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Antibiotic Susceptibility and Carriage Rate of *Salmonella* Serotypes among Healthy Individuals with History of *Salmonella* Infection within One Year in a University Community in Nigeria

Abstract

The increase in the rate of carrier of typhoid fever is on the increase in the recent time and this present a serious threat to the public health. Students in most cases are living in densely populated hostels with poor sanitary conditions. This coupled with poor hygienic practices constitute pre-disposing factors. This study investigates the carriage rate and antibiotic resistance profile of *Salmonella* among students in a tertiary institution who had suffered gastroenteritis and typhoid fever within one year of infection. Seventy four (74) stool samples were obtained from students in the university. Isolates were identified using standard methods, subjected to antimicrobial susceptibility by disc diffusion method. The incidence of the infection was found to be highest among participants of 20 - 24 years age group. Fifty-one (51) faecal samples were positive for enteric pathogens. Six (11.76%) *Salmonella* species were isolated from stool culture and all were *Salmonella paratyphi B* serotype. All *the Salmonella paratyphi B* showed hundred percent (100%) resistance to Ceftazidime, Ampicillin, Amoxicillin clavulanic acid and Cefuroxime but were susceptible to Ofloxacin and Ciprofloxacin. High carriage of *Salmonella* observed from this study calls for proactive action since most of the isolates were resistance to commonly prescribed antibiotic drugs.

Keywords: *Salmonella*; Salmonellosis; Antimicrobial resistance; Gastroenteritis; Typhoid fever

Review Article

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Introduction

Salmonella infection commonly refers to as Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*Salmonella enterica*, and *Salmonella bongori*). Globally, *Salmonella enterica* serotypes causes up to 27 million infections occur per year, with over $2x10^5$ attributable deaths annually, predominantly among children under the age of five years [1]. Within this genus, more than 2,500 serovars have been described [2,3]

Although all serovars may be regarded as potential human pathogens but majority of the infection is caused by few serovars. Salmonellosis is an important health problem and a major challenge worldwide having greater significance in developing countries [4]. It also contributes to negative economic impacts due to the cost of surveillance investigation, treatment and prevention of illness [5].

Salmonella organisms are aetiological agents of diarrhea and systemic infections in humans. Enteric fever (typhoid or paratyphoid fever) is a systemic infection caused by several *Salmonella enterica* serotypes including *Salmonella typhi* and *Salmonella paratyphi* A, B, or C. *Salmonella enterica* serovar *typhi* (*S. typhi*) is a human restricted pathogen [6,7]. The incidence of typhoid fever remains very high in impoverished areas and the emergence of multidrug resistance had made the situation worse [8]. This group of microorganisms adapts to a wide range of foods, becomes endemic and causes high morbidity with a wide spectrum of clinical manifestations such as diarrhea, nausea, abdominal cramps, vomiting and fever [5]. Transmission is by the faecal-oral route whereby the intestinal contents of an infected animal are ingested with food or water. Human carriers are generally less important than animals in transmission of *Salmonella* strains. Meat, poultry products, dairy products, and fruits and vegetables are primary transmission vehicles; they may be undercooked, allowing the *Salmonella* strains to survive, or they may cross-contaminate other foods consumed without further cooking [9].

Following oral uptake, *Salmonella* are exposed to stressful environments such as low pH, antimicrobial effect of bile, decreasing oxygen supply, normal gut flora and metabolites, cationic antimicrobial peptides present on the surface of epithelial cells. *Salmonella* invades a host cell by delivering into the cytoplasm virulence factors which directly interact with host regulators of actin polymerization which leads to bacterial uptake. It biosynthesizes a virulence capsular polysaccharide named as Vi antigen. Moreover, *Salmonella* avoids vacuole lyses and modulates the early and late endosomal markers presented in the vacuole membrane [10].

Salmonellosis becomes endemic and causes high morbidity with a range of clinical manifestations such as diarrhea, nausea, abdominal cramp, vomiting and fever [11]. The prevalent *Salmonella* infection is typhoid fever, caused by *Salmonella typhi*, which is responsible for life threatening infections in resource-poor nations. However, the true magnitude of salmonellosis is difficult to quantify because the clinical picture is confused with many other febrile illnesses and most typhoid endemic areas lack facilities to confirm the diagnosis [12].

Beta-Lactams and fluoroquinolones are generally used to treat invasive *Salmonella* infections, but emergence and spread of antibiotic-resistant strains are being increasingly notified in many countries. In particular, detection of extended-spectrum β-lactamases (ESBLs) in *Salmonella* serotypes is a newly emerging threat worldwide [13]. Increasing occurrence of antimicrobial resistance in both typhoidal and nontyphoidal *Salmonellae* is a serious public health problem. This aims and objectives of the study are, to isolate and characterize *Salmonella* serotypes among the healthy carriers in the University community, to determine the magnitude and know the circulating serotype of *Salmonella* serotypes among the students and to determine the level of antibiotics resistance of the serotype isolated from the healthy carriers.

Study design and period

A prospective cross-sectional study was conducted to determine the carrier rate of *Salmonella* serotypes among the healthy individual who had recovered from *Salmonella* infections between January 2010 to November 2011.

Study area

The areas for this study were the Ekiti State University community which includes, Student hostels, Iworoko - Ekiti and Ado-Ekiti. Students from the nine faculties in the University were involved in the study (Figure 1).

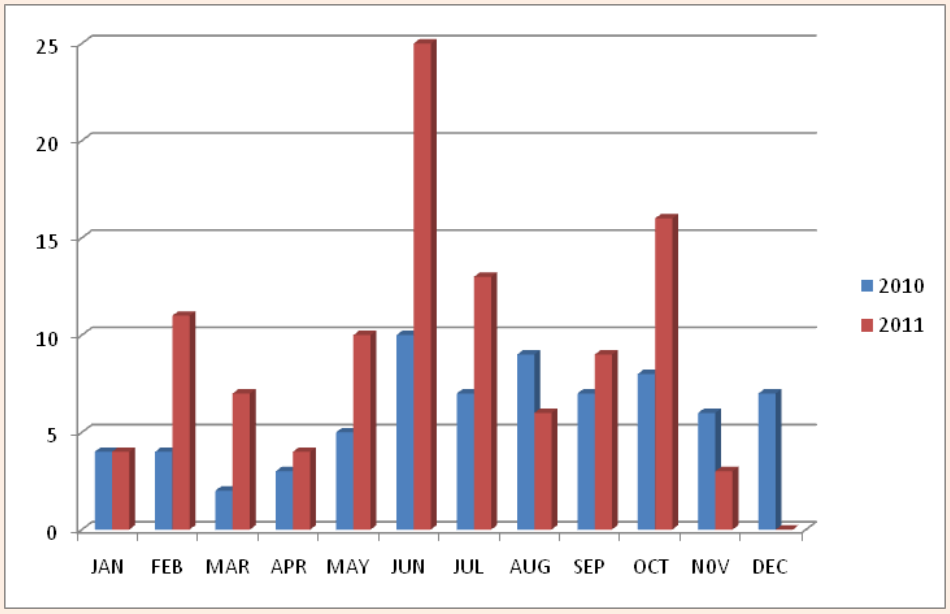

Figure 1: Occurrence of *Salmonella* infection between 2010 and 2011 in the study area.

JAN: January, FEB: February, MAR: March, MAY: May, JUN: June, JUL: July, AUG: August, SEP: September, OCT: October, NOV: November, DEC: December.

Study subjects and population

A total of 1,000 questionnaires were administered across the faculties among the students who were diagnosed at the University Health Centre of gastroenteritis and those who had typhoid fever. Only subjects that gave verbal consents were included in the study. Inclusion criteria were as follows: individuals who were students of the University, regardless of their ages across the faculties in the university, those who were reportedly to be ill of *Salmonella* infections between January 2010 to November 2011 at the university health centre and those who indicated that they had recovered from the *Salmonella* infections.

Collection, handling and culture of specimens

Freshly passed faecal specimens were collected and 1g of the faeces with a sterile wood spatula was transferred and pre enriched into prepared buffered peptone water (1part sample + 9 part buffer) in test tube. Mixed and incubate at 37° C overnight (16-20 hours) [14]. Transferred of approximate 1ml of the pre-enrichment was pipetted into 10ml Rappaport-Vassiliadis soy peptone (RVS) broth and incubated tube at 41.5° C overnight (18-24 hours) [14]. A loop full from the inoculated and incubated RVS broth were emulsified at a point and streaked out on Bismuth Sulphite Agar and incubate at 37° C overnight (18 - 48 hours).

Brown and black colonies growths on plate were picked and subject to biochemical test. Identification of *Salmonella* serotypes was done biochemically as describe by [15].

For the enumeration of other enteric pathogens present in the stool, the samples were differently cultured on MacConkey agar and incubated at 37° C for 24h. Representative colonies were subculture and identified using biochemical and cultural characteristics of the organisms.

Serotyping of *Salmonella* isolates

Serologic identification of *Salmonella* serotypes were performed using Wellcolex colour *Salmonella* test kit. Suspected *Salmonella* colonies from the culture plate were carefully emulsified in 200µl of sterile normal saline in a suspension tube. Holding the bottle vertically, re-suspended latex reagent 1 and 2 were dropped into a separate circle on a flat reaction card after shaking vigorously for few seconds. Transferred of approximately 40µl of bacterial suspension to two of the reaction circles containing latex reagent 1 and 2 respectively and mixed. Placed card on a suitable flatbed rotator and run at 150 ± 5 rpm for 2 minutes then switch off and observed for agglutination without removing the card rotator. Positive controls with the positive control reagents (green, blue and red control) were carried out alongside with the latex reagent 1 and 2 respectively without the inoculums. Results are then interpreted according to the manufacturer guidelines for usage of the kit.

Antimicrobial susceptibility of the *Salmonella* isolates

The isolates were standardized according to Bauer et al. [16] and the antibiotic susceptibility testing of *Salmonella* serotypes was performed using the disk diffusion method, the zone of inhibition was measured to the nearest millimeter and results were interpreted according to Clinical and Laboratory Standards Institute [17]. The antibiotics used were obtained from Abtek Biological Ltd, UK with the following concentrations in Kg: ceftazidime (CAZ) (30Kg), cefuroxime (CRX) (30Kg), gentamicin (GEN) (10Kg), ciprofloxacin (CPR) (5Kg), ofloxacin (OFL) (5Kg), amoxicillin/clavulanic acid (AMX/CLAV) (30Kg), nitrofurantion (NIT) (300Kg) and ampicillin (AMP) (10Kg) were tested against the isolates. Multiple antibiotics resistant was determined as resistant to three or more classes of antibiotics tested.

Results

A total of 1000 questionnaire were administered randomly across the nine (9) faculties in the University out of which 826 were filled correctly and 174 were voided during the study. Out of the 826, 180 indicated positives *Salmonella* infections (typhoid and gastroenteritis) within January, 2010 to November 2011. While 646, claimed not to have been diagnosed of the infection. The age and sex of the 180 students who had recovered having been diagnosed of gastroenteritis and typhoid fever is presented in Table 1. The occurrence of gastroenteritis was highest among those who were within the age range 20 - 24 who had 107 (59.44%), then 25 - 29 had 45 (25.00%), 15 - 19 had 24 (13.33%) and 30 - 34 had 4 (2.22%). High prevalence of the infection was found in female with 112 (62.22%) students and male 68 (37.78%) students. The variation of occurrence of typhoid fever and the incidence of gastroenteritis within January 2010 and December 2011 among the 180 students showed that the peak of occurrence was in the month of June which coincides with the rainy season as presented in Table 2.

Table 1: Age and sex distribution of subjects participated in the study.

Sex	Age (Years)						
	15-19	20-24	25-29	30-34	35-39	Total	%
Male	4	38	22	4	0	68	37.78
Female	20	69	23	0	0	112	62.22
Total	24	107	45	4	0	180	100
Percentage	13.33	59.44	25.00	2.22	0	100	

Table 2: Seasonality of occurrence of *Salmonella* infections among the students examined.

Months													
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2010	4	4	2	3	5	10	7	9	7	8	6	7	40
2011	8	15	9	7	15	35	20	15	16	24	9	7	60
Percentage	4.4	8.3	5.0	3.8	8.3	19.4	11.1	8.3	8.8	13.3	5.0	3.8	100

Jan: January; Feb: February; Mar: March; May: May; Jun: June; Jul: July; Aug: August; Sep: September; Oct: October; Nov: November; Dec: December

The Widal test result of the students examined is presented in Table 3. One hundred and twenty seven (127) (70.56%) did not know their titre result, 23 (12.78%) had a titre of 1/80, 24 (13.33%) had 1/160, 4 (2.22%) had 1/320 and 2 (1.11%) had 1/640.

Out of the 180 students who had been diagnosed of gastroenteritis and typhoid fever only 74 students whose consent were obtained gave out their stool sample. Fifty one (51) faecal samples of the stool submitted showed growth on Bismuth Sulphite agar between 24 - 48 hours of incubation while the remaining 21 showed no growth after this period of incubation.

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Table 3: Titre values of the Widal test conducted on the subjects.

Titre	1/80	1/160	1/320	1/640	Total
Frequency	23	24	4	2	180
Percentage	12.78	13.33	2.22	1.11	100

The profile of bacterial pathogens isolated at from the stool of the subjects is presented in Table 4. The pathogens isolated were *Klebsiella* spp 15 (29.41%), *Proteus* spp 10 (19.61%), *Salmonella* serotype 6 (11.76%), *Escherichia coli* 5 (9.80%), *Morganella* spp 3 (5.88%), *Citrobacter* spp 2 (3.92%) while *Shigella* spp, *Edwardsiella* spp, *Yersinia* spp had one isolate each and six were unidentified after the biochemical test [15].

Serologic testing with Wellcolex *Salmonella* test kit showed that the six *Salmonella* spp isolated were *Salmonella paratyphi* B as presented in Table 5. There was red agglutination of the organisms with both latex reagent 1 and 2. The agglutination in reagent 2 showed the presence of virulent (Vi) antigen in the *Salmonella* serotypes isolated.

The percentage resistances of the bacterial pathogens isolated from the 51 students examined were having 100% resistance to ampicillin, amoxicillin/clavulanic acid and cefuroxime while ciprofloxacin and ofloxacin were susceptible with one or two resistances to them as presented in Table 6. Gentamicin, nitrofurantion and ceftazidime had variable resistance percentage. The resistance patterns of the *Salmonella paratyphi* B isolated is presented in Table 7. All were resistance to ceftazidime, ampicillin, Augmentin and cefuroxime. Two of the *S. paratyphi* B had resistant pattern CAZ/AMP/AMX-CLAV/CRX. The entire organisms isolated were having multiple resistance patterns to the class of cephalosporin, penicillin, aminoglycoside and macrolides.

Table 4: Occurrence of bacteria pathogens isolated from stool of subjects with typhoid and gastroenteritis.

Organisms Isolated	Frequency	%
Salmonella serotype	6	11.76
Proteus spp	10	19.61
Shigella spp	1	1.96
Escherichia coli	5	9.80
Klebsiella spp	15	29.41
Citrobacter spp	2	3.92
Molrganella spp	3	5.88
Edwardsiella spp	1	1.96
Providencia spp	1	1.96
Yersinia spp	1	1.96
Unidentified	6	11.76
Total	51	100

Spp: Species

Table 5: Serogroup of *Salmonella* isolated from the students examined.

Isolate	Agglutination		Colour		*Salmonella serotype*	Antigen
	Reagent 1	Reagent 2	Reagent 1	Reagent 2		
1	+	+	Red	Red	*S. paratyphi*	Vi
2	+	+	Red	Red	*S. paratyphi*	Vi
3	+	+	Red	Red	*S. paratyphi*	Vi
4	+	+	Red	Red	*S. paratyphi*	Vi
5	+	+	Red	Red	*S. paratyphi*	Vi
6	+	+	Red	Red	*S. paratyphi*	Vi

Positive (+); Negative (-)

Table 6: The percentage resistance of the bacterial pathogens isolated from the stool of the subjects.

Antibiotics	Pathogens					
	Salmonella spp (n=6)	*Proteus* spp (n=10)	*E. coli* (n=5)	*Klebsiella* spp (n=15)	*Citrobacter* spp (n=2)	*Morganella* spp (n=3)
CAZ (%)	6 (100)	9 (90)	4 (80)	15 (100)	2 (100)	3 (100)
AMP (%)	6 (100)	10 (100)	5 (100)	15 (100)	2 (100)	3 (100)
NIT (%)	3 (50)	3 (30)	4 (80)	7 (46.67)	0	1 (33.33)
AMX/CLAV (%)	6 (100)	10 (100)	5 (100)	15 (100)	2 (100)	3 (100)
OFL (%)	2 (16.67)	0	0	2 (13.33)	0	1 (33.33)
CIP (%)	0	0	0	2 (13.33)	0	1 (33.33)
GEN (%)	2 (33.33)	5 (50)	2 (40)	2 (33.33`)	0	1 (33.33)
CRX (%)	6 (100)	10 (100)	5 (100)	15 (100)	2 (100)	3 (100)

CAZ: Ceftazidime; AMP: Ampicillin; GEN: Gentamicin; CRX: Cefuroxime; NIT: Nitrofurantion; AMX/CLAV: Amoxicillin/Clavulanic acid; OFL: Ofloxacin; CIP: Ciprofloxacin; n: Number

Table 7: Antibiotics resistance pattern of *Salmonella* serotype isolated.

Resistance Pattern	Frequency
CAZ, AMP, GEN, CRX	2
CAZ, AMP, NIT, AMXCLAV, CRX	2
CAZ, AMP, AMX/CLAV, CRX	1
CAZ, AMP, NIT, AMXCLAV, OFL, CRX	1

CAZ: Ceftazidime; AMP: Ampicillin; GEN: Gentamicin; CRX: Cefuroxime; NIT: Nitrofurantion; AMX/CLAV: Amoxicillin/Clavulanic acid; OFL: Ofloxacin

Discussion

Epidemiological investigation of salmonellosis and carriage rate in developing countries like Nigeria is difficult because of the very limited scope of the studies and lack of coordinated surveillance systems. Out of the 1000 questionnaire administered in this study, 180 (18%) were positive for *Salmonella* infections with high symptoms of headache, fever, weakness, vomiting, loss of appetite, stooling and cold.

Female were more susceptible to the infection than the male counterpart with 122 (62.22%) and 68 (37.78%) respectively in this study, which is in contrast to similar findings by Okonko et al. [18] but Zailani et al. [19] found no influence of age and sex on the distribution pattern of *Salmonella* species in Ile - Ife.

The highest occurrence of *Salmonella* infections were found in 20 - 24 years age group from the participant studied. This also agrees with Liilian et al. [20] which find a significant prevalence of *Salmonella* infection within this age group. Mengistu et al. [21] also reported that *Salmonella* were more prevalent among adult age greater than 15 years.

Akinyemi et al. [22,23] saw in his study in Nigeria that typhoid fever was prevalent during the wet season. This study also shows that highest prevalence of the infection was found within spring and summer seasons (May and October) usually referred as the rainy season with the peak of the disease occurring in June. Typhoid fever is water and food borne disease, and an average temperature of 35° C during the rainy season provides the optimum conditions for the growth of bacteria. Similar findings in India, Shina et al. [24] also found highest incidence of typhoid in the monsoon season.

The Widal test is rapid and sensitive in the diagnosis of *Salmonella* infections (typhoid and paratyphoid). 16.66% were having active infections with the significant of 1/160 and above as presented in Table 3, this significant is in line with Udeze et al. [25] and Liilian et al. [20] in diagnosis of asymptomatic students at Ilorin. 127 (70.56%) knew not their titre result and this can increase the spread of the infection due to their ignorance about the titre results.

In the present study a total of 51 entropathogens were isolated from stool (Table 4). The overall prevalence of *Salmonella* in this study was 8.11% (six *Salmonella* serotypes) this also conformed to the work of Abdullahi, 2010 in Kano State with the incidence of 13.50% also with the work of Cajetan et al. [26] with prevalence of 2.3% out of 400 samples.

The isolates resistant to four or more separate classes of antimicrobials were defined as multidrug resistant [27]. The incidence of resistance (i.e. resistance to two drugs) and multidrug-resistance (i.e. resistance to four or more drugs) of all *Salmonella* strains isolated is presented in Table 6. *Salmonella* sensitivity was highest to ciprofloxacin (100%) and ofloxacin (83.33%) similar to Bahnass et al. [28] which reported that *Salmonella* strains were susceptible to ciprofloxacin likewise observed by Cajetan et al. [26]. The sensitivities of the other antibiotics were as follows: gentamicin (66.67%) and nitrofuratoin (50%) while cefuroxime, amoxicillin/clavulanic acid, ampicillin, and ceftazidime were resistant to by the *Salmonella* species isolated.

Conclusion

Conclusively, the high prevalence of salmonellosis needed to be monitored since most of the species isolated were resistance to most of the commonly available antibiotics and also the provision of portable water for drinking should be at its climax since the organisms is mainly transmitted by water [29,30]. The epidemiology of salmonellosis in Nigeria had to be well explored since the serotypes and resistance pattern of the isolates may vary greatly in different geographical areas and there is a need for the development of guidelines for antibiotic treatment in this area.

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