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Antimicrobial Susceptibility Profile of *Salmonella enterica* of the Blood Culture Isolates Amongst Febrile Patients: One and Half Year Hospital Based Retrospective Study

Kapil Singh, Kirti Nirmal*, Seema Gangar and Shukla Das

Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden, Delhi – 110095, India

**Corresponding author*

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A B S T R A C T

Enteric fever, which is caused by *Salmonella enterica serovar Typhi/ Paratyphi A, B, and C* is an endemic febrile illness in India. The objective of this retrospective study was to analyse the patterns of drug resistance in *Salmonella enterica* isolates in blood culture among febrile patients. A tertiary care institution conducted a one and a half years retrospective analysis (July 2022–Dec 2023). An observational, prospective study screened febrile malaria-negative adults and children for typhoid fever. Venous blood samples were aseptically obtained, inoculated in blood culture bottles, and processed using automated equipment (BacT/Alert 3D, BioMerieux) according to standard protocols. *Salmonella antisera* for slide method (Bacterial Antisera Denka Seiken Tokyo, Japan) serotype grouping verified probable enteric fever isolates. Kirby-Bauer disk diffusion was used to test antimicrobial susceptibility under Clinical and Laboratory Standards Institute 2023. A total of 29,211 blood culture samples were received in the bacteriology laboratory from one and half year. Among them, 17994 samples (61%) tested positive for blood culture. Amongst febrile patients, 0.3% (49 isolates) of the 17994 blood culture samples tested positive for *Salmonella enterica*. Female patients were more compared to male (F>M). *Salmonella enterica* serotype *Typhi* 6 (12.24%) was more prevalent from serotyping. Which were 80% resistant to Ciprofloxacin, 25% with Cefixime and 14% with Chloramphenicol. *Salmonella enterica serovars Typhi* blood culture isolates were 100% sensitive to ceftriaxone, imipenem, and azithromycin. This study highlights the importance of ongoing assessment and careful utilization of antimicrobials, taking into account the constantly evolving situation. Monitoring antibiotic resistance in *Salmonella enterica* is of utmost significance.

Introduction

Enteric fever, often known as typhoid fever, is a major public health issue worldwide, mostly affecting South Asia. India has a particularly high incidence of this disease. Typhoid is unique to humans and causes malaise, fever, stomach pain, transitory rash, splenomegaly, hepatomegaly, bradycardia, and leucopoenia. Intestinal haemorrhage and perforation are the main consequences. Approximately 11-21 million episodes of enteric fever occur annually on a global scale, leading to a mortality rate of 120,000-160,000. The precise burden of the disease is uncertain due to inadequate diagnosis and the presence of several forms of the disease. However, by extrapolating regional statistics, it is estimated that there are 586 instances of typhoid/paratyphoid per 100,000 person-years in India (Revised_EOI_Typhoid_diagnostics_220923.pdf, 2024). *Salmonella enterica serovar Typhi (S. Typhi)* causes 10.9 million cases and 116.8 thousand deaths annually, where else *Salmonella enterica serovar Paratyphoid (S. Paratyphi)* is a similar form of bacteria that also causes enteric fever. It affects over 3.4 million individuals per year and leads to 19.1 thousand deaths.

Antibiotics have proven useful in treating enteric fever; nevertheless, rising global antibiotic resistance has compounded the situation. *S. Typhi* the bacteria that causes typhoid fever has acquired resistance to several antibiotics, including three primary drugs. Parts of Asia and Africa have a high prevalence of multidrug-resistant bacteria. Severely drug-resistant strain of *S. Typhi* has arisen in Sindh, Pakistan. This strain is resistant to Fluoroquinolones and third generation cephalosporins, limiting the available antibiotic options to Azithromycin and expensive intravenous carbapenem medicines.

Antimicrobial resistance is on the rise worldwide, and this includes the development and dissemination of multidrug-resistant strains of *S. Typhi*, which may be efficiently treated with antibiotics but has made matters worse. Identifying antibiotic susceptibility testing for individual patients before initiating antibiotic therapy is crucial to prevent the future spread of resistant *S. Typhi* (Revised_EOI_Typhoid_diagnostics_220923.pdf, 2024). Low middle income countries (LMICs) in South Asia, sub-Saharan Africa, and Latin America are prone to enteric diseases, particularly enteric fever, due to their poor water quality, uneven sanitation, and hygiene conditions (Antillón *et al.,* 2017).
For the detection of potential carriers responsible for acute enteric fever epidemics and to ascertain the etiological agent, early and accurate diagnosis of typhoid fever is essential (Gopalakrishnan *et al.,* 2002). Blood culture is considered the gold standard for diagnosis, with a diagnostic yield of 70-75% during the first week of sickness and dropping by 20-30% later in the course of the disease (Krishna *et al.,* 2011).

Materials and Methods

This retrospective study was conducted in the Microbiology Department of a tertiary care hospital in Delhi, North India during July 2022 to December 2023 after obtaining approval from the Institutional Ethics Committee (GTBHEC 2024/P-203).

Inclusion criteria

The study on malaria negative febrile patients was conducted in which adult and pediatrics patients were screened for typhoid fever and suspected patients were enrolled in the study, then blood sample were collected and tested for confirmation of the disease.

Physicians evaluated patients for the clinical symptom of typhoid fever, which is a fever lasting two or more days before admission, together with other clinical signs of typhoid fever, in the absence of any other known febrile disorders. However, this research did not include individuals who were diagnosed with other known febrile illnesses or those who had received antibiotic therapy for their symptoms within two weeks before arriving at the hospital.

Blood culture processing

Blood for culture was collected under strict aseptic conditions. All the blood specimens received in the microbiology laboratory for culture and sensitivity were inoculated for enrichment in PF plus aerobic/ FA plus culture vials and were processed using automated systems (BacT/Alert 3D, Biomerieux) as per standard protocols.

Immediately after the bottle was marked positive in the system, Gram staining was performed on the smear made from the bottle content and a subculture from the bottle was made using blood agar and MacConkey agar plates. The organisms were presumptively identified on the basis of colony morphology and Gram stain, from the

culture plates after overnight incubation. Gram-negative bacilli were further identified common microbiological tests, and biochemical assays and Salmonella species isolates from suspected cases of enteric fever were confirmed from *Salmonella antisera* for slide method (Bacterial Antisera Denka Seiken Tokyo, Japan) for grouping of serotype.

Antimicrobial susceptibility testing was done in accordance with Clinical and Laboratory Standards Institute 2023 (CLSI). Antibiotics discs processed through Kirby–Bauer's disc diffusion method in which antibiotics : Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Azithromycin (15µg) and Cefixime (5µg) (Hi-Media Laboratories) were tested (CLSI, 2022).

Data management and statistical analysis

Data recording was carried out using the MS Excel spreadsheet program. Categorical variables were summarized using frequencies and percentages. To compare groups in terms of categorical data, the Chi-square test was employed. A p-value of less than 0.05 was considered as the threshold for statistical significance.

Results and Discussion

One and half year retrospectively blood culture clinical isolates of febrile patients from July 2022-December 2023 were collected and analyzed. Overall 29211 blood culture samples were received in bacteriology laboratory for blood culture isolation.

Out of this, 17994 (61%) samples were blood culture positive. Out of 17994 blood culture samples, 49 isolates (0.3%) was culture positive for *Salmonella enterica* amongst febrile patients. Among 49 culture positive isolates 24 (49%) were males and 25 (51%) were females (F>M), which was statistical not significant. (p>0.08, paired t test) Blood culture positive age group were predominant in 1 to 69 years. 15 (31%) isolates were from age group 10 to 19 years. More than 40 years of the age group patients has less number of febrile patients in our study. The age group were statistical significant (p<0.03, chi-square test) (Table 1).

Out of the 49 positive isolates, 31 (63%) were from inpatient care and 18 (37%) from outpatient care. Moreover, in the present study, typhoid fever was more prevalent in Medicine Department 32 (65%) followed by peadiatrics populations. (P<0.05, Chi-square test) (Table 2).

Salmonella enterica isolates were confirmed from *Salmonella antisera* through stereotyping showed that 40 (81.63%) of these isolates were *S. enterica serotype Typhi,* 6 (12.24%) isolates followed by *S. enterica serotype paratyphoid A* and 3 (6.12%) isolates were *S. enterica serotype paratyphoid B*. This was statistical not significant (P>0.08, chi-square test) (Figure 1).

Amongst antimicrobial susceptibility patterns, *S. Typhi* revealed the highest resistance rate for Ciprofloxacin 39(80%) followed by Cefixime 13(25%), Imipenem 5(10%) and Chloramphenicol 7 (14%). Ceftriaxone, Imipenem and Azithromycin were 100% susceptible amongst *Salmonella enterica serovars Typhi* blood culture isolates (Figure 2).

Enteric fever poses a significant public health concern, particularly in developing nations where a considerable portion of the population's lack of access to clean drinking water is alarming. The situation is made worse by *Salmonella enterica serovar Typhi/Paratyphi's* declining antibacterial arsenal and rising resistance to antibiotics. Consequently, the disease's management is becoming more challenging.

The current study found that the prevalence of *S. Typhi* among febrile illness patients at tertiary care government hospitals was 0.3%. This finding was much lower than the India Council of Medical Research (ICMR) 2022 report 3.3%,6 3.28% in 10-year meta-analytical study, (Talukder *et al.,* 2023) 2.7% in recent study conducted in India and 0.3% observed in tertiary care hospital (Nirmal *et al.,* 2023). In the other study the positivity of blood culture for *Salmonella enterica* varied from 0.7% to 1.3%, (Gupta, 2023) while in a study conducted by Biswas *et al.,* (2022) the positivity varied from 0.4% to 1.7% (2017–2022) (Biswas *et al.,* 2022). The overall prevalence of this finding was significantly lower compared to the numbers reported in the study conducted in Central Ethiopia 4.1%, (Murray *et al.,* 2008) Lalitpur 4.1%, (Bell and Peeling, 2006) Shashemene Ethiopia 5% (Laxminarayan *et al.,* 2016) and Indonesia 15.5% (Wongsrichanalai *et al.,* 2007). Similar results have been reported in India with a prevalence rate of 2.5% (WHO, 1999) and in Nepal with a prevalence rate of 1.2% (Banoo *et al.,* 2006).

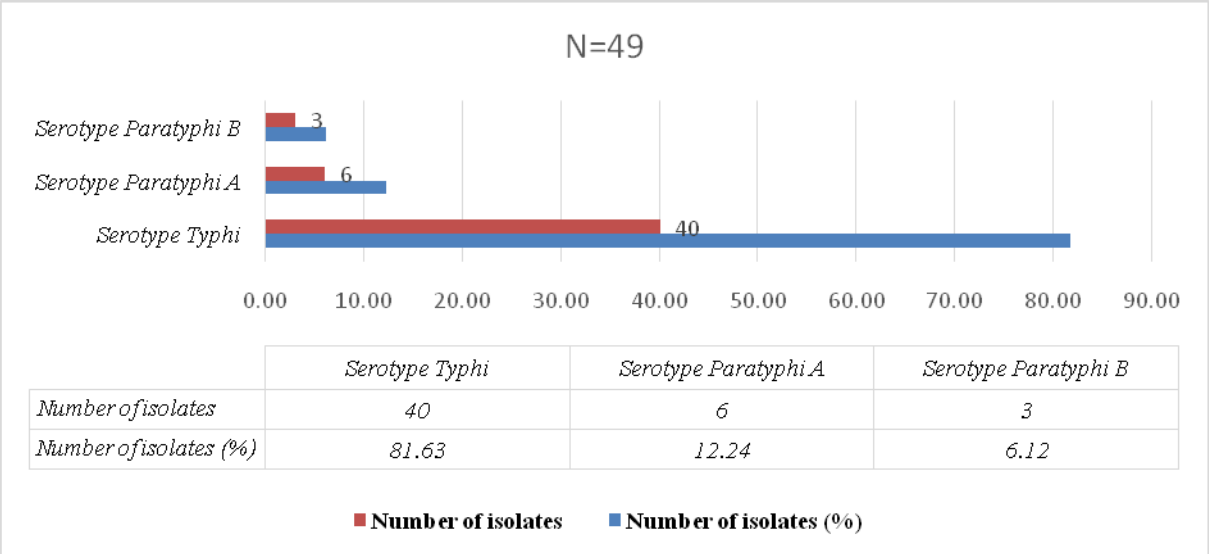
Table.1 Age and Gender profile of *Salmonella enterica* blood culture isolates amongst febrile patients in the study group. (n=49)

Age Range (Years)	Male n= 24 (%)	Female n= 25 (%)	Total n=49 (%)
1-9 Years	4 (8)	9 (18)	13 (26.5%)
10-19 years	9 (18)	6 (12)	15 (31%)
20-29 years	6 (12)	5 (10)	11(22.4%)
30-39 years	2 (4)	2 (4)	4 (8.1%)
40-49 years	1 (2)	1 (2)	2 (4.0%)
50-59 years	1(2)	1(2)	2 (4.0%)
60-69 years	1(2)	1(2)	2 (4.0%)

Table.2 Location wise distribution of *Salmonella enterica* blood culture isolates amongst febrile patients in the study group. (n=49)

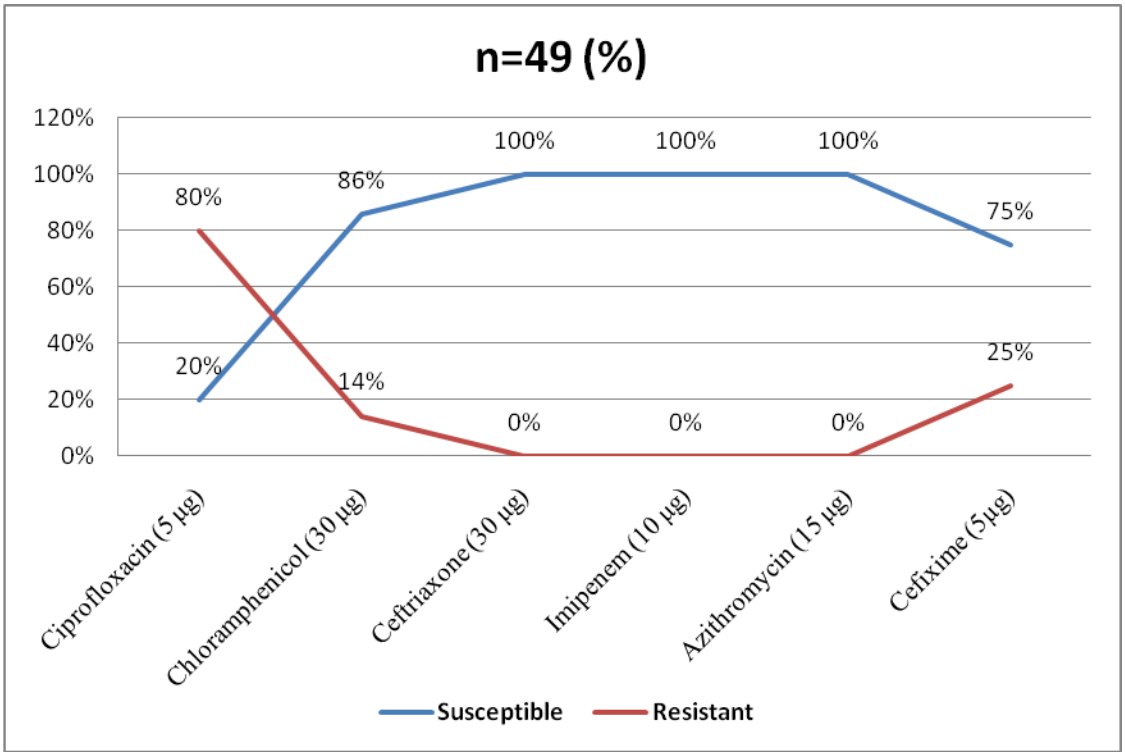
Departments	Location type	n=49 (%)	
Medicine	Out-patients	12 (24.49)	**P<0.05, Chi-square test)**
	In-patients	20 (41%)	
Paediatrics	Out-patients	6 (12.24)	
	In-patients	10 (20.41)	
Obstetrics & Gynaecology	Out-patients	0	
	In-patients	1 (2.04%)	

Figure.1 Frequency of *Salmonella enterica* serovars blood culture isolates amongst febrile patients. (n=49)



	Serotype Typhi	*Serotype Paratyphi A*	*Serotype Paratyphi B*
Number of isolates	*40*	*6*	*3*
Number of isolates (%)	*81.63*	*12.24*	*6.12*

Figure.2 Antimicrobial susceptibility pattern of *Salmonella enterica* serovars in blood culture isolates amongst febrile patients in the study group.



Possible explanations for this discrepancy include differences in the study population, the length of time between surveys, or the research district's geographical location. Differences in the methodology used to conduct the laboratory research also has an impact on the final outcome. In the present study, 15 cases, accounting for 31% of the total, were observed in individuals aged between 10 and 19 years. A total of 24 isolates, accounting for 48.97% of the total, were obtained from male patients. Similarly, 25 isolates, representing 51.02% of the total, were obtained from female patients. Our study does not show any disparity based on gender. Our research also identified forty isolates (81.63%) of *Serovar typhi* and nine isolates (18.36%) of *Serovar paratyphi*, which is comparable to a recent study conducted in northern India (Patel and Shah, 2023).

Over the last twenty years, resistance to ampicillin, cotrimoxazole, and chloramphenicol has emerged on a global scale, rendering fluoroquinolones and third-generation cephalosporins the preferred medications for the treatment of enteric fever. *S. enterica serovar Typhi/Paratyphi* isolates that have been linked to treatment failures resulting from reduced susceptibility to ciprofloxacin have been reported in numerous studies from different regions of the world, which have cast doubt on the efficacy of ciprofloxacin as an empirical option for the management of enteric fever (Threlfall *et al.,* 1992; Pang *et al.,* 1995). In our study, *S. Typhi* revealed the highest resistance rate for Ciprofloxacin 39(80%) followed by Cefixime 13(25%), Imipenem 5(10%) and Chloramphenicol 7 (14%). Ceftriaxone, Imipenem and Azithromycin were 100% susceptible amongst *Salmonella enterica serovars Typhi* blood culture isolates. A similar finding was reported in a study done in Ahmedabad, India *Salmonella sp*. remained sensitive to chloramphenicol, amoxicillin, Ceftriaxone and co-trimoxazole (100, 96.72, 100 & 98.36%, respectively) (Patel and Shah, 2023).

Another antibiotic that is particularly successful for the treatment of enteric fever in all age groups and may be conveniently taken orally is azithromycin, which belongs to the macrolide class. Our study found that all isolates tested were susceptible to azithromycin, which is consistent with a few other reports from various regions of the country (Gupta *et al.,* 2013; Malini *et al.,* 2020). On the other hand, this is in direct opposition to the

reports that Taneja and his colleagues have published. They found that 28% of *S. Typhi* isolates and 21.7% of *S. Paratyphi A* isolates were resistant to azithromycin (Taneja *et al.,* 2021). However, Dutta *et al.,* (2014) from Kolkata found that 28.1% of *S. Typhi* and 21.8% of *S. Paratyphi A* isolates were azithromycin resistant (Dutta *et al.,* 2014).

In contrast to our study Antimicrobial Resistance research and Surveillance Network (AMRSN) Annual Report 2022 shows the susceptibility of *Salmonella typhi* Azithromycin 97.4%, Cefixime 94.9%, Cefotaxime 94.4%, Ceftriaxone 96.1%, Chloramphenicol 95%, Ciprofloxacin 2.2% (AMRSN_Annual_Report_2022.pdf, 2024).

This study highlights the importance of ongoing assessment and careful utilization of antimicrobials, taking into account the constantly evolving situation. Monitoring antibiotic resistance in *Salmonella enterica* is of utmost significance. Infections such as enteric fever are one example of those that present difficulties in terms of antibiotic resistance. Furthermore, it is crucial to uphold Salmonella active surveillance of resistance at a global and cross-sectoral scale.

Author Contribution

Kapil Singh: Investigation, formal analysis, writing—original draft. Kirti Nirmal: Validation, methodology, writing—reviewing. Seema Gangar:—Formal analysis, writing—review and editing. Shukla Das: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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