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Original Article

Antimicrobial Susceptibility Patterns of Salmonella enterica Serovar Typhi Among Febrile Patients at a Tertiary Care Hospital in Peshawar, Pakistan

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Abstract

Background: Typhoid fever, caused by Salmonella enterica serovar Typhi, remains a major public health challenge worldwide, particularly in low- and middle-income countries such as Pakistan. The indiscriminate use of antibiotics has contributed to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, significantly limiting effective treatment options and increasing the burden on healthcare systems.

Objective: To determine the hospital-based prevalence and antimicrobial susceptibility pattern of Salmonella enterica serovar Typhi among febrile patients at Northwest Teaching Hospital, Peshawar, with particular emphasis on resistance to third-generation cephalosporins, fluoroquinolones, and commonly prescribed antibiotics.

Methodology: This descriptive cross-sectional study was conducted from September 2021 to September 2023 and included 127 febrile patients presenting with suspected enteric fever. Blood cultures were performed for pathogen identification, followed by antimicrobial susceptibility testing using the modified Kirby–Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Data were analyzed using SPSS version 25.

Results: Out of 127 enrolled patients, 122 were included in the final analysis. Salmonella enterica serovar Typhi was isolated in 102 (83.6%) cases. The highest resistance rates were observed for co-trimoxazole 96 (94.1%), chloramphenicol 94 (92.1%), and ampicillin 92 (90.1%). In contrast, all isolates showed complete sensitivity to meropenem 102 (100%), while azithromycin demonstrated a sensitivity of 80 (78.4%).

Conclusion: The study demonstrates a high prevalence of Salmonella enterica serovar Typhi and a concerning level of antimicrobial resistance among febrile patients in Peshawar. While resistance to first-line and commonly used antibiotics is widespread, meropenem and azithromycin remain effective therapeutic options. These findings underscore the urgent need for continuous antimicrobial surveillance, rational antibiotic use, and strengthened public health interventions to curb the spread of resistant typhoid fever.

Keywords: Antimicrobial Resistance, Antibiotic Susceptibility, Peshawar, Salmonella Typhi, Typhoid Fever.

Introduction

Typhoid fever is a systemic infectious disease caused by Salmonella enterica serovar Typhi, characterized by prolonged fever and a spectrum of gastrointestinal and systemic manifestations. Globally, an estimated 21 million cases of typhoid fever occur annually, with a disproportionately high burden reported in South Asian countries, including Pakistan [1]. Factors such as poor sanitation, unsafe drinking water, overcrowding, and limited access to healthcare contribute to sustained transmission in endemic regions.

Over the past two decades, the emergence of antimicrobial-resistant Salmonella enterica serovar Typhi has posed a significant threat to effective disease management. The widespread and often inappropriate use of antibiotics has accelerated the development of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, particularly in Pakistan [2]. Recent national and international reports highlight Pakistan as a major epicenter of XDR typhoid, raising serious concerns for both local and global public health systems [3]. According to the World Health Organization, a substantial proportion of reported typhoid cases in Pakistan involve highly resistant strains, severely limiting available treatment options [4].

The therapeutic challenge is further compounded by the fact that azithromycin remains the last widely available oral antibiotic effective against XDR Salmonella Typhi, while carbapenems are often reserved for severe cases due to cost and stewardship concerns [5,6]. The detection of XDR Salmonella Typhi among international travelers returning from Pakistan underscores the global implications of this emerging resistance crisis [5,6].

Salmonella enterica serovar Typhi is a Gram-negative, flagellated, encapsulated bacterium possessing characteristic O and Vi antigens that play a key role in its virulence and immune evasion [2]. Humans serve as the sole reservoir, facilitating sustained transmission through fecal–oral routes, particularly in settings with compromised sanitation [3]. Although the pathogen has been extensively studied, evidence suggests that Salmonella Typhi is a relatively recent human-adapted pathogen, with its evolutionary emergence estimated at approximately 50,000 years ago [7–8]. The bacterium was first identified in the early nineteenth century by Karl Eberth, marking a major milestone in the understanding of enteric fever [9].

Historically, typhoid fever carried a mortality rate of 10–30% in the pre-antibiotic era. Advances in antimicrobial therapy dramatically reduced mortality to less than 1%; however, the resurgence of resistant strains threatens to reverse these gains [10,11]. Recent studies from Pakistan report alarming resistance patterns, with high rates of fluoroquinolone non-susceptibility and increasing resistance to third-generation cephalosporins, while resistance to azithromycin remains relatively low [12–14].

Despite the growing body of literature, regional data on antimicrobial susceptibility patterns remain essential for guiding empirical therapy and informing antimicrobial stewardship programs. Peshawar, as a major urban center with a high patient influx, represents a critical setting for monitoring resistance trends. Therefore, this study aimed to evaluate the hospital-based prevalence and antimicrobial susceptibility profile of Salmonella enterica serovar Typhi among febrile patients at Northwest Teaching Hospital, Peshawar.

Methodology

Study Design

This study employed a descriptive cross-sectional design to determine the hospital-based prevalence and antimicrobial susceptibility pattern of Salmonella enterica serovar Typhi among febrile patients.

Ethics

Ethical approval was obtained from the Institutional Review Board (IRB) of Northwest General Hospital and Research Centre, Peshawar (Approval No. NwGH/Res/Ethical approval/1518, dated 06-07-2021). Written informed consent was secured from all participants or their legal guardians prior to enrollment.

Participants were informed about the study objectives, procedures, potential risks, and confidentiality of data. All procedures were conducted in accordance with the Declaration of Helsinki and institutional ethical guidelines.

Setting

The study was conducted in the Department of General Medicine, Northwest General Hospital and Research Centre, Peshawar, a tertiary-care teaching hospital that serves a large urban and peri-urban population of Khyber Pakhtunkhwa. Data collection spanned September 2021 to September 2023, allowing assessment of antimicrobial susceptibility trends over an extended period.

Participants

Febrile patients of either gender aged ≥5 years presenting with suspected enteric fever were screened for eligibility.

Inclusion criteria comprised patients with a documented fever (≥38°C) and laboratory confirmation of Salmonella Typhi on blood culture. Patients younger than five years were excluded due to difficulties in obtaining adequate blood volumes and diagnostic variability in this age group. Patients who had received systemic antibiotics within 72 hours prior to blood sampling were excluded to minimize false-negative culture results, although prior antimicrobial exposure outside this window was documented. Patients with confirmed alternative infectious diagnoses (such as malaria, dengue, or urinary tract infections) and those with immunocompromised conditions were also excluded. Initially, 127 eligible patients were enrolled; however, five were excluded due to incomplete demographic or laboratory data, resulting in 122 participants included in the final analysis.

Variables

The primary outcome variable was antimicrobial susceptibility and resistance pattern of Salmonella enterica serovar Typhi. Independent variables included demographic characteristics (age, gender, education, occupation, marital status, residence), environmental factors (source of drinking water), clinical history (previous enteric fever and prior antimicrobial use), and microbiological findings from blood cultures.

Data Sources/Measurement

Venous blood samples (5 mL) were collected aseptically using standard protocols and processed for blood culture. Identification of Salmonella Typhi was carried out using conventional microbiological and biochemical methods. Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK). Interpretation of zone diameters followed the Clinical and Laboratory Standards Institute (CLSI) M100, 31st edition (2021) guidelines. Antibiotics tested included ampicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, azithromycin, cefotaxime, ceftriaxone, amikacin, gentamicin, meropenem, and piperacillin–tazobactam. Quality control was ensured using standard ATCC strains (Escherichia coli 25922, Pseudomonas aeruginosa 27853, and Staphylococcus aureus 25923). Data were recorded using a structured proforma and entered into a secured database.

Bias

To reduce selection bias, non-probability consecutive sampling was used to enroll all eligible patients presenting during the study period. Measurement bias was minimized by using standardized laboratory procedures, validated antimicrobial discs, and CLSI interpretive criteria applied uniformly to all isolates. Data entry errors were minimized through cross-checking and validation before analysis.

Study Size

The sample size was calculated using OpenEpi software, assuming a prevalence of 94.4% for Salmonella Typhi among Salmonella isolates based on prior literature, with a 95% confidence level and ±4% margin of error, yielding a required sample of 127 participants. After exclusions, 122 complete cases were analyzed.

Quantitative Variables

Continuous variables such as age were summarized using mean and standard deviation, while categorical variables were expressed as frequencies and percentages. Antimicrobial susceptibility outcomes were categorized as susceptible, intermediate, or resistant according to CLSI breakpoints.

Statistical Methods

Data analysis was performed using SPSS version 25. Descriptive statistics were used to summarize demographic, clinical, and microbiological variables. Results were presented in tables with corresponding frequencies, percentages, and 95% confidence intervals where applicable. No inferential statistical comparisons were performed due to the descriptive nature of the study.

Results

Participants

A total of 127 febrile patients were initially enrolled in the study. However, five participants were excluded from the final analysis due to incomplete demographic or laboratory data. Consequently, data from 122 participants were analyzed, yielding an overall response rate of 96%. All included participants fulfilled the predefined inclusion criteria and had complete clinical, demographic, and microbiological records suitable for outcome assessment.

Descriptive Data

The study population comprised patients aged 5 to 60 years, with the largest proportion belonging to the 31–45 years age group (36.8%), followed by those aged 16–30 years (27.8%). Males constituted the majority of participants (62.2%), while females accounted for 37.7%. Most participants were literate (63.1%), unmarried (58.1%), and resided in urban areas (64.7%). Regarding occupational status, self-employed individuals (29.5%) and students (25.4%) represented substantial proportions of the cohort. Environmental assessment revealed that 73.7% of participants consumed piped water, whereas the remainder

relied on river or well water. A prior history of enteric fever was reported by 27% of participants, and 62.2% had a documented history of antimicrobial use, although not within 72 hours preceding blood culture sampling (Table 01).

Outcome Data

Blood culture analysis confirmed Salmonella enterica serovar Typhi infection in 102 out of 122 participants (83.6%). Antimicrobial susceptibility testing demonstrated marked variability across tested agents.

All isolates (100%) were sensitive to meropenem, indicating preserved efficacy of carbapenems. High sensitivity was also observed for azithromycin (78.4%), while moderate sensitivity was noted for ceftriaxone (41.7%), co-trimoxazole (45.0%), and cefotaxime (34.3%). In contrast, very low sensitivity was observed for chloramphenicol (7.8%), ampicillin (9.8%), and gentamicin (15.6%) (Table 02), reflecting widespread resistance to conventional first-line therapies.

Main Results

The antimicrobial resistance profile revealed alarmingly high resistance rates among Salmonella Typhi isolates. Resistance was most pronounced for chloramphenicol (92.1%) and ampicillin (90.1%), followed by ciprofloxacin (78.4%), gentamicin (84.3%), and trimethoprim (74.5%).

Resistance to third-generation cephalosporins was also substantial, with 58.8% resistance to ceftriaxone and 65.6% to cefotaxime, highlighting reduced effectiveness of commonly used empirical therapies. Notably, resistance to azithromycin remained relatively low (21.5%), and no resistance to meropenem was detected (Table 03).

These findings collectively indicate a high burden of multidrug-resistant and extensively drug-resistant Salmonella Typhi strains among febrile patients at the study site.

Table 1: Demographic and clinical characteristics (n=122).

Parameters	Categories	N (%)
Age	5-15	12 (9.83%)
	16-30	34 (27.8%)
	31-45	45 (36.8%)
	46-60	31 (25.4%)
Gender	Male	76 (62.2%)
	Female	46 (37.7%)
Education	Illiterate	45 (36.8%)
	Literate	77 (63.1%)
Marital Status	Married	51 (41.8%)
	Single	71 (58.1%)
Occupation	Gov't Employee	23 (18.8%)
	Self-employed	36 (29.5%)
	Student	31 (25.4%)
	Housewife	32 (26.2%)
Residence	Urban	79 (64.7%)
	Rural	43 (35.2%)
Water Supply	Pipe	90 (73.7%)
	River/Well	32 (26.2%)
History of Enteric Fever		33 (27%)
History of Antimicrobial (not within the last 72 hours)		76 (62.2%)
Salmonella enterica serovar Typhi		102 (83.6%)

Table 2: Antibiotics sensitivity pattern of Salmonella enterica serovar Typhi separated from blood (n=102)

Antibiotic	n (%)	95% CI
Chloramphenicol	8 (7.8%)	4.0%–14.7%
Ampicillin	10 (9.8%)	5.4% - 17.1%
Ciprofloxacin	22 (21.5%)	14.7%–30.5%
Ceftriaxone	42 (41.7%)	32.1%–50.9%
Azithromycin	80 (78.4%)	69.5%–85.3%
Meropenem	102 (100%)	96.4%–100.0%
Co-trimoxazole	46 (45.0%)	43.3% - 47.5%
Trimethoprim	26 (25.4%)	18.0%–34.7%
Piperacillin–Tazobactam	36 (35.2%)	26.7%–44.9%
Amikacin	35 (34.3%)	25.8%–43.9%
Gentamicin	16 (15.6%)	9.9%–24.0%
Cefotaxime	35 (34.3%)	25.8%–43.9%

Table 3: Antibiotics Resistance pattern of Salmonella enterica serovar Typhi separated from blood cultures (n=102)

Antibiotic	n (%)	95% CI
Chloramphenicol	94 (92.1%)	85.3%–96.0%
Ampicillin	92 (90.1%)	82.9%–94.6%
Ciprofloxacin	80 (78.4%)	69.5%–85.3%
Ceftriaxone	60 (58.8%)	49.1%–67.9%
Azithromycin	22 (21.5%)	14.7%–30.5%
Meropenem	0 (0%)	0.0%–3.6%
Co-trimoxazole	56 (54.9%)	52.2% -56.3%
Trimethoprim	76 (74.5%)	65.3%–82.0%
Piperacillin–Tazobactam	66 (64.7%)	55.1%–73.3%
Amikacin	67 (65.6%)	56.1%–74.2%
Gentamicin	86 (84.3%)	76.0%–90.1%
Cefotaxime	67 (65.6%)	56.1%–74.2%

Discussion

The present study evaluated the antimicrobial susceptibility patterns of Salmonella enterica serovar Typhi among febrile patients presenting to a tertiary care hospital in Peshawar and provides important insights into the growing burden of antimicrobial resistance in this region. The isolation rate of Salmonella Typhi in this study was 83.6%, indicating a substantial contribution of enteric fever to febrile illness in the study population. This finding reinforces the continued public health significance of typhoid fever in Pakistan, particularly in urban and peri-urban settings with compromised sanitation and water safety.

Demographic analysis revealed a higher prevalence of infection among males (62.2%) and adults aged 31–45 years (36.8%). Similar gender predominance has been reported in studies from Peshawar, Islamabad, and Karachi, suggesting increased exposure risk among adult males due to occupational mobility and outdoor activities [15–17]. While several studies have documented a higher burden among children, particularly those under 12 years of age [15,18], the predominance of adult cases in the present study highlights regional variability and underscores the need for age-stratified surveillance strategies.

The most concerning finding of this study is the alarmingly high level of antimicrobial resistance among Salmonella Typhi isolates. Resistance to first-line antibiotics co-trimoxazole (94.1%), chloramphenicol (92.1%), and ampicillin (90.1%) confirms the widespread circulation of multidrug-resistant (MDR) strains. These findings are consistent with reports from Pakistan documenting escalating resistance trends over the past two decades [13,14]. The high resistance to ciprofloxacin (78.4%) further

limits oral treatment options and aligns with previous studies reporting fluoroquinolone resistance exceeding 90% in some settings [8,19].

Equally concerning is the substantial resistance to third-generation cephalosporins observed in this study, with ceftriaxone resistance reaching 58.8%. This trend mirrors national data reporting a steady rise in cephalosporin-resistant and extensively drug-resistant (XDR) Salmonella Typhi strains [15,20]. The reduced efficacy of ceftriaxone, once a cornerstone of typhoid treatment, represents a critical challenge for empirical therapy in endemic regions.

In contrast, meropenem demonstrated 100% sensitivity against all isolates, reaffirming its role as a reliable therapeutic option for severe or complicated XDR typhoid cases. This finding is consistent with multiple national and international studies reporting preserved carbapenem efficacy [8,18]. Azithromycin also showed relatively high sensitivity (78.4%) with low resistance (21.5%), supporting its continued use as an oral treatment option, particularly for uncomplicated cases. However, emerging reports of azithromycin resistance highlight the need for cautious and judicious use to preserve its effectiveness [18].

Environmental and behavioral factors likely contribute significantly to the observed disease burden and resistance patterns. The high proportion of participants consuming piped water (73.7%) suggests possible contamination of municipal water supplies, a well-documented risk factor for typhoid transmission in Pakistan. Additionally, the widespread history of prior antimicrobial use (62.2%) reflects common practices of self-medication and inappropriate prescribing, which accelerate resistance development. Studies have also documented the presence of resistant Salmonella strains in poultry and livestock, raising concerns about zoonotic and food-borne transmission pathways [18].

Collectively, these findings emphasize the urgent need for strengthened antimicrobial stewardship, continuous microbiological surveillance, and integrated public health interventions. Without coordinated efforts including vaccination, sanitation improvement, and regulation of antibiotic use the continued spread of MDR and XDR typhoid poses a serious threat to both national and global health systems [11–13].

Limitation and future direction

This study has certain limitations that should be acknowledged. Its single-center design and relatively small sample size limit the generalizability of the findings to other regions of Pakistan. Additionally, the cross-sectional nature of the study precludes assessment of causal relationships or temporal trends in antimicrobial resistance. Molecular characterization of resistance mechanisms was not performed, which could have provided deeper insights into the genetic basis of observed resistance patterns. Future research should focus on multi-center and longitudinal studies to better capture regional and temporal variations in resistance trends. Incorporation of molecular and genomic techniques is recommended to identify resistance determinants and track the transmission of XDR strains. Further evaluation of vaccination impact, water and sanitation interventions, and alternative therapeutic strategies is also essential to address the escalating challenge of drug-resistant Salmonella Typhi.

Conclusion

This study demonstrates a high burden of Salmonella enterica serovar Typhi infection among febrile patients in Peshawar, accompanied by alarming levels of resistance to commonly used antibiotics. The widespread resistance to first-line agents and declining efficacy of third-generation cephalosporins highlight the growing prevalence of multidrug-resistant and extensively drug-resistant strains. While meropenem and azithromycin remain effective treatment options, their use must be guided by antimicrobial stewardship principles to prevent further resistance development. The findings underscore the critical importance of continuous surveillance, rational antibiotic prescribing, vaccination programs, and improvements in sanitation and water safety to curb the spread of resistant typhoid fever and reduce its public health impact.

Authors' Contributions:

All authors meet the ICMJE criteria for authorship.

Author 1: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 2: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 3: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 4: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.
Author 5: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Author 6: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

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