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Carrier Status of S*almonella* Species Infection among Students in a Tertiarry Institution

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Abstract Salmonellosis is a major challenge to public health due to its persistence and high rate of recurrence. This study investigated *Salmonella* infections and associated risk exposures among tertiary students. This cross-sectional study enrolled 100 undergraduate students. Their personal health information and feeding habits were collected using a questionnaire. Fecal specimens were collected and analyzed bacteriologically. The isolates were subjected to antibiotics susceptibility test. Of 100 students enrolled, 38% had Salmonella species. The prevalence rate was Salmonella *typhi*, 29%, *Salmonella. paratyphi A*, 6%, and *Salmonella enteritidis*, 3%. The females accounted for 27% and males 11% of the isolates. The age group of 20-24 years had the highest infection rate. The participants that were not previously diagnosed of *Salmonella* species infection had a higher carriage rate of 26% than those who were previously diagnosed of typhoid fever (12%), 48% of the students, do not regularly practice hand washing and 63% never had symptoms of typhoid fever. The students patronize food vendors and this accounted for 34% of the *Salmonella* isolates. Self-medication was associated with the prevalence of *Salmonella* species (P=0.000; 95% CI). The *Salmonella* isolates were resistant to Amoxicillin, Cefuroxime and Nitrofurantoin. The carrier rate of *Salmonella typhi* was high due to antibiotic abuse.

Keywords: *typhoid, carrier, self-medication, community co-habitation, antibiotics*

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1. Introduction

Salmonellosis remains a major health challenge world-wide because of its high carrier rate and infectious outcome [1]. The disease, typhoid and paratyphoid fever collectively known as enteric fever are caused by systemic infection with *Salmonella enteritica* subspecies *enteritica* serovars Typhi and Paratyphi A, B and C [2]. Typhoid and Paratyphoid fever, the prevalent *Salmonella* infections, primarily causes bacteremia febrile illnesses with prolonged fever, headache and malaise. On the other hand, non-typhoidal *Salmonella* species infections mostly induce diarrheic illness and less of bacteremia. The non-or-ineffective treatment of typhoid and paratyphoid fevers results in altered mental states (termed the typhoid state), ileus and gastrointestinal bleeding, intestinal perforation, septic shock and death [2]. The global Burden of *Salmonella* infection is enormous. Typhoidal salmonellosis accounts for over 27 million cases, resulting in 220,000 deaths per annum. With the introduction of antimicrobial therapy, the mortality rate of typhoidal salmonellosis saw a sharp decline; however, with the emergence of resistant strains, a 7% increase have been observed [3]. In 2017, it was estimated that 14.3 million people worldwide were infected with typhoid and paratyphoid fever with children and the elderly being more affected [4]. The Global burden of disease [4] estimated that more than 135,000 deaths that occur worldwide was due to typhoid and paratyphoid fevers.

On the other hand, non-typhoidal salmonellosis accounts for 93.8 million foodborne illness, with 155,000 deaths per annum [5]. A 25% mortality rate has been reported in Africa following infection with non-typhoidal salmonellosis [6].

The major route of transmission of typhoidal salmonellosis is fecal-oral, usually following the ingestion of food and water contaminated by human faeces (carriers) [7]. Non-typhoidal Salmonellosis is transmitted predominantly via food contaminated with animal faeces and spread of the organism (particularly in developed countries) is facilitated in part due to increase in the centralization and industrialization of food supply chain [8]. Ingestion of contaminated animal products such as milk, eggs, poultry, ground beef or dairy products and increasingly food products such as chocolate, peanut butter are important sources of infection and major outbreaks [9].

In endemic regions, typhoidal salmonellosis affects children aged 5-19 years and young adults [10]. On the other hand, a bimodal age distribution of non-typhoidal salmonellosis with peaks occurring in children and elderly

has been observed [10]. However, a global study conducted in 2010, revealed that Africa has the highest incidence (about 57%) of this disease, and that infants, children and young adults were mostly affected [11].

With particular emphasis on young adults, studies on *Salmonella* infections tends to be quite limited. However, a common observation has shown that the disease cuts across children, adolescents and adults. Hence, it has been suggested that these age associations are influenced by several factors [10]. A major factor which associates adolescents with *Salmonella* infections is their feeding/eating habit. In consideration of important lifestyle changes, young adults tend to modify their feeding habits in terms of variety and frequency of intake [12]. Eggs and other egg related meals, for example,. Ice-cream, yoghurt, cakes, salad dressings, meat (both home prepared and those sold outside the home) and Diary products are very popular in the diets of adolescents. In addition, young adults heavily consume vended food and snacks (many find it cheap and tasty), unsafe water, locally prepared drinks such as. zobo, kunu; of which the hygienic safety cannot be guaranteed [13]. Amongst young adults there is an inconsistency in their level of hygiene and hand washing practice after using the toilet and during food handling. Moreover, due to their productive and active nature, they interact more with the environment- a major vehicle for transmission of Salmonellosis (via their activities and the food and water prepared and consumed within such environment) and with other individuals (reservoir of *salmonella* infections) [14].

The infections and associated re-occurrence of Salmonellosis continues to be a burden among the populace. The students of tertiary institutions are at high risk mostly due to living conditions especially water source and feeding routines. Some may be carriers of *Salmonella* species, without being aware of such mutual existence. The indiscriminate use of antibiotics tends to suppress the manifestation of the disease, Salmonellosis, thereby rendering such individuals as carriers of the bacterium. Thus, early identification of individuals harboring these bacteria is a step towards its control. The goal of this study was to determine the carrier status of *Salmonella* species among students of a tertiary institution. The study also assessed the risk exposures for acquiring the bacteria, and evaluated the antibiotics susceptibility patterns.

2. Materials and Method

Study area: The study was conducted at University of Nigeria, Enugu Campus (UNEC) located in Enugu State, Nigeria which is made up of young adults majorly. A major health challenge visible in the hostels is student overpopulation thereby over-stretching the available common facilities.

Study population and design: This was a cross-sectional study that enrolled undergraduate students of the institution, either living in the hostel or off-campus. The inclusion criteria consist of those students that do not present with any noticeable clinical symptom of febrile illness or were receiving treatment for typhoid fever and malaria. On the other hand, individuals who were on medication for any illness were excluded from the study. A structured questionnaire was administered to obtain information on demographic characteristics, feeding habits, personal hygiene and antibiotics usage.

Sample and Data collection: Consecutive sampling frame was used to select study participants. These participants were interviewed and completed a structured questionnaire. Participants who met the criteria for this study were provided with an appropriately labeled, clean, dry, wide necked specimen container for sample collection. The participants were advised to provide fresh fecal specimens under aseptic conditions preferably those passed in the mornings and with the aid of a clean applicator spoon incorporated into the lid of the specimen container transfer a spoonful of the specimen into the labeled specimen container provided taking care not to contaminate the neck or outside of the container with the sample. In addition, the participants were advised to avoid contaminating the fecal sample with urine. The stool samples collected from these participants in the mornings were transported to the laboratory immediately for processing and analysis.

2.1. Laboratory Procedure

General Macroscopy and examination: The stool sample was examined macroscopically noting its colour, consistency, presence of mucus and blood. Presence of ova of parasites was examined using standard methods [15].

Bacterial culture: The stool samples were analyzed in accordance with standard bacteriological methods [16]. The stool samples were inoculated unto Salmonella Shigella Agar (SSA), MacConkey agar and Deoxycholate citrate agar; part of the samples were inoculated onto Selenite F broth, all were incubated for 24 hours at 37^0c. The selenite F broth culture was further sub-cultured onto SSA and deoxycholate Agar at 37^0c for 24 hours for confirmation of isolates of Salmonella and the results of both the direct culture and subcultures were noted. The stool-culture plates with growth were identified by morphological characteristics, gram stain and biochemical tests [16].

Serotyping: Pure cultures of all the suspected isolates plated on nutrient agar was used. All the isolates were subjected to tests using commercially available polyvalent O and H (phase 1 and 2) antisera (Bio-Rad, UK). All the positive isolates were further subjected to further testing using monovalent O and H antisera. For *S.typhi*, monovalent O antiserum group D and monovalent H antiserum group C (Bio-Rad, UK) was used. Since *S.typhi* strains may possess Vi antigens, the O antigens were detected after destruction of the Vi antigen by boiling of cultures for 10 minutes. For *S. paratyphi A*, monovalent O antiserum group A and monovalent H antiserum group a (Bio-Rad, UK) was used for confirmation. For *S. enteritidis*, monovalent O antiserum group D and monovalent H antiserum group g, m (Bio-Rad, UK) was used for confirmation. All tests were performed according to the manufacturer's instructions.

Antibiotics susceptibility test: Kirby Bauer disc diffusion technique was used. The antibiotics discs used included: Nitrofurantoin (100mcg), Ceftriaxone

(30mcg), Gentamicin (10mcg), Ciprofloxacin (10mcg), Chloramphenicol (10mcg), Ofloxacin (10mcg), Pefloxacin (10mcg), Cefuroxime (10mcg), Streptomycin (30mcg) and Amoxicillin (30mcg) Susceptibility patterns was recorded quantitatively by measuring the diameters to the nearest millimeters using a meter rule, following the interpretative chat of the Kirby-Bauer Sensitivity Test Method [17]. The results were interpreted as sensitive, intermediate or resistant.

ETHICAL CLEARANCE: Ethical approval to conduct this study was obtained from the Ethics Committee, University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu. A written informed consent was obtained from all the participants of this study.

2.2. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software version 21.0 was used for data entry and analysis. The validity of data collected was ensured by double entry and random checks for errors. Descriptive statistics and use of Tables was applied to compute the data and Chi-square was used to compare the variables. Significance was taken at $P \leq 0.05$.

3. Results

A total of 100 students participated in this study. They comprised of 31 (31%) males and 69 (69%) females with a mean age of 20.9 ± 2.1 (age range 18-29 years) and a median age of 20 years. Of the 100 participants, 38% (38/100) had isolates of *Salmonella* species. The Salmonella species isolated were *Salmonella typhi* 29% (29/100), *Salmonella paratyphi A*, 6% (6/100) and *Salmnella enteritidis*, 3% (3/100) (Table 1).

Table 1.Profile of Salmonella species Isolated

Isolate	f (%)
Salmonella typhi	29(29)
Salmonella paratyphi A	6(6)
Salmonella enteriditis	3(3)
Total	38(38)

Demographics and Personal Hygiene of the Participants: The females had more *Salmonella* species isolates than males 27% (27/100) and 11% (11/100) respectively, while the age group of 20-24 years had the highest number of isolate 27% (27/100). Both age and sex were not statistically significant. Also, the participants that were previously diagnosed of typhoid fever had 12% (12/100) of *Salmonella* species as against those that never had typhoid fever 26% (26/100). Of the 12% (12/100) of participants previously diagnosed of typhoid fever, *Salmonella typhi* was detected from all of them. The students that never had or was previously diagnosed of typhoid fever for the past six months had 17% (17/100) of *S. typhi*, 6% (6/100) of *S. paratyphi A* and 3% (3/100) *S.enteritidis* respectively.

Table 2. Demographic and Clinical Characteristics of the Participants

Variable	N	No of isolate (%)	X^2	P-value
Sex			**0.121**	**0.728**
Male	31	11(11)		
Female	69	27(27)		
Age group (years)			**0.011**	**0.994**
15-19	24	9(9)		
20-24	71	27(27)		
25-29	5	2(2)		
Previously diagnosed of Typhoid fever in the past six months			**1.930**	**0.165**
Yes	24	12(12)		
No	76	26(26)		
Self prescribed antibiotic use			**22.924**	**0.000**
Yes	32	23(23)		
No	68	15(15)		
Hand washing practice using Soap			**2.497**	**0.287**
Yes	44	13(13)		
No	8	4(4)		
Sometimes	48	21(21)		
Symptoms observed			**5.284**	**0.259**
Abdominal cramps	20	9(9)		
Diarrhea	8	4(4)		
Nausea	2	2(2)		
Vomiting	7	3(3)		
No response	63	20(20)		

Table 3. Assessment of feeding habits among the Participants

Variable	N	No of Isolates (%)	X^2	P-value
Do you normally cook?			**0.091**	**0.762**
Yes	91	35(35)		
No	9	3(3)		
Patronize food Vendors?			**0.014**	**0.906**
Yes	89	34(34)		
No	11	4(4)		
Number of times food vendors are patronized per day			**0.520**	**0.471**
Once	31	10(11.2)		
≥Twice	58	24(27)		
How often do you consume pastries			**0.795**	**0.672**
Always	16	7(7)		
Sometimes	53	18(18)		
Rarely	31	13(13)		
Source of drinking water			**0.724**	**0.696**
Tank water	23	9(9)		
Satchet water	72	28(28)		
Tank and Satchet water	24	9(9)		
Washing of fruits and vegetables before eating			-	-
Yes	100	38(38)		
No	0	0(0)		

Table 4. Logistic Regression for Significant Variable

	Do you normally take antibiotics on your own when you feel illness				
Odds Ratio (OR)	OR value	Standard Error	P-value	95% C.I FOR OR	
				Lower limit	Upper limit
	9.030	0.49	0.000	3.456	23.593

The use of antibiotics without prescription was assessed and it showed that 23% (23/100) of *Salmonella* species isolated were from those that practiced self medication while 15% (15/100) of the isolates were from the subjects that depend on a physician's prescription. This variable was statistically significant (P=0.000). The Logistics Regression Analysis indicated that 87.7% of the students that practiced self medication were more likely to harbor *Salmonella* species with OR=9.030, 95% CI: 9.030 (3.456-23.953) (Table 4).

The practice of hand washing after visiting the toilet indicated that majority of the students 48% (48/100) do not regularly practice hand washing as 21% (21/100) *Salmonella* species isolation rate was obtained, while those that consented to the practice had a 13% (13/100) isolation rate.

The clinical symptoms observed by the participants include abdominal cramps, diarrhea, vomiting and nausea while majority of the participants 63% (63/100) do not observe any noticeable symptom (Table 2).

Assessment of feeding habits: Table 3 showed the feeding habits of the participants. Of 91% (91/100) of students who cook their own food, 35% of the *Salmonella* species were isolated. In addition, the students (those that do not cook) do at times patronize food vendors, these categories of people accounted for 34% (34/100) of the *Salmonella* isolates. The numbers of times these students patronize food vendors were categorized and those that patronize food vendors once per day had 11.2% of Salmonella isolates.

The consumption of pastries as a major meal or in between meals as widely practiced by the students was assessed and the result indicated that those who casually consume any of these pastries accounted for 25% (25/100) of the isolates as against those who rarely consume these pastries with 13% (13/100).

The source of drinking water indicates that there was no statistical difference between the use of sachet water and tank water in that both sources were simultaneously used by the students

Antibiotic Susceptibility Pattern of the Isolates: The antibiotic susceptibility patterns indicated that *S. typhi* was sensitive to Gentamicin, Ciprofloxacin, Ofloxacin, Pefloxacin, Chloramphenicol, Ceftriaxone, and resistant to Amoxicillin (96.6%) and Cefuroxime (100%).

On the other hand, the six isolates of *S. paratyphi A* were all resistant to Amoxicillin and Cefuroxime while the three isolates of *S.enteritidis* were all resistant to Nitrofurantoin and Cefuroxime (Figure 1).

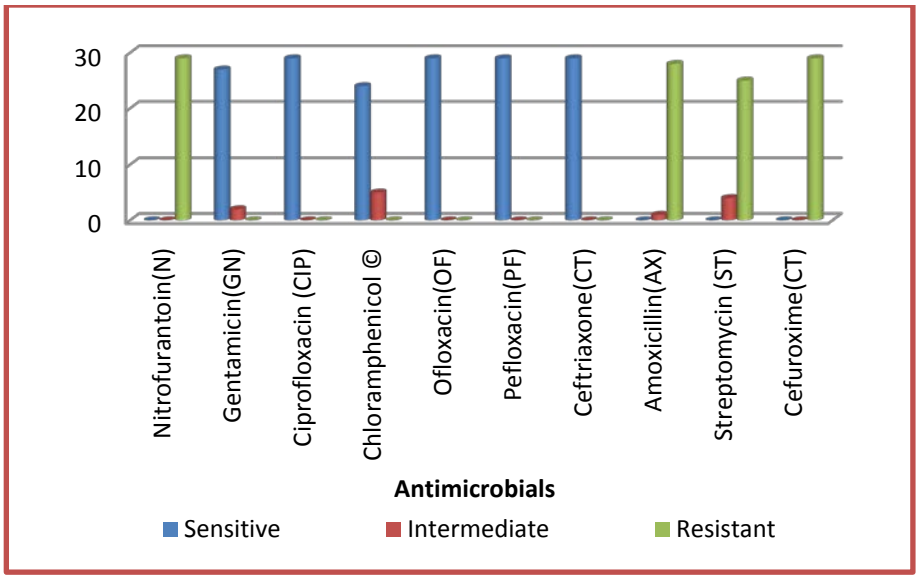

Figure 1a. Antimicrobial susceptibility pattern of Salmonella typhi

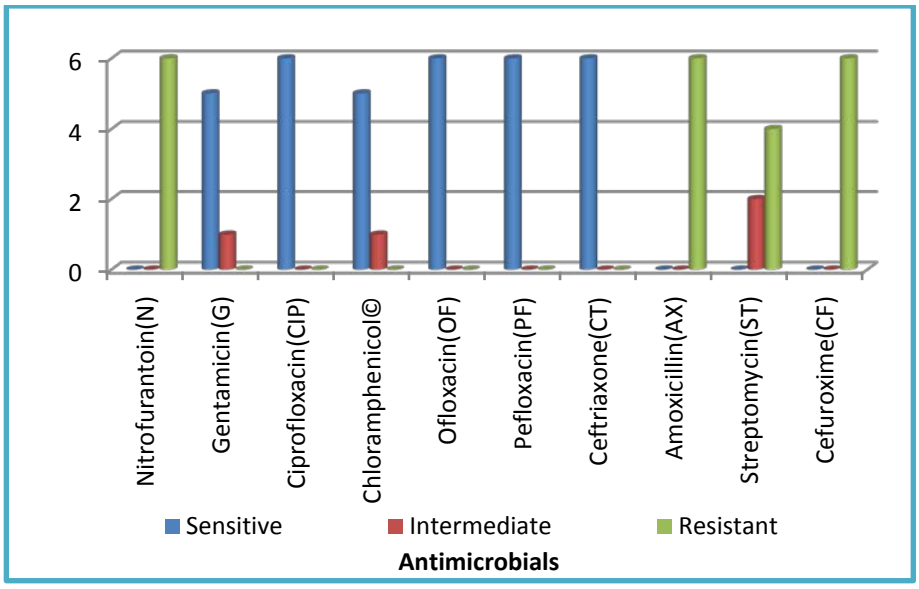

Figure 1b. Antimicrobial susceptibility pattern of Salmonella paratyphi

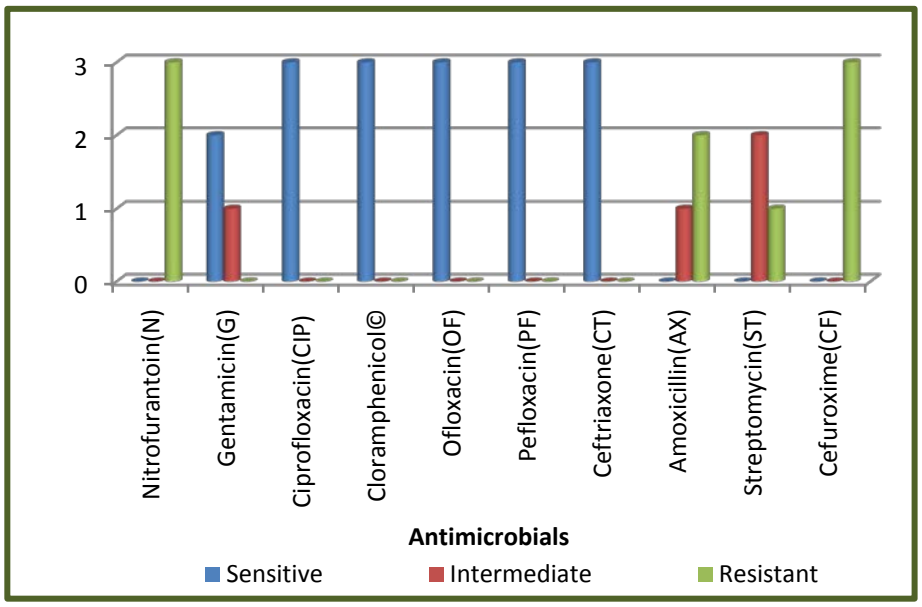

Figure 1c. Antimicrobial susceptibility pattern of Salmonella enteriditis

4. Discussion

The pattern of students living and feeding habits is a complex problem within the society in that, there is an element of communal life which leads to series of interactions like eating together, sharing of toilet systems and other features. This type of living creates an avenue for typhoidal, para-typhoidal and nontyphoidal Salmonellosis and most of all, a carrier status within the student population. The current study analyzed the carrier status of *Salmonella* species among students in a university setting.

The prevalence rate of 38% obtained from the participants indicated a high carrier status among the students. In a similar study, [18] reported an overall prevalence of 36.78% among undergraduate students in Owerri, Nigeria. The prevalence rate could probably be due to the low level of hygiene within the hostels and poor feeding routines of the students as evidenced in their lifestyles.

Three (3) *Salmonella* species were identified in this study. *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella enteritidis*. The high isolation rates of *S. typhi* was similar to the findings of [19] who reported a high prevalence rate of typhoidal disease. In contrast, [18] reported the predominance of *Salmonella typhimurium* followed by *S. paratyphi* and *S. typhi*.

The participants that were not previously diagnosed of *Salmonella* species infection had a higher carriage rate, 26%, than those who were previously diagnosed of typhoid fever 12%. In contrast, [20] reported that those who were previously diagnosed of *S. typhi* had a higher frequency of *Salmonella* species infection amongst food handlers in Ethiopia. This difference may be due to the constant contact food handlers have with food thereby transmitting or getting re-infected with the bacterium, whereas in this study, the students who were previously diagnosed of *Salmonella* infection may have received proper therapy or indulge in self- medication in order to eradicate the disease. From this study, those that were previously diagnosed with *Salmonella typhi* accounted for 12% of the isolates which was indicative of a carrier status. This is in line with studies which suggested that even after recovery from typhoid fever, some individuals continue to excrete the bacilli in stool; and that antibiotic therapeutic interventions do not absolutely eliminate carriage. As a result, such individual would continue to excrete the pathogen in their faeces as carriers [21]. Moreover, majority of the participants 63% who accounted for 20% of the isolates did not observe any noticeable symptoms. Asymptomatic carriers pose a major public health problem as these individuals carry the bacterium for an unspecified period of time ranging from days to years without any noticeable symptom, and shedding viable bacilli in their faeces thereby serving as a reservoir of the disease [22]. Hence, carriers amongst students would not only serve as a source of infection, but would also maintain the chain of infection leading to the persistence of the disease within the student population.

The highest isolation rates were obtained in the age group of 20-24 years, which was similar to the findings of [23], who reported a high prevalence in this age bracket. These findings suggests that in areas endemic with Salmonellosis, young adults present with high infection rates [8,24]. Females had a higher case of Salmonella carrier status accounting for about 27% of the isolates in contrast to males 11% which contradicts the work of [25] which showed males were more affected than females. However, from this study, both age and sex were not statistically significant, and this is corroborated by [26] who reported that age and sex distribution had no influence on *Salmonella* species infection in Ile-Ife.

This study revealed that those who practiced self- medication with antibiotics accounted for 23% of *Salmonella* species isolated in comparison to the 15% isolated from those who depended on a Physician's prescription. In addition, this variable was statistically significant (P=0.0001) implying that it was an important risk factor in the prevalence of *Salmonella* species amongst the students. Furthermore, the Logistics regression analysis indicated that 87.7% of the students who practiced self medication with antibiotics were more likely to harbor *Salmonella* species: OR=9.030, 95%CI: 9.030(3.346-23.953) which was in line with the statement by [27]. The lack of strict restriction on drug purchases [28] and the availability of most antibiotics within drug stores expose students to the indiscriminate use of drugs without prescription. The lack of regulatory policies on drug sale and purchase within the University ensures the availability of controlled antibiotics on the counter [29]. Thus, the improper treatment of this disease may have contributed to the high carrier status of *Salmonella* species within the student population.

The practice of hand washing with soap after visiting the toilet was accessed and it was observed that students who do not practice adequate hand washing were more infected with the disease. In addition, students that cook their own food were more infected. [30] recorded an overall low prevalence rate of *Salmonella* infection amongst food handlers in Ethopia and attributed the findings to be due to a better practice of personal hygiene after visiting the toilet. This finding revealed a strong association between personal hygiene and food safety. Most students are in the habit of either keeping long nails or for the females fixing nails for beautification purposes. The improper grooming coupled with a lack of good hand washing practice all constitute poor hygienic practices seen amongst students. When personal hygiene is poor, the safety of food consumed may not be guaranteed since the major constant route of Salmonellosis is fecal-oral [31,32].

In addition, the consumption of pastries and other vended foods is a major characteristic of the feeding routines amongst students. From this study, students (both those who cook and those who do not) patronize food vendors, and this category had 34% of *Salmonella* isolates. In addition, students who casually consume pastries as a meal or in between meals had 25% isolation rate as against 13% from those who rarely consume these pastries. The high rate of *Salmonella* species isolated from the students who consume vended food may be due to improper handling and preparation of the food or pastries by these food vendors. The role of food vendors in public health and transmission of Salmonellosis has been emphasized continually as they are a great source of the disease [33,34]. Furthermore, the high isolation rate

obtained from those who largely consumed pastries may also be attributed to *Salmonella enteritidis* which accounted for about 3% of the total *Salmonella* species isolated. *Salmonella enteriditis* is mostly associated with poultry and its derivatives, especially eggs which have been implicated as the source of many outbreaks of Salmonellosis [35]. The ability of the bacterium to infect poultry especially laying hens without any noticeable disease has assisted in its spread [35]. As such, eggs and egg related meals like salad dressings, Ice cream, pastries like cake, chicken pie can also serve as a source of the disease. The fact that *Salmonella enteriditis* is zoonotic poses a major problem as it can lead to a wide spread of Salmonellosis.

The relationship between source of drinking water and the prevalence of *Salmonella* infection was indicated in the study. The students attributed source of drinking water to the use of either bagged sachet water or tank water. Although, there was no statistical difference between the use of sachet water and tank water in that both sources were used simultaneously by the students, those who consumed only sachet water were more in number and they accounted for about 28% of the *Salmonella* isolates. The high preference of sachet water amongst the students may be due to the belief that since it is properly packaged, it must have undergone careful processing and treatment, hence "pure" when compared with tank water whose source is largely unknown. The high number of isolates obtained could be due to poor water quality (indicative of fecal contamination), a sign of minimal or no treatment of this water source. This was in line with the work of [36] who did a bacteriological water quality analysis on sachet packed drinking water in Nigeria. Students who are regular consumers of locally prepared drinks like Zobo, Kunu (because of its affordability) are more prone to Salmonellosis especially if unsafe water were used. Moreover, the use of contaminated water to wash food produce like fruits and Vegetables increases the chances of getting infected with the bacterium. [34], in their work recorded a high level of *Salmonella* species isolate from food handlers who consumed untreated water when compared with those who consumed treated water. Thus water remains a major vehicle for its transmission since the bacterium is highly ubiquitous in nature [8].

Salmonella infections can only be tackled properly when appropriate therapeutic agents are administered particularly with the incidence of multi drug resistance on the rise. Therefore, there is a need to determine the susceptibility pattern of antimicrobials before treatment. The antimicrobial susceptibility testing carried out in this study revealed an incidence of resistance to the antibiotics used. The fluoroquinolones (Ciprofloxacin, Ofloxacin, Pefloxacin) and Ceftriaxone showed maximum antimicrobial effect against all the *Salmonella* isolates (100%) which was similar to [37] and [38].

All the *Salmonella* species isolated showed a high level of resistance to Amoxicillin, with *S. typhi* (96.6%), *S. paratyphi A* (100%) and *S. enteriditis* (66.7%). This was similar to the work of [14]. Moreover, the isolates showed 100% resistance to Cefuroxime, in agreement with [23] and 100% resistance to Nitrofurantoin which contradicts that of [30] who reported a 46.2% resistance of isolates to Nitrofurantoin.

The high level of resistance to some of these drugs could be due to the development of resistance genes by this organism either via plasmid transfer or mutation in its chromosome [27]. Indiscriminate use of antibiotics may be an important factor.

In conclusion, there was a high *Salmonella* species carrier status among the students which may enhance the circulation of the bacterium within the student population. Counseling units within the University environs be established to enlighten the students on drug usage and its complications.

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