Original Article

Prevalence and Antibiogram Pattern of Typhoidal Salmonella at a Tertiary Care Hospital in Western Maharashtra

Meghna Chauhan[1], Sandeep Ninawe[1], Neha Singh[1], R Mahesh Reddy[1], Sunita Patil[1,*]

[1]Department of Microbiology, Armed Forces Medical College, Pune, Maharashtra, India

***Correspondence:** drsunipat@gmail.com

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Abstract

Background: Infections from Salmonella Typhi and Paratyphi cause a life-threatening systemic febrile illness which is collectively known as enteric fever. The burden of disease is high in many underdeveloped countries due to lack of hygiene and poor access to safe drinking water. Similarly, the treatment of the disease remains a challenge due to the global spread of multidrug-resistant Salmonella strains. An accurate diagnosis with an appropriate antibiotic is necessary for effective treatment as resistance can vary from region to region over time.
Methods: A retrospective study was conducted to check the prevalence of Typhoidal Salmonella from a tertiary care center. Data from January 2023 to December 2024 of blood culture bottles collected from clinically suspected cases of enteric fever were retrieved and analyzed. Isolation and antibiotic susceptibility testing of Salmonella species was carried out by VITEK 2 COMPACT, an automated instrument at the department of Microbiology.
Results: A total data from 10,333 paired blood culture bottles were collected from suspected enteric fever cases. The prevalence of Typhoidal Salmonella was 1.21%. The study also revealed a changing antibiotic resistance pattern by Salmonella species with increased resistance to ciprofloxacin and increased sensitivity to chloramphenicol as well as cotrimoxazoles.
Conclusion: The study highlights the importance of antimicrobial resistance monitoring under the Anti-microbial Resistance Research Network across India.

Keywords: Salmonella; Enteric fever; Antibiogram; Multidrug-resistant Salmonella

Introduction

Enteric fever, or Typhoid fever, is a serious bloodstream infection primarily characterized by fever, headache, malaise, and other general symptoms. It is a potentially life-threatening systemic illness caused by *Salmonella enterica* subspecies *enterica* serovar Typhi, also referred to as *Salmonella* Typhi [1, 2]. Paratyphoid fevers, caused by Paratyphi A, B, and C, are clinically similar and can exhibit comparable severity in their complications [3, 4].

An estimated 11 million cases of typhoid and 3.5 million cases of paratyphoid occur worldwide each year, causing over 100,000 deaths, predominantly in low- and middle-income countries [5, 6]. According to a study by Jon et al. (2023), India recorded approximately 10 million cases of typhoid fever in 2021, making it the country with the highest burden of typhoid worldwide [7].

Despite the significant burden, systematic surveillance is limited, and there is considerable variation in incidence both within and between countries [8]. The true incidence of *Salmonella* infections may be obscured by the widespread use of antibiotics [9]. Resistance to azithromycin, which became prevalent after the rise of fluoroquinolone resistance, and emerging resistance to third-generation cephalosporins are growing concerns [10].

Historically, the first-line treatments for enteric fever included ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. However, with the rise of multidrug-resistant (MDR) *S. Typhi* in the 1970s, fluoroquinolones became the standard treatment for typhoid fever. Since 2000, due to reduced efficacy of ciprofloxacin, ceftriaxone and azithromycin have increasingly been used for complicated and uncomplicated typhoid fevers, respectively. Additionally, the combination of ceftriaxone and azithromycin has proven to be highly effective for treating initial episodes of complicated typhoid fever [1]. According to the WHO, the term MDR describes resistance to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol, while XDR describes resistance to chloramphenicol, ampicillin, co-trimoxazole, and fluoroquinolones, as well as third-generation cephalosporins.

The study of the antibiogram of *Salmonella* is a necessity to understand the changing antibiotic resistance pattern in order to guide effective treatment and to modify existing antibiotic policy. This study aims to determine the prevalence and antibiotic susceptibility pattern of *Salmonella* species at a tertiary care center in Maharashtra in comparison with Antimicrobial Resistance Surveillance and Research Network (AMRSN) India data.

Materials and Methods

A retrospective study was conducted in a tertiary care hospital in Maharashtra, India. Blood culture data was retrieved from January 2023 to December 2024 from clinically suspected enteric fever cases. The diagnosis of enteric fever was based on symptoms of fever with headache, myalgia, nausea, vomiting, and clinical signs of relative bradycardia, Roth spots, and hepatosplenomegaly.

As per the records, all the blood samples were processed in the clinical microbiology laboratory according to standard operating procedures.

Sample collection: Blood samples were collected in a pair of blood culture bottles (BACT/ALERT® Culture Media Bottles, Biomerieux) before the administration of antibiotics from patients admitted with suspected enteric fever. The positive blood culture bottles were further processed for bacterial identification using MacConkey agar, TSI, and the VITEK 2 ID system.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was done by using the VITEK 2 COMPACT as well as the disk diffusion method, according to the guidelines of the Clinical Laboratory Standards Institute (CLSI M100 33rd edition). All the antibiotic discs were obtained from HI-MEDIA (Himedia, India). The disk diffusion test was done for the following antibiotics: azithromycin (AZM, 15 μg), chloramphenicol (C, 30 μg), cotrimoxazole (SXT, 25 μg), ampicillin (AMP, 10 μg), and ciprofloxacin (CIP, 5 μg). A standard inoculum, which was adjusted to 0.5 McFarland, was swabbed onto Muller-Hinton agar (Himedia, India). After dispensing the discs on the plate, they were incubated at 35–37°C for 18–24 hours. The reference strain *Escherichia coli* ATCC 25922 was used as a control for each batch of drugs.

VITEK 2 Antibiotic susceptibility testing: For antibiotic susceptibility testing, the AST N 405 panel was used. The following antibiotics are tested in this panel: Amikacin, Amoxicillin-clavulanic acid, Cefepime, Cefoperazone-salbactam, Ceftriaxone, Cefuroxime, Ciprofloxacin, Colistin, Ertapenem, Gentamicin, Imipenem, Meropenem, Piperacillin-tazobactam, and Trimethoprim/sulfamethoxazole.

The antimicrobial susceptibility data was compared with the ICMR Antimicrobial Resistance Surveillance Network (AMRSN) data for the corresponding year, 2023. The ICMR collects data from different tertiary care hospitals across India.

IEC approval: Institute Ethics Committee approval was obtained.

Results

A total of 10,333 paired blood culture bottles were collected from suspected enteric fever cases. After 24-48 hours of incubation in the BACT/ALERT blood culture instrument, a total of 63 (1.21%) bottles flagged positive for growth. Analysis of the clinical data of the flagged bottles revealed that 44 (66.84%) isolates were from male patients and 19 (30.16%) were from female patients.

Microbiological culture of flagged bottles on MacConkey agar showed pure growth of non-lactose fermenting colonies (Figure 1). Species identification based on the VITEK 2 COMPACT, as well as using biochemical reactions, revealed the isolation of *Salmonella* Typhi, Paratyphi A, and Paratyphi B (Figure 2).

Of these 63 samples, 49 were identified as *Salmonella enterica* serotype Typhi, 10 were identified as *Salmonella enterica* serotype Paratyphi A, and 04 were identified as *Salmonella enterica* serotype Paratyphi B (Table 1) (Figure 3).

Antibiotic susceptibility testing (Table 2) (Figure 4) revealed higher sensitivity to chloramphenicol (96.83%), cotrimoxazole (98.41%), ampicillin (95.24%), azithromycin, and ceftriaxone (92.06%), but poor sensitivity to ciprofloxacin (4.76%). No multidrug-resistant or extensively drug-resistant *Salmonella* isolates were found.

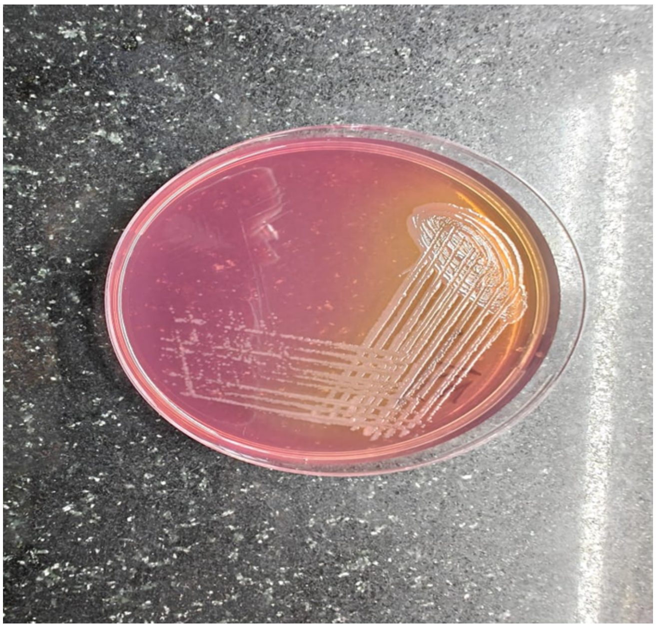

Figure 1: Non-lactose fermenting growth obtained on MacConkey agar.

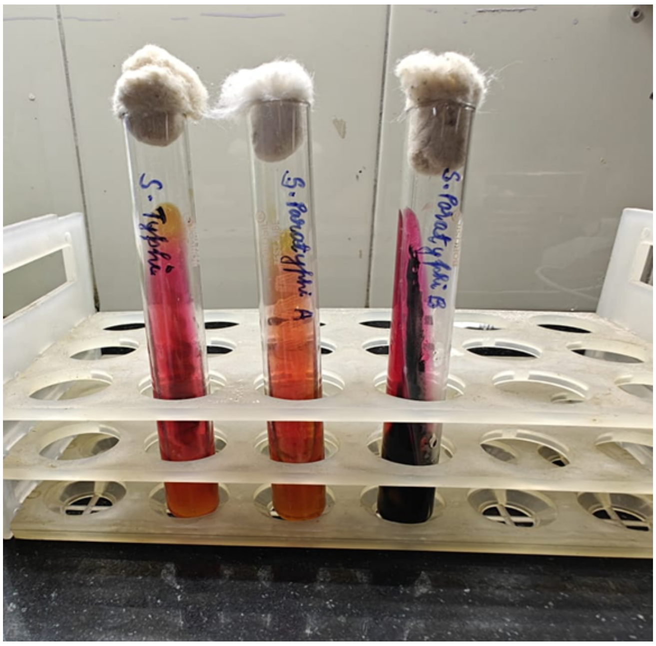

Figure 2: Triple sugar iron agar test for typhoidal *Salmonella* species. Note: Tube 1: *Salmonella* Typhi - K/A (Alkali/Acid) with no gas and specks of H2S on TSI. Tube 2: *Salmonella* Paratyphi A - K/A (Alkali/Acid) with gas and no H2S on TSI. Tube 3: *Salmonella* Paratyphi B - K/A (Alkali/Acid) with abundant H2S on TSI.

Table 1: *Salmonella* species isolated from blood culture bottles.

Total sample	Flagged positive	*S. enterica* Typhi, n (%)	*S. enterica* Paratyphi A, n (%)	*S. enterica* Paratyphi B, n (%)
N=63	49 (77.78%)	10 (15.87%)	04 (6.35%)	

Discussion

Typhoid fever is a major public health concern in India as well as worldwide. Indian data on the prevalence of *Salmonella* reveals substantial heterogeneity across the country, with a high prevalence in urban areas.

According to India's AMRSN annual report for 2023, a total of 1,338 Typhoidal *Salmonella* were isolated from blood across India, with a predominance of *Salmonella* Typhi over Paratyphi A and B [11]. Similarly, we report a total of 63 (1.21%) Typhoidal *Salmonella* with a predominance of *Salmonella* Typhi (49) over Paratyphi A (10) and B (4) during the study period.

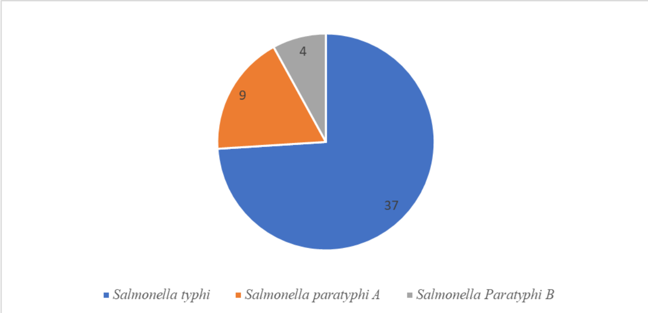

Figure 3: Distribution of various Typhoidal *Salmonella* isolated from blood.

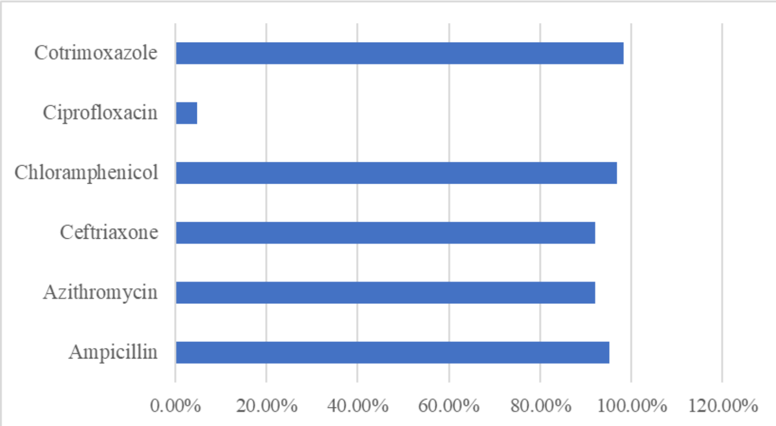

Figure 4: Percentage susceptibility pattern of *Salmonella* species.

Table 2: Antibiotic sensitivity pattern of *Salmonella* species.

Bacterial isolate	AMP	AZM	CRO	C	CIP	SXT
N=63	60 (95.24%)	58 (92.06%)	58 (92.06%)	61 (96.83%)	03 (4.76%)	62 (98.41%)
S. enterica Typhi (n=49)	47 (95.92%)	45 (91.84%)	46 (93.88%)	48 (97.96%)	01 (2.04%)	48 (97.96%)
S. enterica Paratyphi A (n=10)	09 (90%)	09 (90%)	09 (90%)	10 (100%)	01 (10%)	10 (100%)
S. enterica Paratyphi B (n=04)	04 (100%)	04 (100%)	03 (75%)	03 (75%)	01 (25%)	04 (100%)

Note: AMP, Ampicillin; AZM, Azithromycin; CRO, Ceftriaxone; C, Chloramphenicol; CIP, Ciprofloxacin; SXT, Trimethoprim-Sulfamethoxazole.

Table 3: Comparison of antimicrobial susceptibility pattern of *Salmonella* Typhi isolates.

Antibiotics	*S.* Typhi AMRSN National data	*S.* Typhi *	*S.* Typhi (Balaji et al 2021)
Ampicillin	97.5%	95.92%	97.3%
Azithromycin	97.4%	91.84%	99.9%
Ceftriaxone	96.8%	93.88%	100%
Chloramphenicol	98.2%	97.96%	96.1%
Ciprofloxacin	2.1%	2.04%	2.1%
Trimethoprim-sulfamethoxazole	96.8%	97.96%	96.4%

Note: * Denotes *Salmonella* isolates from the current study. Comparison of *Salmonella* study isolates was done with AMRSN data and a multicentric study from 19 different centers across India.

According to the AMRSN report for 2023, the antimicrobial susceptibility results for *Salmonella* Typhi revealed that 97% of isolates were susceptible to ampicillin, 96.8% were susceptible to trimethoprim-sulfamethoxazole, and 98% were susceptible to chloramphenicol. Additionally, 3rd generation cephalosporins exhibited a susceptibility rate of 96.8% in 2023, whereas fluoroquinolones showed a susceptibility rate of only 2%. Azithromycin, on the other hand, exhibited a susceptibility rate of 97% during the same period [11] (Table 3).

In our study, the antimicrobial susceptibility results for *Salmonella* Typhi revealed that 97.96% of isolates were susceptible to chloramphenicol and cotrimoxazole, 95.92% were susceptible to ampicillin, 91.84% were susceptible to azithromycin, 93.88% were susceptible to ceftriaxone, and 2.04% were susceptible to ciprofloxacin (Table 2 and 3).

A comparison of the AMR pattern with AMRSN data, as well as data from a multicentric study by Balaji et al. (2021) [1], revealed similar results. All the *Salmonella* isolates revealed poor sensitivity to ciprofloxacin and very good sensitivity

Table 4: Comparison of antimicrobial susceptibility pattern of *Salmonella* paratyphi A.

Antibiotics	*S.* Paratyphi A AMRSN National data	*S.* Paratyphi A*	*S.* Paratyphi A (Balaji et al 2021)
Ampicillin	100%	90%	100%
Azithromycin	-	90%	–
Ceftriaxone	98.9%	90%	100%
Chloramphenicol	98.9%	100%	98.9%
Ciprofloxacin	6.9%	10%	6.9%
Trimethoprim-sulfamethoxazole	100%	100%	100%

Note: * Denotes *Salmonella* isolates from the current study. Comparison of *Salmonella* study isolates was done with AMRSN data and a multicentric study from 19 different centers across India.

to chloramphenicol as well as cotrimoxazole. However, another recent multicentric study by Sahai et al. (2025) reported increased resistance to third-generation cephalosporins [12].

According to the AMRSN report for 2023, *Salmonella* Paratyphi A exhibited high susceptibility rates to various antimicrobial agents. Ampicillin and cotrimoxazole were 100% sensitive, while ceftriaxone and chloramphenicol exhibited 98.9% sensitivity [11]. However, susceptibility to ciprofloxacin, a fluoroquinolone, was notably low at only 6.9% during the same period (Table 4).

In our study, the antimicrobial susceptibility results for *Salmonella* Paratyphi A revealed that 100% of isolates were susceptible to chloramphenicol and cotrimoxazole, 90% of isolates were susceptible to ampicillin, azithromycin, and ceftriaxone, and only 10% of isolates were susceptible to ciprofloxacin (Table 2 and 4).

A comparison of AMR data obtained from the current study with AMRSN data, as well as with data from Balaji et al. [1], revealed similar results with poor susceptibility to ciprofloxacin.

In our study, the antimicrobial susceptibility for *Salmonella* Paratyphi B revealed 100% susceptibility to ampicillin, azithromycin, and cotrimoxazole, 75% susceptibility to ceftriaxone and chloramphenicol, and 50% susceptibility to ciprofloxacin (Table 2). However, no comparative data is available for *Salmonella* Paratyphi B in the AMRSN report for 2023 [11].

The study highlights the circulation of all three *Salmonella* species with a changing pattern of antibiotic resistance, with no multidrug-resistant *Salmonella* isolates. The re-emergence of sensitivity to chloramphenicol and cotrimoxazole was noted in *Salmonella* species, as also observed by Varghese et al. (2013) [13]. The drug cotrimoxazole remains a good oral option, with IV ceftriaxone reserved for admitted patients with enteric fever. Chloramphenicol stands as the best alternative to ceftriaxone in multidrug-resistant *Salmonella* isolates.

To manage the spread of antibiotic-resistant *Salmonella*, it is essential to ensure appropriate antimicrobial use based on regular AMR surveillance, implement antibiotic stewardship programs, and develop and enforce relevant antibiotic policies. Antimicrobial stewardship will be the important key to preventing emerging resistance in *Salmonella* isolates.

Conclusion

The prevalence of Typhoidal *Salmonella* in our study was low at 1.21%. The study revealed a changing antibiotic resistance pattern by *Salmonella* species with good sensitivity to cotrimoxazole, ceftriaxone, and chloramphenicol and increased resistance to ciprofloxacin. Chloramphenicol stands as the best alternative to ceftriaxone in multidrug-resistant *Salmonella* isolates.

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Conflicts of Interest None declared.

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