ORIGINAL ARTICLE

Multidrug-Resistant and Extensively Drug-Resistant *Salmonella Typhi* and *Salmonella Paratyphi*: A Cross-Sectional Study at Provincial Headquarter Hospital, Gilgit

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ABSTRACT

Objective: To determine the antimicrobial susceptibility of *Salmonella typhi* and *Salmonella paratyphi* to recommended antibiotics and detect the frequency of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella* isolates.

Study Design: A cross-sectional study.

Place and Duration of Study: This study was conducted at the Microbiology Laboratory of Provincial Headquarter Hospital, Gilgit, Pakistan, from January 2024 to December 2025.

Methods: A total of 208 blood cultures with confirmed growth of *Salmonella typhi* and *Salmonella paratyphi* were included using non-probability convenience sampling. Blood cultures that grew non-lactose fermenters were further identified by colony morphology and the Analytical Profile Index (API 20E). The Kirby-Bauer disc diffusion method was used for antimicrobial sensitivity testing. The antibiotic zone diameters were reported as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.

Results: Most of the patients in our study were 1-10 years of age (45.7%), followed by 11-20 years (34.6%). The majority of the patients were males (63%). Out of 208 isolates of *Salmonella*, 168 (80.8%) were identified as *Salmonella typhi*, and 40 (19.2%) were *Salmonella paratyphi*. The antimicrobial sensitivity pattern showed high resistance to ampicillin (92.8%), trimethoprim-sulfamethoxazole (81.7%), chloramphenicol (90%), cefixime (84.1%), ceftriaxone (80.2%), and ciprofloxacin (95.7%). Out of a total of 208 isolates, 134 (64.4%) of the isolates were MDR, and 84 (40.3%) were XDR. Six (2.9%) isolates were azithromycin-resistant, and 2 (1%) were meropenem-resistant.

Conclusion: Antimicrobial resistance to first-line and second-line antibiotics is very high in *Salmonella typhi* and *Salmonella paratyphi*. *Salmonella Typhi* exhibited significantly greater resistance to first- and second-line drugs than *Salmonella Paratyphi*. Around 64.4% of the isolates are multidrug-resistant, and 40.3% are extensively drug-resistant. Meropenem and azithromycin showed a good sensitivity pattern against these isolates.

Keywords: *Antimicrobial Drug Resistance, Salmonella, Salmonella Paratyphi, Salmonella Typhi.*

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Introduction

Salmonella is recognized as the most prevalent foodborne pathogen worldwide. It causes zoonotic infections involving poultry and humans. The infection can be acquired through contaminated water, the use of undercooked meat & poultry products, handling of raw meat, and animal trade.[1] Enteric fever is a fatal, bloodstream infection caused by the serotypes of *Salmonella enterica*, *Salmonella typhi*, and *Salmonella paratyphi*. It is the leading

cause of morbidity and mortality in low-to-middle-income countries. If not properly treated, it can lead to serious complications from the second week onwards. The Global Burden of Disease reported 9.3 million patients of enteric fever annually worldwide, resulting in 100,000 deaths. In addition, the economic burden associated with the illness is also high. Enteric fever in prevalent in South Asia, making up 62% of the global cases.[2] The disease manifests with high-grade fever, malaise, abdominal pain, loss of appetite, constipation, and hepatosplenomegaly. Complete blood counts reveal leukopenia and thrombocytopenia. The complications associated with the disease are intestinal bleeding, perforation, anemia, disseminated coagulation disorder (DIC), and shock. The mortality of the infection is very high in children as compared to other age groups.[3] The route of transmission is fecal-oral. Lack of clean drinking water, improper sanitation, and unsafe food handling practices are major contributors to illness. Food handlers who are silent, chronic carriers of the infection serve as a continuous source of infection.[2,3] Enteric fever is a preventable disease. The preventive measures include using safe drinking water, eating properly cooked food, practicing good hygiene, especially handwashing, screening food handlers, and vaccination. Two types of vaccines available against the disease are Ty21a and Vi capsular polysaccharide vaccines. Ty21a is an oral, live-attenuated vaccine, whereas the Vi capsular vaccine is an injectable subunit vaccine.[2]

The diagnosis of the disease is challenging, with blood culture being the gold standard, but its sensitivity is low.[3] The treatment of choice is antibiotics, but due to rising antimicrobial resistance (AMR), many antibiotics are ineffective against typhoidal strains of *Salmonella*. In the 1940s to 1970s, the mainstay of treatment for enteric fever was chloramphenicol, co-trimoxazole, and ampicillin. These are referred to as first-line drugs against *Salmonella typhi* and *Salmonella paratyphi*. Later on, these organisms developed resistance to first-line drugs. At that time, fluoroquinolones and cephalosporins emerged as effective treatment options for enteric fever. These are the second-line drugs for *Salmonella*. Until that time, enteric fever was effectively managed through improved hygiene, vaccination, and second-line medications. In 2016, *Salmonella* isolates emerged that were resistant to these second-line antibiotics. At that time, enteric fever developed into a critical public health threat, placing considerable strain on healthcare systems. These isolates were first reported from Pakistan, Bangladesh, India, and Nepal.[4] Azithromycin and meropenem are the only antibiotics left to treat enteric fever caused by XDR strains. However, they should be used cautiously and prescribed only according to antimicrobial susceptibility testing results. Strict adherence to the full course of treatment is essential to avoid antimicrobial resistance. Developing countries also face challenges in managing the high cost of these medications. The genes conferring resistance to first-line drugs are blaTEM-1 (ampicillin), catA1 (chloramphenicol), sul1, and dhfR7 (Co-trimoxazole). The genes associated with resistance to fluoroquinolones include gyrA, gyrB, parC, parE, and qnrS. Furthermore, the presence of CTX-M genes encoding extended-spectrum beta-lactamases (ESBLs) has rendered these strains resistant to third-generation cephalosporins.[5] The isolates of *Salmonella* resistant to azithromycin have emerged. Although the resistance is low, the situation is quite alarming. Meropenem has shown promising results against enteric fever, with minimal resistance reported so far. However, its intravenous administration, dosing three times a day, and high cost remain significant concerns.[6,7]

In Pakistan, cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella* are on the rise. The first patient with XDR *Salmonella* was reported in Sindh in 2016, when isolates from positive blood cultures were found to be non-responsive to standard treatment. Since then, Pakistan has been struggling with MDR and XDR strains of *Salmonella*. They are not only a major threat to the country, but can also spread worldwide through outbreaks.[7] Over-the-counter availability of antibiotics & their misuse, unjustified prescribing of antibiotics, and non-compliance are the factors contributing to high antimicrobial resistance among *Salmonella* isolates. Pakistan ranks third among the countries where antibiotic consumption is very high. Antibiotic misuse is strongly associated with the development of bacterial resistance, a problem that is well-documented and considered a major public

health concern. The global use of antibiotics increased by approximately 36%. If this trend continues and resistance is not effectively controlled, it is estimated that antimicrobial resistance could cause up to 10 million deaths annually by 2050.[8]

Antimicrobial resistance in *Salmonella* is rising with each passing day, making it a serious issue. Resistance to first-line and second-line drugs is common, leaving us with azithromycin and meropenem as the drugs of last resort. Many studies have reported the antimicrobial susceptibility pattern of *Salmonella* isolates; however, there was a need for local data from our setup. The healthcare dynamics of Gilgit are quite different. Public awareness regarding timely access to healthcare facilities is limited, and available healthcare resources are also inadequate. Additionally, self-medication and treatment by unauthorized healthcare providers are common, which may influence antimicrobial resistance patterns. So, this study was conducted to determine the antimicrobial susceptibilities of *Salmonella Typhi* and *Salmonella Paratyphi* and to detect the frequency of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella* isolates.

Methods

This cross-sectional study was conducted at the Microbiology Laboratory of the Provincial Headquarters Hospital, Gilgit, Pakistan, from January 2024 to December 2025. After obtaining ethical approval from the hospital's Ethical Review Board, vide letter no: 1104/PHQ/2024, dated: 06th January 2024. The sample size was estimated using a 95% confidence interval, a 5% margin of error, and an expected MDR *Salmonella* frequency of 32%, based on a study conducted in Islamabad.[9] A total of 208 blood cultures with the confirmed growth of *Salmonella typhi* and *Salmonella paratyphi* were included using nonprobability convenience sampling. Inclusion criteria were patients of all ages and genders who presented with clinical features suggestive of enteric fever and had positive blood cultures. Samples with contamination or growth of other bacterial species were excluded from the study.

The patients' blood specimens were collected aseptically and inoculated into blood culture bottles containing appropriate culture media. These blood culture bottles were received in the Microbiology laboratory and incubated at 35°C for 5 days. The subcultures were inoculated on the 2nd and 5th day on blood and MacConkey agar. Blood cultures that grew non-lactose fermenters were initially identified by colony morphology and Gram staining. Further biochemical identification was carried out using the Analytical Profile Index (API 20E). The Kirby-Bauer disc diffusion method was used for antimicrobial sensitivity testing (AST). Antibiotic discs were placed on Mueller-Hinton agar plates and incubated at 35°C for 16–18 hours. The diameters of the inhibition zones around each antibiotic disc were measured in millimeters. The zone diameters of antibiotics were reported as sensitive, intermediate, and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2023.[10] All cultures and AST results were interpreted and reported by an expert microbiologist. The following antibiotics were used for AST: chloramphenicol (C), ampicillin (AMP), trimethoprim-sulfamethoxazole (SXT), ceftriaxone (CRO), cefixime (CFM), ciprofloxacin (CIP), meropenem (MEM), and azithromycin (AZM 15 µg). Ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol are the first-line drugs against *Salmonella*, whereas ciprofloxacin and ceftriaxone are the second-line drugs. The first-line drugs resistant Salmonella isolates are called MDR, whereas the isolates resistant to all first-line drugs, ceftriaxone and ciprofloxacin, are labeled as XDR *Salmonella*.[11]

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 27 was used. For numerical variables such as age, the mean and standard deviation were used. Categorical variables such as gender, ward, *Salmonella* species, and AST were presented as frequency and percentage. The AMR pattern of *Salmonella typhi* and *Salmonella paratyphi* was compared using the Pearson Chi-Square/Fisher extract test. The *P*-value of <0.05 was significant.

Results

The mean patient age was 15.6 ± 9.35 years in our study. Most of the patients were 1-10 years of age (45.7%), followed by 11-20 years (34.6%) and 21-30 years (15.4%). The majority of patients were male (63%), whereas 37% were female. Around 73.6% of

the patients were from the outpatient (OPD) department, 18.7% from the Pediatrics ward, and 7.7% from the Medical ward (Table 1).

Out of 208 isolates of *Salmonella*, 168 (80.8%) were identified as *Salmonella typh*i, and 40 (19.2%) were *Salmonella paratyphi*. The antimicrobial sensitivity pattern showed high resistance to ampicillin (92.8%), trimethoprim-sulfamethoxazole (81.7%), chloramphenicol (90%), cefixime (84.1%), ceftriaxone (80.2%), and ciprofloxacin (95.7%). Out of a total of 208 isolates, 134(64.4%) were MDR, and 84 (40.3%) were XDR. Six (2.9%) isolates were resistant to azithromycin, and 2 (1%) strains were meropenem-resistant (Figure 1).

Table 1: Demographic variables of the patients

Variable	Frequency (Percentage)
Age Groups	
1-10 years	95 (45.7%)
11-20 years	72 (34.6%)
21-30 years	32 (15.4%)
31-40 years	5 (2.4%)
41-50 years	2 (1%)
51-60 years	1 (0.5%)
71-80 years	1 (0.5%)
Gender	
Male	131 (63%)
Female	77 (37%)
Ward	
OPD	153 (73.6%)
Pediatrics Ward	39 (18.7%)
Medical Ward	16 (7.7%)

Table 2: Comparison of AMR patterns of *Salmonella typhi* and *Salmonella paratyphi*

Antibiotic	AMR Pattern	*Salmonella typhi* (N = 168)	*Salmonella paratyphi* (N = 40)	Total (N = 208)	*Chi-Square* test value	*P*-value
Ampicillin	Sensitive	6 (3.6%)	9 (22.5%)	15 (7.2%)	17.215	<0.0001*
	Resistant	162 (96.4%)	31 (77.5%)	193 (92.8%)		
Chloramphenicol	Sensitive	11 (6.5%)	10 (25%)	21 (10%)	12.0610	0.0005*
	Resistant	157 (93.5%)	30 (75%)	187 (90%)		
Trimethoprim-Sulphamethoxazole	Sensitive	22 (13.1%)	16 (40%)	38 (18.3%)	15.587	0.0001*
	Resistant	146 (86.9%)	24 (60%)	170 (81.7%)		
Cefixime	Sensitive	18 (10.7%)	15 (37.5%)	33 (15.9%)	17.282	< 0.0001*
	Resistant	150 (89.3%)	25 (62.5%)	175 (84.1%)		
Ceftriaxone	Sensitive	23 (13.7%)	18 (45%)	41 (19.8%)	19.915	< 0.0001*
	Resistant	145 (86.3%)	22 (55%)	167 (80.2%)		
Ciprofloxacin	Sensitive	4 (2.4%)	5 (12.5%)	9 (4.3%)	-	
	Resistant	164 (97.6%)	35 (87.5%)	199 (95.6%)		
Azithromycin	Sensitive	163 (97%)	39 (97.5%)	202 (97.2%)	-	
	Resistant	5 (3%)	1 (2.5%)	6 (2.9%)		
Meropenem	Sensitive	166 (98.8%)	40 (100%)	206 (99%)	-	
	Resistant	2 (1.2%)	0 (0%)	2 (1%)		

**Statistically Significant*

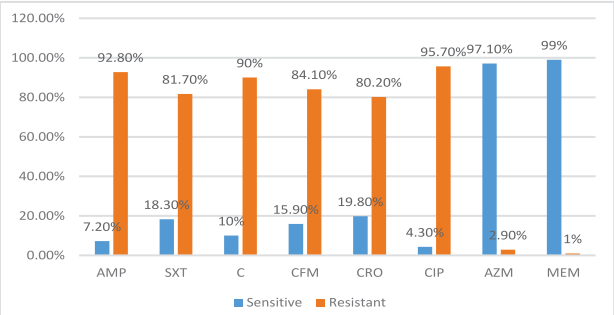

Fig.1: Overall antimicrobial susceptibility pattern of *Salmonella* isolates

When the AMR patterns of *Salmonella typhi* and *Salmonella paratyphi* were seen separately, *Salmonella typhi* isolates showed significantly higher resistance to ampicillin, chloramphenicol, trimethoprim-sulphamethoxazole, cefixime, ceftriaxone, and ciprofloxacin. The resistance of *Salmonella typhi* to azithromycin and meropenem was also higher, but the *P*-value was not significant. These results are shown in Table 2.

Discussion

Antimicrobial resistance in *Salmonella* is a significant threat to public health, especially in developing countries. The mean patient age was 19.1 ± 8.75 years in our study. Most of the patients were 1-10 years of age (45.7%), followed by 11-20 years (34.6%) and 21-30 years (15.4%). Sixty-three percent of the patients were males. The mean age was 25.5 years in a study. The majority of them were male.[12] In another study, 42% of the patients were 40.04±15.79 years, with most of the patients 20-40 years of age and male (63.1%).[13] In another study, 36.8% of the patients were 31-45 years old, with the majority of males (62.2%).[14]

Our results showed that 80.8% of isolates were identified as *Salmonella typhi* and 19.2% as *Salmonella paratyphi*. Similar to our study, another study reported that 79.5% of *Salmonella* were *Salmonella typhi*, and the remaining 20.5% were *Salmonella paratyphi*.[12] Another study reported 80% *Salmonella typhi* and 20% *Salmonella paratyphi*.[15]

In our study, the antimicrobial sensitivity pattern showed high resistance to ampicillin (92.8%), trimethoprim-sulphamethoxazole (81.7%), chloramphenicol (90%), cefixime (84.1%), ceftriaxime (80.2%), and ciprofloxacin (95.7%). Out of a total of 208 isolates, 134 (64.4%) were MDR, and 84 (40.3%) were XDR. Azithromycin and meropenem showed good sensitivity, at 97.1% and 99%, respectively. Our study demonstrated high resistance to first- and second-line antibiotics among *Salmonella*. This may be attributed to a prolonged fever history and prior antibiotic use with inadequate dosage or non-compliance, including self-medication or treatment from an unauthorized healthcare worker. Most patients presented to the hospital after persistent symptoms, with multidrug resistance. Similarly, another study showed 100% resistance of *Salmonella* to ampicillin, amoxicillin, cefotaxime & ciprofloxacin and 95.8% resistance to ceftriaxone. No resistance was shown by azithromycin and meropenem.[16] A study from Islamabad reported 83.1% resistance to ampicillin, 75.6% to chloramphenicol, and 56.1% to cotrimoxazole, 83.3% to ciprofloxacin, and 68.3% to ceftriaxone. Meropenem showed sensitivity of 98.3%, with 1.7% strains producing intermediate zones of inhibition. Alarmingly, resistance to azithromycin was seen in 7.1% of the *Salmonella* isolates. Thirty-two percent of the isolates were MDR, and 27% were XDR.[9] A multicenter study from Pakistan reported 70% MDR and 47.1% XDR isolates of *Salmonella*. None of the isolates were meropenem-resistant, and only 0.45% were azithromycin-resistant.[17] Another study from Karachi revealed 72% XDR *Salmonella* isolates; all of these isolates were meropenem and azithromycin-sensitive.[18]

Other studies also reported MDR and XDR *Salmonella*, but the figures were not as high as in our study. Umair M et al. showed resistance to amoxicillin, co-trimoxazole, and ciprofloxacin in 57.6%, 61.4%, and 62.7% of the isolates at the Pakistan Institute of Medical Sciences, respectively. Ceftriaxone and imipenem showed lower rates of resistance, at 4.4% and 3.8%, respectively. The frequency of MDR *Salmonella* was 44.6%.[12] Sattar A et al. reported the sensitivity to various antibiotics as follows: ampicillin (60%), chloramphenicol (67%), sulphamethoxazole (53%), ceftriaxone (84%), ciprofloxacin (16%), meropenem (96%), and azithromycin (100%). Forty-seven percent of the *Salmonella* were MDR, and 16% were XDR.[15] In a study conducted among children in Sialkot, co-trimoxazole showed 77.6% sensitivity, ceftriaxone 75.8%, and ciprofloxacin 16%. Azithromycin and meropenem showed sensitivities of 95.2% and 96.7%, respectively. Around 24% of the *Salmonella* strains were MDR and XDR.[19]

A study from Nepal showed that a large proportion of *Salmonella* isolates were sensitive to first- and second-line drugs, except for ciprofloxacin: ampicillin (93.93%), cefixime (90.9%), chloramphenicol (100%), and cefotaxime (54.5%). Around 87.87% of the strains were ciprofloxacin-resistant. But azithromycin resistance was high, with 58% sensitivity.[20] In a study conducted at Mayo

Hospital, *Salmonella* was highly resistant to ampicillin (90.5%). The sensitivity to ceftriaxone and ciprofloxacin was 53.6% and 66.7%, respectively. In contrast to other studies, in which *Salmonella* exhibited high resistance to ciprofloxacin, this study reported greater sensitivity to ciprofloxacin. Azithromycin was sensitive in 83.3% of the cases, and imipenem in 85.7% of the cases. The reported resistance to azithromycin and imipenem is alarming, as these are drugs of last resort for treating enteric fever. Around 28.57% and 14.28% of the *Salmonella* strains were MDR and XDR, respectively.[13] The antimicrobial resistance profile of *Salmonella* isolates in a study by Ahmad S et al. showed 90.1% resistance to ampicillin, 74.5% to trimethoprim, 78.5% to ciprofloxacin, and 58.8% to ceftriaxone. Meropenem showed no resistance, whereas azithromycin showed 21.5% resistance.[14] The results of a systematic review and meta-analysis showed 80% resistance to ampicillin, 64% to ciprofloxacin, 7% to azithromycin, and 2% to meropenem. Around 29% of the strains were MDR, and 25% were XDR.[21] A study from Sindh, Pakistan, reported 30.7% MDR and 7.7% XDR *Salmonella* isolates from blood cultures. None of the isolates was azithromycin resistant.[22] Yousaf M et al. revealed 91.47% resistance to ampicillin, 81.9% to trimethoprim-sulphamethoxazole, 83.7% to cefotaxine, and 96.4% to meropenem.[23] A study reported the antimicrobial resistance pattern of *Salmonella* isolates worldwide. Globally, 33.6% of the isolates are MDR and 21.9% are XDR. Resistance to ampicillin is 36.2%, trimethoprim-sulfamethoxazole is 36.7%, chloramphenicol is 26.2%, ciprofloxacin is 21.8%, ceftriaxone is 7.2%, azithromycin is 5.7%, and meropenem is 2.3%. In Asia, resistance to azithromycin (6%) and meropenem (11.9%) was high.[24]

A significantly higher resistance to first- and second-line drugs was observed in *Salmonella* typhi than in *Salmonella paratyphi*. The resistance of *Salmonella typhi* to azithromycin and meropenem was also higher, but the *P*-value was not significant. This might be attributed to fewer strains resistant to these antibiotics, which makes it hard to show significance. A systematic review and meta-analysis from Pakistan revealed that 80% of *Salmonella typhi* isolates were resistant to ampicillin, 64% to ciprofloxacin, 7% to azithromycin, and 2% to meropenem. *Salmonella paratyphi* showed less resistance with 34% resistance to ampicillin, 51% to ciprofloxacin, 4% to azithromycin, and 2% to meropenem.[21] In contrast, the CMH, Quetta study demonstrated comparably high resistance rates in both *Salmonella typhi* and *Salmonella paratyphi*, particularly to ceftriaxone and ciprofloxacin, suggesting a narrowing AMR gap between the two serovars.[25]

Unnecessary prescriptions and the use of antimicrobials should be avoided. Blood cultures of all febrile patients must be sent to the laboratory for culture & sensitivity, and antibiotics should be prescribed judiciously based on the antimicrobial sensitivity report. We must strengthen our surveillance system and monitor antibiotic-resistant cases of enteric fever. A multicenter trial should be conducted in the future with a larger sample size, and antimicrobial susceptibility should be interpreted using minimum inhibitory concentrations (MICs) along with the disc diffusion method. Molecular characterization of antimicrobial resistance genes should also be performed to provide insights into the genetic basis of antimicrobial resistance.

Conclusion

Antimicrobial resistance to first- and second-line antibiotics is very high in *Salmonella typhi* and *Salmonella paratyphi*. Around 64.4% of the isolates are multidrug-resistant, and 40.3% are extensively drug-resistant. *Salmonella typhi* exhibited significantly greater resistance to first- and second-line drugs than *Salmonella paratyphi.* Meropenem and azithromycin showed a good sensitivity pattern against these isolates.

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Author Contributions

SN: Conception, design of the work, and approval for final submission
UA: Data acquisition, curation, statistical analysis, and approval for final submission
AA: Validation of data, interpretation, write-up of results, and approval for final submission
HS: Manuscript writing for methodology design, investigation, and approval for final submission
MRC: Revising, editing, supervising for intellectual content, and approval for final submission
AM: Writing the original draft, proofreading, and approval for final submission
AA is the nominated guarantor and takes full responsibility for the overall content and integrity of the work

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