Prevalence of Salmonella typhi in among febrile patients in a tertiary care hospital of South West Rajasthan

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ABSTRACT

Background: Salmonella enterica, serotype typhi, remains the predominant Salmonella species causing enteric fever in India. The mode of Salmonella typhi transmission is considered to be predominantly vehicle-borne through contaminated water or food. In India, the incidence of Salmonella typhi occurs between the months of April and June (dry season) followed by July and September (monsoon season). Typhoid fever may be difficult to distinguish clinically from other febrile illnesses and if left untreated, intestinal, neuropsychiatric, and other complications develop in some patients. Objective: The aim of this study was to determine the prevalence of S. typhi in bloodstream infections and its antimicrobial susceptibility pattern among patients with febrile illness. Methodology: Febrile patients admitted in the hospital who were prescribed blood culture tests and whose samples were sent to microbiology laboratory were included in the study. All blood samples (average 5 mL for adults and 2-3 mL for pediatric age group) were immediately inoculated into Bac-T ALERT aerobic blood culture bottles containing sodium polyethanol sulfonate as an anticoagulant (0.025%). If growth was isolated, isolated colony characteristics of growth and Gram stain were assessed. On Gram staining, typical nonlactose fermenting Gram negative bacilli were further subjected to species identification and detection of antimicrobial susceptibility pattern on the VITEK2. Results: In this study period, a total of 511 blood culture (paired) samples were processed, out of which 47 isolates of Salmonella were obtained. Among these isolates, 33 (70.23%) were from males, and 14 (29.77%) were from females. Amongst these, 35 (74.4%) patients were from rural, 8 (17%) were from subrural, and 4 (8.5%) were from urban areas. Out of the total 47 isolates of Salmonella, 42 (89.36%) were Salmonella typhi, 2 (4.25%) were Salmonella paratyphi A and B each, and 1 (2.12%) was Salmonella enterica. Antimicrobial susceptibility pattern of Salmonella isolates revealed that all the isolates of Salmonella species were highly susceptible (95%-100%) to third generation cephalosporins (ceftazidime, ceftriaxone, cefepime, cefoperazone-sulbactam) and other higher antibiotics such as betalactamase inhibitors - piperacillin tazobactam (95%-100%) and Ticarcillin-clavulanic acid (100%). They were also highly susceptible (100%) to carbapenems (imipenem, meropenem, doripenem, and ertapenem) but showed a fairly decreased susceptibility was towards nalidixic acid with 15% for Salmonella typhi and 50% for other Salmonella isolates. Conclusion: Surging drug-resistant Salmonella enterica cases, the level of resistance was not as high as predicted in our study population. Multidrug-resistant (MDR) trends may vary; therefore, drug susceptibility testing side-by-side to empirical therapy is mandatory, especially in developing countries where there is a practice of self-medication.

Keywords: Cephalosporins, fluoroquinolones, Salmonella

Introduction

Enteric fever is prevalent world over and continues to be a major public health problem in developing countries. Annual mortality from typhoid has increased by 39% between 1990 and
Infections with *S. typhi* are responsible for approximately 20 million new cases of typhoid each year, globally. In India, *Salmonella enterica* serotype Typhi, remains the predominant *Salmonella* species causing enteric fever.

The mode of *Salmonella typhi* transmission is considered to be largely indirect and predominantly vehicle-borne through contaminated water or food. The role of water as a vehicle for typhoid fever has been appreciated since the late 1800s. The risk for infection is high in low- and middle-income countries where typhoidal *Salmonella* is endemic and that have poor sanitation and lack of access to safe food and water. Its epidemiology is also affected by seasonal variations. In India, the peak incidence of *Salmonella* typhi occurs between the months of April and June (dry season) followed by July and September (monsoon season). Typhoid fever may be difficult to distinguish clinically from other febrile illnesses and if left untreated, intestinal, neuropsychiatric, and other complications develop in some patients.

Culturing the bacteria from body fluids is the definitive test for the diagnosis of typhoid fever although inconclusive serological methods such as the Widal test are commonly employed in many health care settings. A specific diagnosis of typhoid requires access to a competent laboratory that can process blood cultures and such laboratories are uncommon in resource-poor regions. Microbiologic culture of blood or bone marrow remains the mainstay of laboratory diagnosis.

Moreover, *Salmonella* species are increasingly evolving antimicrobial resistance to several commonly used antimicrobial agents. Although fluoroquinolones (FQ) are the drugs of choice to treat invasive *Salmonella* infections, decreased susceptibility to ciprofloxacin is increasing quickly worldwide. The problem of MDR *Salmonella* has increased the challenge in the management of the disease in endemic regions by increasing morbidity and mortality as well as the cost of treatment. However, to control the spread of typhoid fever, surveillance for *S. typhi* and the assessment of antimicrobial susceptibility is essential. Therefore, the aim of this study was to determine the prevalence of *S. typhi* in bloodstream infections and its antimicrobial susceptibility pattern among patients with febrile illness in the South-Western part of Rajasthan wherein there are challenging lifestyle habits, and lack of awareness about personal hygiene is also prevalent.

### Materials and Methods

#### Study area, duration and design

A cross-sectional retrospective hospital-based study was performed in a multispecialty tertiary care center in South-Western Rajasthan for a period of 17 months from 1 March, 2019 to 31 August, 2020.

#### Study population and inclusion criteria

All the febrile patients enrolled in the hospital who had been prescribed blood culture tests by the treating physicians with their samples received in the microbiology laboratory were included in the study.

#### Exclusion criteria

All the patients not consenting for blood culture, who had received antibiotics within 1 week before presentation, or those presenting on more than one time during the study period were excluded from the study.

#### Sample size and ethical consideration

A total of 511 patients blood samples (paired) were collected and received in the Central Lab Microbiology Department for blood culture during the study period. Written informed consent was taken, and ethical clearance was taken from the ethical committee of the hospital.

#### Procedure

Under all aseptic precautions by using 70% alcohol followed by povidone iodine and then subsequently with alcohol (Triple Swab technique), all blood samples were collected (venous site). Blood culture bottles containing brain heart infusion broth were immediately cleaned with 70% alcohol, and the collected blood samples (average 5 mL for adults and 2–3 mL for pediatric age group) were immediately sent to the microbiology lab for further processing. These blood culture bottles were further inoculated into an automated 3D blood culture system BacT ALERT which contained 0.025% of sodium polysulfonate as an anticoagulant for detection of growth, which gives color-coded alarms when detected positive. These bottles were incubated for a period of a maximum of 5 days after which the sample was labeled sterile.

On bottles flagging positive, Gram-stain was done, and the microorganism was identified microscopically. A positive flagged bottle was sub-cultured on Blood agar and Mac Conkey agar subsequently and incubated overnight at 37°C following standard procedures. If any bacterial growth was isolated, along with colony characteristics Gram stain was assessed. All those showing typical nonlactose fermenting Gram negative bacilli were further subjected to species identification and detection of antimicrobial susceptibility pattern on the VITEK 2 which is a fully automated Advanced Expert Phenotypic System.

A 0.5 McFarland suspension of the colonies was prepared and processed following standard operating procedures. Antimicrobial susceptibility pattern and the extended-spectrum beta-lactamase (ESBL) status were determined by the latest Clinical and Laboratory Standard Institution guidelines.

#### Results

During the study period, a total of 511 blood culture (paired) samples were processed, out of which 47 isolates of *Salmonella* were obtained. Among these isolates, 33 (70.23%) were from males and 14 (29.77%) were from females. Amongst these, 35 (74.4%)
patients were from rural, 8 (17%) were from subrural, and 4 (8.5%) were from urban areas. Out of the total 47 isolates of *Salmonella*, 42 (89.36%) were *Salmonella typhi*, 2 (4.25%) were *Salmonella paratyphi A* and *B* each, and 1 (2.12%) was *Salmonella enterica* [Table 1].

The antimicrobial susceptibility pattern of the *Salmonella* isolates revealed that all the isolates of *Salmonella* species were highly susceptible (95%–100%) to third-generation cephalosporins (ceftazidime, ceftriaxone, cefepime, cefoperazone-sulbactam) and other higher antibiotics such as betalactamase inhibitors – piperacillin tazobactam (95%–100%) and ticarcillin–clavulanic acid (100%). They were also highly susceptible (100%) to carbapenems (imipenem, meropenem, doripenem, and ertapenem) but showed a fairly decreased susceptibility was towards nalidixic acid with 15% for *Salmonella typhi* and 50% for other *Salmonella* isolates. The sensitivity pattern of *Salmonella* isolates for FQ was also found to be greatly reduced for ciprofloxacin, which was only 34% for *Salmonella typhi*, and all other isolates of *S. paratyphi A*, *S. paratyphi B*, and *Salmonella enterica* were fully resistant (100%) to it. Whereas for levofloxacin, there was intermediate susceptibility (50%–60%) amongst all.

For cotrimoxazole, fairly good sensitivity (70%) was observed amongst *Salmonella typhi* isolates and *Salmonella paratyphi A* and *B*, and *Salmonella enterica* were found to be fully susceptible (100%) to it as depicted in [Table 2].

### Discussion

*Salmonella* serovar *typhi* and *Salmonella* serovar *paratyphi A* are the major agents of enteric fever. Changing trends of antimicrobial susceptibility pattern has been observed throughout different geographic regions of India which mandates constant surveillance and evaluation.

In the current study, out of the total *Salmonella* spp. isolated, the majority (89.36%) were *Salmonella typhi* followed by *Salmonella paratyphi A* and *B* (4.25% each). These findings were similar to the findings of the study done by Patil et al. in 2019 which reported *S. typhi* isolates three times higher than *S. paratyphi A* isolates (76.5% vs. 23.5%). Altogether a study done by Misra et al. in 2016 stated that *S. typhi* was the predominant isolate at 67%, followed by 18% *S. paratyphi A* and 10% were of *S. typhimurium*. Another study done by Khadka et al. reported *S. typhi* (68.8%) as a major isolate followed by *S. paratyphi A* (31.1%). The majority of the *Salmonella* isolates were from the rural patients which probably can be attributed to the poor faeco-oral hygiene and water sanitation conditions in these areas.

### Table 1: Various *Salmonella* spp. isolated from blood samples

| Organism                      | No. of isolates |
|-------------------------------|-----------------|
| *Salmonella typhi*             | 42              |
| *Salmonella paratyphi A*       | 2               |
| *Salmonella paratyphi B*       | 2               |
| *Salmonella enterica*          | 1               |

### Table 2: Sensitivity pattern of *Salmonella* species

| Antibiotics                | *S. typhi* (n=42) | *S. paratyphi A* (n=2) | *S. paratyphi B* (n=2) | *S. enterica* (n=1) |
|----------------------------|-------------------|------------------------|------------------------|---------------------|
| Amikacin                   | 0 (0%)            | 0 (0%)                 | 0 (0%)                 | 0 (0%)              |
| Gentamicin                 | 0 (0%)            | 0 (0%)                 | 0 (0%)                 | 0 (0%)              |
| Ciprofloxacin              | 14 (34%)          | 0 (0%)                 | 1 (50%)                | 0 (0%)              |
| Levofloxacin               | 25 (60%)          | 1 (50%)                | 1 (50%)                | 0 (0%)              |
| Piperacillin/Tazobactam    | 39 (95%)          | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Ceftazidime                | 39 (95%)          | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Cefepime                   | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Cefoprazone/Sulbactam      | 39 (95%)          | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Ceftriaxone                | 39 (95%)          | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Imipenem                   | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Meropenem                  | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Doripenem                  | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Ertapenem                  | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Ticarcillin/Clavulanic acid| 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Aztreonam                  | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Trimethoprim/Sulfamethoxazole| 29.4 (70%)       | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Minocycline                | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Colistin                   | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
| Nalidixic acid             | 6 (15%)           | 1 (50%)                | 1 (50%)                | 1 (100%)            |
| Ofloxacin                  | 42 (100%)         | 2 (100%)               | 2 (100%)               | 1 (100%)            |
and Misra et al\textsuperscript{11} stated that 15% of *Salmonella typhi* and 16.7% of *Salmonella paratyphi* A isolates were reported susceptible to Ciprofloxacin.

However, a fairly good sensitivity pattern was observed for Cephalosporins (95%–100%) which is similar to findings reported in the studies done by Patil et al\textsuperscript{9} In a study by Khadaka et al\textsuperscript{10} all the isolates showed good sensitivity patterns to third-generation cephalosporins. Furthermore, in the current study, Cotrimoxazole showed 70% sensitivity to *Salmonella typhi* isolates, whereas Salmonella paratyphi A and B and *Salmonella enterica* were fully susceptible (100%) which is very much similar to the findings stated by Khadaka et al\textsuperscript{10} (100%).

The susceptibility towards Nalidixic acid in the current study was greatly reduced to 15% for *Salmonella typhi* and 50% for *Salmonella paratyphi* A and B which is similar to the findings reported by Misra et al\textsuperscript{11} with 12.6% susceptibility and by Khadaka et al\textsuperscript{10} with 6.7% susceptibility Rahman MA et al\textsuperscript{12} also with 15.4%.

**Conclusion**

This study concludes the re-emergence of susceptibility pattern of *Salmonella* strains to various broad-spectrum antibiotics. A major concern about FQ-decreased susceptibility and cephalosporins such as cefepime, ceftriaxone on the other hand being, more susceptible, emphasizes the need for continuous evaluation and judicious use of antimicrobials, considering the ever-changing landscape. A surge in drug-resistant *Salmonella enterica* cases and even the level of resistance was not as high as predicted in our study population. Looking at the trends of multidrug resistance scenarios, thus drug susceptibility testing side-by-side to empirical therapy is mandatory, especially in developing countries where there is a practice of self-medication. Referring to our findings, higher susceptibility of *Salmonella enterica* to the conventional anti-typhoidal drugs was attributed compared to macrolides and fluoroquinolones. Therefore, reconsideration of these antibiotics as implicated therapies could be useful in clinical management. Henceforth, more prospective studies are warranted or should be encouraged to correlate the clinical outcome of treatment based on *in vitro* antimicrobial susceptibility patterns of *Salmonella* isolates in typhoid cases.

**Limitation of study**

Among self-medicated cases, FQ-sensitive isolates might have failed to grow contributing to the lower incidence; therefore, the exact incidence of the disease could be even greater than observed. Besides, lacking the molecular laboratory set-up (presumed as a necessity for high-quality data in clinical studies) in our settings was another drawback as the blood culture has limited sensitivity.

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**Conflicts of interest**

There are no conflicts of interest.

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