Original Research Article

Antibiotic Susceptibility Patterns of Blood Salmonella Isolates Causing Enteric Fever within Enugu, Nigeria.

ABSTRACT

Background: Salmonella enterica enterica serovar typhi/paratyphi is the causative agent of enteric (typhoid) fever, a life-threatening systemic illness, significantly impacting global health especially the developing nations. Although enteric fever can be treated effectively with antibiotics, it is becoming increasingly challenging due to the rising incidence of drug resistance. **Aim**: The aim wasinvestigate the prevalence and antibiotic susceptibility patterns of Salmonella isolates from typhoid fever patients in order identify the multidrug-resistant trends.
Methodology: The study was carried out between March and June, 2024. Venous blood samples were obtained from 200 randomly selected subjects, aged 5-70 years made up of 95 males and 105 females. The samples were allowed to coagulate and the clots were aseptically inoculated in bile salt broth and incubated for 18-48 hours at 37°C and then sub-cultured unto *Salmonella- Shigella* Agar. The suspected colonies were identified by biochemical tests involving the urea hydrolysis and typical reaction in Triple Sugar Iron Agar. Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion method. The antibiotics used included pefloxacin, ofloxacin, azithromycin, levofloxacin, cefotaxime, sparfloxacin, ciprofloxacin, amoxacillin, augmentin and gentamicin.

Results: This study showed that 95 (47.5%) samples were positive for *Salmonella entericaenterica* species, predominantly Salmonella typhi (54.7%). A higher infection rate was observed among males (68.4%) and individuals aged 16-26 years (64.7%). Antibiotic susceptibility testing showed high sensitivity to pefloxacin and azithromycin (100% each) and levofloxacin(94.7%), while resistances were noted against amoxacillin and augmentin with the rates of 68.4% and 63.2%, respectively
Conclusions: This study emphasizes the importance of proper diagnosis and antimicrobial susceptibility testing to guide effective treatment in each patient. The findings also highlight the emergence of multi-drug resistance (MDR) Salmonella typhi/paratyphi, necessitating revised treatment protocols and stringent antibiotic stewardship. However, the high rates of sensitivity to fluroquinolones antibiotics make allowance for choices and alternatives during treatment.

Keywords: salmonella; antibiotics; enteric fever, susceptibility

1. INTRODUCTION

Salmonella enterica ~~*enterica*~~ *serovar typhi* is the causative agent of enteric fever, commonly known as typhoid fever. It is an important cause of a variety of illnesses such as salmonellosis and death worldwide with an estimated 10.9 million new infections and about116, 800 typhoid-fever-related deaths occurring annually (1). Typhoid fever disease is more prevalent in developing nations where there are poor sanitary conditions and is endemic in Asia, Africa, Latin America, the Caribbean, and Oceania; but 80% of cases are recorded in Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, or Vietnam (2). Typhoid fever disease and antibiotic resistance still poses notable health challenge in Nigeria; it is still a major health issue.

There are various species of *Salmonella* distributed in diverse environments and can cause infection in humans and animals. Approximately 1600 serotypes of *Salmonella* have been placed into the sub-species enterica according to White-Kauffmann-Le Minor classification scheme (3). Salmonella are classified into two groups, which are typhoidal and non-typhoidal strains. Typhoidal strains which include *Salmonella typhi* and *Salmonella paratyphi* A, B, and C serotypes can cause illnesses exclusively in humans while non-typhoidal can infect both humans and animals causing a variety of illnesses such as enteric fever, sepsis gastroenteritis, and salmonellosis (3). With the infection classified into typhoidal and non-typhoidal types, still, the severity of the infection in humans varies depending on the serotype of the bacteria and the immune status of the host (4). Enteric fever is a life-threatening systemic infection caused by *Salmonella enterica* serovar Typhi and Paratyphi A, B, and C (5). The disease caused by *Salmonella paratyphi* A is similar to *Salmonella typhi,* but it is less severe. There is a broad range of possible symptoms of enteric fever, from severe illness with sepsis and organ failure to milder symptoms of diarrhea and low-grade fever. The major presentation of enteric fever includes fever, fatigue, abdominal pain, and constipation. If left untreated, enteric fever can lead to serious complications such as delirium, confusion, intestinal bleeding, perforation of the intestines, and even death within a month of the start of the illness(6).Although some of this infection can be treated effectively with antibiotics, the management of this infection is becoming increasingly challenging due to the rising incidence of drug resistance.

Antimicrobial resistance occurs due to the adaptations of infectious microorganisms to the exposure of antimicrobial agents. The resistance in the organism results from genetic changes, either by the acquisition of foreign genes through a plasmid or a chromosomal mutation (7). Quorum sensing and biofilm formation which may play roles in Salmonella persistence and resistance. Biofilm-associated *Salmonella* may exhibit increased resistance to antibiotics, complicating eradication efforts (8). *Salmonella* employs efflux pump systems to expel antibiotics from the bacterial cell. Efflux pumps contribute to intrinsic and acquired resistance mechanisms, making it challenging to combat antibiotic-resistant strains (9). The typhoidal *Salmonella* have, overtime, developed resistance to a wide range of antibiotics, including first-line treatments like ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Epidemiological studies shows that multi-drug resistance *Salmonella* serotypes are more virulent than susceptible strains, and they increase the severity of infection. The MDR strains of *Salmonella have* emerged as a serious public health, particularly in certain regions of the world. The Indian subcontinent, Africa, and Southeast Asia have seen a significant rise in multi-drug resistance *Salmonella typhi* infections, likely due to a combination of factors, including poor sanitation, lack of access to clean water, overuse/misuse of antibiotics, and ineffective healthcare (7). In Nigeria and most other African countries the true burden of typhoid is likely to be underreported because those affected do not always seek health care at hospitals. Furthermore, given the time required to

confirm bacteremia microbiologically, patients with febrile symptoms are usually given antibiotics before laboratory confirmation of typhoid which may also influence the data (10).

Several disposing factors indicate the severity of typhoid fever, which are necessary for the evaluation of its treatment, prevention, and mitigation. This information is crucial in managing the economy of healthcare and its resources, which are important for the regulation of public health (3). Treating *Salmonella enterica* infections successfully may be challenging due to the worldwide increase in antibiotic resistance. Therefore, antibiotic susceptibility testing is a cornerstone of antimicrobial stewardship programs which supports the responsible use of antibiotics to preserve their effectiveness for future generations. The objective also includes reducing indiscriminate usage of antimicrobial agents and promoting selective antimicrobial utilization based on culture results (11). A mere Widal method screening of patients and prescribing antibiotics without carrying out prior sensitivity tests may be a risky. Therefore, updated information on antibiotic susceptibility patterns of isolates is essential to many facets of clinical management and public health initiatives. This presence study focuses on isolation of the *Salmonella enterica* from enteric fever patients, evaluation of antibiotics susceptibility and assessment of multiple antimicrobial drugs resistant trends. The research also evaluates the efficacy of existing antibiotics and potential alternative treatments, while, possibly, exploring interventions to prevent or reduce antimicrobial resistance in these pathogens.

2. MATERIAL AND METHODS

2.1 Setting and Study Area

The study was conducted between a period of March – June 2024. It was conducted in Enugu State in the eastern part of Nigeria. The state shares boundaries with Abia and Imo states to the south, Ebonyi State to the east, Anambra State to the west, Benue State to the northeast and Kogi State to the northwest. It is located at $6^{o}30'$ North of the Equator, and $7^{o}30'$ east of the Latitude. Enugu State had a population of 3,267,837 people at the census held in 2006 (12).

2.2 Population and Sampling

The population was randomly selected from subjects visiting the clinics and clinical laboratory within Enugu urban and peri-urban. Before the sampling informed consent forms were filled by the participants and personal data information were obtained by filling the laboratory forms. The selection/inclusion criteria for subjects involved, the clinician's tentative diagnosis of typhoid and the symptoms reported by the patients. The typical symptoms taken into account during the selection process included continuous fever and headaches with or without abdominal discomfort. Patients with other known cause of pyrexia were excluded from the studies. The age of the subjects varied from below 2 years old to 70 years old. Out of the total population of 200 subjects selected, the male subjects' population was 95(47.5%) while the females were 105(52.5%).

Non duplicate samples were obtained from the subjects. With a sterile pipette, about 5 - 10mls of venous blood were ascetically aspirated from each subject and put into plain sterile test tubes labeled with each subject's name, specimen number, age and gender. The samples were put in ice boxes and immediately transported to the Bacteriology Laboratory in Microbiology Department of University of Nigeria, Nsukka.

2.3. Laboratory Analysis

2.3.1 Culture and Identification of Isolates

The blood samples were left in the test tubes covered with plastic stoppers and allowed to clot at standing position in the test tube racks. After the coagulation the samples were centrifuged and the serum was aseptically separated from the clot. The isolation of salmonella organisms from blood was performed using a clot culture technique. The culture of the blood was carried out using clot culture technique (13). This technique involves lysing the blood clots in order to free the trapped organisms.

The clots were transferred into sterile universal containers and vortexed to lyse the clot. The lysed blood clot was thereafter transferred into 20 mls of Brain Heart Infusion broth(Oxoid, UK) prepared in sterile bottles and incubated at 37°C for 48 hours. The turbidity of the broth cultures signified bacteria growth. The broth cultures were thereafter sub-cultured by aseptically picking with wire loop and streaking on *Salmonella- Shigella* Agar (oxoid, UK). The subcultures were then incubated at 37°C for 18-24 hours. The agar plates were examined for characteristic appearance and colour of salmonella colonies. The presence of grayish non-lactose fermenting colonies with a black center indicated presence of salmonella and further confirmation through biochemical testing were carried out for accurate identification. Colonies best suitable for further biochemical testing were selected.

2.3.2Identification of isolates

Biochemical tests were carried out on the positive isolates which included urease test. The test organisms were inoculated by streaking on the entire slope surface of the urea agar. The tubes were capped and incubated at 37°C for up to 48 hours. Tubes were thereafter examined for change of colour from plain to pale yellow colour, which indicated urease negative typical of salmonella.

The triple sugar iron test was done to determine the ability of an organism to ferment a specific carbohydrate incorporated in a basal growth medium, with or without the production of carbondioxide (CO_2), and hydrogen sulfide (H_2S) production. This test is used, for the identification of enteric pathogens. Triple Sugar Iron (TSI) agar medium was prepared accordingly and dispensed into sterilized tubes placed at slanted positions and allowed to solidify. The isolated colonies were inoculated by carefully stabbing and streaking on the agar using a sterile wire loop. The tubes were incubated at 37°C for 18 to 24 hours. After incubation, the TSI agar media were checked for blackening and change in color from amber to red at the bottom (butt) of the tube and pale yellow at the slant. Blackening of the medium and cracks or bubbles in the agar were also examined for, which indicate the formation of H_2S and CO_2 gas respectively. *Salmonella* (depending on species) showed an alkaline (red) slant with or without gas production and an acidic (yellow) butt with or without blackening and CO_2 formation.

2.3.3 Determination of antibiotic susceptibility of *Salmonella* isolates

Antimicrobial susceptibilities of the *Salmonella* isolates were tested by using modified Kirby–Bauer disc diffusion of Clinical Laboratory Standard Institute(14).The antibiotics used were pefloxacin(30ug), ofloxacin (10ug), azithromycin(10ug), levofloxacin(20ug), cefotaxime (30ug), sparfloxacin(10ug), ciprofloxacin(10ug), amoxicillin(30ug), augmentin(15ug),and gentamicin(10ug). A sterile normal saline solution with test organisms grown for 24 hours was prepared and adjusted to achieve~~achieve~~ the same turbidity level as the 0.5 McFarland standard. The suspension was inoculated and evenly distributed over the entire surface of

solid Mueller-Hinton agar(HiMedia Laboratories, India). The antibiotic multi-discs were placed precisely at the center of the seeded plate using sterilized forceps, which were used to pick the discs from the cartridge and position them on the plates. The agar plates were kept at a temperature between 35 and 37°C for 24 hours. During this time, the antimicrobial substance spread from the disc into the medium. As a result, the growth of the organism was inhibited at a distance from the disc that corresponded to its sensitivity to the added antimicrobial substance. Resistant strains showed minimal or no inhibition zones. The size of the inhibition zones was determined by measuring from one edge to the other edge of the zone using a metric ruler placed on the plate.

2.4 Statistical Analysis

Statistical calculations were carried out using IBM SPSS Windows 21 version to test for significance at 95% confidence interval. Descriptive statistics (frequencies and percentages) and one sample T-test were used to describe variables, calculate means, standard deviations and standard error of means. Statistical significance was defined as a P-value less than 0.05.

3. RESULTS

The colonies suspected of being *Salmonella* speciesdisplayed characteristic growth patterns of smooth circular black-centered colonies which is a typical morphology characteristic of salmonella species. All the salmonella isolates had urease negative reactions in urea agar. Subsequently, in the TSI media, all the suspected salmonella isolates displayed acidic production by fermentation of sugar at the butt of the tubes, alkaline reactions in the slant. Samonella Typhi produced black H_2S gas without patches of CO_2 while Salmonella Paratyphi A produced CO_2 without H_2S. Salmonella Paratyphi B and C produced both H_2S and CO_2. The rate of salmonella isolated from the blood samples was 47.5%. Out of the total number of males (95) and females (105), 68.4% and 28.5% were positive, respectively. The subjects in the age group 16-26 years had the highest prevalence of infection (64.7%), followed by those in age group 5-15 years (54.5%). The prevalence in the age group 27-37 years was low, followed by that of 60-70 years, which was the least. Those in age group 38-48 and 49-59 years had 25.0% rates of salmonella infection each. The prevalence distributions by age and gender are shown in the table 1

Table 2 shows the distribution of salmonella serotypes among the isolates, with the percentage rates of each serotype indicated. *Salmonella Typhi* was 54.7%, *Salmonella Paratyphi* A was 29.5% while those depicting either *Salmonella Paratyphi* B or C in the TSI test reaction constituted the remaining 15.8%.

Table 3 details the susceptibility and resistance rates of various antibiotics tested against *Salmonella Typhi* and *Salmonella Paratyphi* isolates. The results revealed that pefloxacin and azithromycin had complete effectiveness with 100% sensitivities and zero resistances. levofloxacin, ciprofloxacin, and ofloxacin also demonstrate high efficacy, with sensitivity rates above 88%. sparfloxacin and gentamicin had sensitivity rates of 84.2% each. Conversely, amoxicillin and augmentin show low sensitivity rates of 31.6% and 36.9% respectively, indicating significant resistance. Cefotaxim has a moderate performance with a sensitivity rate of 55.8%.

Table 1: Prevalence of *Salmonella enterica* var typhi/paratyphi by gender and age

Variables	Total No. of Subjects N (%)	No. of Subjects with positive result N (%)
Gender:		
Male	95(47.5)	65(68.4)
Female	105(52.5)	30(28.6)
Age:		
5-15	55(27.5)	30(54.5)
16-26	68(34.0)	44(64.7)
27-37	30(15.0)	10(33.3)
38-48	20(10.0)	5(25.0)
49-59	20(10.0)	5(25.0)
60-70	8(2.5)	1(12.5)
Total	**200 (100)**	**95 (47.5)**
Std. Deviation	1.87	
Std. Error Mean	.76	
P value	.006*	

~~Variables~~	~~Number of positive result (%)~~	~~Total number (%)~~
~~**Gender:**~~		
~~Male~~	~~65(68.4)~~	~~95(47.5)~~
~~Female~~	~~30(28.6)~~	~~105(52.5)~~
~~**Age:**~~		
~~5-15~~	~~30(54.5)~~	~~55(27.5)~~
~~16-26~~	~~44(64.7)~~	~~68(34.0)~~
~~27-37~~	~~10(33.3)~~	~~30(15.0)~~
~~38-48~~	~~5(25.0)~~	~~20(10.0)~~
~~49-59~~	~~5(25.0)~~	~~20(10.0)~~
~~60-70~~	~~1(12.5)~~	~~8(2.5)~~
~~**Total**~~	~~**95(47.5)**~~	~~**200(100)**~~
~~**Std. Deviation**~~	~~1.87083~~	
~~**Std. Error Mean**~~	~~.76376~~	
~~***P* value**~~	~~.006*~~	

Table 2: Percentage rate of Isolated Serotypes

Isolated Organisms	Number of occurrence (%)
Salmonella typhi	52(54.7)
Salmonella paratyphi A	28(29.5)
Salmonella paratyphi B/C	15(15.8)
Total	**95(100)**

Table3: Rate of Susceptibility/Resistant of the antibiotics tested against *Salmonella typhi/paratyphi* isolates

Antibiotics	Sensitivity rates N (%)	Resistant rates N (%)
Levofloxacin	90(94.7)	5(5.3)
Cefotaxim	53(55.8)	42(44.2)
Sparfloxacin	80(84.2)	15(15.8)
Ciprofloxacin	86(90.5)	9(9.5)
Amoxicillin	30(31.6)	65(68.4)
Augmentin	35(36.9)	60(63.1)
Gentamicin	80(84.2)	15(15.8)
Pefloxacin	95(100)	0(0.0)
Ofloxacin	84(88.4)	11(11.6)
Azithromycin	95(100)	0(0.0)

4.0 DISCUSSION

This study was conducted to examine the prevalence of susceptibility pattern of Salmonella Typhi/Paratyphi isolates from among enteric fever patients within Enugu State, Nigeria. The study identified *Salmonella enterica* ~~*enterica*~~ serovar Typhi as the most prevalent serotype in comparison to *Salmonella enterica* paratyphi A, B & C. This is quite comparable with the research of Sabeetha et al., (13) who also recorded 63.6 % and 36.4% ratesof S. *Typhi and S. Paratyphi* A, respectively using clot culture technique. This finding is also consistent with previous studies that have shown *S. Typhi* to be the primary cause of enteric fever in humans (1). Although there is no established reason for serotype variation in enteric fever, the higher incidence of S.*enterica serovar Typhi* might be due to its ability to spread more easily through contaminated water, which can transmit the bacteria even at low concentrations (inoculums) whereas *S. enterica serovarParatyphi* requires a larger amount of bacteria cells to cause infection, which is more commonly achieved through contaminated food(1).

Although there was no association conducted between gender and the incidence of disease, our study revealed an interesting trend. While our study found no link between gender and the likelihood of developing the disease, we observed a notable disparity in the number of cases between males and females, with males accounting for 68.4% of cases and females accounting for 28.5%. This is in accordance with the findings of the previous studies (15).While there is no biological reason why males are more prone to enteric fever, a logical explanation suggests that their increased exposure to outdoor environments, where contamination levels are often high, contributes to the higher incidence. Additionally, males' greater likelihood of eating outdoors, consuming commercially prepared food, and having a

more relaxed attitude towards illness may also contribute to the higher number of infections among males.

In this study, there was significant difference (*P*=.006) in the rates of infection among the different age groups. However, patients of the age group 16-26years were the most affected by enteric fever, followed by the age group 5-15years.This is in accordance with the findings of the previous studies reported in Kathmandu valley, Nepal (16).The higher concentration of disease burden among younger adults may be attributable to their active social life where the habit of eating out is very likely.

The susceptibility pattern of Salmonella Typhi/Paratyphi isolates to various antibiotics was investigated in this study. The results show that pefloxacin, azithromycin, and levofloxacin were highly effective against the isolates, with sensitivity rates of 100%, 100%, and 94.7%, respectively, this is in accordance with the previous studies that have found azithromycin to be effective against uncomplicated enteric fever (17). Ofloxacin and ciprofloxacin also showed good activity, with sensitivity rates of 89.5% each. The high sensitivity to fluoroquinolones (pefloxacin, ofloxacin, levofloxacin, and ciprofloxacin) is also in agreement with previous studies (18). However, in contrast, Marhajan et al, (2021) in Nepal, recorded low susceptible to oflaxacin and levofloxacin (15% and 20%, respectively) and no susceptibility to ciprofloxacin. In this study the excellent activity of azithromycin(100%) was found to be consistent to 97% also obtained in Nigeria by Jumbo et al (19). It was also consistent with efficacy reported against salmonella species (20). Gentamicin and sparfloxacin had moderate activity, with sensitivity rates of 84.2% each. In contrast, cefotaxim, amoxacillin, and augmentin showed relatively low sensitivity rates. The high resistance rates to, amoxacillin, and augmentin poses concerns as these antibiotics are commonly used in the treatment of typhoid fever here. Their resistance could however be attributed to the excessive and inappropriate use of these antibiotics, such as using them as over-the-counter medications or taking incomplete treatment courses, leading to the selection and dissemination of resistant strains. While antibiotics constitute the major treatment for salmonellosis, the emergence of antibiotic resistance and the rise of multidrug-resistant (MDR) *Salmonella* strains have prompted the development for alternative antibiotic (21).

CONCLUSION

This study demonstrates the vital role of antimicrobial susceptibility testing and accurate identification in ensuring effective antibiotic treatment. The MDR trend and varied susceptibility patterns observed among *the* salmonellaisolates highlight the need for personalised treatment approaches to combat the escalating issue of antibiotic resistance. This research emphasizes the importance of responsible antibiotic use and informed treatment decisions. By prioritizing accurate identification and susceptibility testing, healthcare providers can improve treatment outcomes and reduce the risks associated with antibiotic resistance. The findings of this study have significant implications for the management of typhoid fever infections and emphasize the need for ongoing research and surveillance to inform evidence-based treatment strategies.

CONSENT (WHEREEVER APPLICABLE)

Authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

According to international standards and the university standards written ethical approval has been collected and preserved by the author(s)

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