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PREVALENCE AND DRUG-RESISTANCE PATTERNS OF *SALMONELLA ENTERICA* SEROVAR TYPHI IN CLINICAL ISOLATES FROM KARACHI, PAKISTAN USING MALDI-TOF MS AND VITEK 2 SYSTEM

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ABSTRACT

Background

Salmonella enterica serovar Typhi is a causative agent of typhoid fever. In Pakistan, it remains a major public health burden due to the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Karachi, being one of the largest metropolitan regions of the country, suffers to experience a significant burden of enteric fever caused by antimicrobial-resistant strains.

Objective

This study aimed to determine the prevalence of Salmonella enterica serovar Typhi in different clinical specimens collected from diagnostic laboratories in Karachi, Pakistan, and to evaluate the distribution of MDR, XDR, and non-MDR/non-XDR isolates using advanced automated identification and antimicrobial susceptibility testing systems.

Methodology

A cross-sectional study was conducted using clinical isolates (n = 115) obtained from multiple diagnostic laboratories during 2023 in Karachi after informed consent and ethical consideration of available anonymized patient records. Clinical information regarding specimen source and laboratory history was documented. High throughput identification and biochemical characterization of isolates were carried out using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) and the VITEK 2 automated microbial identification system. Antimicrobial susceptibility testing (AST) was carried out using the VITEK 2 system according to the Clinical & Laboratory Standards Institute (CLSI). Isolates were categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR), or non-MDR/non-XDR based on observed resistance profiles against standard antibiotics.

Results

The study demonstrated that blood samples constituted the highest proportion of Salmonella enterica serovar Typhi isolates (74.78%), followed by stool (16.52%), urine (7.83%), and bone marrow (0.87%) specimens, confirming bloodstream infection as the predominant clinical presentation of typhoid fever in the studied population. MALDI-TOF MS and VITEK 2 showed high concordance for species-level and serotype-level identification, respectively. Serotypes were successfully confirmed by antigen-antibody

latex agglutination test. Antimicrobial susceptibility profiling revealed an alarming prevalence of resistant strains among clinical isolates. Our findings demonstrated a predominant prevalence of XDR Salmonella enterica serovar Typhi isolates in blood samples, while multi-drug-resistant strains were found mainly among blood and stool specimens. Non-MDR/non-XDR isolates were significantly fewer in number as compared to XDR. Collectively, amicrobial-resistant phenotypes surpassed susceptible isolates. Thus, indicating a high occurrence of antimicrobial-resistant Salmonella enterica serovar Typhi strains in Karachi.

Conclusion:

Our study displayed substantial prevalence of MDR and XDR Salmonella enterica serovar Typhi among clinical isolates in Karachi, Pakistan. The blood culture appeared as the primary source of recovery. The growing problem of Salmonella enterica serovar Typhi infection poses a serious therapeutic and public health challenge. There is a need for continuous surveillance, authentic and rapid diagnostic facilities, antibiotic stewardship programs, and enhanced infection prevention stratagems at the national level for the prevention and control of resistant phenotypes of Salmonella enterica serovar Typhi.

Keywords: *Salmonella enterica serovar Typhi, MALDI-TOF MS, VITEK 2 compact system, extensively drug-resistant, multidrug-resistant*

INTRODUCTION

Salmonella enterica serovar Typhi causes a systemic infection, typhoid fever. It remains one of the major public health challenges in endemic regions due to insufficient sanitation and inadequate access to hygienic food and water. The disease affects densely populated low- and middle-income countries. Notwithstanding advances in diagnostics and therapeutics, *Salmonella enterica* serovar Typhi persists as a significant cause of morbidity, majorly due to the emerging antimicrobial resistance. [1]

Pakistan serves as a country with the highest burden of typhoid fever; particularly, Karachi holds its position as an epicenter due to its population density, mobility, and diverse healthcare setup. [2] During the last decade, the clinical management of typhoid fever has been altered due to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Salmonella enterica* serovar Typhi. The world witnessed the first XDR *Salmonella enterica* serovar Typhi outbreak, which occurred in Hyderabad and Karachi during 2016–2018. This emergence rapidly sped through transboundary movements. Clonal extension of a single dominant ancestry carrying plasmid-mediated resistance was proved by genomic analyses of the emerging strains. This is further verified by the subsequent rapid dissemination across countries and continents. [3]

A sustained increase in antimicrobial resistance was observed in the post-outbreak period with continued circulation of XDR strains alongside MDR and non-resistant phenotypes. Unfortunately, the COVID-19 pandemic additionally exaggerated the situation in an indirect manner due to augmented empirical antibiotic usage, interrupted healthcare systems, abridged surveillance projects, and rife self-medication, contributing to high selective pressure and hastening resistance tendencies in many bacterial pathogens, including *Salmonella enterica* serovar Typhi. This has raised up apprehensions about the long-standing sustainability of existing treatment options, predominantly oral antibiotics, which are progressively ineffective against resistant infections. [4]

Precise and rapid identification of *Salmonella enterica* serovar Typhi has consequently become indispensable for epidemiological investigation and clinical management. In addition, traditional culture-based methods are reliable; these are time-consuming and may cause delay in targeted therapy.

In the recent advancement of diagnostic approaches, Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

(MALDI-TOF MS) and the VITEK 2 compact system have been placed as rapid and accurate identification and characterization systems. In addition, the Vitek 2 compact system performed efficiently for the determination of minimum inhibitory concentration-based antimicrobial susceptibility in accordance with established breakpoints of antibiotics for each group of organisms [5].

The clinical isolates remain a core source for active surveillance studies, evaluation of existing resistance patterns, prevalence, and phylogenetic analysis, hence leading to the establishment of countermeasures and health policies at the national level.

The aim of the study is to determine the prevalence of *Salmonella enterica* serovar Typhi in clinical isolates and antibiotic resistance patterns using cutting-edge technologies, thus aiming to contribute to local surveillance data and antimicrobial stewardship programs.

Methodology

Study Design and Ethical Considerations

Our research was a cross-sectional study, approved by the Institutional Review Board. Good laboratory practices, ethical regulations, and the Declaration of Helsinki were compliant, and confidentiality was ensured throughout the study. Informed consent permitting the anonymous data for research purposes was obtained from parents or legal guardians at the time of sample collection. No direct accessibility to the patient was applied.

Source of Isolates and Data Collection

The isolates were acquired from different diagnostic laboratories located at Karachi during 2023. The isolates were isolated from clinical samples received for routine diagnosis. Specimens include blood, stool, urine and bone marrow. The data was collected from available laboratory records in an anonymous manner. Duplicate entries were excluded to avoid redundancy.

Primary Processing and Storage of Bacterial Isolates

All isolates were handled in a BSL-2 lab in compliance with relevant biosafety regulations. The acquired isolates were subcultured on 5% Blood Agar. Long-term preservation was achieved by preparing bacterial stocks in Tryptic Soy Broth (TSB) supplemented with 15% glycerol and keeping them at −80°C with proper cataloguing. [6]

Identification of Isolates

All isolates were identified through protein mass fingerprinting using the VITEK® MS MALDI-TOF MS system (bioMérieux, France; database version 3.2.0). the manufacturer's guidelines were followed. The quality control strain of *Escherichia coli* ATCC 8739 was used with test isolates simultaneously for calibration and validation. [7]

Characterization of Isolates

All isolates were characterized biochemically using the VITEK® 2 compact system (bioMérieux, France; database version 9.02) and GN identification cards (bioMérieux, France). The bacterial isolates were subculture on Tryptone Soya Agar (TSA) and processed in accordance to the user manual. Automatically, the report was generated from the 9.02v version of the database after 10 h of incubation. [8]

Serotype Confirmation

The serotypes of all isolates were confirmed with a commercial antiserum kit (Cat No: BD Difco™ Becton, Dickinson and Company (BD), US) following Kauffmann–White classification scheme for Salmonella O and H antisera. [9]

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the VITEK 2 system and AST-GN81 cards (bioMérieux, France) according to manufacturer guidelines. The isolates were tested against commonly used antibiotics including amoxicillin/clavulanate, cefazolin, ceftazidime, ceftriaxone, cefepime, meropenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, tetracycline and trimethoprim/sulfamethoxazole. Results were interpreted as susceptible, intermediate, or resistant based on established clinical breakpoints of and Clinical & Laboratory Standards Institute (CLSI). [8]

Antibiotic Resistance Categorization

Based on antimicrobial susceptibility testing (AST) profiles, isolates were categorized according to standard resistance definitions. Multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi strains were defined as isolates resistant to the first-line antibiotics ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Extensively drug-resistant (XDR) *Salmonella enterica* serovar Typhi strains exhibited additional resistance to fluoroquinolones and third-generation cephalosporins, thereby severely limiting available therapeutic options. [10]

Data Analysis

Data were assembled and evaluated to determine the prevalence of *Salmonella enterica* serovar Typhi diagonally in various types of clinical specimens. The MDR, XDR, and non-resistant prevalence was determined according to specimen source. Graphic statistical analysis was used to summarize prevalence and resistance patterns.

Results

A total (n=115) of *Salmonella enterica* serovar Typhi clinical isolates were obtained from routine clinical specimens predominantly blood (74.78%), followed by stool (16.52%), urine (7.83%) and bone marrow (0.87%) samples. Isolates were successfully grown on blood agar plate (Figure 1). The study demonstrated that blood samples constituted the highest proportion of *Salmonella enterica* serovar Typhi isolates followed by stool, Blood specimens accounted for the highest recovery rate, indicating that bloodstream infection remains the primary clinical manifestation of typhoid fever in the studied population (Table 1, Figure 2). The frequency of blood isolates is found 80% in children aged ≤10 years. No significant difference was observed in the prevalence of Salmonella typhi or XDR with respect to gender.

All isolates were successfully identified at genus as *Salmonella enterica species* using MALDI-TOF MS, while VITEK 2 compact systems demonstrating high agreement between both diagnostic platforms and supporting their reliability for rapid serotype-level identification in routine clinical microbiology. The serotypes were confirmed with 100% accuracy through serological testing using overnight-grown cultures on Mueller–Hinton agar. Confirmation was performed using a commercially available latex agglutination kit based on specific antigen–antibody reactions (Figure 3).

Antimicrobial susceptibility profiling revealed a significant burden of resistance among the isolates. The distribution of resistance phenotypes showed a dominance of MDR and XDR strains compared to non-resistant isolates. XDR isolates were most frequently associated with blood samples, while MDR isolates were distributed across both blood and stool specimens. Non-MDR/non-XDR isolates were comparatively fewer and were observed across all specimen types in lower proportions (Table 2, Figure 4).

Overall, the data verified high prevalence of resistant phenotypes of *Salmonella enterica* serovar Typhi in Karachi. This displayed an ongoing public health concern leading to therapeutic limitations and the need for continuous surveillance programs.

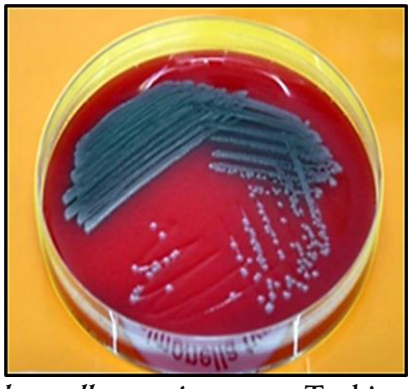

Figure 1 ***Salmonella enterica*** **serovar Typhi on blood agar**

Table 1. Frequency of *Salmonella enterica* Serovar Typhi Isolates by Clinical Specimen Type

Specimen Type	Number of Isolates (n)	Percentage (%)
Blood	86	74.78
Stool	19	16.52
Urine	9	7.83
Bone marrow	1	0.87
Total	**115**	

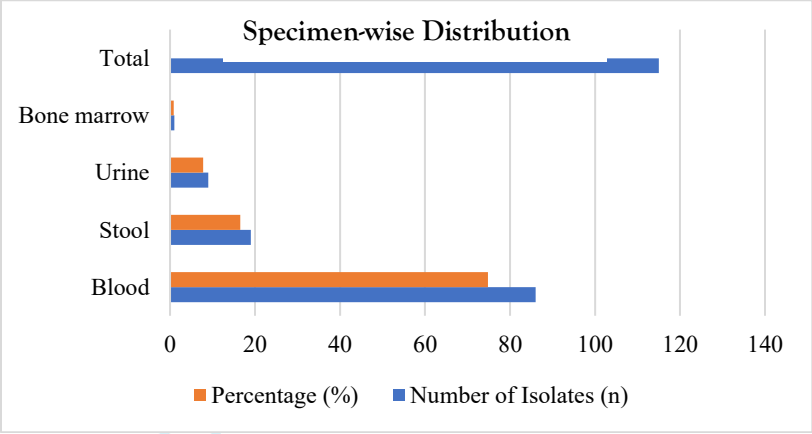

Figure 2 Frequency of *Salmonella enterica* Serovar Typhi Isolates

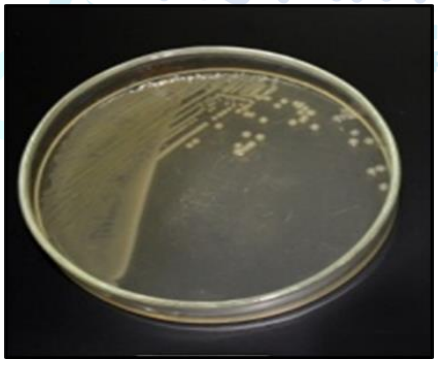

Figure 3 *Salmonella enterica* serovar Typhi on Mueller Hinton Agar

Table 2: Antimicrobial Resistance Profile

Resistance Category	n (%)				
	Blood	Stool	Urine	Bone marrow	Total
XDR	63 (73.3%)	2 (10.5%)	3 (33.3%)	0 (0%)	68 (59.1%)
MDR	14 (16.3%)	6 (31.6%)	2 (22.2%)	1 (100%)	23 (20.0%)
Non-MDR / Non-XDR	9 (10.5%)	11 (57.9%)	4 (44.4%)	0 (0%)	24 (20.9%)
Total	**86 (100%)**	**19 (100%)**	**9 (100%)**	**1 (100%)**	**115 (100%)**

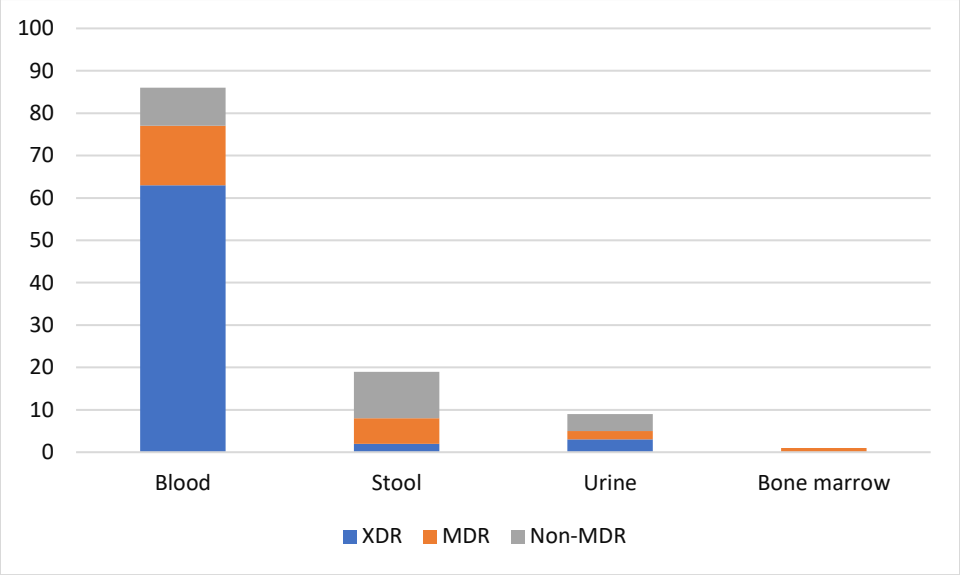

Figure 4 ***Salmonella enterica*** **serovar Typhi Clinical Isolates Resistance Pattern**

Discussion and Conclusion

We found a significantly high burden of XDR *Salmonella enterica* serovar Typhi during 2023 in Karachi, Pakistan. Karachi is a metropolitan city and is known as a typhoid-endemic region. The escalated prevalence of XDR and MDR strains of *S. typhi* provides reason for its existence, recurrent outbreaks, and failure of antibiotic treatment.

Blood specimens submitted for culture showed the highest percentage of XDR isolates, demonstrating their bigger diagnostic value in detecting active bacteremia during the early course of infection. In comparison, stool and urine samples established a lower frequency of isolation and a comparatively high fragment of non-XDR / non-MDR strains. This is consistent with sporadic shedding and later phases of disease progression. We have a limited bone marrow sample, although it is the most sensitive specimen, but it is not feasible for epidemiological interpretation due to its very small sample size. A study from Pakistan (2017–2019) on substantial blood-culture datasets showed 57–64% of *S. typhi* isolates were XDR, with a particularly high burden in Sindh. That study also reported that children aged 2–14 years were the most affected age group. [11,12] Another study from Lahore and other cities presented XDR magnitudes of ~46–48% among blood isolates, with an additional 24–25% MDR strains. [13,14]

In our study, high prevalence of resistance to first-line antibiotics, including ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, along with reduced susceptibility to fluoroquinolones, was principally displayed. Unfortunately, a substantial proportion of isolates carry resistance to first-line agents, fluoroquinolones, and third-generation cephalosporins.

The high resistance against the first and second lines of antibiotics imposes a threat for clinical management and narrowing treatment options. It necessitates the frequent use of high concentrations of antibiotics and last-choice drugs, which lead to increased treatment cost, risk of additional resistance selection, toxicity, and organ failure [13].

During COVID-19, a study from Peshawar was conducted on pediatric samples and reported a significant proportion of XDR blood isolates. [4] This could be due to the overuse and misuse of antibiotics at that time period. Other research findings from South Asia also highlighted the similar factors along with insufficient hygiene. [15]

In conclusion, our findings confirm the sustained and progressive prevalence of XDR *Salmonella enterica* serovar Typhi among clinical isolates, particularly in blood samples at Karachi, Pakistan. Continuous laboratory surveillance, standardized antibiotic treatment, and infection control

strategies are essential to mitigate the spread of resistant phenotypes and to preserve the effectiveness of remaining therapeutic options.

REFERENCES

1. Buzilă ER, Dorneanu OS, Trofin F, Sima CM, Iancu LS. Assessing *Salmonella typhi* pathogenicity and prevention: The crucial role of vaccination in combating typhoid fever. International Journal of Molecular Sciences. 2025 Apr 23;26(9):3981.
2. Ishtiaq A, Khalil S, Khalil S, Ahmed F, Ahmad B, Ghaffar A, Khalid M, Azam SM, Imran M. Prevalence of Typhoid Fever among Different Socio-Demographic Groups in District Bahawalnagar, Pakistan: Prevalence of Typhoid Fever. Pakistan Journal of Health Sciences. 2023 Nov 30:138-43.
3. Ahmad J, Khan MA, Atif M, Rahman ZU, Nasir A, Ahmad HM, Haroon A, Sarwar A, Khan MA, Fazal N. Incidence and antimicrobial resistance profile of multi-drug resistant Salmonella typhi isolated from blood samples in Khyber Pakhtunkhwa, Pakistan. Diagnostic Microbiology and Infectious Disease. 2025 Sep 26:117122.
4. Taleb MH, Elmanama AA, Taleb AH, Tawfick MM. Pre-and post-COVID-19 antimicrobial resistance profile of bacterial pathogens, a comparative study in a tertiary hospital. The Journal of Infection in Developing Countries. 2023 May 31;17(05):597-609.
5. Nganga AK, Mweu MM. Diagnostic accuracy of three tests for *Salmonella typhi* infection in adult patients presenting to health facilities in an informal urban settlement in Nairobi, Kenya. Preventive Medicine Reports. 2025 Oct 15:103278.
6. Sunarno, Nursofiah S, Hartoyo Y, Amalia N, Febrianti T, Febriyana D, Saraswati RD, Puspandari N, Sariadji K, Khariri, Rukminiati Y. Long-term storage of bacterial isolates by using tryptic Soy Broth with 15% glycerol in the deep freezer (-70 to-80℃). InIOP Conference Series: Earth and Environmental Science 2021 Nov (Vol. 913, No. 1, p. 012070). IOP Publishing.
7. Brauge T, Trigueros S, Briet A, Debuiche S, Leleu G, Gassilloud B, Wilhelm A, Py JS, Midelet G. MALDI-TOF mass spectrometry fingerprinting performance versus 16S rDNA sequencing to identify bacterial microflora from seafood products and sea water samples. Frontiers in Marine Science. 2021 Apr 28;8:650116.
8. Akter F, Shamimuzzaman M. Detection of invA Gene by PCR and Identification with Susceptibility Pattern Evaluation by VITEK 2 of Salmonella Isolates. Journal of Applied Biological Sciences. 2024 Oct 1;18(3):235-52.
9. Neupane DP, Dulal HP, Song J. Enteric fever diagnosis: current challenges and future directions. Pathogens. 2021 Apr 1;10(4):410.
10. Fatima G, Kazmi SS, Kainat S. XDR/MDR Salmonella: An experience from a tertiary care hospital, Karachi, Pakistan. International Journal of Infectious Diseases. 2020 Dec 1;101:37.
11. Qamar FN, Yousafzai MT, Qazi I, Qureshi S, Bar-Zeev N, Sultana S, Jawwad M, Hotwani A, Irfan S, Memon MA, Iftikhar I. Trends of enteric fever and emergence of extensively drug-resistant typhoid in Pakistan: population-based laboratory data from 2017–2019. InOpen Forum Infectious Diseases 2025 Apr (Vol. 12, No. 4, p. ofaf106). US: Oxford University Press.

12. Qamar FN, Yousafzai MT, Dehraj IF, Shakoor S, Irfan S, Hotwani A, Hunzai MJ, Thobani RS, Rahman N, Mehmood J, Hemlock C. Antimicrobial resistance in typhoidal Salmonella: surveillance for enteric fever in Asia project, 2016–2019. Clinical Infectious Diseases. 2020 Nov 1;71(Supplement_3):S276-84.
13. Zakir M, Khan M, Umar MI, Murtaza G, Ashraf M, Shamim S. Emerging trends of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella typhi* in a tertiary care Hospital of Lahore, Pakistan. Microorganisms. 2021 Nov 30;9(12):2484.
14. Jabeen K, Saleem S, Jahan S, Nizamudin S, Arshad F, Huma ZE, Raza SM, Mehmood M, Roman M, Haq FU. Molecular characterization of extensively drug-resistant *Salmonella enterica* Serovar Typhi clinical isolates from Lahore, Pakistan. Infection and Drug Resistance. 2023 Dec 31:2987-3001.
15. Khan M, Tu-Zahra F, Gul A, Saleem MZ, Riaz M, Gul W. Extensively Drug-resistant *Salmonella enterica* serovar Typhi in pediatric population during CoVID-19 pandemic. International Journal of Infectious Diseases. 2022 Mar 1;116:S4.