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Antibiotic susceptibilities of *Salmonella* species prevalent among children of 0-5 years with diarrhea in Katsina state, Nigeria

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ABSTRACT

This study was conducted to assess the prevalence of *Salmonella* species among children having diarrhea in Katsina State, Nigeria. A total of 220 diarrhea stool samples of children aged five years and below (0-5 years) were collected and screened for *Salmonella* species using culture technique. Presumptively positive isolates were further screened biochemically and serologically, using Microgen™ Enterobactericeae ID kit and Microgen™ *Salmonella* rapid confirmatory latex agglutination test kit. Antibiotic susceptibility of confirmed isolates was carried out and resistance patterns of the isolates determined. The highest incidence was observed in children of 13-24 months of age and the least in children of 0-12 months of age. There was a higher prevalence in male than female children. All the isolates screened were resistant to Ampicillin and Amoxicillin and of these, 90.9% were resistant to Amoxicillin-clavulanic acid and 45.5% were resistant to Nalidixic acid. However, 100% were sensitive to Gentamicin, 90.9% were sensitive to Ciprofloxacin and Cefotaxime and 9.09% showed reduced susceptibility to Ciprofloxacin. Both the occurrence of *Salmonella* in children and their resistance to multiple antibiotics as observed are of public health significance. The vein of this study underscores the importance of routine monitoring of the incidence of *Salmonella* and continued health education of caregivers.

KEY WORDS: ***Antibiotic resistance; Prevalence of Salmonella; Childhood diarrhea; Katsina State; Antibiotics susceptibility of Salmonella***

INTRODUCTION

Diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). It is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene.

Diarrheal diseases caused by enteric infections remain a leading global health problem. Two to four billion episodes of infectious diarrhea have been estimated to occur annually in developing

countries, resulting in 3 to 5 million deaths.[1] It is a common cause of infant deaths in developing countries, especially where safe drinking water and adequate sanitation and hygiene is unavailable.[2,3] It remains the second leading cause of death among children under five globally.[4] Diarrhoeal disease kills 2,195 children every day (about 801,175 each year), accounting for 1 in 9 child deaths worldwide.[5] As at the end of 2015, Nigeria still ranked second among the top 15 countries with high child mortality due to diarrhea and pneumonia.[6]

The major causes of diarrheal illness include, among others, limited access to or poor quality of water, poor food hygiene, and sanitation. Reports from different parts of the world have implicated various pathogens including Parasites (*Giardia lamblia*, *Entamoeba histolytica*), bacteria (*Escherichia coli*, *Salmonella* species, *Shigella* species, *Campylobacter jejuni*, *Klebsiella species*, *Enterobacter species* e.t.c.) and viruses like the Rotavirus, with the outbreak of infantile diarrheal disease[7-12], all of which are known to be endemic in essentially all developing countries. However in developing countries like Nigeria, infantile diarrhea disease is grossly under-reported and the incidence under-estimated, this is attributed to poverty and ignorance among the affected group who constitute up to 80% of the population of the area[13], the lack of coordinated epidemiological surveillance system, inadequacy of laboratory facilities for culture and unsafe water from all sources contribute significantly to the global burden of disease.[14]

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. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths.[15] Although infections with non-typhoidal *Salmonellae* usually cause self-limiting diarrheal illness, serious sequelae, including meningitis, sepsis, and death, may occur, especially among infants and elderly persons.[16]

In recent years problems related to *Salmonella* have increased significantly, both in terms of incidence and severity of cases of human salmonellosis. Socio-demographic factors (age, education, income etc.), environmental and sanitation factors (poor access to a good water source and poor sanitation) and climatic factors (rainfall, temperature and humidity) are thought to be related to incidence and spatial distribution of diarrhea.[17] The frequency and gravity of these infections are affected by hygienic conditions, malnutrition, and the excessive use of antibiotics that select for multi-drug resistant strains.[18]

In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. New concerns have been identified as since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antibiotics, including first-choice agents for the treatment of humans, such as chloramphenicol and co-trimoxazole, and the third-generation cephalosporins, have emerged and are threatening to become a serious public health problem[19] (**Table 1**). Although Fluoroquinolones have been found to be efficacious both *in vitro* and *in vivo* in the treatment of severe *Salmonella*-associated illnesses, strains with reduced susceptibility to ciprofloxacin among travelers have been reported in some parts of the globe[20]. This resistance results from the use of antimicrobials both in humans and animal

husbandry. Multi-drug resistance to "critically important antimicrobials" is compounding the problems.

Table 1: Review of occurrence of antibiotic resistant Salmonella phenotypes in Nigeria

Author (year)	Location	Antibiotic (% of resistant isolates)
Olowe *et al.* (2007)	Osogbo	AML (93.3), SXT (93.3), AMP (86.9), STP (82.6), CIP (30.4)
Ifeanyi *et al.* (2010)	Abuja	AML (55.6), AMC (55.6), CPL (55.6), CFR (55.6)
Sule *et al.* (2012)	Anyigba	PFX (100), CIP (100), AMC (100), GEN (100), SXT (100), AMP (100), CPL (20.0), NAL (20.0), STP (40.0), OFL (10.0)
Ajibade (2013)	Ekiti-State	STP (100), CHL (100), CTX (100), AMP (100), NAL (100), GEN (96.0), CIP (69.0), TET (100), SXT (100)
Moses *et al.* (2014)	Ile-Ife	NIT (100), CFZ (97.2), GEN (94.2), AML (52.2), AMC (55.1), CFZ (89.9), SXT (44.9)
Galadima and Kolo (2014)	Minna	CHL (100), STP (100)

Key: STP-Streptomycin, AML-Amoxycillin, AMC-Amoxycilin-clavulanic acid, CPL-Cephalexin, CFR-Cefuroxime, PFX-pefloxacin, AMP-Ampicillin, NIT-nitrofurantoin, CFZ-ceftriazone, GEN-gentamicin, CHL- chloramphenicol, NAL-Nalidixic acid, CTX-Cefotaxime

METHODOLOGY

Study Population

The study was hospital and clinic based. The study population included infants and young children of five years and below who reported to the hospitals or clinics with diarrhea symptoms or gastroenteritis. The flowchart of the study is outline in **Figure 1**.

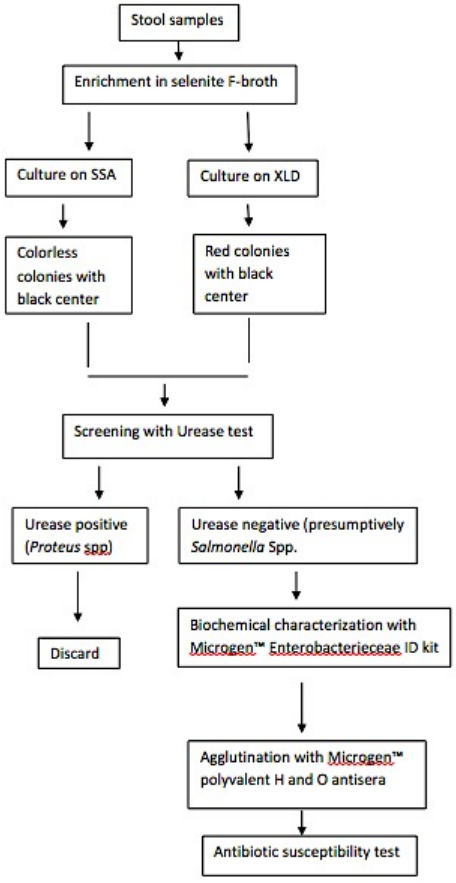

Figure 1: Flowchart of Methodology

Keys: SSA - Salmonella/Shigella agar; XLD - Xylose Lysine Deoxocholate

Control subjects: These were children of the same age group, who had no history of diarrhea, at least three weeks preceding sampling.

Inclusion criteria: Children aged below 5 years, presenting with acute diarrhea attending public and private hospitals within the study area and who have given consent.

Exclusion criteria: Include children above 5 years, of both sexes and lack of consent.

Operational definition: Diarrhea case in this study was defined as a child passing loose or watery stool or a bloody stool three or more times in 24-hour period as reported

by parents. The control subjects were children presented for illnesses other than diarrhea and with no history of diarrhea in the three weeks preceding sampling. Histories were taken from each child from informed and consented parents/caregivers before sample collection by the attending qualified health worker. All the relevant data (demographic, clinical, and laboratory data) were recorded.

Ethical Consideration: Ethical approval was obtained from the Katsina State Ministry of Health (*Ref No: Perm. Sec. / 065-35554*) and the ethics committee of each hospital under study before the commencement of the study. In the course of this research, individuals' anonymity was maintained, good laboratory practice/quality control was ensured and every finding was treated with utmost confidentiality and for the purpose of the research only.

Sample Collection

About 5-10g of stool was scooped into well-labeled sterile wide mouth universal sample bottle using plastic spoon. Where it was not possible to obtain stool, rectal swab was made using sterile swabs and placed in physiological saline in properly labeled sample bottles. Sample collection forms were filled for each sample. This contained information such as: color of stool, texture (formed, semi-formed, uniform, fluid) and presence of blood, mucus, pus in samples. All samples were transported in ice packs to the Bacteriology laboratory, Department of Microbiology Ahmadu Bello University, Zaria for analysis.

Microbiological analysis of stool samples

A loopful of liquid stool or fecal suspension was enriched in selenite F broth for 24 hours at 37°C and then sub-cultured on MacConkey agar (Oxoid) and *Salmonella Shigella* agar (Oxoid). The plates were incubated at 37°C for 24 hours. The resultant isolates were purified for further tests. Non lactose fermenting organisms on MacConkey agar (MCA) and *Salmonella Shigella* agar (SSA) with colorless or pale colonies were purified on Xylose Lysin Deoxocholate (Oxoid) agar, and stored on Nutrient agar (Oxoid) slants for further studies. Media preparations were carried out according to manufacturer's instructions.

Biochemical characterization of isolates

All isolates were biochemically characterized using the Microgen^{TM} GnA+B - ID System for Enterobacteriaceae. Tests were performed strictly following the manufacturer's instructions.

Serological identification of *Salmonella* isolates

Serology tests were performed on all isolates positive for *Salmonella* by biochemical characterization using Microgen^{TM} *Salmonella* kit; a rapid confirmatory latex agglutination test for *Salmonella*. All tests were performed according to the manufacturer's instructions.

Determination of antibiotic susceptibility pattern of isolates

Isolates from both water and stool samples, earlier characterized were tested for susceptibility to the following 10 antibiotics, commonly used in humans for the treatment of Gram negative pathogens; Gentamicin (10µg), Ampicillin (10µg), Amoxycillin (30µg), Trimethoprim/Sulphamethoxazole (1:19) (25µg), Ciprofloxacin (5µg), Nalidixic acid (30µg), Tetracycline (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Amoxicillin/clavulanic acid (2:1), (Oxoid).

Antimicrobial susceptibility testing was performed for all *Salmonella* isolates using the disk diffusion method and results were interpreted using the criteria of Clinical and Laboratory Standards Institute guidelines.[21]

Briefly, each stored isolate was sub-cultured on SS agar (Oxoid). When a pure culture was obtained, a loopful of bacteria was taken and transferred to a tube containing 2 ml 0.85% saline (pH 7.2) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of a McFarland unit of 0.5 to standardize the inoculum size; 0.1ml of the suspension was transferred onto the surface of Mueller Hinton agar (Oxoid) and evenly spread using a sterile bent glass rod. The inoculated plates were left at room temperature to dry for 10-15 minutes. With the aid of disk dispenser, a set of antibiotic disks was then placed gently on the surface of the Mueller Hinton agar (3 antibiotic discs per plate). The discs were gently pressed onto the surface of the medium with sterile forceps to ensure firm contact and incubated at 37^{o}C for 24 hours. Diameters of the zone of inhibition around the antibiotic discs were measured to the nearest millimeter using a rule and the isolates were classified as sensitive (S) or resistant (R), based on CLSI break points. Intermediates were reported as isolates with reduced susceptibility.

RESULTS

A total of 220 stool samples of children aged five years and below, presenting with diarrhea, were collected and investigated for *Salmonella* infection along with 22 control samples from apparently healthy children within the study area. Of the 220 study subjects, 119 (54.1%) were males and 101 (45.9%) were females.

Prevalence of *Salmonella* species among the study population

Figure 2 shows the overall prevalence of *Salmonella* species among the study subjects within the study area. Out of 220 samples collected from children with diarrhea, 11 were positive for *Salmonella* species, giving the overall prevalence of 5.0% in the area.

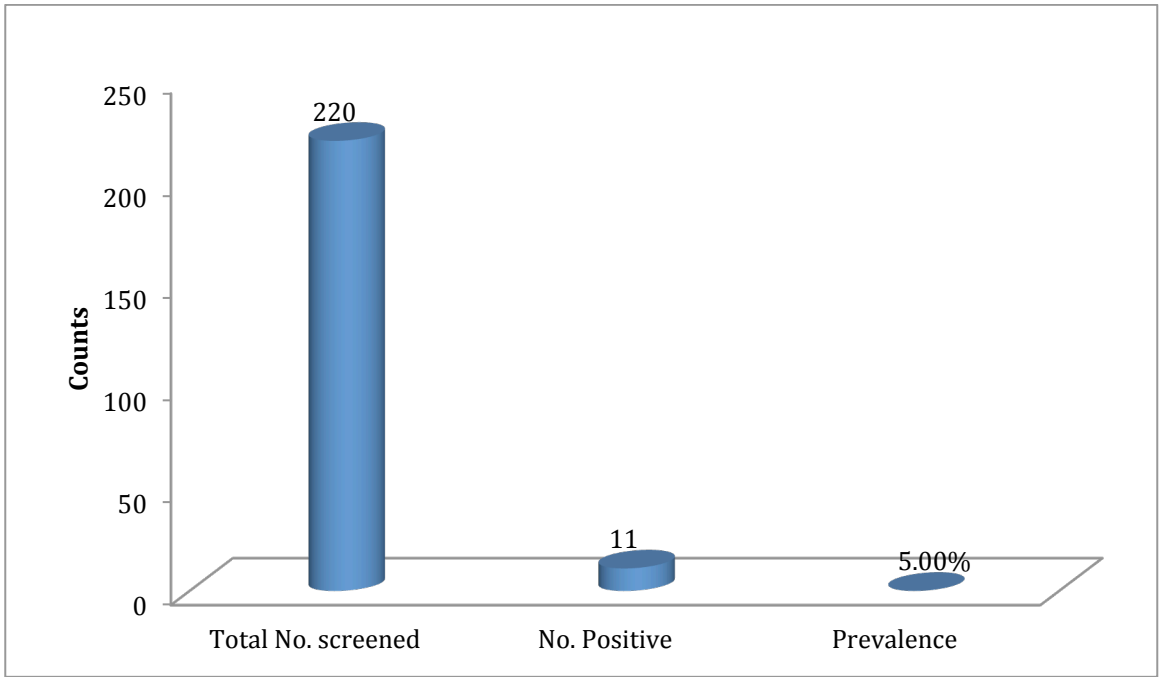

Figure 2: Prevalence of *Salmonella* species among the study population

Prevalence of *Salmonella* spp. according to age and gender of the study population

Table 2 shows the prevalence of *Salmonella* species in relation to age and gender of the study subjects. A high frequency of isolation occurred among children aged 13-24 months and 25-36 months (36.4%), and a 0.0% prevalence among 0-12 months old. Male children were more affected than their female counterparts. There was a statistically significant difference in the occurrence of *Salmonella* with respect to age and gender.

Antibiotic susceptibility of *Salmonella* isolates

Table 3 shows the result of antibiotic susceptibility of *Salmonella* isolates where the organisms showed 100% resistance to the β-lactams: Ampicilin, Amoxycillin and 90.9% resistance to Amoxicillin-clavulanic acid and 63.6% of isolates were resistant to Sulphamethoxazole Trimethoprim. The isolates were, however, 100% susceptible to the aminoglycoside - Gentamicin, 90.9% and 45.5% susceptible to the quinolones (Ciprofloxacin and Nalidixic acid) respectively. A sensitivity of 63.6% to Chloramphenicol was also observed.

Antibiotic resistance pattern of *Salmonella* isolates

Table 4 shows the patterns of antibiotic resistance by *Salmonella* isolates in the study. Two isolates showed resistance to three antibiotics. One isolate was resistant to 4 antibiotics, 2 were resistant to 5 antibiotics, 3 isolates showed resistance to 6 antibiotics, and 3 isolates showed resistance to 7 antibiotics. Various antibiotic resistance patterns were exhibited by the isolates as shown in the table.

Table 2: Prevalence of *Salmonella* spp. according to age and gender of the study population

Age group	Male		Female		Total	
	No. Examined	No. (%) positive	No. Examined	No. (%) positive	No. Examined	No. (%) positive
0-12	37	0(0.0)	25	0(0.0)	62	0(0.0)
13-24	40	2(18.2)	53	2(18.2)	93	4(36.4)
25-36	15	3(27.3)	21	1(9.0)	36	4(36.4)
37-48	15	1(9.0)	9	1(9.0)	24	2(18.2)
49-60	3	1(9.0)	2	0(0.0)	5	1(9.0)
Total	**110**	**7(63.6)**	**110**	**4(36.4)**	**220**	**11(100)**

$\chi^2 = 1.454$, $P \leq 0.05$, $df = 4$

Table 3: Antibiotic susceptibility of *Salmonella* species isolated from diarrheic stools

Antibiotic	Susceptibility of isolates to antibiotics (n = 11)		
	Susceptible	Intermediate	Resistant
Ampicilin	0	0	11(100%)
Amoxycillin	0	0	11(100%)
Amoxicillin Clavulanic acid	0	1(9.09%)	10(90.9%)
Gentamicin	11(100%)	0	0
Sulphamethoxazole Trimethoprim	4(36.4%)	0	7(63.6%)
Chloramphenicol	7(63.6%)	1(9.09%)	3(27.3%)
Cefotaxime	10(90.9%)	0	1(9.09%)
Tetracycline	2(18.2%)	0	9(81.8%)
Ciprofoxacin	10(90.9%)	1(9.09%)	0
Nalidixic acid	5(45.5%)	4(36.4%)	2(18.2%)

Table 4: Antibiotic resistance pattern of *Salmonella* species isolated from diarrheic stool samples

No. of isolates	No. of antibiotics resistant to	Resistance pattern
1	3	AMP, AMC, CTX
1	4	AMP, AMC, TET, AML
2	6	AMP, AMC, CTX, TET, SXT, AML
2	7	AMP, AMC, CTX, TET, SXT, CHL, AML
1	5	AMP, AMC, TET, SXT, AML
1	5	AMP, AMC, CTX, TET, AML
1	6	AMP, AMC, TET, NAL, SXT, AML
1	7	AMP, AMC, TET, NAL, SXT, CHL, AML
1	3	AMP, CTX, AML

Key: AMP- Ampicilin; AML- Amoxycillin; AMC- Amoxicillin Clavulanic acid; SXT- Sulphamethoxazole Trimethoprim; CHL- Chloramphenicol; CTX- Cefotaxime; TET- Tetracycline; NAL- Nalidixic acid

DISCUSSION

Diarrheal diseases caused by enteric infections remain a leading global health problem. It is a common cause of infant deaths in developing countries, especially where safe drinking water and adequate sanitation and hygiene is unavailable.[2,3] Identification of the enteropathogens involved in diarrheal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease.[22] In this study, a prevalence of 5.0% was observed for *Salmonella* species among 220 children with diarrhea. This low prevalence reflects the fact that diarrhea disease in children has multiple etiologies ranging from viruses, parasites and other bacteria agents. These have been reported with varying prevalence around the world including Nigeria. Ogbu *et al*[23] reported the occurrence of Rotavirus (23.3%), *E. coli* (15.4%), *Salmonella* species (11.3%), *Klebsiella* species (7.3%) and *Enterobacter* species (9.6%) as the most predominant etiological agents of diarrhea children in Abakaliki south-east Nigeria. *Shigella, Yersinia enterocolitica*, *Entamoeba histolytica* and *Giadia lamblia* were also recovered in that study. Similarly, Okolo *et al* reported the occurrence of *E. coli, Salmonella Campylobacter* and *Shigella* species among children in Anyigba North Central Nigeria.[24] Ifeanyi *et al* observed *E. coli, Salmonella* Typhi, *Klebsiella pneumoniae*, *Staphylococcus aureus* among others[25]; and Akingbade *et al*[26] isolated *E. coli, Salmonella* and *Shigella*. However, Korie *et al* observed only Enteropathogenic *E. coli* from their report in Enugu.[27]

There was no case observed among the 22 control subjects included in the study, indicating a low carrier rate of the agent in children of this age (≤5years), which is probably due to their lower immune status, and virulence of *Salmonella* species. However, carrier cases have been reported among primary school pupils in a study in Akure, Nigeria.[28]

The occurrence of *Salmonella* species in this study is in conformity with the findings from Bissau, Guinea Bissau[29], Hong Kong[30], Sao Paulo, Brazil[31], Abakaliki, South–eastern Nigeria,[23] and Anyigba, North Central Nigeria[24].

The prevalence of *Salmonella* species (5.0%) in the study subjects agree with the reports of similar studies in Lagos[32], Bangladesh[33], Korea[34], and Ghana.[35] However, the prevalence rate is higher than that obtained by Ifeanyi *et al*[25] who reported a prevalence of 3.2% from a study in Abuja, Nigeria. This could be due to the significant difference in infrastructural development, socioeconomic status and educational level of people living in the area of study (Abuja). This report however, shows lower *Salmonella* prevalence than that reported by Ogbu *et al.* (11.3%),[23] Duru *et al*[36] reported a prevalence of 10.0%, Ike and Damola[37] reported 10.7%, while Galadima and Okolo observed a prevalence of 16.6%.[3]

The disparity may be attributable to differences in study design, patients' selection, differing environmental conditions and behavioral patterns of people in those regions. Occurrence of diarrhea has been associated with factors including lack of education of mother, lack of exclusive breastfeeding, poor nutritional status, immunization status, personal hygiene, overcrowding, garbage disposal, source of water supply, and toilet facility, which are also predominant in the study area.[38]

The result from this study showed that the majority of *Salmonella* species were detected among children of between the ages 13-24 months and 25-36 months (36.0%). Abdullahi *et al*[39] observed the highest prevalence among children between 20-24 months of age. Similarly, Okolo *et al*[24] reported the prevalence among children between ages 13-24

months (28.1%). These findings could be due to combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of foods that may be contaminated with fecal bacteria and direct contact with human or animal faeces when the infant starts to grow. This age bracket also represent a very active stage in the growth and development of children, thus corresponding to the period when the children's contact with the environmental pathogens increases dramatically[36], typically due to ignorance on the rudiments of aseptic or hygienic practices.[40] All these, including the high virulence of *Salmonella* and its low infective dose make exposed children more vulnerable to infections. Detection of *Salmonella* diminished in the age bracket 37-48 months (19.2%). This may signify both an improvement in the immune status of the children and their eating habits. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults. The prevalence of diarrhea is also shown to generally reduce as the children grow due to these factors.[38]

Low prevalence in the 0-12 months of age group could be due to breast-feeding. Antibodies in breast milk protect them from infectious agents. Bacteria like the *Salmonella* species, are associated with dairy products, fecally contaminated food or water, hence proper hygiene and sanitation must be practiced to reduce infection by these pathogens. Similar trends in age related prevalence were observed in other reports.[24,25,39]

Male children were more infected than their female counterparts. Female children within the study area are more protected than the males; this could also increase the chances of infection in the male children. Abdullahi *et al* observed an overall *Salmonella* prevalence of 13.67% from a study in Kano, Nigeria, out of which 8.0% were male and 5.67% were female.[39] Similar observations were made in studies by Adkins and Santiago,[41] Al-Jurayyan,[42] and Ngozi and Onyekwe.[43]

The use of antimicrobial agents in the treatment of diarrhea cannot be overemphasized, because specific antimicrobial treatment may be required to supplement supportive anti-dehydration treatment, which is the cornerstone of therapy of acute infantile diarrhea. However, non-adherence to treatment strategies and dubious drug quality and self-medication all favor the emergence of microbial resistance.[44] This was clearly evident from this study where over 90% resistance to three antibiotics (Ampicillin, Amoxicilin, and Amoxicilin Clavulanic acid) by *Salmonella* species was observed.

It has become increasingly important to monitor patterns of resistance as the antibacterial susceptibility of bacterial pathogens which contribute significantly to the burden of infantile diarrhea is declining. The resistance of the *Salmonella* isolates to the antibiotics tested in this study calls for great concern, as it depicts a high prevalence of antibiotic resistance by Salmonella strains to the β-lactam class of antibiotics (Ampicillin, Amoxycillin, Amoxycillin-clavulanic acid, and Cefotaxime) which are frequently used empirically for the treatment of diarrhea and a number of infectious diseases. Similar trends in antibiotic resistance have been reported previously.[22,34,45,46]

According to Ajibade,[47] the susceptibility patterns reported in 2009 from different locations in Ekiti State showed resistance to streptomycin, chloramphenicol, cefepime, nalidixic acid tetracycline and trimethoprim-sulfamethoxazole. This same trend of resistance was repeated in 2010 and 2011, however, the notable change was a significant increase in the resistance to ampicillin from 20% to 100% in Ado Ekiti

and from 39% to 100% at Ikole Ekiti and also Gentamicin from 18% at Ikere-Ekiti in 2009, to 86% and 84% in 2010 and 2011 respectively.

However, all the strains had varying percentage susceptibility to Nalidixic acid (45.5%), Ciprofloxacin (90.9%), Cefotaxime (90.9%) and Chloramphenicol (63.3%). This means that these chemotherapeutic agents are effective in the treatment of diarrhea caused by these pathogens. The use of Ciprofloxacin in young children however, has grave risks as complications involving troubled breathing, swelling of the face, lips, tongue, mouth, or throat, irregular or uneven heartbeat, fainting or seizures can develop as side effects[48].

Resistance, particularly to the commonly available antibiotics poses major health concerns because the most effective chemotherapeutic agents such as Ciprofloxacin are not readily available in most rural and urban communities.

Salmonella strains in this study generally showed 100% susceptibility to the aminoglycoside gentamicin, however, it has been reported that *in vitro* susceptibility of *Salmonella* species and *Shigella* species to aminoglycosides may appear active. However, are not effective clinically and should not be reported as susceptible.[21] The influence of antimicrobial resistance in *Salmonella* species is quite extensive, reaching many areas. Acquisition of resistance genes adds complexity to laboratory diagnosis and complicates therapeutic outcomes. Antimicrobial resistance also affects the therapeutic regimen, leading to considerable public health concerns and substantial economic burden.

Limitations: Some important aspects such as socio-economic, and environmental factors coupled with health, physiological and behavioral risk factors could not be covered in the study.

CONCLUSION

These research findings show that, though there are a number of causative agents of diarrheal diseases, *Salmonella* still remains one of the major and most important bacterial pathogen of diarrhea among children in the study area. Age of children plays a significant role in both their exposure and susceptibility of the study subjects to *Salmonella* infections. The results of antibiotic susceptibility in this study shows a high resistance rate among isolates especially to the β-lactam group of antibiotics making them completely unreliable in the management of *Salmonella* infections in the study area. Multiple antibiotic resistance was observed in 100% of the isolates, this defines the level of significance antibiotic resistance has become to public health especially in child health.

Recommendations: As a public health measure to reduce the disease burden, an integrated package of immunization services and other childcare programs need to be implemented in addition to well focused health education messages to improve treatment–seeking behavior for childhood diarrhea as well as improved personal and environmental hygiene. The need to continue to carry out extensive multi-center studies involving both rural and urban areas to identify all the risk factors precipitating diarrhea will lead to policies on preventive programs globally.

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