



Prevalence and Antimicrobial Susceptibility of *Salmonella* Species Associated with Childhood Acute Gastroenteritis in Federal Capital Territory Abuja, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ICIC conceived and designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BEB, INF, IRN and AAC contributed to the design, literature search and managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: The aim of this study was to isolate and characterize *Salmonella* strains associated with childhood acute gastroenteritis in Nigeria; as well as to evaluate the resistant patterns of the strains to the commonly used antimicrobials agents.

Study Design: Children \leq 5years having diarrhoea characterized by the occurrence of three or more loose or watery stool or at least one bloody loose stool in a 24-hour period were enrolled in the study.

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Methods: The study was conducted between July and December 2008. Samples were pre-enriched in buffered peptone water followed by selective enrichment using selenite cysteine and Rapaport-Vassilidis broths. Isolation and identification was made by inoculating the selectively enriched sample on to Xylose Lysine Deoxycholate agar followed by confirmation of presumptive colonies using different biochemical tests. The CLSI, 2006 method was used for antimicrobial susceptibility testing.

Results: In all the 400 tested samples, 9 (2.3%) were positive for *Salmonella* isolates. Results showed that the children aged 0-5 months had the highest *Salmonella* infection rate of 5 (4.1%), followed by 13-24 months 4 (3.5%), while *Salmonella* infection was not present in the age groups of 25-36 months, 37-48 months, and 49-60 months.

The highest (3.2%) *Salmonella* infection rate was seen among children on solid food followed by those on breast milk (2.5%), while those on a combination of breast milk and formula had no detectable level of *Salmonella* infection.

The study recorded various degrees of resistance to four antimicrobials as observed in amoxicillin, cephalixin, and cefuroxime (55.6%) each, while resistance was observed in 77.8% of the isolates against amoxicillin-clavulanic acid. All isolates were susceptible to ciprofloxacin, nalidixic acid, and Ceftriaxone.

Conclusion: The study reports *Salmonella* species as a potential pathogen isolated from stool samples of children with acute gastroenteritis. The overall resistance level of the isolates to amoxicillin-clavulanic was highest followed by resistance to amoxicillin, cephalixin, cefuroxime giving a cause for concern.

Keywords: Prevalence; gastroenteritis; salmonellosis; antimicrobial agents.

1. INTRODUCTION

Diarrhoea defined as excessive and frequent evacuation of watery faeces, usually indicating gastrointestinal distress or disorder during 3-7 days, is a frequent illness in developing countries and contributes to the deaths of 4.6 million to 6 million children annually in Asia, Africa, and South America. It has been estimated that in the very poor countries of these regions each child suffers up to 15 to 19 episodes of diarrhoea per year [1].

Salmonella species are leading causes of acute gastroenteritis in different countries, especially in the developing countries where substandard hygienic conditions and unsafe water supplies prevails [2]. It is estimated that about 17 million cases of acute gastroenteritis or diarrhoea due to non-typhoidal salmonellosis (NTS), with 3 million deaths are recorded annually [3]. Although the prevalence of *Salmonella* infections is highest in children [4], the real incidence of salmonellosis among children in many countries is not well documented [4].

The emergence of antimicrobial resistance is a matter of concern, and the early identification and effective antimicrobial treatment of cases is an important step in the management of young infants with invasive bacterial infections [5]. Because of the increased resistance to conventional antibiotics, extended-spectrum cephalosporins and fluoroquinolones have become the preferable drugs for the treatment of infections caused by multidrug-resistant *Salmonella* serotypes [6]. Nonetheless, the potential of arthropathy has limited the use of fluoroquinolones in paediatric patients [6]. Therefore, extended-spectrum cephalosporins (example ceftriaxone) are commonly used because of their pharmacodynamic properties and the low prevalence of resistance [7]. Accurate estimates of the burden of diarrhoeal diseases caused by *Salmonella* species and other food borne pathogens remain essential in setting effective public health goals and allocation of resources to reduce disease burden [8]. In recent years, *Salmonella* related diseases have been documented by several clinical-based

studies conducted in different parts of Nigeria [9]. However, dearth of surveillance data on the incidences of *Salmonella* species associated with childhood acute gastroenteritis and its antimicrobial resistance pattern in the Federal Capital Territory Abuja, Nigeria is a major epidemiological issue.

The aim of this study was to isolate, and characterize *Salmonella* strains associated with childhood acute gastroenteritis; and to evaluate the resistance patterns of the strains to the commonly used antimicrobials.

2. MATERIALS AND METHODS

2.1 Sampling of Faeces

The pediatric patients included in the study came from two tertiary hospitals (University of Abuja Teaching Hospital and National Hospital); two secondary hospitals (Maitama and Nyanya Districts hospitals) and one primary health centre (Mpape Health centre), all located in Abuja, Nigeria. Abuja has a total population of 1,405,201, with 740,489 males and 664,712 females, including 281,040 children ≤ 5 years. Predominantly, the inhabitants of Abuja are top politicians, civil servants, traders and the natives who are mostly farmers.

Stool samples of children ≤ 5 years that meet the inclusion criteria of diarrhoea characterized by the occurrence of three or more loose or watery stool or at least one bloody loose stool in a 24-hour period were enrolled in the study. The study lasted from July to December 2008, during which structured questionnaires were completed for each eligible patient and stool samples collected into clean sterile wide mouthed, dry leak proof container in accordance with standard routine procedure. Stool samples were initially stored at 4°C at the hospitals of collections, transported in a cold box and were subsequently analysed the same day of collection. The maximum number of 10 stool samples were collected and analyzed daily. Informed consent was obtained from the parents/guardians of the children after the purpose of the study was explained to them.

2.2 Isolation of *Salmonella* Strains

The isolation of *Salmonella* strains from the fecal samples were performed at the microbiology laboratory, Department of Biological Sciences, University of Abuja using recommended standard microbiology laboratory techniques [10,11]. Briefly, 1 g of faecal sample was pre-enriched in 9 ml of buffered peptone water (BPW) (Oxoid CM509, Basingstoke, England) for 24 hrs at 37°C. A portion (0.1 ml) of the pre-enriched culture was transferred into 10 mL of selenite cysteine (SC) broth (CM0699; Oxoid, Basingstoke, England) and of Rappaport and Vassilidis (RV) broth, which were incubated at 37°C and 42°C for 24 hours, respectively, for further selective enrichment. The selectively enriched cultures were inoculated by spreading 0.1mL on to Xylose lysine deoxycholate (XLD) agar (Oxoid CM0469, Basingstoke, England) and incubated at 37°C for 24-48 hours.

Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish colour and a black center, were sub-cultured by cross-striking on nutrient agar (CM 309; Oxoid, Basingstoke, England) and MacConkey agar (CM0109; Oxoid, Basingstoke, England) for purification. This step was repeated until the colonies in the plate showed morphological homogeneity.

2.3 Identification of *Salmonella* Isolates

Biochemical identification of isolates were performed using Kligler iron agar (KIA), Christensen's urea agar, Simmon Citrate Agar, lysine iron agar (LIA), Voges Proskauer (VP), methyl red (MR), and Indole tests and Peptone water [10]. All media used were from Oxoid, Basingstoke, England. Presumptively identified positive *Salmonella* isolates were confirmed using API 20E system (bioMerieux, Marcy l'Etoile, France). *Salmonella* isolates confirmed by API 20E were further identified by serotyping based on slide agglutination using the antiserum against the somatic O antigen and flagellar H antigens according to the Kauffmann-White scheme (Bio-Rad, Marnes-La-Coquette, France).

2.4 *In vitro* Antimicrobial Susceptibility Testing

Susceptibility testing was performed for Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Cephalexin (30 µg), Cefuroxime (30 µg), Amoxicillin (25 µg), Ceftriaxone (30 µg) and Amoxicillin-clavulanic acid (30 µg) via disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute [12].

2.5 Data Analysis

Data was analyzed using SPSS version 16 Command Syntax Reference (SPSS Inc., Chicago). The Chi-square test was utilized to assess significant differences. A difference was taken as significant at a p-value less than 0.05.

3. RESULTS

All 400 children less than 5 years willingly participated in the study. In total, 9/400 (2.3%) were positive for salmonellae isolates and 3 (1.8%) were in the age group 6-12 months. Results show that children aged 0-5 months had the highest *Salmonellae* infections of 5 (4.1%), followed by 13-24 months with 4 (3.5%), while no *Salmonella* was isolated from the age groups 25-36 months, 37-48 months, or 49-60 months. Males (3.3%) had more *Salmonellae* infection than females (1.4%). The 9 *Salmonella* isolates identified were typed into three serovars: *Salmonella zanzibar* (5), *Salmonella brancaster* (3), and *Salmonella enterica* serovar *enteritidis* (1); all were non-typhoidal *salmonellae*. Symptoms reported by subjects were typical of acute gastroenteritis of NTS infection, which include non bloody diarrhea, abdominal pains (7.2%), nausea (2.0%) and vomiting (1.7%). Feeding type also influenced the rate of infection; children on solid food recorded the highest *Salmonellae* infection of 3.2%, followed by those on breast milk (2.5%), while those on a combination of breast milk and formula milk had no detectable level of *Salmonellae* infection.

Results also showed that, children with watery diarrhoea had the highest *Salmonellae* infection of 2.5%, followed closely by children with mucoid stool 2.2%, while subjects with bloody and loose stool had no detectable *Salmonellae* isolates. In addition, results showed that *Salmonella* was recovered from children with diarrhoea of 4 days (2.8%) than those with shorter diarrhoea duration. Interestingly, (3.1%) of *Salmonella* isolates were recovered from children on antimicrobial therapy, while those who were not on antimicrobial agents, their stool tests came out negative for *Salmonella*. *Salmonella* infections were found more in out-patients than the hospitalized (Table 1). However, there was no statistically significant difference in the occurrence of *Salmonellae* between age groups, sex, symptoms, feeding type, diarrhoea type and duration, drug use and hospitalization.

Table 1. Clinical features and risk factors associated with nine *Salmonella* isolated from diarrhoeagenic children younger than five years in FCT Abuja, Nigeria

Clinical features/ risk Factors	No. tested	No. Positive (%)	P-value*
Age groups (months)			
0-5	49	2(4.1)	} 0.6
6-12	165	3(1.8)	
13-24	116	4(3.5)	
25-36	29	0	
37-48	17	0	
49-60	24	0	
Sex			
Male	180	6(3.3)	} 0.1
Female	220	3(1.4)	
Symptoms			
Nausea	50	1(2.0)	} 0.06
Vomiting	181	3(1.7)	
Abdominal pain	83	6(7.2)	
Body temperature			
≥ 38.0°C	64	0	
Feeding type			
Breast milk	80	2(2.5)	} 0.2
Breast milk + Formula	98	0	
solid food	222	7(3.2)	
Diarrhoea type			
Watery	238	6(2.5)	} 0.8
Mucoid	135	3(2.2)	
Bloody	12	0	
Loose	15	0	
Duration of Diarrhoea			
2 days	312	8 (2.6)	} 0.7
3 days	26	0	
4 days	36	1(2.8)	
No information	26	0	
Drug use			
Antibiotic	64	2(3.1)	} 0.7
supportive treatment only	35	0	
Hospitalization type			
Admitted	59	0	} 0.3
Ambulatory	321	9(2.8)	
No information	20	0	
Characterization of population studied			
Urban	242	7(2.9)	
Peri-urban	158	2(1.3)	

Antimicrobial susceptibility patterns of *Salmonellae* isolated from diarrheic children were tested against seven antimicrobial agents commonly available to determine their susceptibility and resistant patterns (Table 2). Nalidix acid, Ciproflaxacin, and Ceftriaxone were the drugs of choice for *Salmonellae* with susceptibility rates of 100%. The susceptibility

of *Salmonella* isolates to Amoxicillin-clavulanic acid was 22.2%. The resistance pattern to antibiotics used was highest for Amoxicillin, Cephalixin and Cefuroxime 55.6% (Table 2).

Table 2. Antimicrobial susceptibility patterns of nine *Salmonella* isolates from diarrheic children younger than years in FCT Abuja, Nigeria

Antimicrobial agent	No. of susceptible (%)
Amoxicillin 30 µg	4 (44.4)
Amoxicillin-clavulanic acid 20/10 µg	2 (22.2)
Cephalexin 30 µg	4 (44.4)
Cefuroxme 30 µg	4 (44.4)
Ceftriaxone 30 µg	9 (100)
Ciprofloxacin 5 µg	9 (100)
Nalidix acid 30 µg	9 (100)

4. DISCUSSION

This study was conducted to investigate the prevalence of *Salmonella* spp. associated with acute childhood gastroenteritis patients seen in clinics in three urban and two peri-urban hospitals and to evaluate the antimicrobial susceptibility of *Salmonella* isolates to commonly used antibiotics. Infantile gastroenteritis of microbial origin still constitutes a public health problem, with high mortality rate in developing countries and high impact on health costs in industrialized countries [13]. In a study in Abakaliki, south-eastern Nigeria, it was established that 11.3% of cases of acute infantile diarrhoea was associated with *Salmonella* spp. [13], which is higher than the 2.3% recorded in the current study in Abuja.

The finding in this study indicates that *Salmonella* infections were relatively higher in male children than female counterparts in the same age group. This is in agreement with earlier studies by Adkin and Santiago [14] and Ngozi and Onyenekwe [15] also previous reports from Nepal have shown higher prevalence of salmonellosis in males than in females [16]. Higher prevalence rate among males has also been reported among children attending a children hospital in Kathmandu [17]. However high prevalence rates of salmonellosis in females compared to males has been also documented [6].

The nine *Salmonella* isolates were recovered from the age groups less than 24 months. This appeared to be due to many factors. These age groups may have contracted the *Salmonella* infection from close contacts with mother or other caregivers who might have passed the infection. Moreover, hygiene and sanitation are not imminently attainable in these settings due to paucity of portable water and other environmental differentials. Therefore, the vulnerability of these immune deficient age groups could be increased due to unpasteurized milk or food contaminated by the hands of an infected food handler who is a carrier. The findings favourably compares with other studies from Bahia, Brazil that reported higher infection rates among younger children [18]. A report from Kano, Nigeria however, indicates increasing prevalence rates with age [19].

Abdominal pains, nausea and vomiting, watery and mucoid stool were reported as main clinical features associated with Salmonellosis in this study. This is in agreement with Koplan et al. [20] who suggested that the best clinical predictor of positive culture was the combination of fever, and either abdominal pain, with nausea and vomiting [20].

Resistance to antibiotics is becoming more prevalent among *Salmonellae* and other pathogens in poor resource settings. The poor who suffer most of the infection caused by *Salmonellae* bear the cost of extended illness and death brought about by resistance [21]. In our study, none of the 9 *Salmonella* isolates was resistant to Nalidixic acid, different from the studies in Republic of Ireland, which reported resistance in 2.6% [22], and 13.3% of the *Salmonella* isolates [23]. In Owerri South Eastern Nigeria 66.7% of resistance was recorded by Kalu *et al.* [24], other studies in rural Mozambique also reported 3% of resistance to Nalidixic acid [25].

Ceftriaxone is commonly used to treat children with *Salmonella* infections, particularly invasive infections, because of its favorable pharmacokinetic properties and the low prevalence of resistance. In this study Ceftriaxone showed a good antimicrobial activity against the nine *Salmonella* isolates, and it was similar to the study in Ibadan [26].

Ciprofloxacin showed a good antimicrobial activity against the isolates. This is also comparable with the result reported by Akinyemi *et al.* [9] from Lagos, Nigeria, and that reported by Molla *et al.* [16] from central part of Ethiopia. The effectiveness of such drugs like ciprofloxacin may be because it is not widely used in countries like Nigeria, Ethiopia and other African countries [27].

Because specific antimicrobial treatment may be required to supplement other supportive anti-dehydration treatment which is the cornerstone of therapy of acute infant diarrhoea, selective use of antimicrobial agents therefore, cannot be overemphasized. This is vital especially in the developing countries, where inadequate health services, inadequate drug supplies, non-adherence to treatment strategies and dubious drug quality all favour the emergence of microbial resistance [28]. Therefore, it is worthy to note that one outcome of the increased availability and usage of antimicrobial agents for symptomatic treatment of illness has been the emergence of antimicrobial resistance. This was clearly evident from this study where resistance of the *Salmonella* isolates was observed against four commonly used antibiotics (amoxicillin, amoxicillin+ clavulanic acid, cephalixin and cefuroxime). This is of particular concern in the developing world including Nigeria because fewer affordable, appropriate, and effective treatment options such as ciprofloxacin are not readily available in most rural communities.

5. CONCLUSION

This study demonstrated that *Salmonella* isolates are potential pathogens implicated in acute gastroenteritis in children. The overall high resistance level of the isolates to amoxicillin-clavulanic acid, amoxicillin, cephalixin, cefuroxime is a cause for concern.

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COMPETING INTERESTS

The authors declared that they have no competing interests exist.

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