

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *SALMONELLA TYPHI* ISOLATED FROM HUMAN PATIENTS

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Abstract

Salmonella Typhi (S. Typhi) is the pathogen that causes typhoid fever, one of the most significant health-related problems, particularly in regions with inadequately sanitized and limited access to clean water. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) S. Typhi strains has made disease management and treatment outcomes even more difficult. The research aimed to determine the occurrence and distribution of antimicrobial resistance of S. Typhi in human blood. 120 blood samples were collected from suspected typhoid patients aged 5-40 years across different hospitals and clinics. Blood culture was positive in 70 cases (58%), and the most common age group was 11-30 years. The modified Kirby-Bauer disc diffusion method according to CLSI was used to test the antimicrobial susceptibility of 14 S. Typhi isolates. Results showed that 28.6% were MDR, i.e., resistant to the first-line agents (trimethoprim-sulfamethoxazole, ampicillin, or tetracycline), 50% were XDR, resistant to the first- as well as second-generation agents (fluoroquinolones and 3rd-generation cephalosporins), and 3% were multidrug-resistant, i.e., resistant to all first-, second-, and third-generation agents. It is important to note that 21.4 percent of the isolates exhibited abnormal antibiogram patterns, which showed resistance to almost all the antibiotics tested, including azithromycin and carbapenems. Age analysis showed that MDR was dominant in the 5-15 years age and XDR was dominant in the 11-20 years age group and affected both genders. These results demonstrate that there is a pressing necessity to conduct continuous antimicrobial surveillance, address the rational use of antibiotics, enhance sanitation, and mass vaccination to curb the dissemination of highly resistant S. Typhi strains and avoid untreatable typhoid infections.

INTRODUCTION

Typhoid fever is an acute and potentially fatal universal infection in humans that is caused by a Gram-negative rod-shaped bacterium, *Salmonella enterica* serovar *Typhi* (*S. Typhi*), of the Enterobacteriaceae family (Kirt et al., 2024). *S. Typhi* is a distinct pathogen because it is only adapted to humans, which are its only natural reservoir, compared to other *Salmonella* serotypes that possess a wide host range. The infection is fecal-oral and most often is transmitted through the ingestion of contaminated water or food, and is heavily linked to the low hygiene and sanitation standards (Crump et al., 2015). Typhoid fever has remained a huge burden to the global population despite access to better healthcare. The World Health Organization (WHO, 2024) indicates that annually, the number of cases of typhoid fever is estimated between 11-20 million with an estimated number of 128,000-161,000 deaths surrounding the globe (Mohanto et al., 2025). In the past, typhoid was among the leading causes of death in industrialized countries in the early 20th century. Nevertheless, the incidence in those areas was greatly lowered by better water sanitation and the availability of antibiotics. Typhoid fever is still endemic in South Asia, sub-Saharan Africa and some parts of Latin America, where access to safe drinking water and sanitation facilities remains low (Kim et al., 2022). School-aged children and young adults are the most vulnerable to the disease and this leads to a significant social and economic burden in low and middle-income countries (Buckle et al., 2012). Clinical symptoms of typhoid fever in the human body are usually non-specific, and this makes them hard to diagnose early. A typical fear of the disease consists of a slow beginning of persistent high-grade fever, malaise, headache, abdominal pain, anorexia, as well as hepatosplenomegaly. Gastrointestinal symptoms can be very different, including constipation to diarrhea. Severe complications may arise during the second or the third week of illness and include intestinal perforation, gastrointestinal hemorrhage, encephalopathy, myocarditis and septicemia, unless treated (Boakye Okyere et al.,

2025). These complications are linked to morbidity and mortality, and especially in the case of limited resources by the fact because obstruction to effective medical care may be delayed (Parry et al., 2023). Additionally, some individuals might become chronic carriers, carrying *S. Typhi* in their gallbladder and acting as continuing transmitters for the virus throughout their communities. Chloramphenicol, ampicillin and co-trimoxazole were used as first-line therapy for decades. The rise of MDR *S. Typhi* strains, which are resistant to all three drugs in the 1970s, was, however, followed by the proliferation of such strains in Asia, Africa, and Latin America (da Silva et al., 2022). There was the development of fluoroquinolone resistance in the 1990s that caused treatment failure with ciprofloxacin which once acted as a reliable alternative. More recently, strains of *S. Typhi* that are extensively drug-resistant (XDR) have been identified with resistance to first-line agents, fluoroquinolones, and third-generation cephalosporins. Therapeutic possibilities are severely limited to azithromycin and carbapenems with these strains, causing real concerns about the possibility of untreatable typhoid infections in the future (Klemm et al., 2018; Imran et al., 2024). Besides antimicrobial resistance, the diagnosis of typhoid fever is also highly difficult. Blood culture is the ideal standard laboratory evidence of the condition, although it is frequently sensitive, and endemic locations may not always have adequate microbiological facilities (Cosgrove, 2022). This results in the proliferation of clinical diagnoses, which also helps increase resistance and increases the chance of more antibiotic misuse. The measures to manage typhoid fever must integrate improved water quality, hygienic environments, health education, and vaccination programs to be perceived from the public health perspective. The recent development of typhoid conjugate vaccines (TCVs) has promising chances of being effective, especially in children, but they have not been utilized extensively in most endemic regions due to economic and logistical issues (Nampota-Nkombi et al., 2023). *S. Typhi* causes typhoid

fever, which is a leading health concern among individuals across the globe and low-and middle-income countries in particular. The absence of specific clinical signs, the increase of resistance to antibiotics, and the sustainability of the infection because of poor sanitation are important factors that highlight why an integrated approach to control is needed. The goals of the study were to identify *S. Typhi* prevalence in blood samples of human patients with typhoid fever and to determine the antimicrobial resistance (antibiogram) patterns of isolated *S. Typhi*.

Methodology

Sampling and Data Collection

This experiment was carried out over a period of 6 months, where 120 blood samples were taken from suspected patients of typhoid aged 5-40 years. Samples were collected at various hospitals and clinics, and around 20 samples were taken in each hospital. All the blood samples were collected aseptically and were brought to the laboratory to be processed. The samples that were positive for typhoid were inoculated into a blood culture broth to boost bacterial growth. The samples were grown on MacConkey agar and Salmonella-Shigella (SS) agar to study the morphology of the colonies and their fermentation properties. Gram staining was done on colonies in order to observe the morphology of bacteria. Identification of the isolate was done based on the standard biochemical tests, such as catalase, indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, urease, triple sugar iron (TSI), and nitrate reduction tests. The

isolates were tested on the antimicrobial susceptibility test of MDR and XDR *S. Typhi* using the modified Kirby-Bauer disc diffusion test. 8 classes of antibiotics were used, and the zones of their inhibition were measured. Interpretations were done based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

GraphPad Prism software was used to analyze the data. The percentage positivity of *S. Typhi* isolates and frequency of MDR, XDR, and non-traditional antibiogram patterns were determined. Distribution of resistant isolates by age and gender was also assessed.

Results

Out of the 120 blood samples that were taken, 70 (58 males and 62 females) had bacterial growth in the blood culture broth, which was indicative of typhoid positivity. The turbidity in the blood culture broth was monitored as an indication of bacterial development.

Growth in Blood Culture Broth

The bacterial growth in the blood culture broth was enriched by inoculating the typhoid-positive and typhoid-negative samples. The growth of blood bacteria was enriched and improved by the use of blood culture broth. The samples were allowed to incubate at 37°C 24 hours. Among 120 samples, 70 samples were turbid, and these are samples that are typhoid positive, and the percent positivity is 58 percent (Table 1).

Table 1. Distribution of Typhoid Positive Cases Across Hospitals/Clinics

No. of Samples	Typhoid Positive	% Positivity
19	12	63
19	9	47
18	9	50
18	6	33
18	15	83
28	19	68
120	70	58

Antimicrobial Susceptibility Testing for *S. Typhi*

The samples (n=14) were assayed in the presence of 8 antibiotics, and the zone of inhibition was noted as in Table 3. The zone of inhibition was to be measured and the data were to be compared as per the CLSI guidelines, as in Table 2. High resistance to the first-line of drugs such as trimethoprim-sulfamethoxazole, tetracycline, and ampicillin was observed in samples (n=4). N=7 samples were found to be resistant to both the first and second line of medications, which comprise fluoroquinolones and the third generation of cephalosporins, classified as XDR. Table 4 was used to compute percentage

positivity and the data were distributed according to the sensitivity and resistance pattern. In *S. Typhi*, some of the samples depicted a very strange antibiogram pattern as they were resistant to all the antibiotics that were tested, as indicated in Table 4, 5.

Antibiogram Pattern of *S. Typhi* Standards

The zone of inhibition standards demonstrated in Table 2, according to the CLSI guidelines, were compared with the zones of observed antibiotics.

Table 2. Zone of Inhibition (mm) according to CLSI standards for *S. Typhi*

Antibiotics	Conc.	Zone of Inhibition (mm) according to CLSI standards		
		Sensitive	Intermediate	Resistant
Trimethoprim-Sulfamethoxazole	25µg	≥15	11-15	≤10
Amikacin	30µg	≥15	14-16	≤14
Imipenem	10µg	≥25	21-22	≤19
Tetracycline	10µg	≥14	12-15	≤11
Ampicillin	25µg	≥15	13-16	≤12
Azithromycin	15µg	≥12	-	≤12
Chloramphenicol	20µg	≥17	13-17	≤13
Ciprofloxacin	5µg	≥28	21-30	≤22

Table 3. Observed Antibigram Pattern of *S. Typhi*, n=14

antibiotics	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
Imipenem	R	R	R	S	R	S	R	S	R	S	R	I	S	S
Azithromycin	R	R	S	R	S	S	S	S	S	S	S	S	S	R
Amikacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ciprofloxacin	R	R	S	R	R	S	R	R	I	R	R	R	S	S
Chloramphenicol	R	R	S	R	S	R	S	R	R	S	S	R	S	S
Tetracycline	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Trimethoprim-sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Table 4. CLSI Standards for the Antibigram Pattern of *S. Typhi*

Antibiotics	Conc.	Zone of Inhibition (mm)			Observed Zone (mm)	Sensitive (S), Resistant (R), Intermediate (I)
		CLSI standards				
		Sensitive	Intermediate	Resistant		
Trimethoprim-Sulfamethoxazole	25µg	≥15	11-15	≤10	12	I
Amikacin	30µg	≥15	14-16	≤14	6	R
Imipenem	10µg	≥25	21-22	≤19	12	R
Tetracycline	10µg	≥14	12-15	≤11	13	I

Ampicillin	25µg	≥15	13-16	≤12	14	R
Azithromycin	15µg	≥12	-	≤12	7	R
Chloramphenicol	20µg	≥17	13-17	≤13	11	R
Ciprofloxacin	5µg	≥28	21-30	≤22	9	R

Table 5. Percentage Positivity of *S. Typhi* based on Antibigram Pattern (n = 14)

Antibiotics	Conc.	Antibiogram pattern of samples according to CLSI guidelines		
		Sensitive	Intermediate	Resistant
Trimethoprim-Sulfamethoxazole	25µg	0	0	14
Amikacin	30µg	0	0	14
Imipenem	10µg	8	1	5
Tetracycline	10µg	0	0	14
Ampicillin	25µg	0	0	14
Azithromycin	15µg	11	0	3
Chloramphenicol	10µg	5	0	9
Ciprofloxacin	5µg	5	1	8

Drug Resistance Pattern of *S. Typhi*

The pattern of drug resistance as presented in Figure 1 indicates that *S. Typhi* is resistant to the first line and second line of drugs, resulting in the development of MDR and XDR stereotypes, respectively. Percentage positivity according to the sensitivity pattern in Figure 2. The greatest

percentage of extensively drug-resistant (XDR) *S. Typhi* was found in comparison with the MDR, which is introduced in Tables 6 and 7. The presence of an unusual resistance pattern was also identified in 20 percent of isolates, as is presented in Table 8. Age-wise distribution revealed that MDR cases occurred most

commonly among the patients aged 5-15 years (Figure 3), and XDR cases were distributed among the patients aged 15-25 years (Figure 4).

Figure 5 shows the total percentage distribution of MDR, XDR, and unusual patterns of resistance.

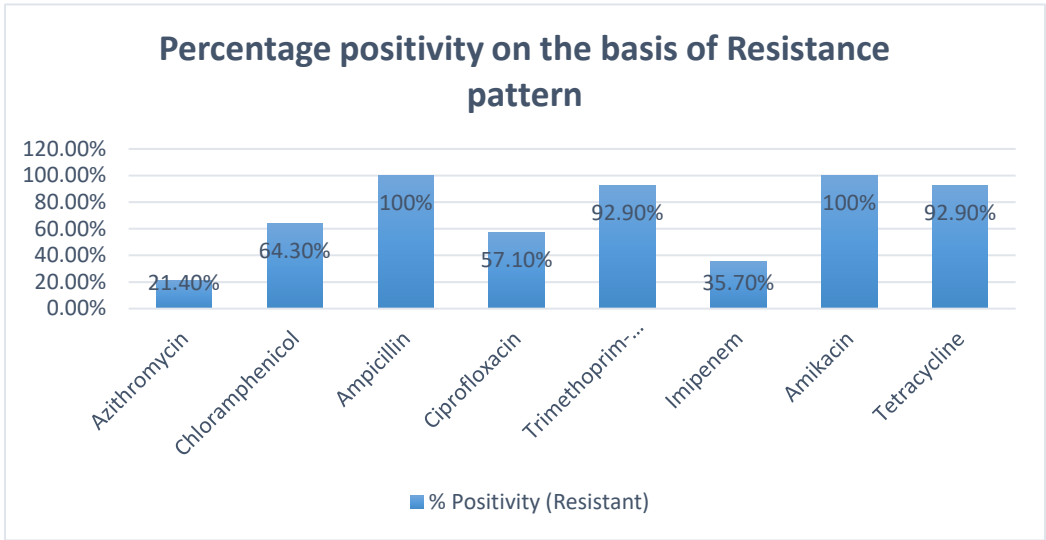


Figure 1. Percentage Positivity of *S. Typhi* isolated from Human Blood based on Drug Resistance Pattern

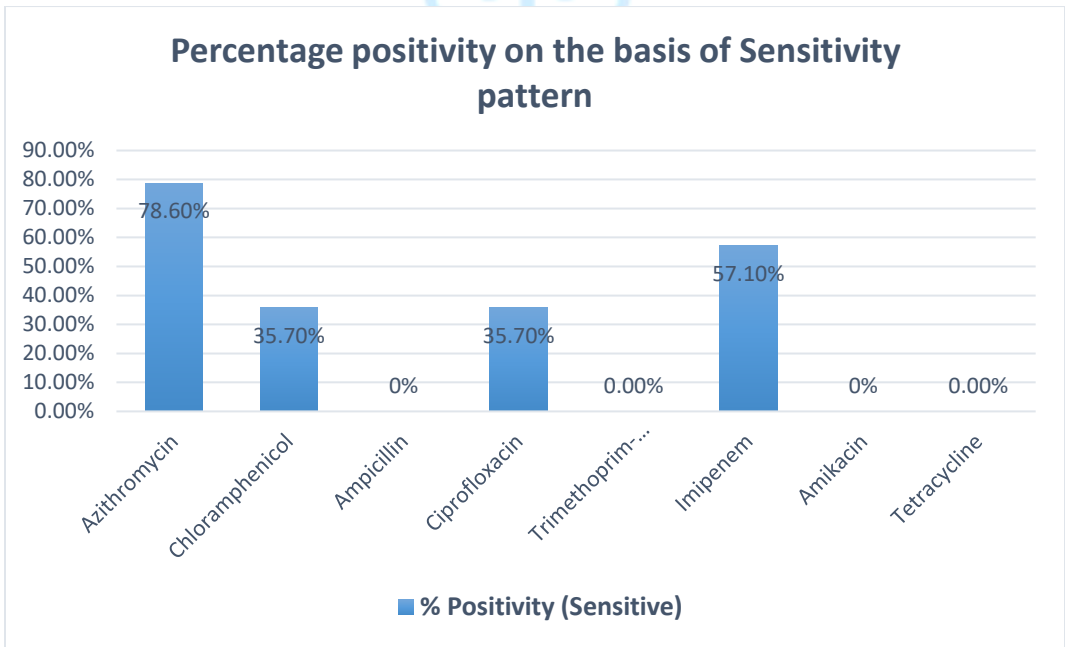


Figure 2. Percentage Positivity of *S. Typhi* isolated from Human Blood based on the Sensitivity Pattern

Table 6. Percentage Positivity of MDR *S. Typhi* isolates (n=14)

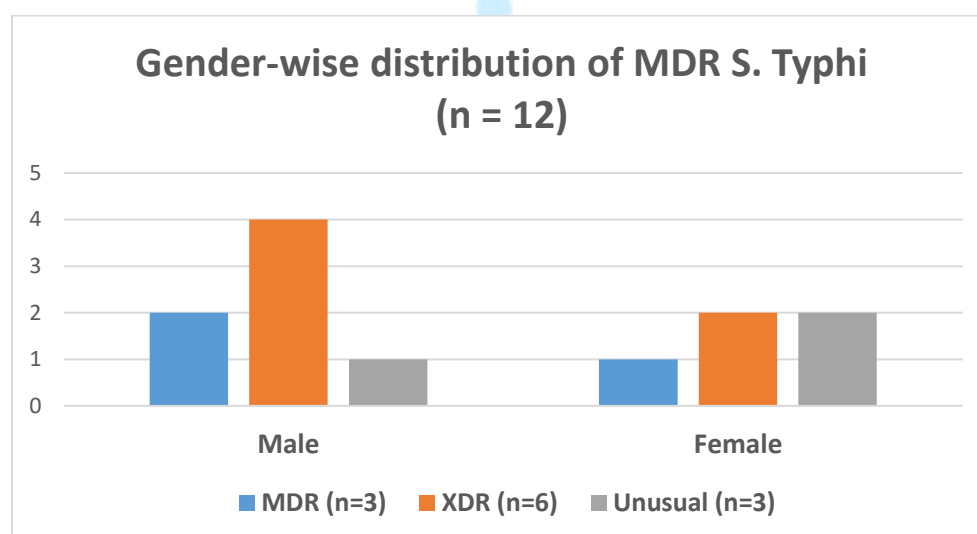
Total number of <i>S. Typhi</i> resistant to first line of drugs n=14	Percentage positivity
4	28.6%

Table 7. Percentage Positivity of Extensively Drug-Resistant *S. Typhi*

Total number of <i>S. Typhi</i> resistant to second line of drugs n=14	Percentage positivity
7	50%

Table 8. Percentage Positivity of Unusual Antibigram Pattern in *S. Typhi* Isolates (n=14)

Total number of <i>S. Typhi</i> showed unusual antibiogram pattern n=14	Percentage positivity
3	21.4%

Figure 3. Gender-wise Distribution and Percentage Positivity of Multi-Drug Resistant *S. Typhi* Isolated from Human Blood

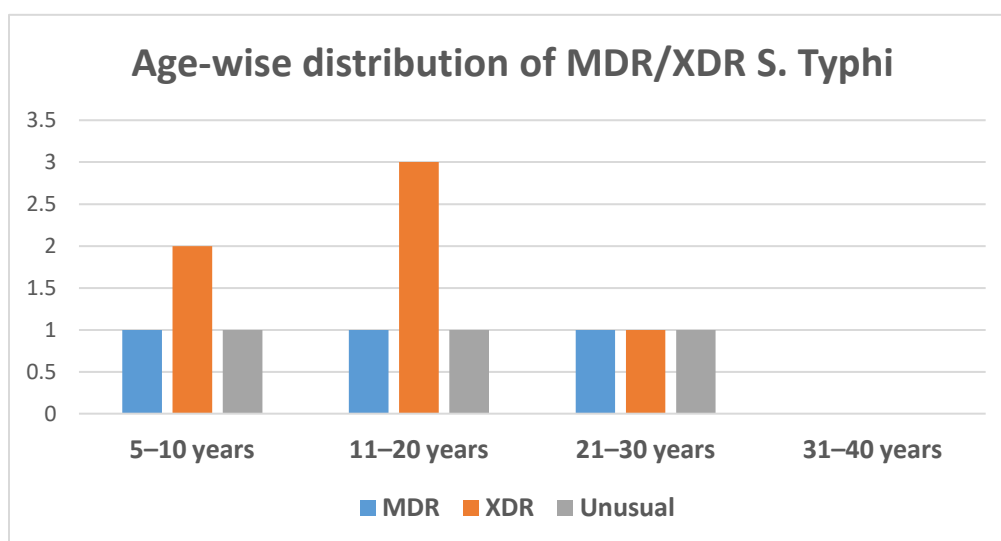


Figure 4. Age-wise Distribution and Percentage Positivity of *S. Typhi* Isolated from Human Blood

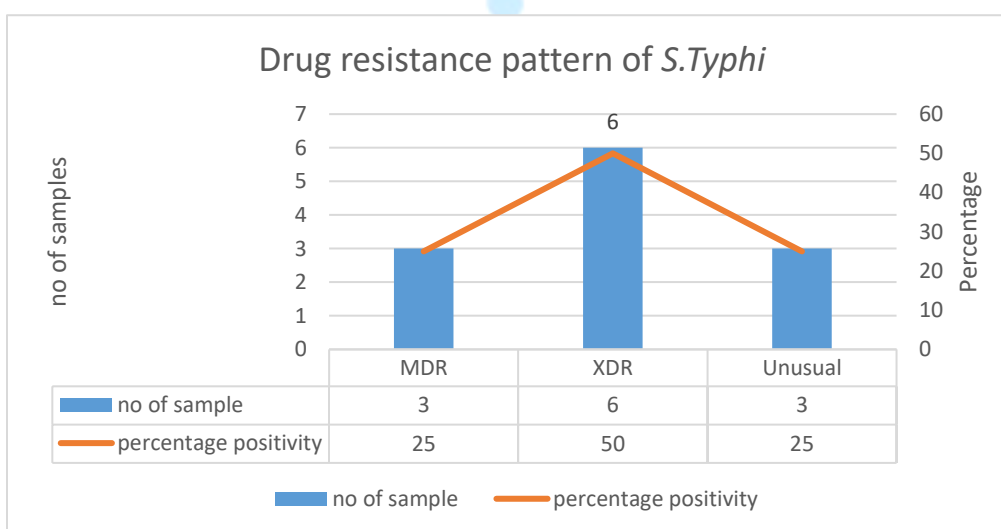


Figure 5. Percentage Positivity of *S. Typhi* Isolated from Human Blood based on Drug Resistance Pattern

Discussion

Salmonella Typhi remains a serious health concern, particularly in underprivileged and inadequately sanitary environments. Typhoid fever, a disease caused by *S. Typhi*, is spread through a fecal-oral route and can affect children, teenagers, and even adults, although school-going children and young adults are most vulnerable (Buckle et al., 2012; Crump et al., 2015). Clinical findings are usually non-specific, such as continuous high-grade fever, headache, malaise, abdominal pain, anorexia, and

hepatosplenomegaly. Unattended severe complications may result in intestinal perforation, gastrointestinal hemorrhage, septicemia, and encephalopathy, which are the causes of serious morbidity and mortality (Parry et al., 2023). The current research involved 120 blood samples of suspected typhoid patients aged between 5-40 years of age in various hospitals and clinics. Out of them, 70 samples (58% positive), were confirmed to be positive with *S. Typhi* through the use of blood culture. The age

distribution showed that there was a higher sample in the 11-30 years age category, which correlates with the epidemiological pattern of typhoid incidence being higher in this group. Male (58) and female (62) samples were equally susceptible to the disease, gender-wise. Concerning high levels of resistance were observed in the antimicrobial susceptibility patterns of 14 *S. Typhi* isolates. Four isolates had MDR *S. Typhi*, which is a resistance to first-line antibiotics (trimethoprim-sulfamethoxazole, ampicillin, tetracycline) (28.6%). In 7 isolates (50%), the XDR *S. Typhi* serotype that is resistant to both first- and second-generation cephalosporins as well as the fluoroquinolones and third-generation cephalosporins was detected. Moreover, 3 isolates (21.4%) had an uncharacteristic pattern of antibiogram and were resistant to almost all antibiotics tested, including azithromycin and carbapenems (meropenem and imipenem). These results show the severe spread of highly resistant *S. Typhi* strains in the study area. The age distribution of the resistant isolates indicated that MDR isolates were mainly detected in patients between 5-15 years of age, and the XDR isolates were focused on the 11-20 years group. There were also abnormal patterns of resistance in various age groups (5-30 years). Men were the predominant MDR (2/3) and XDR (4/6) and women were the predominant unusual antibiogram patterns (2/3).

This infection is a sign that both genders and other age groups have also been affected by the distribution, and this shows the broad spectrum of the impact of the drug-resistant *S. Typhi*. The high incidence of resistance is consistent with regional reports of the development and spread of MDR and XDR of *S. Typhi* in Asia and Africa. Drug-resistant bacteria spread through the H58 haplotype to cause rapid resistance to the first-line antibiotics and fluoroquinolone-based antibiotics (Mashe et al., 2021). The possibility of developing atypical antibiograms can be connected with the misuse of antibiotics and the simultaneous evolution of genes resistant to antibiotics, including azithromycin and carbapenem resistance genes. The trends show that, in addition to the challenge of controlling

typhoid fever in endemic regions, there is the risk of untreatable infection in favor of further propagation of resistance. Overall, the present study confirms the existing data of the widespread occurrence of typhoid fever (58) among suspected patients and the risk of gradually growing drug resistance. MDR and XDR *S. Typhi* made up 28.6 and 50% of the isolates, respectively, and odd patterns of resistance were found in 21.4 % of the isolates. These findings focus on the significance of continual monitoring of antimicrobial resistance, the responsible application of antibiotics, improved hygiene, and vaccination to exterminate the propagation of highly resistant special *S. Typhi* forms.

Conclusion

In conclusion, the study revealed a high prevalence of typhoid fever (58% among suspected cases) along with an alarming increase in MDR (28.6%) and XDR (50%) *S. Typhi* isolates in the study area. The presence of abnormal patterns of antibiograms (21.4%) also reflects the steadily more complex character of the antimicrobial-resistant infections. MDR was more common in 5-15 year olds and XDR in 11-20 year olds, and in both males and females, they had higher rates of MDR and XDR. The results indicated acquired first-line antibiotic resistance (MDR) and even fluoroquinolone and third-generation cephalosporins resistance against *S. Typhi* (XDR). The existence of XDR strains was tested through the evaluation of zones of inhibition, which were not susceptible to first- and second-generation drugs, including ampicillin, tetracycline, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporin drugs. The most effective and sensitive antibiotics among the tested antibiotics were azithromycin.

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