were used to analyze discrete variables. Statistical analysis was done using Microsoft Excel. The data are represented in terms of frequency and percentages.

III. RESULTS

Several enteric bacterial pathogens were recovered from the children. *Shigella* spp. has the highest frequency of 168 (3022%), *S. typhi* constituted 140 (25.18%), *E. coli,* 92(16.55%), *Vibrio cholerae*, 64 (11.51%) while *Vibrio parahemolyticus* had the least frequency of 40 (7.9%) as shown in Table 1.

Table 2 shows the age and sex distribution of the enteric bacterial pathogens among the subjects. Males (58.99%) had higher infection rate than females (41.01%). The study of the age distribution of the enteric bacterial pathogens shows that the 0-1 age group had 258 (46.40%), the 2-3 age group had 180 (32.37%) while the 4-5 age group had the least prevalence of enteric bacterial pathogens (21.22%).

Table 3 shows the antibiotic resistance pattern of the bacterial isolates to various antibiotics. S. typhi had the highest resistance to chloramphenicol (100%), 50% to cefuroxime and 16.7% each to amoxicillin and ceftazidime respectively but 100% susceptibility to cefuroxime, peflacin, cefotaxime and ofloxacin. Most enteric bacteria tested showed high resistance rate to chloramphenicol and ceftazidime but were highly susceptible to almost all the other antibiotics.

Table 1: Occurrence of bacterial isolates among infants with diarrhoea .

S/N	Organism	Frequency	Percentage
1.	*Shigella* spp.	168	30.22
2.	*Salmonella typhi*	140	25.18
3.	*Escherichia coli*	92	16.55
4.	*Vibrio cholerae*	64	11.51
5.	*Proteus* spp.	52	9.35
6	*Vibrio parahaemolyticus*	40	7.19
	TOTAL	**556**	**100**

Table 2: Age and sex distribution of infants with enteropathogens**.**

Age (in months)	*Salmonella typhi*		*Shigella* spp		*Escherichia coli*		*Vibrio cholera*		*Proteus* spp		*V. parahaemolyticus*		
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Total
0-1	35	20	50	41	29	10	20	2	18	11	12	10	258
2-3	29	20	25	20	16	16	12	20	7	2	6	7	180
4-5	20	16	22	10	11	10	6	4	6	8	4	11	118
Total	84	56	97	71	56	36	38	26	31	21	22	18	556

Table 3: Antibiotic resistance pattern of bacterial enteric bacterial pathogens isolated**.**

Organism	No tested	CL	CRO	AMC	PEF	CAZ	CTX	CXM	OFX
Salmonella typhi	**72**	**72(100)**	**0(0)**	**12(16.7)**	**0(0)**	**12(16.7)**	**0(0)**	**36(50)**	**0(0)**
***Shigella* spp.**	**60**	**60(100)**	**0(0)**	**0(0)**	**0(0)**	**48(80)**	**0(0)**	**0(0)**	**6(10)**
Escherichia coli	**48**	**48(100)**	**12(25)**	**36(75)**	**0(0)**	**36(75)**	**24(50)**	**36(75)**	**0(0)**
Vibrio cholera	**40**	**24(60.9)**	**0(0)**	**8(20)**	**0(0)**	**16(40)**	**0(0)**	**0(0)**	**0(0)**
Vibrio parahemolyticus	**24**	**16(66.7)**	**(0)**	**8(33.3)**	**0(0)**	**16(66.7)**	**0(0)**	**0(0)**	**8(33)**
***Proteus* spp.**	**24**	**24(100)**	**12(50)**	**24(100)**	**0(0)**	**12(50)**	**24(100)**	**24(100)**	**0(0**

Legend: CL- Chloramphenicol, CRO-Cefuroxime, AMC-Amoxicillin, PEF- Peflacin, CAZ-Ceftazidime, CTX-Cefotaxime, CXM-Cefuroxime, OFX-Ofloxacin

IV. DISCUSSION

*Corresponding Author: Dauphin Dighitoghi Moro

Diarrheal diseases are the leading cause of hospital admissions and deaths occurring in children especially during their first year of life world-wide [2]. Diarrhea still remains one of the major health related problems in Nigeria especially among infants with high mortality rates with some enteric bacterial pathogens, viruses and intestinal parasites as the etiologies [8].

Loss of water and electrolytes from the body often results in dehydration which can be fatal in young children especially those in poor health and are malnourished in developing countries [18].

This study showed that infantile diarrhea of bacterial etiology is still a common occurrence in Nigeria and other developing countries with 258(92.1%) out of the 280 infants harboring one, two or three enteric bacterial pathogens.

Invasive cases of non-typhoidal Salmonellae are frequently reported in infants and young children with a higher risk of secondary complications such as bacteremia and meningitis. Increase of multidrug resistance (MDR) among non-typhoidal *Salmonella* spp. is a serious problem worldwide, due to widespread use of traditional antibiotics in human and veterinary medicine [19]. Published studies on childhood diarrhea are relatively few in Nigeria, therefore, the pathogen spectrum and their antibiotic resistance patterns requires investigation.

High morbidity and mortality are associated with diarrhea in Iraq, particularly among children <5years, which is predominantly due to serious challenges facing the delivery of basic public health and environmental sanitation services [20].

A higher likelihood of isolation of non-typhoidal *Salmonella* from children diarrheal cases in this research was associated to source of water, presence of domestic animals in household, education level of caregivers and hygiene practices [21]. Phenotypic and genotypic characterization of *Salmonella* resistance to some clinically important antimicrobial agents emphasizes the need to carry out long term monitoring. This work bridges the gap in research on the frequency of enteric bacterial pathogens and their antibiotic resistance which can be useful to public health authorities in diarrheal control generally in children.

The bacterial strains were slightly more diverse among female than their male counterpart may have contributed to their higher bacterial infections associated with diarrhea among the female.

Shigella spp., the most commonly encountered bacterium was isolated from 60% of the infants constituting 30.2% of the total bacterial isolates in this study. This finding is significant because it is the causative agent of dysentery which is often a later stage in the progression of diarrhea. This finding agrees with that reported by Niemogha et al. [22]. According to Ronald [23], the genus Shigella is genetically close to *Escherichia* as organisms both genera undergo genetic recombination with each other. *Shigella* also produces an endotoxin and neurotoxin which exhibit enterotoxic effects. The high prevalence and incidence of both *Shigella* spp. and *Escherichia coli* may have thus aggravated the high prevalence and severity of infantile diarrhea encountered in this study.

Our finding *on Shigella* spp. closely matched to a greater extent in a report in Ghana by Ashie et al [24] that *Shigella* spp. were generally more commonly isolates in children below 2 years. Dysentery outbreaks are more common especially in developing countries where hygiene are sanitation are questionable.

The high incidence of *Salmonella typhi* observed in this study is quite alarming. This calls for serious concern because *S. typhi* is an exclusive human pathogen and could be fatal especially among infants with low immune status which may lead to septicemia. *S typhi* may therefore have contributed to the high number of deaths usually recorded from infantile diarrhea. Similar findings have also been reported in Nigeria and elsewhere [2, 22]. The occurrence of *E.coli, Vibrio cholera*, *Proteus* spp and *Vibrio parahemolyticus* with relative frequencies of 32.9%, 22.9%, 18.6% and 14.3% respectively in all age groups agrees with an earlier report [25].

Salmonella septicemias in childhood both typhoidal and non-typhoidal Salmonellae reported in this study is a serious problem in many African countries while the emergence of multi-drug resistant Salmonellae over the years present a therapeutic challenge. This emphasizes the need to carry out blood cultures of children with severe diarrhea [22].

The high incidence of *E. coli*, *Vibrio cholerae*, *Proteus* spp. and Vibrio parahaemolyticus with relative frequencies of 32.9%, 22.9%, 18.6% and 14.3% respectively in all age groups agree with that reported earlier [25].

Given the multifactorial nature of diarrheal illness, it is suggested that enteric pathogens co-infections play an important role in gastroenteritis, however, research efforts often focus on a small range of species belonging to a few pathogen groups [21, 26]

The greatest challenge confronting developing countries on diarrhea is that of treatment among children. The line of treatment of infants depends upon the exposure and knowledge of the parents and the health personnel concerned. WHO [12], however, emphasized that rehydration is necessary in all cases and even recommended that ORT be employed prior to chemotherapy especially in children.

*Corresponding Author: Dauphin Dighitoghi Moro

The drugs commonly used in the treatment of infantile diarrhea include trimethoprim-sulfamthoxazole, furazolidine, nalidixic acid and erythromycin .The emergence of multiple drug resistance to drug commonly employed in infantile diarrhea has been reported [22, 27].

Antibiotics susceptibility testing of 286 of the isolates using third generation cephalosporin and quinolones showed that ceftriaxone, augmentin, peflacin, ceftazidime, cefotaxime sodium and ofloxacin were effective against Shigella spp. The high resistance shown by most isolates tested against cephalexin and ceftazidime is quite alarming and calls for concern. The dual problems of lack of drugs for trimethoprim-resistant shigellosis as well as the potential for cartilage damage by fluoroquinolones in children has been reported [28].

Most of the bacterial isolates studied were multiple antibiotic resistant but mechanism has however reported that ceftazidime was very active to all the enteric bacterial pathogens examined. Resistance to antimicrobial agents is a dilemma to the physician, microbiologist and epidemiologist [29].

Employed was however unclear. Resistance to several antimicrobial drugs have been reported by researchers as either plasmid mediated or chromosomally mediated [22, 30].

The highest rate of multiple resistance was found among *Shigella* spp, *Salmonella typhi, Escherichia coli*, *V. parahaemolyticus* and *Proteus* spp. Multiple antibiotics resistance predominant in areas where antibiotics are grossly abused. In Nigeria, antimicrobial agents are easily purchased from the shops without any prescription, a practice which favors the development of antimicrobial resistance [30]. Bacterial etiologies are common and are a significant cause of cholera and gastroenteritis among children below five years in Murang, a county in Kenya. *S. typhimirium* and *S. enterica* are bacterial agents causing diarrhea among children below five years but also the less common Enterobacteriaceae have largely been ignored, but these bacteria should be investigated.

Rational drug use is therefore important in the treatment of acute diarrhea, although management of dehydration should always be considered essential. The WHO [31], has provided definite guidelines for use in cholera and dysentery whereas oral rehydration remains the mainstay in the management of acute watery diarrhea. This should precede all forms of treatment in order to resuscitate such child prior to treatment.

The probable reasons for the high multiple resistance to cephalexin, ceftazidime and cefuroxime sodium observed in the study may include; transfer of highly transposable genetic elements and plasmids from the use of the antimicrobial agents, lack of specific antimicrobial agents in animal feed, poor water quality, poor personal and environmental hygiene and methods of fecal and domestic waste disposal

V. CONCLUSION

Infantile diarrhea of multiple etiology is very high in the study population. The bacterial isolates a very high rate of multiple drug resistance most of antibiotics tested including drugs that are not found over the counter, thus points to be one of the factors culminating in the high incidence of morbidity and mortality to the high morbidity and mortality of infantile diarrhea. The high frequency of selection of *Salmonella typhi*, on-typhoidal *Salmonella* among several other enteric bacteria suggests that these organisms are important causes of diarrhea especially in infants in the study area. Overall, this work fills a gap in research on the frequency of a range of enteric bacterial pathogens and thus could be useful to health authorities in their attempt to controlling diarrheal illness and diarrhea control programs among infants and children in Nigeria and other developing countries.

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Declaration of Conflicting Interests

This is not applicable.

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