

Antibiotic Susceptibility Patterns of Salmonella Isolates from Pediatric Blood Cultures at UCMD-Affiliated Teaching Hospitals

Anum Tahir,¹ Muhammad Naveed,² Mahwish Ejaz,³ Sehr Syed,⁴ Sara Maqsood,⁵ Ammara Gul⁶

ABSTRACT

BACKGROUND & OBJECTIVE: Antibiotic resistance is a global public health threat and the emergence of multidrug-resistant and extensively drug-resistant strains has significantly complicated treatment, resulting in increased morbidity, mortality, healthcare costs and prolonged hospital stays. The objective of this study is to evaluate the patterns of antibiotic susceptibility in *Salmonella Typhi* and *Salmonella Paratyphi A* isolates from pediatric blood culture samples.

Methodology: It is a retrospective observational study conducted on 369 pediatric patients aged 1-15 years with positive blood cultures for *Salmonella* species. Data were analyzed using SPSS software, with Chi-square test was applied to compare resistance patterns between *S. Typhi* and *S. Paratyphi A* against different classes of antibiotics. The study conducted in hospitals affiliated with the University College of Medicine and dentistry Lahore, from January 2022 till June 2024.

Results: Among 369 pediatric patients (mean age 10.3 ± 3.6 years; 66.7% male), 39% of *Salmonella Typhi* isolates were sensitive to both first-line drugs and third-generation cephalosporins, 52.6% were multidrug-resistant, and 0% were extensively drug-resistant. ESBL positivity was observed in 29.3% of isolates, while azithromycin and meropenem retained high activity.

Conclusion: The results demonstrated that *Salmonella Typhi* showed higher resistance as compared *S. Paratyphi A* (52.6% multidrug-resistant isolates and no extensively drug-resistant strains). It is pertinent to note the decrease in cases of extensively drug-resistant strains in the present study after the introduction of TCv in the region. The study highlights the need for continuous surveillance and rational antibiotic use.

KEY WORDS: Typhoid Fever, Salmonella Typhi, Drug Resistance, Multiple, Bacterial Drug Resistance, Typhoid-Paratyphoid Vaccines

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INTRODUCTION

Antimicrobial resistance (AMR) is a threat to public health globally. The World Health Organization (WHO) has estimated that approximately 200,000 deaths in Pakistan may be attributed to AMR-associated infections annually. The widespread misuse of antibiotics across various fields has escalated this crisis manyfold. If there is a lapse in implementation of effective control measures, AMR is projected to cause up to 10 million deaths worldwide annually by 2050.¹

In low- and middle- income countries including Pakistan, antibiotic resistance particularly in *Salmonella* infections poses a serious threat to pediatric health. According to the Bacterial Priority Pathogens List (BPPL) by the WHO, *Salmonella Typhi* has been classified as a "High Priority" pathogen, which classifies organisms that are becoming increasingly resistant, with high transmission rates, are difficult to treat and associated with a disease burden substantially.² *Salmonella Typhi* and *Salmonella Paratyphi* cause typhoid fever, which is

potentially life-threatening illness if inadequately treated. Typhoid fever is estimated to cause over 250,000 deaths annually in developing countries.³

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has significantly complicated treatment, which has resulted in increased morbidity, mortality, healthcare costs, and prolonged hospital stays.⁴ Several studies from South Asia, including Pakistan, have linked irrational antibiotic use; particularly fluoroquinolones to increasing resistance rates. MDR and XDR strains resistant to both fluoroquinolones and third-generation cephalosporins have been reported nationally and internationally, further increasing the clinical and economic burden of enteric fever.^{5,6} Traditional first-line antibiotics such as chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole are now largely ineffective.⁷⁻¹⁰ Currently, azithromycin and carbapenems remain the last therapeutic options for resistant infections; however, their high cost and limited availability restrict their use in resource-constrained settings.¹¹⁻¹⁴

To address the rising burden of drug-resistant typhoid, the Typhoid Conjugate Vaccine (TCV) was introduced in Pakistan one year prior to this study as part of the national immunization program for children aged 6 months to 15 years. Given regional variability in resistance patterns and limited pediatric data, this study aimed to

Correspondence:

Anum Tahir
Assistant professor of Pediatrics,
Medicine and Dentistry, The University of Lahore, Lahore
Email: anumt9991@gmail.com

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evaluate the antibiotic susceptibility patterns of *Salmonella* isolates from blood cultures of pediatric patients at University College of Medicine and Dentistry (UCMD)-affiliated teaching hospitals in Punjab, Pakistan.

METHODOLOGY

This retrospective study included 369 pediatric patients aged 1-15 years with blood culture-confirmed *Salmonella* infection. Blood culture sensitivity reports from patients treated as outpatients or admitted to UCMD-affiliated teaching hospitals between January 2022 and June 2024 were reviewed. Ethical approval was obtained from the Institutional Review Board of The University of Lahore. Patient confidentiality and data security were maintained throughout the study. Consecutive sampling was employed including all eligible pediatric patients according to predefined inclusion criteria within the specified time period. A structured data collection form was used to record demographic variables (age and gender). Patient data including demographics, blood culture results and antibiotic resistance profiles were extracted by accessing electronic medical records and laboratory information systems. To maintain patient confidentiality, all data were de-identified.

Inclusion criteria comprised patients aged 15 years or under having positive blood cultures for *Salmonella* species and complete microbiological data. It is pertinent to mention that only first positive culture from a single infectious episode was included in the study. Patients older than 15 years, with positive blood cultures for organisms other than *Salmonella* or having incomplete microbiological data and patients with immunocompromised status or comorbid conditions were excluded from the study.

Cross-verification of extracted data with original records was included as a quality control measure. Moreover, complete case analysis, along with imputation and sensitivity analysis performed where appropriate.

Initial processing of blood culture specimens was done using the BACTEC automated blood culture system. Positive samples were sub-cultured onto MacConkey's, agar, blood agar and chocolate agar. Sub-culturing was followed by incubation of the samples at 35-37°C for 18-24 hours. Isolates were identified using standard microbiological procedures, after adequate colony growth. Identification included API 20E (bioMérieux), by following the manufacturer's instructions. Final analysis included only confirmed *Salmonella* isolates.

Kirby-Bauer disc diffusion method on Mueller-Hinton agar was performed for antimicrobial susceptibility testing. The testing was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) M100 2025 guidelines. Zone diameters in were measured in millimeters after incubation of plates at 35-37°C for 16-18 hours. Based on CLSI breakpoints, isolates were classified as susceptible, intermediate or resistant.

In this study, typhoid fever was classified according to WHO drug-resistance criteria. Non-resistant typhoid fever is caused by *Salmonella* Typhi and/or *Salmonella* Paratyphi A, B, or C strains sensitive to first-line antibiotics and third-generation cephalosporins, with or without resistance to second-line drugs. MDR typhoid fever is caused by strains resistant to all first-line drugs, with or without resistance to second-line agents, but remaining sensitive to third-generation cephalosporins. XDR typhoid fever is caused by strains resistant to first- and second-line antibiotics as well as third-generation cephalosporins. Extended-spectrum beta-lactamase (ESBL)-producing typhoid fever refers to *S. Typhi* strains resistant to third-generation cephalosporins but potentially sensitive to fluoroquinolones, chloramphenicol, or cotrimoxazole.⁴

Statistical Analysis was performed using IBM SPSS software (version 27). Shapiro-Wilk test was performed for assessing normality of continuous variables. Descriptive analysis included mean \pm standard deviation (for normally distributed variables), median (non-normally distributed variables). Categorical variables were summarized as percentages and frequencies. Inferential analysis was performed using the Chi-square test to compare categorical variables (antibiotic resistance patterns between *Salmonella* Typhi and *S. Paratyphi* A). Assumptions for Chi-square test were assessed prior to analysis. It was ensured that no more than 20% of cells had expected counts below five by examining expected cell frequencies. p-value of <0.05 was considered statistically significant.

RESULTS

A total of 369 patients aged 1-15 years were included in the study. The mean age was 10.32 ± 3.61 years. Males comprised 66.7% (n=246) and females 33.3% (n=123).

The antibiotic susceptibility of *Salmonella* isolates is shown in Table I. The antibiotic sensitivity pattern showed varying degrees of sensitivity to different classes of antibiotics.

For Trimethoprim, 42% of samples were sensitive, while 52.8% were resistant. For Chloramphenicol, 21.7% were sensitive and 41.4% resistant. The isolates showed varying levels of sensitivity in case of Fluoroquinolone. 14.1% showed sensitivity towards Fluoroquinolone, 39.6% had intermediate sensitivity and 45% samples were resistant. The analysis of 3rd Generation antibiotics showed that 60.2% isolates were sensitive to this class of antibiotics, 0.3% showed intermediate sensitivity and 29.3% were resistant. 93.8% isolates were sensitive to Azithromycin and only 6.0% were resistant. Analysis showed that 95.4% isolates were sensitive to Meropenem and only 0.8% showed resistance patterns. It is pertinent to mention that "Missing data" indicates unperformed, unrecorded, or indeterminate tests, retained separately to ensure transparency.

Comparative analysis of sensitivity patterns of

Table I: Patterns of Antibiotic Susceptibility in Clinical Isolates (n=369)

Antibiotic	Sensitive	Intermediate Sensitivity	Resistant	Missing data	Total no of patients
Trimethoprim	155 (42%)	-	195 (52.8%)	19 (5.1%)	369
Chloramphenicol	80 (21.7%)	-	152 (41.4%)	135 (36.6%)	367*
Fluoroquinolone	52 (14.1%)	147 (39.9%)	167 (45.3%)	2 (0.5%)	369
3 rd Generation	222 (60.2%)	1 (0.3%)	108 (29.3)	38 (10.3%)	369
Azithromycin	346 (93.8%)	-	22 (6.0%)	1 (0.3%)	369
Meropenem	352 (95.4%)	-	3 (0.8%)	14 (3.8%)	369

*Total number of isolates tested for chloramphenicol was 367 due to insufficient growth on 2 samples, all other antibiotics were tested on 369 isolates.

Table II: Comparative Analysis of Antibiotic Susceptibility Patterns of Salmonella Typhi and S. Paratyphi A against Different Classes of Antibiotics

Antibiotic	Organism								p-value
	Salmonella Typhi				S. Paratyphi A				
	S	IS	R	Missing data	S	IS	R	Missing data	
Trimethoprim	33.30%	-	63.20%	3.5	71.40%	-	17.90%	10.70%	<0.001*
Chloramphenicol	19.70%	-	49.60%	30.60%	28.90%	-	13.30%	57.80%	<0.001*
Fluoroquinolone	15.10%	33.40%	50.60%	0.70%	10.70%	61.90%	27.40%	-	<0.001*
3 rd Generation	53.70%	0.40%	36.50%	9.50%	82.10%	-	4.80%	13.10%	<0.001*
Azithromycin	93.00%	-	6.70%	0.40%	96.40%	-	3.60%	0.00%	0.492
Meropenem	97.20%	-	0.40%	2.50%	89.30%	-	2.40%	8.30%	0.08

*p<0.05 is statistically significant

Salmonella Typhi and S. Paratyphi A against different classes of antibiotics is presented in Table II. In case of Trimethoprim, a statistically significant increase in resistance patterns was present among S. Typhi isolates (63.2%) as compared to S. Paratyphi A (17.9%), however S. Paratyphi A showed more sensitivity (71.4% vs. 33.3%; $p < 0.001$). A similar trend was observed in case of Chloramphenicol where S. Typhi isolates showed more resistance (49.6%) as compared to S. Paratyphi A (13.3%, $p < 0.001$). As a larger proportion of S. Paratyphi A isolates were reported as "missing data" (57.8% vs. 30.6%), this may indicate the percentage may vary in larger patient population.

In case of Fluoroquinolones, S. Typhi isolates showed higher resistance (50.6%) when compared with S. Paratyphi A (27.4%) isolates. It was seen that S. Paratyphi A isolates exhibited greater intermediate susceptibility (61.9% vs. 33.4%; $p < 0.001$) which may indicate emerging resistance.

The sensitivity patterns of 3rd generation cephalosporins showed that sensitivity was significantly higher in S. Paratyphi A (82.1%) as compared to S. Typhi (53.7%; $p < 0.001$). Similarly, in case of Azithromycin, a high degree of sensitivity was seen in both organisms (S.

Typhi: 93.0%, S. Paratyphi A: 96.4%), with no significant difference ($p = 0.492$) indicating that both organisms are highly sensitive to Azithromycin. A high degree of sensitivity was observed in both groups against Meropenem (S. Typhi: 97.2%, S. Paratyphi A: 89.3%), and the difference in sensitivity between two groups was not statistically significant ($p = 0.08$) showing high degree of sensitivity of both S. Typhi and S. Paratyphi A.

In order to classify the confirmed cases of typhoid fever by drug resistance categories, based on their antimicrobial sensitivity patterns chi-square test was applied (Table III).

Among the 369 Salmonella Typhi isolates analyzed, 144 (39.1%) were sensitive to both first-line antibiotics and third-generation cephalosporins, representing non-resistant typhoid fever cases. In contrast, 224 (60.7%) isolates exhibited resistance to both classes of antibiotics.

Further analysis revealed that 194 (52.6%) isolates were resistant to the first-line drugs but remained sensitive to third-generation cephalosporins, corresponding to the multidrug-resistant (MDR) typhoid pattern. No extensively drug-resistant (XDR) Salmonella Typhi isolates were detected in this study (0%).

Table III: Classification of confirmed cases of typhoid fever based on antibiotic sensitivity patterns.

Classification	Percentage Sensitivity/Resistance
Non-resistant typhoid fever	39% sensitive to first line drugs and 3rd-generation cephalosporins
Multi-drug-resistant typhoid fever	52.6 % resistant to first-line drugs and sensitive to 3 rd generation cephalosporins
Extensively drug-resistant typhoid fever	0% resistant to 1 st line-, 2 nd line drugs and 3 rd generation cephalosporins
ESBL-positive typhoid fever	29.3% resistant to 3 rd generation cephalosporins but maybe sensitive to chloramphenicol, cortimoxazole or fluoroquinolones

Regarding extended-spectrum β -lactamase (ESBL) production, 29.3% of isolates were resistant to third-generation cephalosporins, indicating potential ESBL-positive *S. Typhi* strains.

DISCUSSION

A total of 369 pediatric patients aged 1-15 years were included in this study, with a mean age of 10.32 ± 3.61 years. Males constituted 66.7% (n=246) and females accounted for 33.3% (n=123) of the cohort.

The results of this study highlight important differences in the patterns of antimicrobial susceptibility between *Salmonella Typhi* and *S. Paratyphi A*. Comparative analysis showed variable susceptibility to different antibiotics. It was noted that *S. Typhi* isolates had significantly higher resistance to first-line antibiotics, in particular to trimethoprim and chloramphenicol, however *S. Paratyphi A* isolates remained comparatively sensitive. Our results align with previously published studies reporting higher resistance rates in *S. Typhi* relative to *S. Paratyphi A* in South Asia.¹⁰⁻¹⁹

A noteworthy observation of the present study was the pattern of resistance to fluoroquinolones. 50.6% of *S. Typhi* isolates were found to be resistant, and 61.9% of *S. Paratyphi A* isolates showed intermediate susceptibility. Earlier studies have reported similar trends, where reduced susceptibility to fluoroquinolones in case of *S. Paratyphi A* particularly has been linked to genetic mutations resulting in reduced drug efficacy.²⁰ This emerging pattern may represent an early warning sign of fluoroquinolone resistance and underscores the need for cautious antibiotics use.

When third-generation cephalosporins are taken into consideration, *S. Typhi* showed lower sensitivity compared to *S. Paratyphi A*. Emergence of extended-spectrum beta-lactamase (ESBL)-producing strains can be considered as an attributing factor for resistance in *S. Typhi*. It has been increasingly reported across the region.²¹ Our findings are in concordance with these reports and point toward a growing concern about multidrug resistance, particularly third-generation cephalosporins.

S. Typhi and *S. Paratyphi A* isolates showed high levels of sensitivity to Azithromycin and meropenem. These sensitivity patterns are consistent with recent data from the region; however, there is also documented evidence of emerging resistance to azithromycin.²³

Although these antibiotics remain reliable treatment options, it is imperative to use them cautiously to preserve effectiveness.

Another interesting observation was the fact that when individual antibiotic resistance patterns were analyzed, the results were suggestive of multidrug- and extensive drug- resistance. Collective categorization of isolates into resistance classes (*Typhoid management guidelines, 2022*),²¹ the proportions of MDR and XDR cases were lower. It was seen that 52.6% of isolates met the criteria for MDR, while there were no XDR *S. Typhi* isolates identified. The results demonstrate that individual antibiotic resistance does not necessarily coincide across all agents which are required to define MDR or XDR status. It is therefore important to have standardized classification systems for accurate epidemiological interpretation.

Another factor that may be taken into consideration is the recent introduction of the Typhoid Conjugate Vaccine (TCV) in Pakistan, which was implemented one year prior to this study. This may explain the comparatively lower MDR rate and no XDR isolates. It has been suggested through modelling studies that TCV introduction could avert up to 75% of antimicrobial-resistant typhoid cases over the period of a decade. Moreover, empirical data from Karachi have demonstrated that following vaccine rollout, there was a reduced use of last-line antibiotics such as carbapenems.²² These observations are suggestive of the fact that early vaccine impact may have contributed to reduced XDR prevalence in our study population however it needs further evaluation.

The present study is not without its limitations that should be addressed. It's a retrospective study which relied on existing records, that may have been incomplete for certain variables. Missing data susceptibility results of a percentage of *S. Paratyphi A* isolates for specific antibiotics may have potentially affected comparative analyses. Patient antibiotic history, vaccine compliance and molecular characterization of resistance mechanisms were unavailable. Additionally, long term impact of TCV could not be assessed as the study was conducted within one year of TCV introduction. It is therefore essential to conduct continued surveillance to confirm these trends and also to ensure sustained control of antimicrobial resistance.

CONCLUSION

The present study gives a comprehensive overview of patterns of antimicrobial susceptibility of *Salmonella* Typhi and *S. Paratyphi A* which cause Typhoid fever amongst the pediatric cohort in Lahore, Pakistan one year after the introduction of the Typhoid Conjugate Vaccine (TCV). The results demonstrated higher resistance of *S. Typhi* overall as compared to *S. Paratyphi A*, particularly in first-line antibiotics and third-generation cephalosporins. It is noteworthy that azithromycin and meropenem remained highly effective against these pathogens. Resistance patterns showed variable results with 52.6% isolates were multidrug-resistant (MDR) and no extensively drug-resistant (XDR) isolates. These results indicate a lower prevalence of XDR in this setting as compared to earlier reports from the region.

The findings of this study underscore the importance of rational use of antibiotics, continued resistance monitoring and widespread use of TCV vaccination to limit the spread and emergence of resistant typhoid strains in Pakistan.

Ethical approval:

Ethical approval was obtained from the Institutional Review Board of The University of Lahore IRB No: ERC/33/24/05 dated 22-07-2024.

Conflict of Interest:

Authors declare no conflict of interest.

Financial Disclosure: None

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Authors' Contributions:

AG & MN: Conceptualization & study design.

ME, SM, AT: Data Collection and manuscript drafting.

AG, SS: Data Analysis and critical review.

SM, MN, AT, AG: Supervision & Manuscript drafting & proof reading.

All authors have read and approved the final version of the manuscript and are responsible and accountable for the accuracy and integrity of the work.

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1. Anum Tahir
Assistant professor of Pediatrics,
The University of Lahore, Lahore.
 2. Muhammad Naveed
Associate Professor of Pediatrics,
The University of Lahore, Lahore.
 3. Mahwish Ejaz
Assistant Professor of Pediatrics,
The University of Lahore, Lahore.
 4. Sehr Syed
Assistant Professor Pathology,
The University of Lahore, Lahore.
 5. Sara Maqsood
Assistant professor of Pediatrics
The University of Lahore, Lahore
 6. Ammara Gul
Senior registrar Pediatrics
The University of Lahore, Lahore