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# Prevalence and Antimicrobial Susceptibility Profile of *Salmonella* spp. Isolated from Blood Cultures of Patients with Suspected Enteric Fever at a Specialized Laboratory in Kashmir, North India

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## ABSTRACT

**Introduction:** Enteric fever, caused by *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A, is a prevalent infection in developing countries. Although highly endemic in India, data on the prevalence and antimicrobial resistance patterns of this pathogen in Kashmir remain scarce. Therefore, this study aimed to determine the prevalence and antimicrobial susceptibility profile of *Salmonella* isolates from blood cultures of patients with suspected enteric fever. **Methods:** A total of 970 blood culture samples from patients with suspected enteric fever were processed at Dr. Qadri's Hematology Center and Clinical Laboratory, Srinagar, between October 2022 and September 2024. Samples were inoculated into BacT/ALERT® culture media. Positive cultures were sub-cultured on blood and MacConkey agar plates, and the resulting isolates were identified and subjected to antimicrobial susceptibility testing using the VITEK® 2 system according to the latest Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** Of the 970 blood cultures processed, 37 (3.8%) were positive for *Salmonella* spp., with 23 (62%) isolates identified as *S. Typhi* and 14 (38%) as *S. Paratyphi* A. All isolates showed 100% susceptibility to Cefepime, Piperacillin/Tazobactam, Carbapenems, Azithromycin, Tetracyclines, Co-trimoxazole, and Polymyxins. Susceptibility to Ceftriaxone and Tigecycline was 97.3%. In contrast, susceptibility rates were markedly low for the fluoroquinolones Ciprofloxacin (2.7%) and Levofloxacin (5.4%). No multidrug-resistant (MDR) isolates were detected in this study. **Conclusion:** This study confirms that *Salmonella enterica* remains a significant cause of bloodstream infections in this resource-limited setting. However, the high prevalence of fluoroquinolone resistance, despite the absence of MDR strains, represents the primary therapeutic challenge in managing these infections. Therefore, extensive surveillance, enhanced diagnostics, and treatment protocols guided by antimicrobial susceptibility testing (AST) reports are crucial to optimize patient outcomes and combat emerging resistance in our region.

## INTRODUCTION

*Salmonella*, a genus in the family *Enterobacteriaceae*, is a facultatively anaerobe, motile, Gram-negative bacilli with over 2,500 serovars (serotypes). Taxonomically, the genus *Salmonella* (*S.*) contains two species, *Salmonella enterica* and *Salmonella bongori*, with the former responsible for almost all human infections [1]. Typhoidal *Salmonella* include serovars such as *Salmonella enterica* serovar Typhi, *S. enterica* serovar Paratyphi A, *S. enterica* serovar Paratyphi B, and *S. enterica* serovar Paratyphi C. These serovars cause febrile illnesses known as typhoid and paratyphoid fever, which are collectively known as enteric fever [2]. Globally, 11 to 18 million cases of



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enteric fever are reported annually, with an estimated mortality rate of approximately 1% [3]. Typhoidal *Salmonella* are primarily transmitted via the fecal-oral route. The risk of bloodstream infection remains high in developing nations where typhoidal *Salmonella* are endemic and where poor sanitation and limited access to hygienic food and safe water are prevalent [4]. Children and young adults are more commonly affected [5, 6]. After entering via the fecal-oral route, *S. Typhi* first colonizes the distal ileum, followed by bloodstream (primary bacteremia), liver, spleen, bone marrow, and gallbladder, leading to bacteremia, which can progress to sepsis [7]. The infection typically presents with fever, headache, malaise, chills and rigors, abdominal pain, constipation or diarrhea, and a transient maculopapular rash (rose spots) [8]. If left untreated, complications typically develop between the second and fourth week, including intestinal bleeding and perforation, and encephalopathy, resulting in high mortality [9]. Both *S. Typhi* and *S. Paratyphi* A are associated with relapse, reinfection, and chronic carriage [10]. Effective prevention of enteric fever involves safe food and water precautions, frequent handwashing, especially before meals, and typhoid vaccination [11]. In India, the typhoid vaccine is currently not part of the Universal Immunization Programme (UIP), whereas the World Health Organization (WHO) recommends its inclusion in routine vaccination programs to prevent and control the spread of enteric fever in endemic regions [12].

The laboratory-based diagnosis of enteric fever remains challenging, as the currently available serological, culture-based, and molecular tests do not provide an ideal balance of sensitivity and specificity [13, 14]. The definitive diagnosis is established by isolating *Salmonella enterica* from samples, typically from blood or bone marrow. A positive culture enables antimicrobial susceptibility testing (AST), slide agglutination tests, and molecular confirmation. While blood culture has a sensitivity of 40% to 80%, bone marrow culture is more sensitive (>80%) but is also more invasive and painful to perform. As a result, empirical antibiotic therapy is often initiated in patients with suspected enteric fever [15]. The volume of blood collected for culture is crucial, as it directly impacts the diagnostic yield. The optimal time for blood culture collection is during the first two weeks of illness, when the bacterial load is highest [16].

Treatment with appropriate antibiotics reduces the mortality associated with enteric fever from 10–15% to <1% and dramatically shortens the fever duration from weeks (3–4) to just a few days (3–5) [17]. Early-diagnosed enteric fever is usually managed with a single antibiotic [8]. However, the emergence of antibiotic-resistant *S. Typhi* and *S. Paratyphi* A has drastically reduced the number of effective treatment options in many regions. Until the mid-1970s, the first-line antibiotics used for managing enteric fever cases were Chloramphenicol, Ampicillin, and Co-trimoxazole. However, by the late 1980s, widespread resistance to these drugs emerged due to the spread of IncHI1 plasmids, which conferred simultaneous resistance to all three antibiotics. Strains with this resistance profile were defined as MDR *S. Typhi* and remain widely disseminated. Although the renewed susceptibility to these first-line antibiotics is a positive development, it has been accompanied by a concurrent rise in Fluoroquinolone resistance [18]. In response to the emergence and rapid dissemination of MDR *Salmonella*, Fluoroquinolones such as Ciprofloxacin were recommended for treating enteric fever. However, resistance to these drugs developed rapidly. Reduced susceptibility to the Fluoroquinolone class is often associated with Nalidixic acid resistance (NAR). As a result, NAR is used as a surrogate marker to predict treatment failure with Fluoroquinolones, even when the Clinical and Laboratory Standards Institute (CLSI) criteria for Fluoroquinolone susceptibility are met [19, 20]. In regions where both multidrug resistance and Fluoroquinolone resistance are prevalent, Azithromycin and third-generation Cephalosporins are often used to treat enteric fever. However, there is growing concern as *S. Typhi* strains resistant to extended-spectrum Cephalosporins have emerged. Specifically, *S. Typhi* isolates have been found to produce extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases. In contrast, Azithromycin resistance in typhoidal *Salmonella* is extremely rare [21]. Extensively drug-resistant (XDR) strains of *S. Typhi* and *S. Paratyphi* A are those that exhibit resistance to all first-line antibiotics, Fluoroquinolones, and third-generation Cephalosporins. The therapeutic options for these XDR isolates are limited, but often include Azithromycin, Carbapenems, and Tigecycline [22].

This study aimed to determine the prevalence and antimicrobial susceptibility profile, including minimum inhibitory concentrations (MICs), of *Salmonella* isolates recovered at a specialized laboratory in Srinagar, Kashmir, North India.

## MATERIAL AND METHODS

**Study design.** This retrospective, cross-sectional study was conducted at Dr. Qadri's Hematology Center and Clinical Laboratory, a specialized clinical laboratory in Srinagar, North India. The study analyzed data from all blood cultures collected between October 2022 and September 2024 from patients with suspected enteric fever to determine the prevalence and antimicrobial susceptibility profile of *Salmonella* spp.

**Study population.** The study population comprised patients of all ages and both genders referred by clinicians with a clinical suspicion of enteric fever. Exclusion criteria were recent antibiotic use (within the last 14 days) or a confirmed alternative diagnosis for which a cause of fever was identified through clinical assessment or rapid



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diagnostic tests (*e.g*., dengue, malaria, viral hepatitis, or urinary tract infections).

**Sample collection.** A total of 970 whole blood specimens were collected from patients with a clinical suspicion of enteric fever. Following standard venipuncture techniques and WHO guidelines, blood was aseptically inoculated directly into BacT/ALERT® (bioMérieux) culture bottles (10 mL for adults and 4 mL for pediatric patients) [23]. All inoculated bottles were transported to the laboratory and incubated within 2 hours of collection [24]. Incubation and monitoring were performed using the bioMérieux BacT/ALERT® 3D system, a fully automated instrument that utilizes colorimetric $CO_2$ detection method.

**Culture and identification.** All inoculated BacT/ALERT® culture bottles were aerobically incubated at 37 °C for up to 5 days [25]. Bottles flagged as positive by the system were immediately subcultured onto Blood agar and MacConkey agar. Bottles remaining negative after the 5-day incubation underwent a terminal subculture. Plates were incubated aerobically at 37°C for 18–24 hours. Following incubation, plates were inspected for characteristic non-lactose fermenting colonies, which were then subjected to Gram staining. Preliminary identification of *Salmonella* spp. was based on colony morphology, Gram stain results, and a panel of biochemical tests, including oxidase and urease negative reactions, a catalase positive test, and characteristic results for Methyl Red (MR), Voges-Proskauer (VP), citrate utilization, and Triple Sugar Iron (TSI) tests. Serotyping was performed via slide agglutination using *Salmonella* polyvalent O antisera, followed by testing with monovalent O:2 and O:9, and Vi antisera to differentiate key serovars [26]. Concurrently, isolate identification was confirmed using the VITEK® 2 Compact system with the Gram-negative identification (GN) card.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility testing of *Salmonella* isolates was performed using the VITEK® 2 Compact system with the AST-N405 card. Quality control (QC) was performed for each batch of tests by testing American Type Culture Collection (ATCC) strains of *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The instrument underwent regular calibration and timely maintenance according to the manufacturer's guidelines. Minimum inhibitory concentrations (MICs) were interpreted based on the Clinical and Laboratory Standards Institute M100, 33rd edition guidelines [27]. The MIC values generated by the VITEK® 2 system were used to categorize isolates as susceptible (S), intermediate (I), or resistant (R) according to these clinical breakpoints.

**Data analysis.** Data were entered and managed in Microsoft Excel (Microsoft Corp., Redmond, WA) and analyzed using IBM SPSS Statistics, version 27 (IBM Corp., Armonk, NY). The chi-square ($\chi^2$) test was used to assess associations between categorical variables. A *P*-value < 0.05 was considered statistically significant.

## RESULTS

Of the 970 non-duplicate blood culture samples processed, 37 (3.8%) were positive for *Salmonella* spp. (Table 1). Among these 37 *Salmonella* isolates, 62.2% (23/37) were identified as *S. Typhi* and 37.8% (14/37) as *S. Paratyphi* A (Figure 1). The patients with positive cultures were predominantly male (29/37, 78.4%), with a mean age of 19.6 years (median: 16 years; range: 1–43 years). The most frequently affected age group was children aged 0–11 years (32.4%), followed by adolescents aged 12–18 years (29.7%), young adults aged 19–34 years (21.6%), and older adults aged ≥35 years (16.2%) (Table 2).

The time to positivity (TTP) for the positive cultures was also recorded. Of the 37 positive cultures, 5 (13.5%) were flagged by the BacT/ALERT® system within 24 hours, 23 (62.2%) within 48 hours, 6 (16.2%) within 72 hours, 2 (5.4%) within 96 hours, and 1 (2.7%) at 120 hours of incubation (Figure 2).

The antimicrobial susceptibility profile of *Salmonella* isolates to different antimicrobial classes is summarized in Table 3. All isolates (100%) were susceptible to Cefepime, Carbapenems, Piperacillin/tazobactam, Azithromycin, Tetracyclines, Co-trimoxazole, and Polymyxins. High rates of susceptibility were also observed for Ceftriaxone and Tigecycline (97.3%). Conversely, a high level of Fluoroquinolone resistance was observed, with susceptibility rates of only 2.7% for Ciprofloxacin and 5.4% for Levofloxacin (Figure 3).

According to Centers for Disease Control and Prevention (CDC) guidelines, *Salmonella* isolates with concurrent resistance to Ampicillin, Chloramphenicol, and Co-trimoxazole are classified as MDR. MDR isolates that are also resistant to third-generation Cephalosporins and Fluoroquinolones are defined as XDR. In this study, no MDR or XDR isolates were detected.

**Table 1.** Baseline demographic and microbiological characteristics of the study population.

| Characteristic | Category | N (%) |
|---|---|---|
| **Patient demographics (n = 970)** | | |
| | Male | 621 (64.0) |
| | Female | 349 (36.0) |
| **Blood culture results (n = 970)** | | |
| | Culture negative | 933 (96.2) |
| | Culture positive | 37 (3.8) |
| **Distribution of *Salmonella* isolates (n = 37)** | | |
| | *S. Typhi* | 23 (62.2) |
| | *S. Paratyphi* A | 14 (37.8) |





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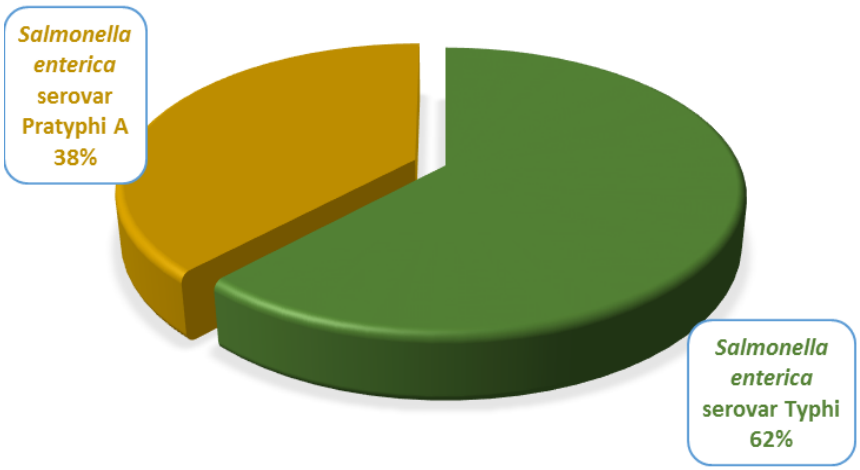


**Fig. 1.** Distribution of *Salmonella* serovars isolated from blood culture specimens.

**Table 2.** Basic characteristics of patients who had culture-positive enteric fever.

| Variable | Categories | Total cases (%) | *Salmonella* species | | *P*-value[a] |
|---|---|---|---|---|---|
| | | | *S. Typhi* | *S. Paratyphi* A | |
| Gender | Male | 29 (78.4) | 16 | 13 | 0.191 |
| | Female | 8 (21.6) | 7 | 1 | |
| Age group | Childhood (0-11 years) | 12 (32.4) | 8 | 4 | 0.926 |
| | Adolescence (12-18 years) | 11 (29.7) | 8 | 3 | |
| | Young adults (19-34 years) | 8 (21.6) | 6 | 2 | |
| | Older adults (≥35 years) | 6 (16.2) | 1 | 5 | |
| Total | | 37 (100.0) | 23 | 14 | |

[a] *P*-values calculated using Chi-square test.

**Table 3.** Antimicrobial susceptibility profile and CLSI breakpoints for *Salmonella* spp. isolates (n = 37).

| Antimicrobial class | Antimicrobial agent | S MIC breakpoints (µg/mL) [a] | S n (%) | I MIC breakpoints (µg/mL) | I n (%) | R MIC breakpoints (µg/mL) | R n (%) |
|---|---|---|---|---|---|---|---|
| Penicillin | Ampicillin | ≤ 8 | 37 (100) | 16 | 0 | ≥ 32 | 0 |
| Extended-spectrum Cephalosporins | Ceftriaxone | ≤ 1 | 36 (97.3) | 2 | 0 | ≥ 4 | 1 (2.7) |
| | Ceftazidime | ≤ 4 | 34 (91.9) | 8 | 0 | ≥ 16 | 3 (8.1) |
| | Cefepime | ≤ 2 | 37 (100) | 4–8 | 0 | ≥ 16 | 0 |
| Carbapenems | Ertapenem | ≤ 0.5 | 37 (100) | 1 | 0 | ≥ 2 | 0 |
| | Imipenem | ≤ 1 | 37 (100) | 2 | 0 | ≥ 4 | 0 |
| | Meropenem | ≤ 1 | 37 (100) | 2 | 0 | ≥ 4 | 0 |
| β-lactam/β-lactamase inhibitor combinations | Amoxicillin/Clavulanate | ≤ 8.4 | 35 (94.5) | 16.8 | 2 (5.4) | ≥ 32.16 | 0 |
| | Piperacillin/Tazobactam | ≤ 8.4 | 37 (100) | 16.4 | 0 | ≥ 32.4 | 0 |
| | Cefoperazone/Sulbactam | ≤ 16 | 33 (89.2) | 32 | 2 (5.4) | ≥ 64 | 2 (5.4) |
| Fluoroquinolones | Ciprofloxacin | ≤ 0.06 | 1 (2.7) | 0.12–0.5 | 6 (16.2) | ≥ 1 | 30 (81.1) |
| | Levofloxacin | ≤ 0.12 | 2 (5.4) | 0.25–1 | 10 (27) | ≥ 2 | 25 (67.6) |
| Aminoglycosides[b] | Gentamicin | ≤ 4 | - | 8 | - | ≥ 16 | 37 (100) |
| | Amikacin | ≤ 16 | - | 32 | - | ≥ 64 | 37 (100) |
| Macrolide | Azithromycin | ≤ 16 | 37 (100) | - | - | ≥ 32 | 0 |
| Tetracyclines | Tetracycline | ≤ 4 | 37 (100) | 8 | 0 | ≥ 16 | 0 |
| | Minocycline | ≤ 4 | 37 (100) | 8 | 0 | ≥ 16 | 0 |
| Folate pathway inhibitors | Trimethoprim-sulfamethoxazole | ≤ 20 | 37 (100) | - | - | ≥ 4.76 | 0 |
| Monobactam | Aztreonam | ≤ 4 | 31 (83.8) | 8 | 2 (5.4) | ≥ 16 | 4 (10.8) |
| Polymyxins | Colistin | ≤ 2 | 37 (100) | - | - | ≥ 4 | 0 |
| | Polymyxin B | ≤ 2 | 37 (100) | - | - | ≥ 4 | 0 |

- [a]MIC breakpoints based on CLSI M100 guideline (33rd edition); percentages shown in parentheses.
- [b]As per CLSI guidelines, *in vitro* susceptibility to aminoglycosides does not reliably predict clinical efficacy for systemic *Salmonella* infections and is therefore not reported as susceptible.
- "-" indicates not defined"
- S, Susceptible; I, Intermediate; R, Resistant.



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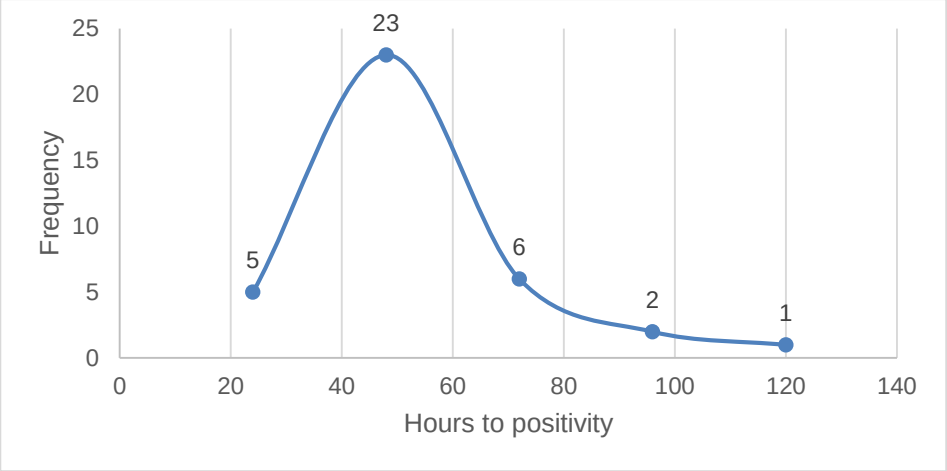


**Fig. 2.** Distribution of time to positivity (TTP) for the 37 *Salmonella*-positive blood cultures, as detected by the BacT/ALERT® automated culture system.

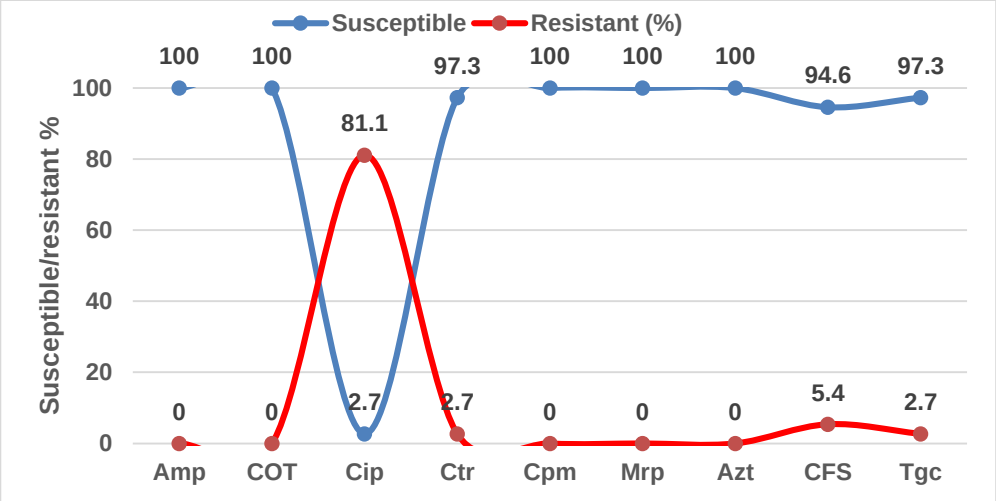


**Fig. 3.** Antimicrobial susceptibility profile of the 37 *Salmonella* spp. isolates. Abbreviations: Amp, Ampicillin; COT, Co-trimoxazole; Cip, Ciprofloxacin; Ctr, Ceftriaxone; Cpm, Cefepime; Mrp, Meropenem; Azt, Aztreonam; CFS, Cefoperazone/Sulbactam; Tgc, Tigecycline.

## DISCUSSION

Enteric fever poses a considerable disease burden in developing countries, with South Asia being a particular hotspot, where the estimated incidence exceeds 100 cases per 100,000 population. Mogasale *et al.* (2014) classified the incidence of enteric fever as low (<10 cases per 100,000 annually), middle (10–100 cases per 100,000 annually), and high (>100 cases per 100,000 annually). Annually, approximately seven million people in South Asia are affected by enteric fever, resulting in around 75,000 deaths. The highest incidence rate is reported in Pakistan (at 493.5 cases per 100,000 population), followed by India (with 360 cases per 100,000 population annually) [28, 29].

The overall prevalence of culture-positive enteric fever among suspected cases in this study was 3.8%. This prevalence rate is lower than those reported in other similar studies across India, which range from 7.6% to 18.2% [30, 31]. The higher prevalence rate reported in these studies can likely be attributed to factors such as inadequate sanitation, contaminated food and water supplies, and limited typhoid vaccine coverage. The relatively low prevalence of enteric fever in Kashmir may be due to a combination of factors, including environmental advantages such as a cooler climate and cleaner water sources; improved sanitation and hygiene practices such as boiled water consumption; lower population density; and a robust public healthcare system. In our study, 62% of enteric fever cases were caused by *S. Typhi*, and 38% were caused by *S. Paratyphi* A. This finding is consistent with most published reports, where *S. Typhi* is the predominant causative agent isolated; for example, studies by Gautam *et al.* (2002) (81% *vs.* 19%) and Patil *et al.* (2019) (76.5% *vs.* 23.5%) also reported a predominance of *S. Typhi* [31, 32].

The demographic data from our study revealed two key findings: a significant male predominance (78.4%) among patients and a high disease burden in younger age groups,





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with children (32.4%) and adolescents (29.7%) being the most affected**.** These findings are broadly consistent with a study by Park *et al.*, who reported a similar male predominance (63%) and found that the highest incidence (45.4%) occurred in the 5–19-year-old age group. Beyond the age of 20, the incidence declines, likely due to the development of immunity resulting from previous clinical or sub-clinical infections [33]. These findings suggest that current vaccination strategies warrant reassessment, particularly as they highlight the urgent need to implement routine vaccination for children and younger age groups, where enteric fever is significantly more common.

In the current study, a high level of resistance to Fluoroquinolones was observed among *Salmonella* spp., with only 2.7% of the isolates found susceptible to Ciprofloxacin and 5.4% to Levofloxacin. Similar high resistance rates have been reported in studies by Bhumbla *et al.* (2022) [30], Patil *et al.* (2019) [32], and Asghar *et al.* (2024) [34]. In contrast, high susceptibility was maintained to extended-spectrum Cephalosporins, including Ceftriaxone (97.3%) and Cefepime (100%), as well as the macrolide Azithromycin (100%). These results closely align with those reported by Patil *et al.* [32], who also documented 100% susceptibility to Ceftriaxone, Cefixime, and Azithromycin among *S. Typhi* and *S. Paratyphi* A isolates.

The absence of MDR and XDR isolates among the *Salmonella* isolates in our study is particularly noteworthy, though these findings may not be generalizable to the entire region due to the study's limited scope, especially in light of the global rise in antimicrobial resistance (AMR) and the emergence of such isolates in neighboring countries, particularly Pakistan [34, 35]. The absence of MDR and XDR isolates in our sample suggests that the *Salmonella* strains circulating in the study area may not yet be significantly impacted by the selective pressures that drive the development and spread of MDR phenotypes. This underscores the importance of continued surveillance and effective antimicrobial stewardship to prevent the emergence of resistance. Furthermore, the absence of MDR strains offers a promising context for the management of *Salmonella* infections in the region, as effective treatment options, such as third-generation cephalosporins and azithromycin, remain highly viable. Continuous monitoring of antimicrobial susceptibility trends is therefore essential to detect any shifts that could compromise public health and guide future therapeutic strategies.

In conclusion, it is noteworthy that in many endemic regions, *S. enterica* commonly causes bloodstream infections that can be hard to differentiate from other febrile illnesses, often resulting in high mortality if not diagnosed and treated promptly. Bacteriological culture of blood and/or bone marrow remains the cornerstone of laboratory diagnosis for *S. enterica* infections. However, antimicrobial resistance has become a significant challenge, beginning with resistance to first-line treatments such as Chloramphenicol, Ampicillin, and Co-trimoxazole. Over time, reduced susceptibility to Fluoroquinolones, and eventually resistance, has emerged, as was demonstrated in this study. Our findings support the use of third-generation Cephalosporins and Azithromycin as effective treatment options for enteric fever. Prompt diagnosis and treatment are essential to prevent life-threatening complications associated with this infection. It is important to acknowledge, however, that this study has several potential limitations, including a modest sample size, its retrospective nature, and the potential for selection bias, as it only includes patients referred to a specialized private laboratory, which may not fully represent the broader community prevalence of *Salmonella*.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

## DATA AVAILABILITY

The summary datasets generated and analysed during the current study are included within this published article. The raw data are not publicly available due to patient privacy restrictions.

## FUNDING

No funding was received for this manuscript.

## AI DISCLOSURE

No artificial intelligence (AI) tools were used in the creation or analysis of this manuscript.

## AUTHORS' CONTRIBUTIONS

SK: Conceptualization, Writing – original draft. UA: Conceptualization, Writing – original draft, Supervision. TM: Data curation, Formal analysis. TM: Data curation, Formal analysis. MIQ: Writing – review & editing, Supervision. MP: Software, Data curation. WAR: Software, Data curation. All authors read and approved the final manuscript.

## ETHICS STATEMENT

The study was approved by the institutional ethics committee at Dr. Qadri's Hematology Center and Clinical Laboratory, Srinagar on 18-10-2024, Ref. No: IEC-REF/QHCCL/05/2024.



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