

Susceptibility Pattern of Salmonella Typhi and Paratyphi A in Blood Cultures in a Tertiary Care Public Hospital

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ABSTRACT

Objective: To identify recent susceptibility patterns of *Salmonella enterica* subspecies *enterica* serovar Typhi (S. Typhi) and Paratyphi A in a government hospital in Taxila Cantt and to determine the frequency of Multi-Drug Resistant (MDR) and Extensively Drug-Resistant (XDR) S. Typhi.

Study Design: Cross-sectional study.

Place and Duration of Study: Pathology Department, HIT Hospital, Taxila Cantt, Pakistan, from Jan 24 to Jan 25.

Methodology: Samples of blood cultures from patients suspected of having enteric fever were received in the microbiology section, HIT Laboratory, Taxila Cantt. Isolates of *Salmonella* Typhi and Paratyphi A from positive blood cultures were included in the study. Antibiotic susceptibility was tested based on CLSI guidelines (2024).

Descriptive statistics were shown as Mean $\pm$ SD.

Results: In this study, out of the 64 positive blood cultures for Typhoidal *Salmonella* from patients (median age 20), 95.3% were *Salmonella* Typhi (61) and 4.7% were *Salmonella* Paratyphi A (03). In 71.9% of cases, CRP was positive. After using appropriate antimicrobials, fever took around five days to settle. Out of the total *Salmonella* isolated from blood culture, including both species of *Salmonella* Typhi and Paratyphi A, Azithromycin and Meropenem showed 100% susceptibility, as compared to first-line antibiotics with % resistance of 92, 90.6, and 87.5 for Ampicillin, co-trimoxazole, and chloramphenicol, respectively. Ciprofloxacin and Ceftriaxone demonstrated substantial resistance rates of 97% and 76.6%, respectively.

Conclusions: This study showed significant antimicrobial resistance to multiple antibiotics in both *Salmonella* Typhi and Paratyphi A isolates, with an alarmingly high number of MDR and XDR strains of *Salmonella* Typhi, thus highlighting the importance of antimicrobial stewardship and adherence to effective prevention strategies.

Keywords: Antimicrobial Susceptibility, Blood Culture, Extensively Drug-Resistant (XDR), Multidrug Resistance (MDR).

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INTRODUCTION

Enteric fever is caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (S. Typhi) and Paratyphi (S. Paratyphi) A, B, and C. Significantly targeting the developing countries, especially of South East Asia, it has become a medical concern globally.¹ In previous cohort studies, the “typhoid fever” incidence was reported to be between 428 and 1,062 cases per 100,000 (0.428%–1.062%) in Lalitpur, Nepal, and between 635 and 1,135 cases (0.635%–1.135%) in Dhaka, Bangladesh, with variations observed over time and depending on the estimation method used.² In Pakistan, a study highlighted an incidence rate of 573.2 per 100,000 among children aged 2-5 years and 451.7 per 100,000 among children aged 2-15 years.³

People who don't have access to clean food, drinking water, poor hygiene, and inadequate sanitation are more likely to get infected.⁴ Symptoms

include a slow-rising fever, rising to 39- 40°C, and abdominal symptoms (nausea, vomiting, abdominal pain, and diarrhea). Fever is the only presenting symptom in children who are less than 5 years of age.⁵ Soft, tender hepatosplenomegaly has been observed on abdominal examination, usually in children.⁶

Multidrug-resistant (MDR) typhoid refers to resistance to first-line antibiotics: Ampicillin, Co-trimoxazole, and Chloramphenicol. Recently, extensively drug-resistant (XDR) typhoid has also been reported in Pakistan. XDR typhoid is defined as MDR along with resistance to Fluoroquinolones and third-generation Cephalosporins such as Ceftriaxone.³

Chloramphenicol was originally the preferred antibiotic for treating typhoid fever; however, due to the development of resistance and side effects, other antibiotics such as Ampicillin (which inhibits bacterial cell wall synthesis) and Ciprofloxacin (which inhibits bacterial protein synthesis) were used instead.⁷ In recent years, since 2016, a concerning trend has emerged with resistance to multiple antibiotics such as

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Fluoroquinolones (Ciprofloxacin), Ampicillin, Third-Generation Cephalosporins (Ceftriaxone), and Sulfonamides in isolates of Salmonella Typhi, leaving Azithromycin and Carbapenems as the only options for effective treatment. This has led to difficult treatment of Salmonella Typhi, especially in 3rd-world countries where resistance rates are higher and there is easier access to last-resort antibiotics, leading to more resistant infections.⁸ This includes the rise and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of Salmonella Typhi across Pakistan. In Lahore, Pakistan, a high percentage of S. Typhi isolates were found to be XDR (46.1%) and MDR (24.5%).⁹

Typhoidal Salmonella, particularly Salmonella Typhi, poses a critical challenge as one of the primary causes of infections in the bloodstream around the globe, especially in developing countries.¹⁰ It is a serious global health challenge that increasing incidents of MDR and XDR Salmonella Typhi infections are being reported, demanding our attention. This study is focused on Salmonella Typhi and Salmonella Paratyphi A isolates from blood cultures, evaluating their current susceptibility patterns, placing a strong emphasis on their antimicrobial resistance profiles and the associated inflammatory markers. Understanding these factors is essential for combating the increasing threat of these dangerous pathogens.

The purpose of this study was to highlight the susceptibility pattern of Salmonella isolates to various antibiotics used for the treatment of enteric fever and to find the association of infectivity of Salmonella species with inflammatory mediators.

METHODOLOGY

This cross-sectional study was performed at the Pathology Department of HIT Hospital, Taxila Cantt, Pakistan, from Jan24 to Jan 25, after the approval of the Institutional Review Board (IRB) /Institution Review Committee (IRC) vide Reference number 01 dated 25/04/2025. Sample size was calculated using the WHO sample size calculator, taking a confidence level of 95%, margin of error of 5%, and reported prevalence of 43%.¹¹ The estimated sample size came out to be 64. Sampling was done using a non-probability consecutive sampling technique.

Inclusion Criteria: Patients of either gender below the age of 70 years with positive blood cultures for Typhoidal Salmonella submitted from both inpatient

and outpatient departments of medicine and paediatric specialties were included in the study.

Exclusion Criteria: Repeat specimens from the same patients were excluded from this study.

Commercially available blood culture bottles were used for the inoculation of all blood samples submitted for bacterial culture and sensitivity, and they were incubated at 35±2°C in an automated BacT/ALERT blood culture system (BioMérieux). Samples that flagged positive were sub-cultured onto both differential MacConkey agar (Oxoid, UK) and enriched sheep blood agar plates (Oxoid, UK). Colony morphology, biochemical testing with the API 20E system (BioMérieux, France), and serotyping using specific antisera were used to identify the isolates. Antibiotic susceptibility was assessed by the Kirby-Bauer disk diffusion method according to the CLSI guidelines (2024). The antibiotic discs of Ampicillin (10µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg), Chloramphenicol (30 µg), Ciprofloxacin (5µg), Ceftriaxone (30 µg), Meropenem (10µg), and Azithromycin (15µg) were used on Mueller-Hinton agar. Zone diameters were interpreted in line with CLSI standards. Quality control for antibiotic discs was evaluated using reference strains of E. coli ATCC 25922, P. aeruginosa ATCC 27853 (for testing meropenem), and S. aureus ATCC 25923 (for azithromycin assessment in S. Typhi).

Other data from the patients were also collected, which included their gender and age, along with some inflammatory markers for correlation with positive or negative blood culture outcomes, such as the total leukocyte count, C-reactive protein levels, Platelet count, and presence or absence of fever.

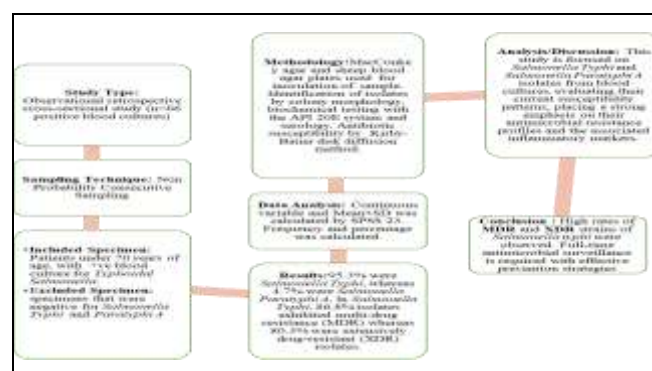


Figure-1: Flow Diagram showing Phases of Study

Data analysis was done using SPSS (Statistical Package for the Social Sciences) version 23.00. The

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Shapiro-Wilk test was applied to assess the normality of data distribution. Since the data were not normally distributed, the median and interquartile range (IQR) were calculated for continuous variables, including age, total leukocyte count (TLC), platelet count, and fever duration. Frequencies and percentages were used to summarize categorical variables such as gender, age groups, Salmonella species, strain type (XDR/MDR), and CRP status. The Mann-Whitney U test was used to compare TLC and platelet counts between CRP-positive and CRP-negative cases. A p -value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 64 patients were included in this study, with 33(51.6%) males and 31(48.4%) females. The majority (70.3%) were aged 12–50 years, while 26.6% were under 12 years, and only 3.1% were above 50 years. The median age of the patients was 20 years (IQR: 23–11), and the median total leucocyte count (TLC) was 6.70 (IQR: 8.20–4.78). The median platelet count was 242,000 (IQR: 359,000–192,000). Fever lasted a median of 5 days (IQR: 6–4 days) before subsiding after the initiation of antibiotics, as shown in Table-I.

Table-I: Baseline Characteristics (n=64)

Demographic Variables		n (%)
Gender	Male	33 (51.6%)
	Female	31 (48.4%)
Age Group	<12 Years	17 (26.6%)
	12-50 years	45 (70.3%)
	>50 years	2 (3.1%)
Salmonella Species	Salmonella Typhi	61 (95.3%)
	Salmonella Paratyphi A	3 (4.7%)
Median, IQR		
Age (Years)		20.00 (23.00-11.00)
TLC (109/L)		6.70 (8.20-4.78)
Platelet Count (109/L)		242.00 (359.00-192.00)
Duration of Fever (Days)		5.00 (4.00-6.00)

Inflammatory marker analysis showed that 46(71.9%) patients were CRP-positive, while 18(28.1%) were CRP-negative. Although CRP was positive in the majority of patients with positive blood culture results, there was no significant association between CRP-positive cases and low TLC and platelet counts (Table-II).

Table-II: Comparison of TLC and Platelet Counts Between CRP-Positive and CRP-Negative Cases

Parameters	Study Groups		p-value
	CRP positive (n=46) median(IQR)	CRP negative (n=18) median(IQR)	
TLC (109/L)	6.90(8.20-5.10)	6.10(8.30-3.92)	0.24
Platelet Count (109/L)	249(359-200.50)	209(389-174)	0.32

Among the isolates, 95.3% were Salmonella Typhi, whereas 4.7% were Salmonella Paratyphi A, indicating a predominance of S. Typhi in the study population. Among Salmonella Typhi, a significant proportion of cases (75.4%) were extensively drug-resistant (XDR), while 13.1% exhibited multi-drug resistance (MDR) (Table III).

Table-III: Strain Type of Salmonella Typhi (n=61)

Strain Type of Salmonella Typhi	n(%)
Extensively Drug-Resistant (XDR)	46(75.4%)
Multi-Drug Resistant (MDR)	08(13.1%)

High resistance to several antimicrobials is revealed by the susceptibility pattern of Salmonella isolates (n=64) (Table-IV). Out of a total of 64 isolates, including both Salmonella paratyphi A and Salmonella Typhi, there were no fully susceptible cases, with resistance percentages of 92%, 90.6%, and 87.5% to Ampicillin, Co-trimoxazole, and Chloramphenicol, respectively. Among all Salmonella isolates, only 02 isolates exhibited susceptibility to Ciprofloxacin (3.3%), observed in Salmonella Typhi species only, whereas the remaining 62(97%) isolates, including both Salmonella paratyphi A and Salmonella Typhi, showed resistance to Ciprofloxacin. Likewise, 23.4% of isolates showed susceptibility to Ceftriaxone, while 76.6% of isolates showed resistance. Among 61 isolates of Salmonella Typhi, only 12 isolates were sensitive to Ceftriaxone, with a sensitivity of 19.7% (Table-V). On the other hand, the most effective medications against these isolates were Azithromycin and Meropenem, which demonstrated 100% susceptibility. This demonstrates notable resistance to widely used antibiotics, except for Azithromycin and Meropenem. The susceptibility pattern of both Salmonella Typhi and Salmonella paratyphi A separately against antibiotics is shown in Table-V.

Table-IV: Pattern of Susceptibility of Salmonella species including both Salmonella Typhi and Paratyphi A Against Antimicrobials (n=64)

	Resistant	Susceptible
Ampicillin	59 (92%)	5 (7.8%)
Cotrimoxazole	58 (90.6%)	6 (9.4%)
Chloramphenicol	56 (87.5%)	8 (12.5%)
Ciprofloxacin	62 (97%)	2 (03%)
Ceftriaxone	49(76.6%)	15(23.4%)
Meropenem	0	64 (100.0%)
Azithromycin	0	64 (100.0%)

Table-V: Pattern of Susceptibility of Salmonella Typhi and Paratyphi A against Antimicrobials Separately (n=64)

Antibiotics	Salmonella Typhi (n=61)		Salmonella Paratyphi A(n=03)	
	Resistant	Susceptible	Resistant	Susceptible
Ampicillin	58 (95%)	03 (05%)	01(33.3%)	02(66.7%)
Cotrimoxazole	58 (95%)	03 (05%)	0	03 (100.0%)
Chloramphenicol	56 (92%)	05 (08%)	0	03 (100.0%)
Ciprofloxacin	59(96.7%)	02(3.3%)	03 (100%)	0
Ceftriaxone	49(80.3%)	12(19.7%)	0	03(100.0%)
Meropenem	0	61 (100.0%)	0	03(100.0%)
Azithromycin	0	61 (100.0%)	0	03(100.0%)

DISCUSSION

In our study, we observed a striking trend: 70.3% of patients were between 12 and 50 years of age, highlighting the alarming prevalence of typhoid fever among adolescents and young adults. Only a small fraction of older adults (3.1%) and children under 12 years (26.6%) were affected, reinforcing the notion that Salmonella Typhi infections predominantly affect those in the working-age population, especially in endemic areas, where prevalence is more than 9 million cases and more than 110 000 attributable deaths per year globally.¹²

Our study included 51.6% male and 48.4% female participants. Ishtiaq *et al.* reported that the incidence of this illness is higher in females (51.46%) than in males (48.54%).¹³ This underscores the need for targeted interventions and robust awareness campaigns to confront this critical health challenge directly. For Salmonella Typhi, our study found that 75.4% were XDR isolates and 13.1% were multidrug-resistant (MDR) isolates. This alarming trend signals a dramatic rise in antimicrobial resistance among Salmonella Typhi isolates, with high resistance to first-line antibiotics, including Ampicillin, Cotrimoxazole, and Chloramphenicol, at 95%, 95%, and 92%, respectively, according to our study. Akram *et al.* highlighted that unregulated antibiotic use has resulted in a significant increase in extensively drug-resistant (XDR) Salmonella Typhi in regions such as South Asia and sub-Saharan Africa.¹⁴

Meropenem is a broad-spectrum antibiotic used as a last-resort treatment option. We found 100% sensitivity to Meropenem and Azithromycin, indicating that these antibiotics are likely to remain effective for treating typhoid fever.¹⁵ Resistance to Azithromycin has been reported in a study conducted from 2016 to 2018, in which, amongst 2,519 isolates of Salmonella Typhi and 506 isolates of Salmonella Paratyphi A, 59 Salmonella Typhi and 45 Salmonella Paratyphi A were potentially resistant isolates in a

study conducted by Sajib *et al.*¹⁶ Irrespective of this, the global increase in Carbapenem resistance is a serious concern, emphasizing the need for careful usage and management of these important antibiotics.¹⁷

The inflammatory marker C-reactive protein (CRP) was positive in a notable 71.9% of cases, underscoring the robust systemic inflammatory response commonly observed in typhoidal infections. This important finding underscores the value of CRP as a key biomarker for both diagnosis and continuous monitoring.¹⁸ Despite CRP showing a positive association with Salmonella infections, there is no linkage between total leukocyte count (TLC) and platelet count and the clinical severity of CRP-positive and CRP-negative cases, with p-values of 0.243 and 0.317, respectively, as reported by Jabeen *et al.*¹⁹ Thus, accurate markers should be established and studied to diagnose and assess the severity of these infections.

Watkins *et al.* reported that XDR Salmonella Typhi have shown a significant proportion of isolates resistant to multiple first-line antibiotics.²⁰ This trend has been documented in India as well, with emerging XDR strains resisting nearly all available treatments, including cephalosporins.²¹ This demanding crisis has led to clinical challenges, including limited treatment options, risks of prolonged morbidity, treatment failure, and complications. Therefore, it is necessary for global health bodies to implement decisive measures to address this health crisis and develop an effective management plan.

LIMITATIONS OF STUDY

This study is limited to one of the government hospitals in Pakistan. Other secondary and tertiary care units can also be targeted for more accurate representation of local epidemiological data and better clinical outcomes.

CONCLUSIONS

It was observed strikingly high rates of MDR and XDR strains of Salmonella Typhi in this study, with 100% susceptibility only to two last-resort antibiotics, including Azithromycin and Meropenem. These observations emphasize that there is an urgent need for full-time antimicrobial surveillance to keep in check the resistance rates of critical infectious pathogens. This, in turn, can be used as a guide by clinicians to direct judicious antibiotic use and reduce the spread of resistant pathogens by strengthening infection prevention and control policies in endemic regions.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NT & SN: Data acquisition, data analysis, critical review, approval of the final version to be published.

RB & MN: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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