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Original Article

Blood-Culture Isolation and Antimicrobial Resistance Patterns of Salmonella Typhi among Patients with Suspected Typhoid Fever in Peshawar, Pakistan

Jamila Hiader[1], Sofia Bano[2], Bibi Maryam[3], Maham Iqbal[4], Muhammad Usman[5], Aiman Waheed[6*]

[1]Assistant Professor (Microbiology), Institute of Pathology and Diagnostic Medicine, Khyber Medical University (KMU), Peshawar, Pakistan

[2,3,4,5]Undergraduate students (BS Microbiology), Institute of Pathology and Diagnostic Medicine, Khyber Medical University (KMU), Peshawar, Pakistan

[6*]Research Associate, Institute of Pathology and Diagnostic Medicine, Khyber Medical University (KMU), Peshawar, Pakistan

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Corresponding Author:

Aiman Waheed

Research Associate, Institute of Pathology and Diagnostic Medicine, Khyber Medical University (KMU), Peshawar, Pakistan

Email: draimanwaheed.ibms@kmu.edu.pk

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ABSTRACT

Background: Typhoid fever remains an important public health challenge in South Asia, where increasing antimicrobial resistance in Salmonella enterica serovar Typhi (S. Typhi) complicates empirical treatment and disease control. Local laboratory-based surveillance is essential for characterizing circulating resistance patterns and informing antimicrobial selection.

Objective: To determine the frequency of blood-culture isolation of S. Typhi among patients clinically suspected of typhoid fever and to characterize the antimicrobial susceptibility patterns of recovered isolates in Peshawar, Pakistan.

Methodology: A laboratory-based cross-sectional study was conducted at the Khyber Medical University–Public Health Reference Laboratory, Peshawar, from October to December 2025. Seventy blood specimens obtained from clinically suspected typhoid patients were processed using standard blood-culture, microbiological, and biochemical procedures. Confirmed S. Typhi isolates were subjected to antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute recommendations. Descriptive statistics were used to summarize participant characteristics, bacterial isolation, and antimicrobial susceptibility profiles.

Results: S. Typhi was isolated from 48 of 70 blood specimens (68.6%), making it the predominant organism recovered. Participants aged 1–10 years constituted the largest age group (54.3%), while males represented 54.3% of the study population. Among S. Typhi isolates, the highest resistance was observed to ampicillin (87.5%), cefotaxime (81.3%), trimethoprim–sulfamethoxazole (81.3%), chloramphenicol (62.5%), and ciprofloxacin (52.1%). Conversely, high in vitro susceptibility was observed for tetracycline (97.9%), meropenem (95.8%), and azithromycin (87.5%).

Conclusion: The findings demonstrate substantial antimicrobial resistance among blood-culture-confirmed S. Typhi isolates in Peshawar, particularly to several historically important therapeutic agents. Continued laboratory-based AMR surveillance, antimicrobial stewardship, microbiological confirmation, and integrated typhoid prevention strategies are warranted.

INTRODUCTION

Typhoid fever is a systemic bacterial infection caused by Salmonella enterica serovar Typhi (S. Typhi) and remains an important public health problem in South Asia [1,2]. It is mostly transmitted via the fecal-oral route and is strongly linked to poor water, sanitation, and hygiene practices. Intestinal invasion can lead to systemic spread of S. Typhi, resulting in chronic carriage and febrile illness, and systemic complications. Antimicrobial resistance (AMR) is increasingly complicating the management of typhoid fever. The therapeutic value of several conventional antibiotics has been greatly diminished because of increasing resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. There is increasing resistance to fluoroquinolones and third-generation cephalosporins, and multidrug-resistant and extensively drug-resistant (XDR) S. Typhi have become a significant public health concern [4-6]. XDR S. Typhi has been seen in Pakistan with a significant burden of resistant typhoid. Genomic studies have revealed resistant S. Typhi isolates in the Peshawar region, reinforcing the importance of ongoing AMR surveillance [7,8]. Laboratory-based surveillance can identify local resistance patterns, guide susceptibility-based treatment, and strengthen antimicrobial stewardship. The symptoms of typhoid fever are nonspecific and can be mistaken for other febrile diseases. Blood culture remains an important tool for microbiological confirmation, as a positive culture allows for antimicrobial susceptibility testing. However, serological tests such as the Widal test have important limitations and should not replace culture-based diagnosis when appropriate microbiological facilities are available [9]. Therefore, this study aimed to determine the frequency of blood-culture isolation of S. Typhi among patients clinically suspected of typhoid fever and to characterize the antimicrobial susceptibility patterns of the recovered isolates in Peshawar, Pakistan.

Materials and Methods

Study Design and Setting

A laboratory-based cross-sectional study was conducted at the Khyber Medical University–Public Health Reference Laboratory (KMU–PHRL), Peshawar, Pakistan, from October to December 2025. KMU–PHRL provides bacteriological diagnostic services to government and private healthcare facilities in Khyber Pakhtunkhwa and receives clinical specimens from Peshawar and surrounding districts.

Study Population and Sample Collection

Patients aged 1–70 years who were clinically suspected of having typhoid fever and whose blood specimens were submitted to KMU–PHRL for microbiological investigation were eligible for inclusion. A total of 70 blood specimens were included during the study period. Specimens were referred from government and private hospitals, basic health units, and diagnostic laboratories in Peshawar and surrounding districts of Khyber Pakhtunkhwa. Fresh, adequately labeled blood specimens with sufficient volume for culture were included. Contaminated, hemolyzed, clotted, spilled, or inadequately labeled specimens were excluded. Blood specimens were collected aseptically according to standard blood-culture procedures. Approximately 3–5 mL was collected from pediatric patients and 5–10 mL from adults. Specimens were immediately inoculated into aerobic blood culture bottles and transported to KMU–PHRL for processing [10].

Blood Culture and Bacterial Isolation

An automated BACTEC blood-culture system was used to incubate blood-culture bottles at 37 °C. Blood culture bottles were incubated at 37°C using an automated BACTEC blood culture system. Bottles flagged as positive were processed aseptically, and aliquots of positive broth were subcultured onto Xylose Lysine Deoxycholate (XLD) agar and MacConkey agar. The plates were incubated aerobically at 37°C for 18–24 hours. Bacterial growth was assessed based on colony morphology, followed by Gram staining and biochemical identification [10,11].

Identification of Salmonella Typhi

Presumptive identification of S. Typhi was performed using colony morphology, Gram staining, and conventional biochemical testing [10,11]. Suspected isolates were evaluated using Triple Sugar Iron (TSI) agar, urease, indole, citrate utilization, and oxidase tests. Isolates showing Gram-negative bacillary morphology and biochemical characteristics consistent with Salmonella, including an alkaline slant/acid butt reaction on TSI agar and negative urease, indole, oxidase, and citrate reactions, were identified as S. Typhi.

Antimicrobial Susceptibility Testing

The AST of confirmed S. Typhi isolates was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Antimicrobial agents tested included tetracycline, meropenem, ampicillin, azithromycin, cefotaxime, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, and trimethoprim-sulfamethoxazole. After inoculating Mueller–Hinton agar, antimicrobial disks were placed on the plates, which were then incubated for 18–24 hours at 35–37°C. The diameter of the inhibition zone was measured in millimeters and interpreted as susceptible, intermediate, or resistant based on the CLSI criteria for the specific organism [12].

Statistical Analysis

All the data were recorded, coded, and analyzed using IBM SPSS Statistics 27.0. Categorical variables were presented in frequencies and percentages. The percentage of blood specimens that were positive for S. Typhi was determined by dividing the number of S. Typhi-positive specimens by the total number of blood specimens processed. The AST results were reported as susceptible, intermediate, or resistant for each antimicrobial agent. Two-sided 95% confidence intervals (CIs) were obtained by the Clopper–Pearson exact binomial approach [13]. The study was descriptive, and inferential statistical comparisons were not used.

Results

A total of 70 patients clinically suspected of having typhoid fever were included. There were 38 males (54.3%) and 32 females (45.7%). Participants aged 1–10 years constituted the largest age group (38; 54.3%), followed by 11–20 years (14; 20.0%) and 21–30 years (9; 12.9%).

Participants aged 31–40, 41–50, and 61–70 years accounted for 5.7%, 4.3%, and 2.9%, respectively. No participants were recorded in the 51–60-year group.*S. Typhi* was isolated from 48 of 70 blood specimens (68.6%). *Staphylococcus aureus* was isolated from 17 specimens (24.3%), while *Escherichia coli* was recovered from five (7.1%).Among the 48 *S. Typhi* isolates, the highest susceptibility was observed for tetracycline (47/48, 97.9%; 95% CI: 88.9–99.9%), followed by meropenem (46/48, 95.8%; 95% CI: 85.7–99.5%) and azithromycin (42/48, 87.5%; 95% CI: 74.8–95.3%). The highest resistance was observed against ampicillin (42/48, 87.5%; 95% CI: 74.8–95.3%), followed by cefotaxime (39/48, 81.3%; 95% CI: 67.4–91.1%) and trimethoprim-sulfamethoxazole (39/48, 81.3%; 95% CI: 67.4–91.1%). Resistance was also observed to chloramphenicol (30/48, 62.5%; 95% CI: 47.4–76.0%), ciprofloxacin (25/48, 52.1%; 95% CI: 37.2–66.7%), gentamicin (18/48, 37.5%; 95% CI: 24.0–52.6%), and cefoxitin (17/48, 35.4%; 95% CI: 22.2–50.5%). No meropenem-resistant isolates were detected, while one isolate (2.1%; 95% CI: 0.1–11.1%) was resistant to tetracycline.

Table 1. Demographic Characteristics of Study Participants (n = 70)

Age group (years)	Male, n (%)	Female, n (%)	Total, n (%)
1–10	22 (57.9)	16 (50.0)	38 (54.3)
11–20	7 (18.4)	7 (21.9)	14 (20.0)
21–30	5 (13.2)	4 (12.5)	9 (12.9)
31–40	2 (5.3)	2 (6.3)	4 (5.7)
41–50	1 (2.6)	2 (6.3)	3 (4.3)
51–60	0 (0.0)	0 (0.0)	0 (0.0)
61–70	1 (2.6)	1 (3.1)	2 (2.9)
Total	**38 (54.3)**	**32 (45.7)**	**70 (100.0)**

Values are presented as frequency and percentage. Percentages in the male and female columns are calculated within each sex, whereas percentages in the total column are calculated from the overall study population.

Table 2. Bacterial Species Isolated From Blood Cultures (n = 70)

Bacterial species	Positive isolates, n	Percentage (%)
Salmonella Typhi	48	68.6
Staphylococcus aureus	17	24.3
Escherichia coli	5	7.1
Total	**70**	**100.0**

Values represent the number and percentage of bacterial isolates recovered from the 70 blood specimens processed. Percentages were calculated using the total number of specimens (n = 70) as the denominator.

Table 3. Antimicrobial Susceptibility Profile of *Salmonella Typhi* Isolates (n = 48)

Antimicrobial agent	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
Ampicillin	5 (10.4)	1 (2.1)	42 (87.5)
Azithromycin	42 (87.5)	1 (2.1)	5 (10.4)
Cefotaxime	7 (14.6)	2 (4.2)	39 (81.3)
Cefoxitin	25 (52.1)	6 (12.5)	17 (35.4)
Gentamicin	28 (58.3)	2 (4.2)	18 (37.5)
Chloramphenicol	15 (31.3)	3 (6.3)	30 (62.5)
Clindamycin	36 (75.0)	1 (2.1)	11 (22.9)
Ciprofloxacin	14 (29.2)	9 (18.8)	25 (52.1)
Trimethoprim-sulfamethoxazole	6 (12.5)	3 (6.3)	39 (81.3)
Meropenem	46 (95.8)	2 (4.2)	0 (0.0)
Tetracycline	47 (97.9)	0 (0.0)	1 (2.1)

Values are presented as frequency and percentage among confirmed *S. Typhi* isolates (n = 48). Ant Susceptibility was categorized as susceptible, intermediate, or resistant according to the applicable CLSI interpretive criteria. Percentages may not total exactly 100% because of rounding. The table contains the susceptibility counts reported in the manuscript.

Discussion

The present study provides contemporary laboratory-based evidence of *S. Typhi* isolation and AMR among patients clinically suspected of typhoid fever in Peshawar. S. Typhi was the predominant pathogen recovered from bloodstream infections, and resistance rates were high for some of the conventional antimicrobials, reflecting the ongoing therapeutic challenges of resistant typhoid in Pakistan. Over half of the patients studied were children aged 1–10 years. This observation is consistent with the known susceptibility of children to typhoid in endemic areas. Still, it cannot be used to determine the incidence of typhoid across different age groups or at the population level [1,14]. The finding highlights the need for vaccination, safe water, sanitation and hygiene, and proper antimicrobial use in children [15,16]. The greatest concern was the level of ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole resistance. These agents have traditionally played a significant role in the treatment of enteric fever, but with progressive resistance, their utility has been significantly curtailed [4,5]. The observed pattern of resistance reflects the overall evolution of S. Typhi AMR reported in South Asia and Pakistan [6,17]. The isolates' resistance to cefotaxime was also high (81.3%). Cephalosporin's have traditionally been used as important alternatives after resistance to 1st-line agents and fluoroquinolones. This decreased activity is therefore alarming in the context of the emergence of XDR S. Typhi in Pakistan. Circulation of XDR strains has been confirmed in Peshawar through genomic studies [7,8]. This research supports the need for regional surveillance, not just data from other geographical areas. Resistance to ciprofloxacin was observed in 52.1% of isolates. There has been a gradual erosion of the empirical use of this class of antimicrobials for typhoid fever as fluoroquinolone resistance has increased [18]. Phenotypic susceptibility was assessed in the present study, and molecular determinants of resistance were not investigated; thus, the observed phenotype should not be interpreted as indicative of a specific genetic mechanism. However, the finding has immediate relevance to antibiotic stewardship and to treating patients with the right drug for the right infection. Azithromycin and meropenem demonstrated high in vitro activity, with susceptibility rates of 87.5% and 95.8%, respectively. Azithromycin is an important oral drug used for susceptible typhoid when resistance to conventional agents is prevalent [19]. However, surveillance is still required, as continued use may lead to the selection of azithromycin-resistant organisms. Meropenem showed high activity in this study and is considered a reserve antimicrobial agent rather than a standard choice. Preserving carbapenems for situations in which they are clinically indicated is especially important in settings affected by XDR typhoid[20]. The results also indicate the difference between phenotypic surveillance for AMR and molecular characterization. The present investigation does

not identify resistance genes, plasmid structures, bacterial lineages, or transmission relationships. Whole-genome sequencing studies have demonstrated the additional epidemiological data that genomic surveillance can provide in Peshawar [7]. The future surveillance program should therefore integrate standardized phenotypic AST with molecular testing, particularly for isolates resistant to third-generation cephalosporins, fluoroquinolones, azithromycin, or other clinically important antimicrobials [21].In an environment where empirical therapy for febrile illness is prevalent, blood culture confirmation has added value. The clinical features are not always sufficient to distinguish typhoid from other causes of fever, and blood culture would provide microbiological confirmation and an isolate for susceptibility testing [9,22]. Access to high-quality blood cultures and AST may thus benefit patient care and, at the same time, be used as a tool for regional AMR surveillance. Because the study was conducted at a referral laboratory and included specimens submitted for suspected typhoid fever, the isolation proportion should not be interpreted as the population prevalence or incidence of typhoid fever in Peshawar or Khyber Pakhtunkhwa. Moreover, conventional phenotypic identification and AST cannot determine resistance mechanisms or lineages. Future multicenter studies would provide more detailed epidemiological data with more patients, longer follow-up, and molecular characterization. The study has presented useful baseline data on Peshawar laboratories and shown remarkable resistance in S. Typhi isolates recovered from the study. A regional strategy involving blood culture diagnosis, quality-assured AST, vaccines, antimicrobial stewardship, WASH interventions, and genomic surveillance is required to reduce transmission and track emerging resistance [15,23].

Conclusion

Blood-culture-confirmed *S. Typhi* demonstrated substantial resistance to several conventional antimicrobials in this Peshawar-based laboratory study. High resistance to ampicillin, cefotaxime, trimethoprim-sulfamethoxazole, chloramphenicol, and ciprofloxacin highlights the need for susceptibility-guided therapy and continued AMR surveillance. The preserved activity of azithromycin and meropenem should be monitored carefully to protect remaining therapeutic options. Strengthening microbiological diagnosis, antimicrobial stewardship, typhoid vaccination, WASH interventions, and regional surveillance is essential for controlling resistant typhoid in KP.

Ethical Approval

Ethical approval for the study was obtained from the Khyber Medical University Advanced Studies and Research Board **(KMU-AS&RB) under ERB No. DIR/KMU-AS&RB/CL/001943,** dated July 15, 2024. The study was conducted in accordance with the applicable institutional ethical requirements.

Author Contributions (ICMJE)

Sofia Bano: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft Preparation.
Bibi Maryam: Conceptualization, Supervision, Methodology, Validation, Writing – Review & Editing.
Maham Iqbal: Investigation, Laboratory Analysis, Data Collection, Data Curation, Writing – Review & Editing.
Muhammad Usman: Formal Analysis, Methodology, Validation, Visualization, Writing – Review & Editing.
Jamila Hiader: Supervision, Methodology, Validation, Interpretation of Findings, Writing – Review & Editing.
Aiman Waheed: Conceptualization, Project Administration, Supervision, Scientific Interpretation, Critical Review of the Manuscript, Writing – Review & Editing.

All authors contributed substantially to the conception and design of the study, acquisition, analysis, or interpretation of data; participated in drafting or critically revising the manuscript; approved the final version of the manuscript; and agree to be accountable for all aspects of the work in accordance with the authorship criteria of the **International Committee of Medical Journal Editors (ICMJE)**.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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