WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 18, 9-20. Research Article ISSN 2277–7105

ANTIBIOTIC SUSCEPTIBILITY PATTERN AND PREDOMINANCE OF *SALMONELLA*SPECIES AMONG STUDENTS OF TERTIARY INSTITUTIONS IN IMO STATE

Nwaehiri U. L.* **and Ozurumba A. U.**

Department of Environmental Biology, Federal Polytechnic Nekede, Owerri.

Article Received on
25 August 2023,

Revised on 15 Sept. 2023,
Accepted on 05 Oct. 2023

DOI: 10.20959/wjpr202318-29876

***Corresponding Author**
Nwaehiri U. L.
Department of Environmental Biology, Federal Polytechnic Nekede, Owerri.

ABSTRACT

Salmonellosis is a major challenge to public health due to its persistence and high rate of recurrence. The research was carried out to determine the antibiotic susceptibility pattern and predominance of *Salmonella* species in relation to knowledge, attitudes and practices among students of tertiary institutions in Imo State. Blood and stool specimens were collected from five hundred and ten (510) students schooling in the five tertiary institutions (Federal Polytechnic Nekede, Alvan Ikoku Federal College of Education, Imo State University Owerri, Imo State Polytechnic Umuagwo and Federal University of Technology Owerri) in Imo State. The inoculation and isolation of *Salmonella* species was done using various media followed by biochemical identification to obtain positive isolates before serotyping to obtain Salmonella serogroups. Afterwards, the positive isolates were utilized in testing the antibiotic susceptibility against different antibiotic drugs. Generally, the prevalence rate was in a decreasing order of *S. typhi* (25.3%) >*S. paratyphi A* (12.5%) >*S. enteriditis* (5.3%). FPNO (48.0%) and Imo Poly Umuagwo (45.7%) had the highest prevalence rates while AIFCE (33.8%) had the least prevalence. Ciprofloxacin, pefloxacin, ofloxacin and ceftriaxone showed 100% sensitivity to the three *Salmonella* isolates while sensitivity to gentamicin and chloramphenicol were*S. typhi* (96.6%), *S. paratyphi*(66.6%) and *S. enteriditis* (66.6%). On the contrary, the three *Salmonella* isolates were highly resistant to amoxicillin, cefuroxime, nitrofurantoin and streptomycin. In conclusion, there was high carrier rate of *S. typhi* among students in the tertiary institutions in Imo State probably because of the abuse of antibiotics.

KEYWORDS: *Salmonella* infection, antibiotic susceptibility, students, tertiary institution.

1.0 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*). Salmonellosis remains a major health challenge world-wide because of its high carrier rate and infectious outcome.[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. 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.[4] 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.[5]

The global Burden of *Salmonella* infection is enormous. Typhoidal salmonellosis accounts for over 27 million cases, resulting in 220,000 deaths per annum.[5] 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.[6] 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.[7] The[7] estimated that more than 135,000 deaths that occur worldwide were due to typhoid and paratyphoid fevers.

The major route of transmission of typhoidal salmonellosis is fecal-oral, usually following the ingestion of food and water contaminated by human faeces (carriers).[8] Human carriers are generally less important than animals in transmission of *Salmonella* strains. In endemic regions, typhoidal salmonellosis affects children aged 5-19 years and young adults.[9] On the other hand, a bimodal age distribution of non-typhoidal salmonellosis with peaks occurring in children and elderly has been observed.[9] 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.[5, 10]

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.[11] In particular, detection of extended-spectrum β-lactamases (ESBLs) in *Salmonella* serotypes is a newly emerging threat worldwide.[12] Increasing

occurrence of antimicrobial resistance in both typhoidal and non-typhoidal *Salmonellae* is a serious public health problem.

Theinfection prevails irrespective of any age categories; nevertheless, children of low and middle-income countries havethe highest documented burden.[13] In some countries (Nepal to be precise), the multi-drug resistant (MDR) H58 Salmonella *typhi*seems to have been substituted by non-MDR H58 carryingthe S83F mutation in gyrA and other mutation-associated with reduced susceptibility to fluoroquinolones.[14] Likewise, Salmonella *paratyphi* A frequentlycarries fluoroquinolone non-susceptibility alleles in gyrAand parC; however, none of these isolates were MDR.[15]Nevertheless, the possible transmissibility ofMDR H58 could not be neglected since many casesof MDR H58 had been reported from studies in developing countries.[14]

2.0 METHODS

2.1 Study Area

This cross-sectional study was carried out in five institutions (Federal Polytechnic Nekede, Alvan Ikoku Federal College of Education, Imo State University Owerri, Imo State Polytechnic Umuagwo and Federal University of Technology Owerri) in Imo State. Federal Polytechnic Nekede is located at Nekede and Nekede is a rural area near the city of Owerri, Imo State Nigeria. Federal University of Technology Owerri (FUTO) is approximately 20 away from Nekede while driving and is located at Ihiagwa. Ihiagwa is a rural community located in Owerri West Local Government Area (LGA) in Imo State, Nigeria. The distance between Ihiagwa and Owerri capital city is approximately 12 km apart. Imo State Polytechnic is located at Umuagwo and Umuagwo is a town in the Ohaji/Egbema LGA of Imo State in Nigeria. Alvan Ikoku Federal College of Education (AIFCE) and Imo State University (IMSU) are located within the heart of Owerri capital in Imo State. Owerri is the capital of Imo State in Nigeria, set in the heart of Igbo land.

2.2 Instrument for Sample Collection and Duration of study

Researcher's self-constructed questionnaires were administered to the students to obtain information on demographic characteristics and knowledge, attitude and practices while sample bottles were used to collect stool samples. The study lasted for approximately eleven months, between October 2022 and August 2023.

2.3 Sample Size Determination

The Cochran's formula was used in this study (Cochran, 1977).

Thus, the following formula**, $n = z^2 \times p(1-p) / d^2$;**is suitable for the present study design and therefore will be applied.

Where, n=required sample size.

z=confidence level at 95% (standard value of 1.95)

p=estimated prevalence in the project area **=28%**.[16]

d=margin of error at 5 %(standard value of 0.05).

$n = 1.95^2 \times 0.28(1- 0.28)/0.05^2$

$= 3.8025 \times 0.28 \times 0.72 / 0.0025$

$= 306.6336$

$=307$

The minimum sample size will be 307.

2.4 Ethical Approval: Institutional Review Board approval for this study will be obtained from Federal Medical Center (F.M.C.) Owerri.

2.5 Inclusion/Exclusion criteria

Inclusion criteria: The participants in the study will be;

a) Male and Female students from tertiary institutions in Imo State within the age group of 15-50 years.
b) Students who voluntarily and willingly accept to provide us with data and sample.
c) Students with proven records of Salmonella infection documented in government hospital in the past 2 years, and those presently suffering it.
d) Students that do not present with any noticeable clinical symptom of febrile illness, or not receiving treatment for typhoid fever and malaria.

Exclusion criteria: Exclusion criteria will be as follows.

a) Student suffering other type of infection other than those caused by Salmonella species.
b) Pregnant students and those experiencing menstrual bleeding at that moment.
c) Students on medication for any illness.

2.6 Informed Consent: The participants to the study were issued a consent form which they signed without been compelled. Those who refuse to give in their consent were not victimized or maltreated in any way. All information obtained was treated as confidential.

The students were enlightened and counseled on life style modification practices. Objectives and nature of the study were explained to participants that gave their consent. Information about participant's identity was not included with the other data as only the researcher was permitted to have access to this information. No reference to the participant's identity was made at any stage during data analysis.

2.7 Sample collection

A total of 510 students were screened, 150 from FPNO, 65 from AIFCE, 80 from IMSU, 140 from Imo Poly Umuagwo and 75 from Federal University of Technology, Owerri. The number of students from the various tertiary institutions was chosen based on the population size and the ratio of male: female in the school.

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. Approximately 5-10 ml of blood was collected from the students in sterile BHIB under aseptic precautions. The stool and blood samples collected from these participants were transported to the laboratory immediately for processing and analysis. Then, the blood sample bottles were put in the Bactec machine where it was incubated at 37˚C and agitated continuously.

2.8 Analysis of samples

2.8.1 Inoculation and isolation of *Salmonella* species

Samples were analyzed in accordance with standard bacteriological methods.[17] The stool samples were inoculated unto Salmonella Shigella Agar (SSA), MacConkey agar and Deoxychocolate Citrate Agar (DCA); parts of the samples were inoculated onto Selenite F broth, all were incubated for 24 hours at 37^{o}C. The selenite F broth culture was further sub-cultured onto Kliger Iron Agar (KIA) and Simmon Iron Medium (SIM) at 37^{o}C for 24 hours for confirmation of isolates of Salmonella and the results of both the direct culture and subcultures were noted. The culture plates with growth were identified by morphological

characteristics, gram stain and biochemical tests (gram staining, motility, catalase, indole, citrate, fermentation of sugars, hydrogen sulphide test, urease, methyl red, vogues proskeaur, coagulase, carbohydrate metabolism and oxidase tests).[17]

2.8.2 Serotyping of Salmonella Isolates

Pure cultures of all the suspected isolates plated on nutrient broth were 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.

2.8.3 Antibiotic Sensitivity Testing

Kirby Bauer disc diffusion technique was used. The antibiotics discs used included: Nitrofurantoin (100 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Ciprofloxacin (10 µg), Chloramphenicol (10 µg), Ofloxacin (10 µg), Pefloxacin (10 µg), Cefuroxime (10 µg), Streptomycin (30 µg) and Amoxicillin (30 µg). Susceptibility patterns were recorded quantitatively by measuring the diameters to the nearest millimeters (zone of inhibition) using a meter rule, following the interpretative chat of the Kirby-Bauer Sensitivity Test Method.[18] The results were interpreted as sensitive, intermediate or resistant on the basis of zone of inhibition following the criteria of CLSI guidelines.

2.9 Data ANALYSIS

Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS; version 27.0) and expressed using Tables and graphs. P-value of <0.05 was considered statistically significant.

3.0 RESULTS

Table 1: Distribution of *Salmonella* spp in various schools.

Schools	Total no of students	Students infected with *Salmonella* (%)
FPNO	150	72(48.0)
AIFCE	65	22(33.8)
IMSU	80	34(42.5)
Imo Poly Umuagwo	140	64(45.7)
FUTO	75	28(37.3)

Table 2: Prevalence of various*Salmonella* species in different schools.

Schools	*Salmonella spp* (%)		
	Salmonella typhi	***Salmonella paratyphi A***	***Salmonella enteriditis***
FPNO	48(32.0)	20(13.3)	4(2.7)
AIFCE	12(18.5)	7(10.8)	3(4.6)
IMSU	19(23.8)	10(12.5)	5(6.3)
Imo Poly Umuagwo	36(25.7)	18(12.9)	10(7.1)
FUTO	14(18.7)	9(12.0)	5(6.7)
Total	**129(25.3)**	**64(12.5)**	**27(5.3)**

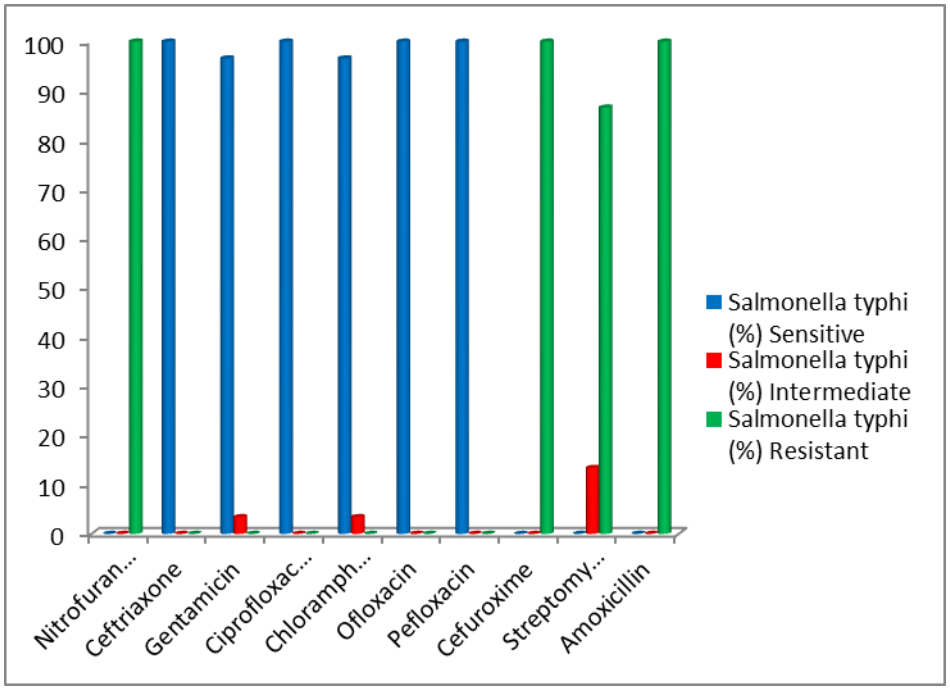

Figure 1: Antimicrobial susceptibility pattern of *S. typhi.*

Bars are mean±S.D of triplicate determinations (n=3).

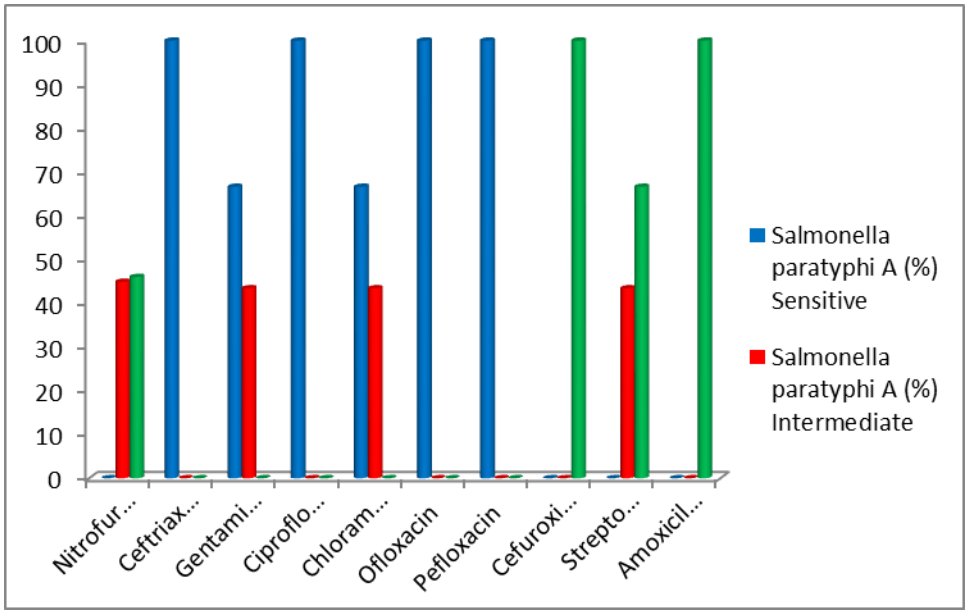

Figure 2: Antimicrobial susceptibility pattern of *S. paratyphi.*

Bars are mean±S.D of triplicate determinations (n=3).

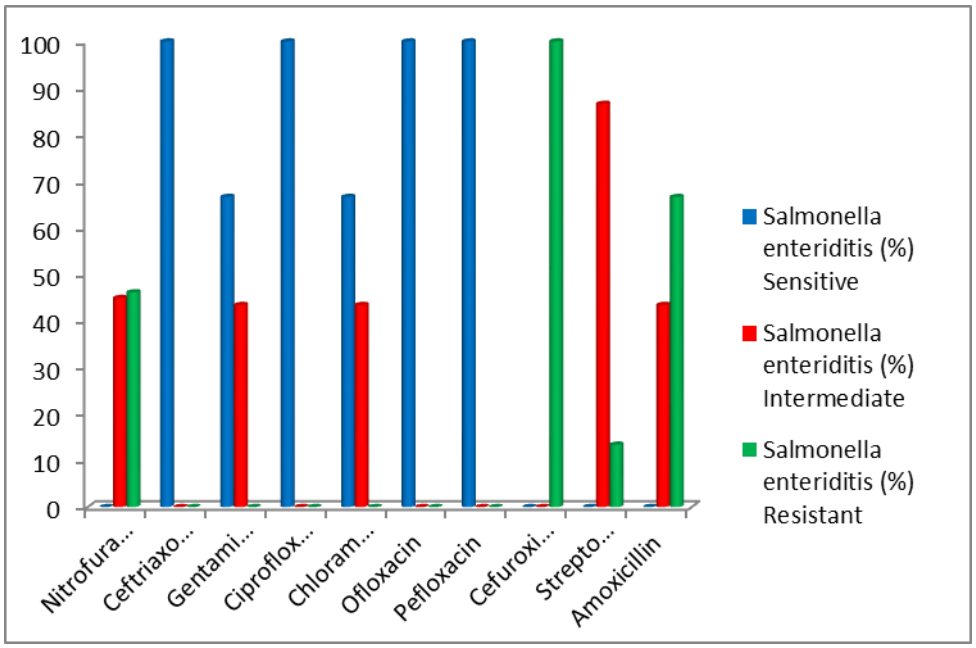

Figure 3: antimicrobial susceptibility pattern of *S. enteriditis.*

Bars are mean±S.D of triplicate determinations (n=3).

4.0 DISCUSSION

The living condition of student on and off the campuses within Imo State calls for concern as they are often jam-packed into spaces that triggers competition and sharing of items. There is anelement of communal life which leads to series ofinteractions like eating together, sharing of toilet systemsand other features. This type of living creates an avenuefor transmission of

the *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella enteriditis* species. Most importantly, it will help to ascertain the carrier rate of the three isolates among the students. This study analyzed antibiotic susceptibility pattern and predominance of *Salmonella* species among students of tertiary institutions in Imo State.

Similar findings on high prevalence of Salmonella infection among the students in tertiary institution (Table 1) was obtained by.[5] The prevalence was higher in FPNO and Imo Poly Umuagwo probably because their population and practices undertaken by the students. Also, these schools are located off the heart of town unlike IMSU and AIFCE.

Three (3) *Salmonella* species were identified in thisstudy- Salmonella typhi, Salmonella paratyphi AandSalmonella enteritidis. The prevalence rates for the various species of Salmonella are *S. typhi* (25.3%), *S. paratyphi A* (12.5%) and *S. enteriditis* (5.3%) making a total of 43.1% from the five schools (Table 2). This percentage indicate a high carrier status among thestudents and higher than similar studies reported by[5] who obtained 38% and[19] who reported an overallprevalence of 36.78% among undergraduate students inOwerri, Nigeria. The prevalence rate could probably bedue to the low level of hygiene within the hostels and poorfeeding routines of the students as evidenced in theirlifestyles. The high isolation rates of S. typhiwas similar to the findings of[20] who reported a highprevalence rate of typhoidal disease; however, it contradicts the findings of[19] who reported the predominance of *Salmonella typhimurium*followed by *S. paratyphi* and *S. typhi*.

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 (Figures 1-3). Ceftriaxone and the fluoroquinolones (Ciprofloxacin, Ofloxacin, Pefloxacin) showed maximum antimicrobial effect against all the *Salmonella* isolates (100%) which was similar to[5],[21] and.[22] The three *Salmonella* isolates were highly resistant to amoxicillin, cefuroxime, nitrofurantoin and streptomycin and this was consistent with the findings of.[23] Specifically, the three Salmonella isolates exhibited 100% resistances to Cefuroxime as observed by.[11] *S. typhi* was 100% resistance to Nitrofurantoin which contradicts that of[24] who reported 46.2% resistance; however, *S. paratyphi A* and *S. enteriditis* were 50% resistance to Nitrofurantoin.

The high level of resistance to some of these drugscould be due to the development of resistance genes by this organism either via plasmid transfer or mutation in itschromosome.[25] Indiscriminate use of antibiotics maybe an important factor.

5.0 CONCLUSION

This study has shown substantial prevalence of *Salmonella species* among students of higher institutions in Imo State particularly in Federal Polytechnic Nekede and Imo State Polytechnic Umuagwo. There was high carrier rate of *S. typhi* among students in the tertiary institutions in Imo State probably because of the abuse of antibiotics, poor personal hygiene and unsafe food practices.

6.0 Funding

The research was funded by Tertiary Education Trust Fund (TETFund) Nigeria through the Institution Based Research (IBR) Project Grant 2022 (TETF/DR &D/CE/POLY/NEKEDE/IBR/2022/VOL. II).

7.0 ACKNOWLEDGEMENT

The authors sincerely acknowledge Tertiary Education Trust Fund (TETFund) Nigeria for the financial support. We also want to acknowledge the management of Federal Polytechnic Nekede Owerri for creating enabling environment for research.

REFERENCES

1. John SG, Joanna MM, Stephen B, Sabina D, Richelle C, Edward T. Salmonella chronic carriage, epidemiology diagnosis & gall bladder persistence. Trends microbial, 2014; 2(11): 648-655.
2. Graziani C, Busani L, Dionisi AM, Lucarelli C, Owczarek S. Antimicrobial resistance in *Salmonella enteric* serovar typhimurium from human and animal sources in Italy. J Vet Microbiol, 2008; 128(3-4): 414- 418.
3. Popoff MF, Bockemuhl J, Gheesling, L. Supplement to the Kauffmann - White scheme. J Res Microbiol, 2001; 154(3): 173-174.
4. Harris JB, Brooks WA. Typhoid and paratyphoid (enteric) fever. In A. J. Magills, E.T. Ryan, D.R. Hill and T. Solomon (Eds.), *Hunter's Tropical Medicine and Emerging Infectious Disease* (9th ed.). Philadelphia, PA: Elsevier, 2013 pp. 568-576.

5. Udeani TK, Emenuga VN, Sunday FC. Carrier Status of S***almonella***Species Infection amongStudents in a Tertiarry Institution. American Journal of Infectious Diseases and Microbiology, 2020; 8(2): 75-82.
6. Eng S-K, Pusparajah P, Ab-Mutalib N-S, Ser H-L, Chan KG, Lee L-H. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science, 2015; 8(3): 284-293.
7. Global Burden of Diseases. Burden Typhoid and Paratyphoid Collaborators. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infectious Diseases, 2019; 19: 369-381.
8. Radhakrishnan A, Als D, Mintz ED, Crump JA, Stanaway J, Breiman RF, Bhutta ZA. Introductory Article on Global Burden and Epidemiology of Typhoid Fever. American Journal of Tropical Medicine and Hygiene, 2018; 99(3): 4-9.
9. World Health Organization. Food and Agriculture Organization of the United Nations. Salmonella (non-typhoidal) WHO, 2018. Retrieved.
10. Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. Global burden of invasive non-typhoidal *Salmonella* disease. Emerging Infectious Diseases, 2015; 21(6): 941-949.
11. Olubukunola OA, Blessing OA, Moses DO, Olawale EC, Adekunle BJ. 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. Journal of Human Virology & Retrovirology, 2016; 3(6): 00113.
12. Ranjbar R, Giammanco GM, Aleo A, Plano MR, Naghoni A. Characterization of the First Extended-Spectrum β Lactamase-Producing Non-typhoidal *Salmonella* Strains Isolated in Tehran, Iran. Foodborne Pathog Dis, 2010; 7(1): 91-95.
13. Britto CD, Dyson ZA, Duchene S, Carter MJ, Gurung M, Kelly DF. Laboratory and molecular surveillance of paediatric typhoidal Salmonella in Nepal: antimicrobial resistance and implications for vaccine policy. Plos Negl Trop Dis, 2018; 12(4): 1–19.
14. Khadka P, Thapaliya J, Thapa S. Susceptibility pattern of *Salmonella enterica* against commonly prescribed antibiotics, to febrile-pediatric cases, in low-income countries. BMC Pediatrics, 2021; 21(38): 1-8.
15. Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)- producing *Salmonella enterica* (serotypes Typhi and Paratyphi a) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis, 2006; 10(6): 434–438.

16. Emele FE, Anaghalu IC, Adimma ED, Okonkwo JEN. Prevalence of *Chlamydia trachomatis* antibodies among infertile subjects in Anambra State, Nigeria. International Journal of Infectious Diseases, 2015; 15: 179.
17. Lyne SG, Michael A, Evelyne K, Dylan RP, Gary WP, NorbertR, Robyn YS, Covinda V. Laboratory diagnosis of Parasites fromthe Gastrointestinal tract. DO1: 2017; 10(1128): 17-25.
18. Clinical and Laboratory Standard Institute (CLSI). Performancestandards for Antimicrobial Disk susceptibility: Approvedstandard (11th edn.). Wayne, PA: Clinical and Laboratorystandards institute. 2012.
19. Udujih HI, Edward UC, Ibe SNO, Udujih OG, Iwuala CC, Kenechukwudozie, QC. *Salmonella* infection among Undergraduate students of Imo State University, Nigeria. International Journal of Health, Safety and Environment, 2017; 3(6): 128-133.
20. Ishaleku D, Sar TT, Houmsou RS. *Salmonella typhi* infection in a tertiary institution in Nasarawa State, Nigeria. Asian Pacific Journal of Tropical Medicine, 2011; 4(2): 137-139.
21. Mijovic G, Andric B, Terzic D, Lopicic M, Dupanovic B. Antibiotic susceptibility of *salmonella* spp: A comparison of two surveys with a 5 years interval. Journal of International Medical Association of Bulgaria-Annual Proceeding (Scientific Papers), 2012; 18(1): 216-219.
22. Taha RR, Alghalibi SM, Saeed-Saleh MG. *Salmonella* spp. in patients suffering from enteric fever and food poisoning in Thamar city, Yemen. Eastern Mediterranean Health Journal, 2013; 19(1): 88-92.
23. Deksissa T, Gebremedhin EZ. A cross sectional study of enteric fever among febrile patients at Ambo hospital: prevalence, risk factor, comparison of widal test and stool culture and antimicrobial susceptibility pattern of isolates. BioMed Central Infectious Diseases, 2019; 19: 288-300.
24. Garedew-Kifelew L, Wondafrash N, Feleke A. Identification of drug resistant *Salmonella* from food handlers at the University of Gondar, Ethopia. BioMed Central Research Notes, 2014; 7: 545-550.
25. Gatechew T. Prevalence of human Salmonellosis in Ethiopia, a systematic review and meta-analysis. BMC infectious Diseases, 2014; 14(88): 6827.