https://doi.org/10.29309/TPMJ/2026.33.04.10077 www.theprofesional.com

ORIGINAL ARTICLE

Evolving trends in the antibiotic susceptibility and resistance pattern of Salmonella species isolated from patients with suspected bloodstream infections in a clinical setting.

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ABSTRACT… Objective: To assess the evolving trends in Salmonella Typhi antibiotic susceptibility and resistance patterns from patients with suspected bloodstream infections in clinical settings. **Study Design:** Cross-sectional study. **Setting:** Department of Pathology, Khyber Teaching Hospital, Peshawar. **Period:** Over 6 months (January 2023 to June 2023). **Methods:** The study was conducted on blood culture and susceptibility samples received in the Department of Pathology, Khyber Teaching Hospital, Peshawar. The automated blood culture system VERSATREK was utilized for initial processing of the blood sample. Pathogens were labeled as Salmonella based on colony characteristics on Blood agar, McConkey agar, Salmonella Shigella agar, biochemical tests, and the API 20E kit. The antimicrobial resistance pattern was mapped out using the Disk Diffusion method as per CLSI M100-Ed33 guidelines version 23. In total, 1170 blood culture samples were analyzed. **Results:** The study revealed that Salmonella Typhi (96%) was the major pathogen, followed by Escherichia coli (2%), Citrobacter spps (1%), and Staphylococcus aureus (1%). Salmonella Typhi demonstrated resistance to Ampicillin (98%), Ciprofloxacin (94%), Chloramphenicol (95%), Ceftriaxone (92%), and Cotrimoxazole (62%). All Salmonella isolates were found sensitive to Meropenem (100%) and Azithromycin (100%). About 56% of the isolates were Extensively Drug-Resistant (XDR). Gender-wise distribution revealed an infection rate of 62% in male patients and 38% in female patients. Patients <20 years (82%) accounted for the majority as compared to those over 20 years (18%) of age. **Conclusion:** Future outbreaks of XDR typhoid are most likely to occur in Pakistan, and it continues to be the dominant circulating strain. While Cotrimoxazole is reemerging with comparatively improved sensitivity, azithromycin and meropenem continue to be available treatment options. The evolving susceptibility pattern of Salmonella isolates demands ongoing surveillance of antibiograms to optimize therapeutic protocols and control the emergence of resistant strains. Public health activities to track and mitigate the spread of XDR S. Typhi should be prioritized in this country.

Key words: Antimicrobial Resistance, Blood Stream, Infection, Salmonella.

Article Citation: Gul A, Khan M, Gul T, Sahar S, Ali M, Shahid A, Khan S. Evolving trends in the antibiotic susceptibility and resistance pattern of Salmonella species isolated from patients with suspected bloodstream infections in a clinical setting. Professional Med J 2026; 33(04):561-566. https://doi.org/10.29309/TPMJ/2026.33.04.10077

INTRODUCTION

Typhoid fever, which causes an estimated 10.9 million new infections and 116,800 deaths annually, is caused by Salmonella enterica serovar Typhi (S. Typhi).[1] Due to inadequate sanitation practices and a scarcity of clean water, Salmonella is spreading alarmingly in developing nations, favoring the fecal-oral route of transmission.[2] Salmonella Typhi causes infection by penetrating the small intestine's epithelial cells. From there, it travels through the bloodstream and infects a number of organs, including the liver, bone marrow, lymph nodes, and spleen. Later, it breaks out of the bloodstream again and manifests as fever.[3]

As per a 2018 study, out of all the Southeast Asian countries, Pakistani citizens are thought to have the highest rate of Salmonella Typhi infection.[4] Therefore, historically, South Asian urban slums with inadequate sanitation have been linked to high prevalence rates of typhoid fever.[5] In sub-Saharan Africa, typhoid fever is a serious issue in both urban and rural areas, according to recent multicenter surveillance studies.[6]

But azithromycin is now the only practical oral antimicrobial for treating typhoid fever in South Asia due to newly circulating extensively drug-resistant (XDR; MDR plus resistance to fluoroquinolones and third-generation cephalosporins) variants of

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Article received on:
13/09/2025
Date of revision:
27/11/2025
Accepted for publication:
29/11/2025

S. Typhi[7] According to many studies, Salmonella serovars that were resistant to cephalosporins and then fluoroquinolones started to appear and spread. Laboratory monitoring data from Karachi, Pakistan, between 2009 and 2011 showed a rise in S. Typhi that was resistant to multiple drugs, while the incidence of rare third-generation cephalosporin resistance was very low, at 0.08%.[8] S. Typhi was referred to as "extensively drug-resistant" (XDR) when it was found to be resistant to five different types of antibiotics, including ampicillin, co-trimoxazole, fluoroquinolones, and third-generation cephalosporins.[9] Presently, XDR S. Typhi is the primary cause of typhoid fever in most of Pakistan; cases have been reported from most provinces.[10]

The sole available treatments for S. Typhi are azithromycin and meropenem due to the infection gaining resistance to first- and second-line antibiotics through chromosomal gene mutations, transposons, and plasmids. This would put additional strain on already overworked health systems and come at a high additional cost to patients and their families.[11] In the future, typhoid could become effectively incurable: Azithromycin-resistant typhoid has independently surfaced in some nations[12], and non-typhoidal Salmonella is resistant to carbapenems.[13] A US study found that 300 travelers returning from Pakistan had XDR Salmonella serovar typhi.[14]

Before then, reports of isolated cases of S. Typhi that produced extended-spectrum beta-lactamases came from various locations. Senegal, Africa, the Netherlands, France, Latin America, Asia, and Europe all reported cases of S. Typhi isolates that were resistant to ceftriaxone.[15,16] During the COVID-19 pandemic, a different study from Peshawar, Pakistan, detailed the first instance of an XDR Salmonella Typhi outbreak that spread widely.[17] Clinical diagnosis is challenging due to the nearly similar clinical presentations of these genetically related salmonella serovars, which include high-grade fever, anorexia, headache, abdominal pain, leukopenia, and tachycardia.[18]

Nevertheless, despite the concerning antimicrobial resistance (AMR) of salmonella species, physicians may not be aware of the changing antibiotic landscape that necessitates continuous antibiogram monitoring. Local antibiogram development will assist physicians in selecting the best course of antibiotic treatment, thereby slowing the spread of AMR. A key strategy for preventing and managing the emergence of antibiotic-resistant salmonella species as well as forecasting the emergence of antibiotic resistance on a national and international scale is laboratory surveillance. Here, we report the extensive rise of XDR typhoid cases in Peshawar, Pakistan and to look into the susceptibility profile of Typhoidal salmonellae that are currently being treated with azithromycin alone, especially XDR S. Typhi.

Rationale: The study seeks to identify trends in resistance, highlight emerging patterns of MDR & XDR strains, and evaluate the effectiveness of current treatment protocols. That will help clinicians make informed decisions about appropriate antimicrobial therapies, and assist in developing more targeted strategies for controlling Salmonella infections. Furthermore, the study will contribute to the growing body of knowledge on antimicrobial resistance (AMR) in enteric pathogens, which is essential for global health efforts aimed at combating the spread of resistance. Ultimately, this research is crucial for improving patient outcomes, informing public health policies, and enhancing the effectiveness of interventions aimed at controlling Salmonella infections in healthcare settings.

METHODS

This cross-sectional study was carried out at the Department of Pathology, Khyber Teaching Hospital, Peshawar, from January 2023 to June 2023. Ethical approval was obtained from the IREB committee (627/DME/KMC-15-08-24) of Khyber Medical College, Peshawar. A total of 1170 blood cultures received from inpatient as well as outpatient departments in the microbiology section for culture and sensitivity were included in the present study. Samples that turned out positive for any microorganism other than Salmonella were excluded. Blood samples were initially analyzed phenotypically using the VersaTREK™ Automated Microbial Detection System. When the bottle was flagged as positive, they were subcultured/inoculated onto blood agar, MacConkey's agar, and Chocolate agar and incubated overnight at 37°C. Isolates strains

were labeled as Salmonella species based on Colony characteristics on agar culture media, Gram staining, conventional biochemical tests, and API 20E (bioMérieux, France). After 24 hours, oxidase-negative, non–lactose fermenters were identified. Antimicrobial susceptibility testing was performed via the disc diffusion Kirby-Bauer method, as described in CLSI M100-Ed33 (https://www.clsi.org) recommendations version 23. The turbidity of the stock culture was adjusted to 0.5 MacFarland and streaked evenly over Muller-Hinton agar plates. A panel of 7 antibiotic discs was utilized, including tested ampicillin (AMP 10µg), cotrimoxazole (SXT 25µg), chloramphenicol (C 30µg), ciprofloxacin (CIP 5µg), ceftriaxone (CRO 30µg), meropenem (MEM 10µg), and azithromycin (AZM 15µg). The diameter of zones of inhibition was interpreted after 24 hours of aerobic incubation as per CLSI guidelines version 23.

Data was entered in Microsoft Excel and analyzed using Descriptive statistics for quantitative variables, and data was presented as frequency and percentages.

RESULTS

Among 1170 blood cultures analyzed, 516 (44%) yielded growth of microorganisms, while 654 (55.6%) yielded no growth. Among positive blood cultures, gram-negative rods accounted for the majority, 512 (99%) of bloodstream infections, while gram-positive cocci were observed only in 4(1%) of the isolates, as shown in Figure-1. Gender wise distribution revealed an infection rate of 310 (62%) in male and 188 (38%) in female patients, Figure-2. Patients <20 years 406 (82%) accounted for the majority, as compared to those over 20 years 92 (18%) of age, Figure-3.

Among gram-negative bacteria, Salmonella Typhi were major pathogens 498 (96%) followed by Escherichia coli 10 (2%), and Citrobacter spps 4(1%). Salmonella Typhi demonstrated resistance to Ampicillin (98%), Ciprofloxacin (94%), Chloramphenicol (95%), Ceftriaxone (92%), and Cotrimoxazole (62%). All Salmonella isolates were found sensitive to Meropenem (100%) and Azithromycin (100%), as shown in Figure-4. Extensively Drug-Resistant (XDR) Salmonella species accounted for 277(56%) of all the infections. The frequency of Multidrug resistance (MDR) isolates was relatively low, 24 (5%), while NON-MDR-NON-XDR isolates were observed in 196(39%) of the cases, Figure-5.

FIGURE-1

Frequency of negative, positive & bacterial isolates in blood samples

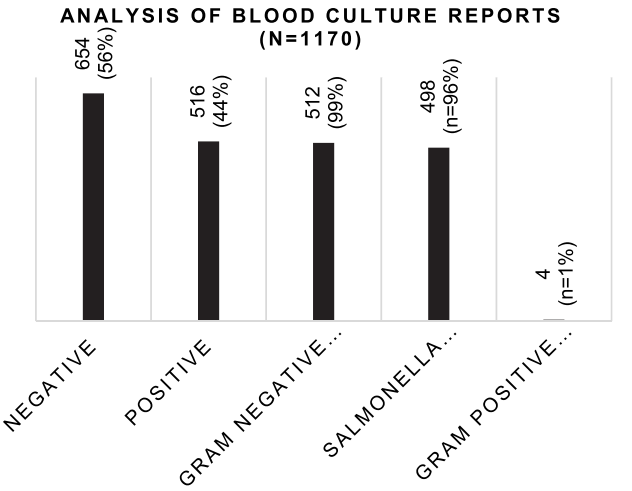
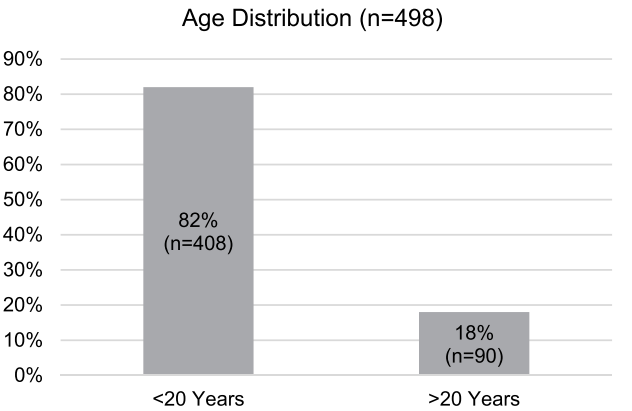


FIGURE-2

Age distribution of salmonella Typhi (n=498)



DISCUSSION

In 2016, the XDR S. Typhi outbreak first affected the province of Sindh, then the province of Punjab, and finally the province of Khyber Pakhtunkhwa. There has been a rise in XDR typhoid cases in Peshawar, with children bearing the majority of the burden and an increasing percentage of ceftriaxone resistance (92%), among Salmonella Typhi cases.

FIGURE-3

Gender wise distribution of salmonella Typhi isolated (n=498)

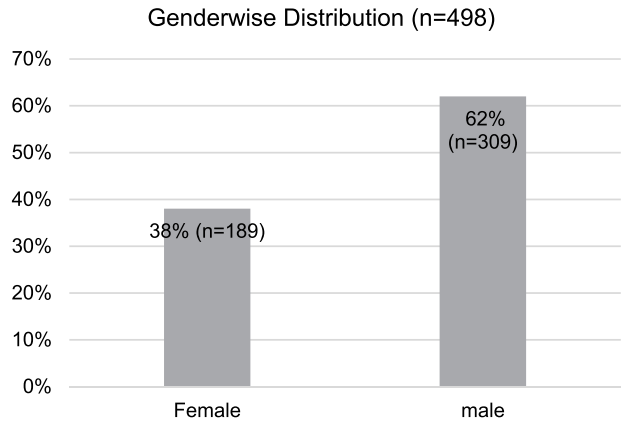


FIGURE-4

Antimicrobial susceptibility pattern of Salmonella Typhi isolated (n=498)

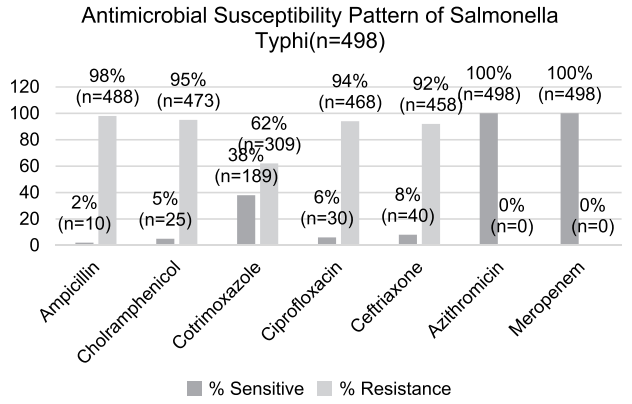
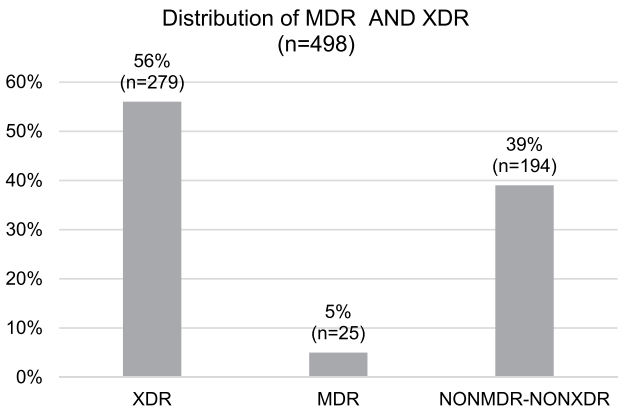


FIGURE-5

% of all Salmonella Typhi isolates from blood culture samples that were Multidrug Resistant (MDR), Extensively Drug Resistant (XDR) & non-MDR/XDR



Comparable to a study in which pediatric patients infected with a strain of S. Typhi resistant to all first- and second-line medications, including the third-generation cephalosporin, accounted for 88% of cases.[17] Five isolates of XDR S. Typhi were found in water sources during a recent outbreak that was reported to be occurring in China.[19] Furthermore, 5,274 cases of XDR typhoid fever were reported to the World Health Organization (WHO) out of 8,188 cases of typhoid fever that were reported in Pakistan between November 2016 and December 2018.[9] Therefore, XDR typhoid will keep getting worse and going unreported as long as proper diagnostics, treatments, and surveillance aren't implemented.

Antibiotic self-medication is widespread in Pakistan and is accessible without a prescription.[20] Furthermore, the number of cases of S. Typhi that are resistant to ceftriaxone is rising, and ceftriaxone is currently the most popular empirical treatment option among medical professionals. This poses a risk because it leads to the frequent use of ineffective antibiotics.[21] Compared to our results, a study from Peshawar that used 100% ampicillin, 100% ceftriaxone, 100% chloramphenicol, and 100% ciprofloxacin reported a slightly higher resistance rate.[17] This finding may be explained by the unjustified and unwarranted use of this antibiotic in Pakistan, where several antibiotics are available over-the-counter, to treat the COVID-19 pandemic.

In contrast, 5% of the Salmonella isolates in our study were MDR, and 56% were XDR cases. A recent study conducted in Lahore found that 121 (67%) of the isolates were MDR and 62 (34%) were XDR. The only oral medication for treating XDR typhoid is azithromycin. Spontaneous resistance to Salmonella species has been reported worldwide, despite the fact that azithromycin resistance in S. Typhi is rare. Global reports of antimicrobial resistance in laboratories and clinical treatment failures have been made.[23] In contrast, carbapenem resistance in Enterobacterales is rare and was most likely initially reported in S. Typhi from Faisalabad, Pakistan. Injectable carbapenems are an additional treatment option.[24]

On the other hand, all of the XDR isolates in our investigation responded positively to azithromycin

and carbapenems. Carbapenem resistance cases are extremely uncommon both internationally and in this region of the world. According to a different study from Punjab, Pakistan, 48% of the isolates were resistant to carbapenems.[25] Between 1972 and 2018, global data from different nations revealed that 2.5% of S. Typhi isolates were resistant to carbapenems.[26] Second-line drug resistance in S. Typhi is a severe global health concern that needs to be closely monitored for both local and global spread. These results also imply that almost all locally circulating S. Typhi will probably eventually acquire molecular markers for resistance to azithromycin and carbapenem, and that this may soon become a worldwide issue. It makes sense to give priority to vaccine interventions in South Asia (while supply is limited) to have the biggest possible impact on disease incidence and AMR, as resistance often originates and spreads from this region.[27] Furthermore, since there are limited treatment options for typhoid fever, there should be a lot of focus on XDR typhoid, which can result in treatment failure, extended hospital stays, and widespread and recurrent transmission of the illness. Furthermore, in order to stop the emergence of new variants that have the potential to spread, we propose that greater focus be given to the prospective identification and treatment of chronic carriers.

CONCLUSION

The prevalence of MDR and XDR S. Typhi in the province of Khyber Pakhtunkhwa is clearly apparent. Nearly all strains of XDR S. Typhi have developed resistance to third-generation cephalosporins as well as first- and second-line antibiotics. Thus, it is imperative to identify the origin of the XDR strains and to bolster surveillance of their dissemination via lab and comprehensive epidemiological studies in the future.

LIMITATIONS

The exact antibiotic regimens used, comorbidities, and specific details of the patients' treatment responses were not available. Our study focused primarily on phenotypic resistance patterns, which might not fully account for the molecular mechanisms of resistance. Whereas Whole Genome Sequencing (WGS) is a powerful tool to identify these mutations in the chromosomal DNA of Salmonella and correlate them with specific resistance profiles.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Amina Gul:** Conceptualization.
2	**Maria Khan:** Methodology.
3	**Tamjeed Gul:** Data entry.
4	**Sana Sahar:** Data analysis.
5	**Momena Ali:** Data collection.
6	**Aliza Shahid:** Revision.
7	**Saba Khan:** Data analysis.