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Antibiotic Susceptibility Pattern of *Salmonella* Species Isolated from Suspected Cases of Typhoid Fever in General Hospital Minna, Nigeria

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Abstract:

Typhoid fever remains a major public health concern in many developing countries due to poor sanitation, inadequate access to clean water, and the increasing emergence of antimicrobial resistance. This study investigated the antibiotic susceptibility pattern of *Salmonella* species isolated from suspected cases of typhoid fever attending General Hospital Minna, Nigeria. Clinical samples, primarily blood and stool specimens, were collected from patients presenting with symptoms suggestive of typhoid fever. Standard microbiological techniques were employed for the isolation and identification of *Salmonella* species, while antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

The findings revealed varying levels of susceptibility and resistance among the isolated *Salmonella* strains to commonly prescribed antibiotics. High resistance rates were observed against older and frequently used antibiotics such as ampicillin, cotrimoxazole, and chloramphenicol, indicating the growing prevalence of multidrug-resistant strains. Conversely, relatively higher susceptibility was recorded for ciprofloxacin, ceftriaxone, and azithromycin, suggesting their continued effectiveness in the treatment of typhoid fever. The emergence of resistant isolates highlights the indiscriminate use and misuse of antibiotics within the community.

This study emphasizes the urgent need for continuous antimicrobial surveillance, rational antibiotic use, and strengthened infection prevention strategies to curb the spread of resistant *Salmonella* species. The results provide valuable baseline data for clinicians and public

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health authorities in selecting appropriate therapeutic interventions for typhoid fever management in Nigeria.

Keywords: Typhoid fever, *Salmonella* species, Antibiotic susceptibility, Antimicrobial resistance, Multidrug-resistant bacteria, Kirby–Bauer disk diffusion, Public health surveillance

Introduction

Salmonellosis caused by members of the genus Salmonella, remains one of the most significant foodborne illnesses worldwide, contributing to substantial morbidity, economic burden, and, in severe cases, mortality (Kemal,2014). It is a digestive system infection caused by *Salmonella* serotypes and is clinically identified by one or more of three main syndromes: septicaemia, acute enteritis, and chronic enteritis affecting both humans and animals, with millions of cases reported globally (Kemal, 2014). Many countries face considerable health challenges due to foodborne salmonellosis (Bayu *et al*., 2013).

Salmonella, a genus of Gram-negative, facultatively anaerobic bacilli belonging to the family *Enterobacteriaceae* are primarily transmitted faeco-orally through the ingestion of contaminated food or water (Eng *et al.,* 2015). Typhoidal serovars, such as S. *typhi* and S. *Paratyphi* A, B, and C are highly adapted to humans and cause systemic infections (typhoid and paratyphoid fever), which can lead to severe complications if untreated. Globally, the burden of *Salmonella* infections is disproportionately high in low and middle-income countries (LMICs), where factors such as inadequate sanitation, poor food safety standards, and limited access to healthcare exacerbate transmission (Tadesse *et al*., 2019; Marchello *et al*., 2022). Similarly, typhoid fever remains endemic in regions with poor water infrastructure, contributing to an estimated 11–21 million cases and 128,000–161,000 deaths annually (WHO, 2023).

A critical challenge in managing salmonellosis is the rising prevalence of antimicrobial resistance (AMR), which complicates treatment and increases healthcare costs. Irrational antimicrobial use is a significant worldwide health threat, particularly in developing countries with inadequate antibiotic regulation (Deb *et al*., 2023). More alarmingly, emerging resistance to fluoroquinolones (e.g., ciprofloxacin) and third-generation cephalosporins (e.g., ceftriaxone) has been reported, leaving few therapeutic options for the management of severe

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infections (NARMS, 2023). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella* strains further threaten global health security, as highlighted by the World Health Organization's (WHO) classification of fluoroquinolone-resistant *Salmonella* as a "high-priority pathogen" for research and intervention (WHO, 2023).

Salmonella typhi isolates demonstrating multidrug resistance such as resistance to fluoroquinolones like ciprofloxacin, macrolides such as azithromycin, and beta-lactams were identified (Adikwu *et al*., 2021) including a study of patients attending military hospitals in Minna (Adabara *et al.*, 2012). An additional investigation involving multidrug-resistant S. typhi from patients suffering from pelvic inflammatory disease in Niger State found resistance to multiple antibiotics such as ofloxacin, nalidixic acid, Augmentin, cephalexin, perfloxacin, and streptomycin (Oyedum *et al*., 2023).

2.0 Materials and Methods

2.1 This study was conducted at the Clinical Microbiology Laboratory of the General Hospital, Minna, a referral hospital that serve the majority of the inhabitants of Minna city. Approval to conduct the study was obtained from the Research Ethics and Publication Committee (REPC) of the hospital while informed consent was obtained from individual participant.

2.2 Samples Collection and Isolation of Clinical Isolates.

Stool samples were collected over a period of four month from 300 participants suspected to have typhoid fever irrespective of the age and gender, in sterile disposable containers. Prior to stool collection, patients were properly guided on how to take samples without contamination. Samples were inoculated on selenite F broth (Difco) using calibrated wire loop and incubated for 24h at 37ºC following which pure cultures were obtained by sub-culturing on Salmonella-Shigella Agar (SSA) (Oxoid) for 24h at 37ºC. Subsequently, agar slants of the pure culture were prepared for further analysis.

2.3 Characterization and Identification of S. Typhi

Gram's staining and biochemical tests of probable *Salmonella* sp colonies were carried out. Biochemical tests employed include oxidase, triple sugar ion (TSI), catalase, indole, motility, methyl red (MR), urease, citrate, glucose, lactose and sucrose tests (Terna *et al.*, 2021)

2.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using modified Kirby-Bauer disk diffusion method to determine the resistance profile of each isolate according to the

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guidelines of the Clinical and Laboratory Standards institute (CLSI, 2024). Pure colonies from fresh cultures were suspended in sterile normal saline adjusted to match the turbidity of 0.5 McFarland standard (1.5 X 10^8 CFU/ml.). The bacterial suspension was gently dispersed onto the surface of already prepared Mueller-Hinton agar (MHA, Oxoid Ltd., UK) plate. The test antibiotic disks which include levofloxacin (20µg), cefotaxime (10µg), sparfloxacin (10µg), ciprofloxacin (30µg), amoxicillin (30µg), augmentin (10µg), gentamycin (30µg), pefloxacin (30µg), terivid (10µg) and azithromycin (12µg) were placed gently on the agar surface using sterile forceps. Plates were incubated at 37°C for 24 hours after which zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI standards (Weinstein *et al*., 2020). Multidrug resistance patterns and multiple antibiotic resistance indices were calculated based on percentage resistance while double disk synergy test was used to screen for the presence of extended beta lactamase enzyme as described by Magiorakos *et al*. (2012).

Result

S. Typhi was recovered only in twenty out of the 300 stool samples collected for the detection of the organism in this study thus resulting in a percentage positivity of 6.67%.

The antibiotic susceptibility test revealed that the isolates were unevenly resistant to the antibiotics commonly prescribed for the treatment of typhoid fever in the study area with the highest resistance against drugs such as amoxicillin (85%), Augmentin (85%), ciprofloxacin (80%) and the least resistance against tarivid (40%), gentamycin (40%), and pefloxacin (25%). The result is presented in table 1 below.

Table 1: Antimicrobial susceptibility profile of suspected *Salmonella* isolates

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
AZ	8(40.0)	0(0.0)	12(60.0)
OFX	11(55.0)	1(5.0)	8(40.0)
PEF	15(75.0)	0(0.0)	5(25.0)
AU	3(15.0)	0(0.0)	17(85.0)
CPX	2(10.0)	2(10.0)	16(80.0)
CN	12(60.0)	0(0.0)	8(40.0)

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LEV	3(15.0)	5(25.0)	12(60.0)
CF	3(15.0)	3(15.0)	14(70.0)
AM	2(10.0)	1(5.0)	17(85.0)
SP	8(40.0)	3(15.0)	9(45.0)

KEY: ST=*Salmonella typhi*; AZ= Azithromycin; OFX= Tarivid; PEF= Pefloxacin; CN= Gentamycin; AU= Augmentin; AM= Amoxicillin; CPX= Ciprofloxacin; SP=Sparfloxacin; CF= Cefotaxime; LEV= Levofloxacin

MULTIPLE ANTIBIOTIC RESISTANCE INDEX

Multiple antibiotic resistance index is not just a primary indicator of the resistance profile of an organism but it also gives insight regarding the level of antibiotic pressure to which an organism may have been exposed. The determination of the antibiotic resistance index for each of the isolates showed a multiple antibiotic resistance indices (MARI) that ranged from 0.2 to 1 as shown in table 2 below.

Table 2. Multiple Antibiotic Resistance Index (MARI) of Individual Isolates

Isolate code	Antibiotic resistant pattern	MARI	No of Antibiotics classes Resisted	Resistance Category
ST1	PEF, AU, CF	0.3	2	Non MDR
ST2	--	0.0	0	Susceptible
ST3	CN, AM	0.2	2	Non MDR
ST4	AZ, OFX, AU, CPX, CN, LEV, CF, AM, SP	1.0	4	MDR
ST5	AU, CPX, LEV, CF, AM, SP	0.6	2	Non MDR
ST6	AZ, AU, CPX, LEV, CF, SP	0.6	3	MDR
ST7	AZ, AU, CPX, LEV, AM, SP	0.6	3	MDR

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ST8	AZ, OFX, AU, CPX, LEV, CF, AM	0.7	3	MDR
ST9	OFX, PEF, AU, CPX, CN, LEV, CF, AM, SP	1.0	4	MDR
ST10	AU, CPX, CN, CF, AM	0.5	3	MDR
ST11	PEF, AU, CPX, CN, CF, AM, SP	0.7	3	MDR
ST12	AZ, OFX, PEF, AU, CPX, CF, AM	0.7	3	MDR
ST13	AZ, AU, CPX, LEV, AM, SP	0.6	3	MDR
ST14	AZ, OFX, AU, CPX, CN, LEV, CF, AM, SP	1.0	4	MDR
ST15	AZ, OFX, AU, CPX, LEV, CF, AM	0.7	3	MDR
ST16	AZ, AU, CPX, LEV, AM, SP	0.6	3	MDR
ST17	OFX, AU, CPX, CN, CF, AM	0.6	3	MDR
ST18	AZ, OFX, AU, CPX, LEV, CF, AM	0.7	3	MDR
ST19	AZ, AU, CPX, CN, LEV, CF, AM	0.7	4	MDR
ST20	AZ, PEF, AM	0.3	3	MDR

The Double Disk Synergy Test (DDST)

The double disk synergy test (DDST) phenotypically detects the presence of extended spectrum beta lactamase enzymes and invaluably helps to monitor the spread of antibiotic resistance especially in institutional settings. From the result of the double disk synergy test, it was observed that none of the isolates probably harboured an extended spectrum beta

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lactamase enzyme as none of the culture plates demonstrated the characteristic shape associated with positive DDST result.

Plate 1: Diagram of a negative double disk synergy test result.

Discussion

The finding of this study that S. Typhi was recovered only in the stool samples of 20 (6.67%) of the 300 patients with enteric symptoms suspected to be suffering from typhoid fever is not significantly different from the reports of many previous researchers. This observation is similar to that of Ahmad *et al*. (2026) where S. Typhi was recovered only in the sample of 341 subjects out of 1,625 suspected enteric fever patients. Similarly, Muche *et al*. (2024) reported that in a study involving 317 volunteers suspected of typhoid fever, only 30% demonstrated positive result following laboratory diagnostic test. These observations once again underscore the importance of definitive diagnosis and laboratory guided antibiotic prescription in the management of typhoid fever cases in health facilities. Untreated typhoid fever cases pose significant risk to patient because of the possible complications but a misused antibiotic is far more dangerous because of far reaching effect on the usefulness of antibiotics and the promotion of antibiotic resistance.

The result of the antibiotic susceptibility test revealed an uneven demonstration of resistance against the antibiotics tested. While antibiotics such as amoxicillin and augmentin were highly resisted, the isolates were relatively susceptible to others such as pefloxacin, gentamycin and ofloxacin. All the isolates resisted, at least, two antibiotics with three isolates

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demonstrating resistance against all the antibiotics tested. However, the result of the double disk synergy test revealed that none of the isolates is possibly possessed the ESBL genes which suggests that the observed resistance may have been through resistance mechanisms other than enzymatic hydrolysis of the antibiotics. A previous study by Eleazar *et al*. (2024) reported a similar trend of resistance by S. Typhi to augmentin (63.1%) and amoxicillin (68.4%). The easy availability of these drugs exposes them to abuse by the populace thereby creating the pressure necessary for bacterial resistance. This assertion is further evidenced by the result of a study conducted in Nepal, in which total resistance to ciprofloxacin was observed by Marhajan *et al*. (2021).

The values recorded for multiple antibiotic resistance index (MARI) in this study for majority of the isolates calls for concern because of the implication not just for the control of S. Typhi but all other Gram-negative pathogens and public health management generally. According to Isaiah *et al*. (2025), high MAR index is a potent indicator of potential treatment failure which translates into increased morbidity, long hospital stays, increased healthcare cost and invariably fatalities.

Conclusion

The findings of this study have demonstrated a high level of antibiotic resistance among S. Typhi isolates to the commonly prescribed antibiotics in the study area which may be due to the misuse of antibiotics. The study therefore underscores the need for the full implementation of antibiotic stewardship as well as the creation of awareness on the dangers of self-medication.

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