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Prevalence and carriage status of *Salmonella typhi* among students of Ekiti State University, Ado-Ekiti, Nigeria

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Abstract

The carriage of *Salmonella*Typhi among Nigerian students is still very high due to very poor hygiene. In this study the prevalence, antibiotic susceptibility of *Salmonella*Typhi from the stool of undergraduate students from a Nigeria university was investigated. *Salmonella* Typhi was isolated from stool samples from 510 subjects. Antibiotic susceptibility test of the isolates was carried out using disk diffusion method. The clinical data (Widal Test) from the University Health Centre records were also collected and analyzed in this study. Out of the isolates, 75.90 % were resistant to ceftazidime, 34.87 % to cefuroxime, 83.59 % to cefixime and 18.46 % to ofloxacin. *Salmonella*Typhi isolated exhibited multiple resistance to at least three of the antibiotics tested. The carriage rate of the *Salmonella*Typhi among undergraduate students of Ekiti State University, Ado-Ekiti was 33.86%. The clinical data showed the rate of the *Salmonella* infection among the undergraduate students to be 58.90%. From the clinical record of the University Health Centre, 935 (40.20%) and 1,391 (59.80%) of the students with symptoms of the enteric fever were male and females respectively and only 1370 tested positive to *Salmonella* antibody. There was a significant difference between the males and the females with the two tails, p-value = 0.023, F=2.931. The needs for proper educated and the good hygiene cannot be overemphasized.

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Introduction

The incidence of food-borne infections is global and these infections are mainly caused by microorganisms, majorly enteric bacteria (CDC, 2005; WHO, 2007). The principal causative agent of food-borne bacterial pathogens include *Salmonella, Campylobacter*, *Listeria,* pathogenic *Escherichia coli, Yersinia, Shigella, Vibrio, Enterobacter*and *Citrobacter*(Emmanuel and Ashley, 2011). The presence of the toxins of *Staphylococcus aureus*, *Clostridium botulinum*and *Bacillus cereus* has also been reported to cause food-borne intoxications (EFSA, 2012).

Salmonella is a Gram-negative facultative rod-shaped bacteriumthat is responsible for bloodstream and acute gastroenteritis which result from food-borne infection or intoxication (Forbes, 2002; Anushtup*et al.,* 2010). *Salmonella* is a microbiota of gastro intestinal tracts of both warm- (birds and mammals) and cold-blooded (reptiles) animals (Abera*et al.,* 2010). This bacterium is a major cause of enteric bacterial illness in man that has been reported to be transferred to humans mainly through food of animal origin including poultry, eggs, dairy products, meat and meat products (Abera*et al.,* 2010).

More than 3,000 serovars of *Salmonella* Typhimurium*, S.* Enteritidisand *S.* Entericahave been reported worldwide (Mead *et al.*, 1999; WHO, 2005). *Salmonella* infections can be typhoidal or non-typhoidal. Serotypes such as *S.* Typhi, *S.* Paratyphi Aand *S.* Paratyphi Bare the main causes of typhoidal salmonellosis that only infect human host (Boyle *et al.*, 2007; EFSA, 2012; Aofe*et al.,* 2014). Typhoid salmonellosis causes enteric fever, a systemic disease of human characterized by fever and abdominal pain. Asymptomatic human (acute, transient and permanent carriers) may spread the disease and the rates of communicability may be enhanced when hygiene is very poor or compromised. Unlike typhoidal salmonellosis, non-typhoidal salmonellosis infects both humans and animals (Gonzalez-Escobedo and Gunn, 2013). Although food-borne, animals are the major reservoir, of salmonellosis, it can be spread from person-to-person via the faecal-oral route in few cases (Muleta, 2001; Brooks *et al.*, 2004; Buchwald and Blaser, 2004; Gomez-Duartz, 2011).

Like the general society, institutions of learning are equally exposed to diverse conditions that encourage the transmission of enteric pathogens. Therefore this study aimed at determining *Salmonella* carrier rate among students of Ekiti State University, Ado-Ekiti and the antibiotic resistance pattern of the isolates.

Materials and methods

Collection and Processing of Samples

A total of 510 non-repeat stool samples were collected from both male and female consented students of Ekiti State University, Ado-Ekiti, Nigeria (EKSU) and examined. The samples were homogenized in sterile distilled water and 1.0 ml of diluted stool samples was plated on MacConkey agar and Plate Count Agar and plated at 37 ^{o}C for 24 h. Faecal samples (1 g) was pre-enriched in 9 ml of buffered peptone water for 24 hrs at 37 ^{o}C after which 0.1 ml of the pre-enriched culture was plated into of Selenite cysteine broth (10 ml) and of Rappaport-Vassilidis broth and incubated for 24 h at 37 ^{o}C and 42 ^{o}C respectively. The enriched cultures were inoculated on to Xylose Lysine Deoxycholate agar and incubated at 37 ^{o}C for 24 h.

Identification of the Organisms

Colonies with circular shape or form, big or small size, entire margin or edge, raised or flat elevation and smooth surface were selected. Gram-negative rods; motile, catalase-positive, oxidase-negative and Simmon's citrate-negative were selected for further biochemical methods of identification. Urase, casein, gelatin and utilization of carbohydrate (arabinose, glucose, glycerol, indole, lactose, mannitol, mentose, starch and sucrose) were carried out on the isolates. The results were interpreted according to Barrow and Feltham (1993).

Antibiotic Sensitivity Testing

The isolates were standardized by growing at 37 ^{o}C in Mueller-Hinton Broth (Oxoid) for 16 h and adjusted

to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625 nm. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (CLSI, 2012). The isolates were tested against the following eight commercial antibiotics (AbtekBiologicals Limited) with their concentrations in microgram: Amocixillin/clavulanic acid (30), cefixime (5), ceftazidime (30), cefuroxime (30), ciprofloxacin (5), gentamicin (10), nitrofurantoin (300) and ofloxacin (5).

Retrospective collection of cases of typhoid infection (enteric fever) in the institution

A retrospectivestudywascarried out to determine the incidence of typhoidfever. Records of all Widal positive diagnosed cases in the UniversityHealth Centre wereretrieved and analyzed. Demography of patients whichincludedsex, time of diagnosiswas obtained.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Scientists (SPSS, version 17). Any significant difference between the values was assessed at 99.95% level of significance (p 0.05).

Results

Five hundred and ten (510) non-repeat stool samples from 186 male and 324 female students within the age range of 16-35 years were collected and examined; sex distribution of these students is presented in Table 1. One hundred and ninety five (38.24%) of the total stool samples were positive for *Salmonella* spp. A total of eighty two (44.09 %) and 113 (34.24 %) stool of male and female respectively were positive for *Salmonella* Typhi.

Table 1. Sex distribution of students examined in the study for the carriage of *Salmonella*.

Sex	Number Examined	Percentage (%)	Number Positive	Percentage (%)
Male	186	36.47	82	44.09
Female	324	63.53	113	34.88
Total	510	100.00	195	38.24

The *Salmonella* Typhi isolates subjected to antibiotic susceptibility test resistant to the antibiotics test at varying degrees. All the isolates were susceptible to gentamicin and nitrofurantoin while 18.60 % of the isolates were resistant to ofloxacin while 26.15 % were resistant to ciprofloxacin. Only 83.59 %, 75.90 % and 55.38 % of the total isolates were susceptible to cefixime, ceftazidime and Amoxicillin/clavulanic acid respectively as shown in Table 2.The phenotype of multiple antibiotic resistance patterns of *Salmonella*Typhi isolated from the stool samples are depicted in Table 3. One hundred and eighteen (118) *Salmonella*Typhi isolated had multiple antibiotic resistances to at least three of the antibiotics tested. It also shows clearly the high resistance of *Salmonella*Typhi to ceftazidime, cefixime and cefuroxime antibiotics that are classes of cephalosporin. Forty one of the isolates were resistant to three antibiotics while fifty nine and eighteen out of the isolates were resistant to four and six antibiotic respectively.

Table 2. Antibiotics resistant pattern of *Salmonella* Typhi isolated from stool sample of undergraduate students.

Antibiotics concentration	Resistant	
	Frequency (n = 195)	Percentage (%)
Ceftazidime (30µg)	148	75.90
Cefuroxime (30µg)	68	34.87
Gentamicin (10µg)	0	0.00
Cefixime (5µg)	163	83.59
Ofloxacin (5µg)	36	18.46
Amoxicillin/clavulanic acid (30µg)	108	55.38
Nitrofurantoin (300µg)	0	0.00
Ciprofloxacin (5µg)	51	26.15

From the clinical record of the University Health Centre, 935 (40.20%) and 1,391 (59.80%) of the people with symptoms of the enteric fever were male and females students respectively. Out of the total number of students that complained of gastrointestinal infections only 477 (34.82%) males and 893 (65.18 %) females were positive for *Salmonella* antibody indicating *Salmonella* infection. A total of 1,370 (58.90 %) were positive out of 2,326 screened as shown in Table 4. There was a significant difference between the males and the females with the two tails, p-value = 0.023, F=2.931.

Table 3. Phenotype of multiple antibiotics resistance pattern of *Salmonella* species isolated against Gram-negative antibiotic discs.

Number of Antibiotics	Resistotype	Number of Occurrence(n = 195)	Percentage (%)
3	CAZ, CRX, CXM	14	7.18
	CAZ, CXM, AMOX/CLAV	27	13.85
	Total	41	21.03
4	CAZ, CXM, OFL, AMOX/CLAV	14	7.18
	CAZ, CXM, AMOX/CLAV, CIP	9	4.62
	CAZ, CRX, CXM, CIP	9	4.62
	CAZ, CRX, CXM, AMOX/CLAV	27	13.85
	Total	59	30.30
6	CAZ, CRX, CXM, OFL, AMOX/CLAV, CPR	18	9.23
	Total	18	9.23

KEYS:CAZ = Ceftazidime, CRX = Cefuroxime, CXM = Cefixime, AMOX/CLAV = Amoxicilin-clavulanic acid, CIP= Ciprofloxacin.

The highest occurrence of *Salmonella* infection observed in this study followed the same distribution among the male and female students. Among the students (both male and female) the occurrence of *Salmonella* was highest among the age bracket of 26-30 year with percentage occurrence of 61.40% (n=280) and 72.46 % (n=521) among the male and female students respectively. Male students within the age range of 31-35 years had the least occurrence (n=32, 29.36 %). Students within the age range of 31-35 years had the least occurrence with male having 29.36 % (n=32) and female 51.16 % (n=66) as shown in Table 5.

Table 4. Sex distribution of the of undergraduate students with enteric fever.

Sex	Number Examined	Percentage (%)	Number Positive	Percentage (%)
Males	935	40.20	477	34.82
Females	1,391	59.80	893	65.18
Total	2,326	100.00	1,370	100.00

The highest occurrence of *Salmonella* was observed in 2014 followed by 2013. In 2011, the least occurrence of *Salmonella* infections was recorded in the health center of the University (Table 6). The occurrence of the salmonella infection was notice in May (74.89 %) and June (70.18 %) of the years considered. The occurrence was also high in January (69.36 %) while August (33.33 %) recorded the least occurrence followed by July (47.66 %) (Table 7).

Discussion

Human salmonella infections constitute a major health concern in Africa like in other resource-poor nations of the world. *Salmonella* has low doubling period and can survive in most food samples (Dugid and North, 1991). One hundred and ninty five

(38.24%) of the total stool samples were positive for *Salmonella*Typhi comprises of 113 female and 82 male. The occurrence of the organisms was higher in female than male. Fentabil (2013) reported that chronic *Salmonella* carrier state occurs most commonly among the middle age women.

Table 5. Age distribution of undergraduate students with enteric fever.

Age	Males			Females			Total		
	Number Examined	Number Positive	Percentage (%)	Number Examined	Number Positive	Percentage (%)	Number Examined	Number Positive	Percentage (%)
16-20	115	42	36.52	195	104	53.33	310	146	47.10
21-25	255	123	48.24	348	202	58.05	603	325	53.99
26-30	456	280	61.40	719	521	72.46	1,175	801	68.17
31-35	109	32	29.36	129	66	51.16	238	98	41.18
Total	935	477	51.02	1,391	893	64.20	2,326	1,370	58.90

All the isolates were susceptible to gentamicin and nitrofurantoin while 18.48 % of the isolates were resistant to ofloxacin. This is similar to the finding of Akyala*et al.* (2013) who reported a low resistance of *Salmonella* spp to gentamicin and nitrofutatoin. The resistant *Salmonella* strains have been reported to be more than 20 times greater in virulence than the antibiotic-susceptible strains (Helms *et al.*, 2003). Abuse of the first-line drug of choice may have led to the selection of resistant strains of *S. typhi* as opined by Olopoenia*et al.* (2000).

Table 6. Year distribution of the cases of enteric fever in EKSU Health Centre.

Year	Males		Females		Total	
	Number Examined	Number Positive	Number Examined	Number Positive	Number Examined	Number Positive
2011	23	11 (47.83)	50	27 (54.00)	73	38 (52.05)
2012	390	204 (52.31)	571	321 (56.22)	961	525 (54.63)
2013	283	138 (48.76)	429	278 (64.80)	712	416 (58.43)
2014	239	124 (51.88)	341	267 (78.30)	580	391 (67.41)
Total	935	477 (51.02)	1,391	893 (64.20)	2,326	1,370 (58.90)

The resistance patterns of the *Salmonella*Typhi isolated from stool samples in this study showed a remarkable high resistance to first line antibiotics. This high resistance among the isolates should be a serious public health concern because antibiotic resistance among pathogenic bacteria could increase fatality. Food and water may be the major vehicle for the transmission of the pathogens as reported by Karshima*et al.* (2013). Multiple antibiotic resistance (MAR) patterns of the isolates showed three different resistotypes in this study. The MAR in *Salmonella*Typhi may be as a result of selective pressure from the unguided and indiscriminate use of antibiotics.

Enteric fever has been reported to be diagnosed by Widal agglutination test: a serologic reaction between the pathogen and the specific antibody produced by the body due to (previous) exposure to the pathogens (Washington and Henry, 1991). This has formed a baseline on which typhoid fever diagnosis has been based for quite some time (Ibekwe*et al.*, 2008). From the clinical data collected from the University Health Centre 935 (40.20%) and 1,391 (59.80%) were male and females students respectively. Female students had a higher carriage of *Salmonella* Typhi and

occurrence of typhoid fever than their male counterpart. However, Dewan*et al.* (2013) reported higher incidence of typhoid fever in male population than female in the Dhaka Metropolitan Area of Bangladesh. Typhoid fever is a common infection in resource-poor nations and lack of potable water, inadequate sewage disposal system and poor personal hygiene the major factors responsible for the spread of the infection.

Table 7. Month Distribution of cases of enteric fever in EKSU Health Center.

Month	Males			Females			Total		
	Number Examined	Number Positive	Percentage (%)	Number Examined	Number Positive	Percentage (%)	Number Examined	Number Positive	Percentage (%)
January	64	36	56.25	10 9	84	77.06	173	120	69.36
February	96	40	41.67	130	90	69.32	226	130	57.52
March	121	48	39.67	187	114	60.96	308	162	52.60
April	115	51	44.35	170	105	61.76	285	156	54.74
May	90	61	67.78	145	118	81.38	235	179	74.89
June	161	101	62.73	219	165	75.34	379	266	70.18
July	93	49	52.69	121	53	43.80	214	102	47.66
August	28	11	39.29	56	17	30.36	84	28	33.33
September	67	32	47.76	85	44	51.76	152	76	50.00
October	29	15	51.72	34	21	61.76	63	36	57.14
November	50	22	44.00	94	57	60.64	144	79	52.78
December	21	11	52.38	41	25	60.98	63	36	57.14
Total	935	477	51.02	1,391	893	64.20	2,326	1,370	58.90

In this study there was a clear seasonality in the distribution of typhoid fever, age and gender differences were also noticed in the clinical data of serological diagnosis collected from the University Health Centre. The occurrence was also high in January (69.36 %) while August (33.33 %) recorded the least occurrence followed by July (47.66 %). This trend was also reported earlier by Dewan*et al.* (2013). We observed the highest occurrence of the enteric fever in the months of May and June. Anyebe*et al.* (2008) reported the seasonal variation of typhoid fever occurrence in Zaria, Nigeria to follow the same pattern. They noticed that in the five-year period they investigated the occurrence of the infection was higher in May and June with 20.2% and 20.7 %, respectively.

In conclusion, female had higher rate of typhoid infections in the higher institution selected for this study. Gentamicin and nitrofurantoin had a better *in vitro* activity on the isolates. Students have to be informed about the risks of misuse of antibiotics and good hygiene has to be promoted. The highest occurrence of the enteric fever was observed among the students with age range of 26 and 30 years followed by those within 21 and 25 years. The least occurrence was recorded among students within 31 and 35 age bracket.

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