



Research Article

Concerning Trend in Ceftriaxone Minimum Inhibitory Concentration (MIC): Implications for the Treatment of Enteric Fever

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A B S T R A C T

Introduction: *Salmonella typhi* and *paratyphi* - related enteric fever still poses serious health risks to people all over the world. Ceftriaxone and azithromycin were recommended as the preferred treatments for enteric fever following the emergence of fluoroquinolone resistance. Several reports of ceftriaxone resistance have also been reported, hence accurate medication susceptibility tracking is essential to maintain the empiric management of enteric fever. This study's objective is to determine the MIC of ceftriaxone in order to help clinicians prescribe the right dosage and stop the emergence of resistance.

Material and Methods: The investigation was carried out in 800 bedded hospital in Ghaziabad using a cross-sectional prospective design. A total of 228 *Salmonella* isolates were included in this investigation. The isolates' antibiotic susceptibility was assessed using the Kirby Bauer disc diffusion method, and the ceftriaxone MIC was calculated using the Broth Micro-Dilution (BMD) method.

Result: Of the 228 *Salmonella* species that were isolated, 64 (28.07%) were *Salmonella paratyphi* A, and 164 (71.92%) were *Salmonella typhi*. Ceftriaxone resistance was found in 33 (20.12%) *Salmonella typhi* strains while it was found in 14 (21.87%) *S. paratyphi* A strains. 0.125 µg/ml was the ceftriaxone MIC₅₀ value for *S. typhi* as well as for *paratyphi* A but the MIC₉₀ value was 8 µg/ml in *S. typhi* and 4 µg/ml in *S. paratyphi* A isolates.

Conclusion: This investigation revealed a concerning rise in the MIC to ceftriaxone and the reemergence of sensitivity to first-line medications.

Keywords: MIC, BMD, Re-emergence, *Salmonella*, MIC₅₀, MIC₉₀



Introduction

The serious public health issue, known as enteric fever, is mostly caused by *Salmonella typhi* and *Salmonella paratyphi*.¹ It is a severe health hazard in developing nations including India, with 12 million cases and 1,30,000 fatalities annually, and children being the most vulnerable.² Antibiotic therapy continues to be the mainstay of management because mortality can reach 30% in untreated or only partially treated cases; it is necessary to reduce mortality to less than 1% with the proper therapy.¹ Since 1990, there has been a 44.6% decrease in the cases of enteric fever reported globally. This decrease can be largely ascribed to improvements in the availability of clean drinking water, good sanitation, and typhoid vaccination uptake, especially among young people.² Enteric fever caused by *S. typhi* is prevalent in India at around 9.7%, and *S. paratyphi* at 0.7%.

Fluoroquinolones have replaced first-line medication (trimethoprim-sulfamethoxazole, ampicillin, and chloramphenicol) as the preferred medications for dealing with typhoid fever after the emergence of Multi-Drug Resistance (MDR).³ However, chromosomal mutations in the Quinolone-Resistance-Determining Regions (QRDRs) of the genes encoding DNA gyrase and topoisomerase IV have resulted in Decreased Ciprofloxacin Susceptibility (DCS) and fluoroquinolone resistance.⁴ Therefore, ceftriaxone for intravenous therapy and azithromycin for oral administration are being utilised more frequently for the empirical management of uncomplicated enteric fever.³ Ceftriaxone is administered intravenously: 100 mg/kg x 7-10 days.⁵ Azithromycin has a 2-3 days half-life and has high intracellular concentration and effective clinical response.⁶ It is administered orally: 10-20 mg/kg x 7-10 days.⁵

Ceftriaxone, which is used either alone or in combination with azithromycin to treat enteric fever in this hospital, should be regularly monitored to spot any escalating *Salmonella* isolates resistance identified by the laboratory. MIC, a key pharmacokinetic parameter, enables dose adjustment to be done to ensure clinical effectiveness. It was required to conduct the current investigation to establish the MIC of ceftriaxone in isolates to understand the *Salmonella* susceptibility and also to identify any discrepancy between the results of ceftriaxone disc diffusion and Broth Micro-Dilution method (BMD).

Materials and Methods

Over a course of 14 months (July 2021-August 2022), the authors conducted the current cross-sectional prospective study at Santosh Medical College in Ghaziabad. Before beginning the investigation, IEC's approval was obtained (Reference No. SU/2021/092).⁴ All study participants provided their written informed permission. Using the

2013 version of MS Excel, the results were statistically analysed in terms of numbers and percentages.

The study comprised 228 *S. enterica* serotypes: typhi (n = 164), and paratyphi A (n = 64), which were found in blood cultures from probable cases of enteric fever. Standard biochemical assays were used to identify the isolates, and slide agglutination with certain antisera for confirmation was performed (CRI, Kasauli).

Inclusion Criteria

- *Salmonella* isolated from blood samples only

Exclusion Criteria

- *Salmonella* isolated from samples other than blood
- Duplicate samples from the same patient

Antimicrobial Sensitivity Testing

The 2021 Clinical and Laboratory Standards Institute (CLSI)⁷ guidelines were followed for interpreting the Kirby-Bauer disc diffusion method's findings. *Escherichia coli* ATCC 25922 was used for quality control.

Minimum Inhibitory Concentration

In this investigation, disodium salt of ceftriaxone (CTR) was used. Following CLSI 2020, MIC was calculated by BMD method as defined by Andrews.⁸ Two-fold dilutions in Cation Adjusted Mueller Hinton Broth (CAMHB) were used in the method on a sterile 96-well microtiter plate with concentrations varying from 0.06 µg/ml to 128 µg/ml. To ensure reproducibility, tests were run in pairs. Quality assurance was carried out by using *Escherichia coli* ATCC 25922.

Interpretation of Results

Positive results showed turbidity in the well while the well was clear in the case of negative results. MIC breakpoint recommended by CLSI for ceftriaxone is as follows:

Sensitive ≤ 1, Intermediate 2, Resistant ≥ 4.

The control strain, *Escherichia coli* ATCC 25922 with a MIC of 0.06 µg/ml, was used.

The ceftriaxone drug concentration in this study that inhibited 50% of the isolates was designated as MIC₅₀, while MIC₉₀ was the concentration that inhibited 90% of the isolates. Following are the calculations for MIC₅₀ and MIC₉₀:

Isolates numbers (n) x 0.5 for MIC₅₀ and Isolates numbers (n) x 0.9 for MIC₉₀.

Results

At an 800 bedded hospital in Ghaziabad, this cross-sectional prospective study was carried out over a period of 14 months. For *Salmonella* spp., 228 samples tested positive in a culture. Of the 228 *Salmonella* species that were isolated, 64 (28.07%) were *Salmonella paratyphi*

A, and 164 (71.92%) were *Salmonella typhi*. With a male preponderance of 66.2%, the age group of 11 to 20 years were the majority (37.7%), followed by that of 21 to 30 years (28.5%). Figures 1 and 2 depict the *Salmonella* isolates resistance pattern.

AMP - Ampicillin, COT - Cotrimoxazole, CH - Chloramphenicol, CTR - Ceftriaxone, CAZ - Ceftazidime, AZM - Azithromycin, NA - Nalidixic acid, PE - Pefloxacin, CIP - Ciprofloxacin, CX - Cefoxitin, IMI - Imipenem.

Out of 164 *Salmonella typhi* tested, 33 (20.12%) were resistant, 9 (5.5%) were intermediate, and 122 (74.4%) were sensitive to ceftriaxone whereas in *S. paratyphi A*, 14 (21.87%) were resistant, 4 (6.3%) were intermediate, and 46 (71.9%) were sensitive to ceftriaxone.

Despite the fact that the Multi-Drug Resistant (MDR)

Salmonella strains rate has decreased in *S. typhi* (4.26%) and *S. paratyphi A* (4.68%), there has been a concurrent rise in decreased ciprofloxacin susceptibility among *Salmonella* isolates, which limits their routine practical usage.

The distribution of MIC trends against ceftriaxone in *S. paratyphi A* (64) and *S. typhi* (164) is shown in Table 1 and Figure 3.

MIC₅₀ value of ceftriaxone was 0.125 µg/ml for *S. typhi* as well as for *S. paratyphi A* isolates but MIC₉₀ value was 8 µg/ml for *S. typhi* and 4 µg/ml for *S. paratyphi A* strains.

When comparing the antimicrobial susceptibility of ceftriaxone, disc diffusion and BMD method showed a high degree of agreement.

For *S. typhi*, the highest MIC was 64 µg/ml, whereas, for *S. paratyphi A*, it was 16 µg/ml.

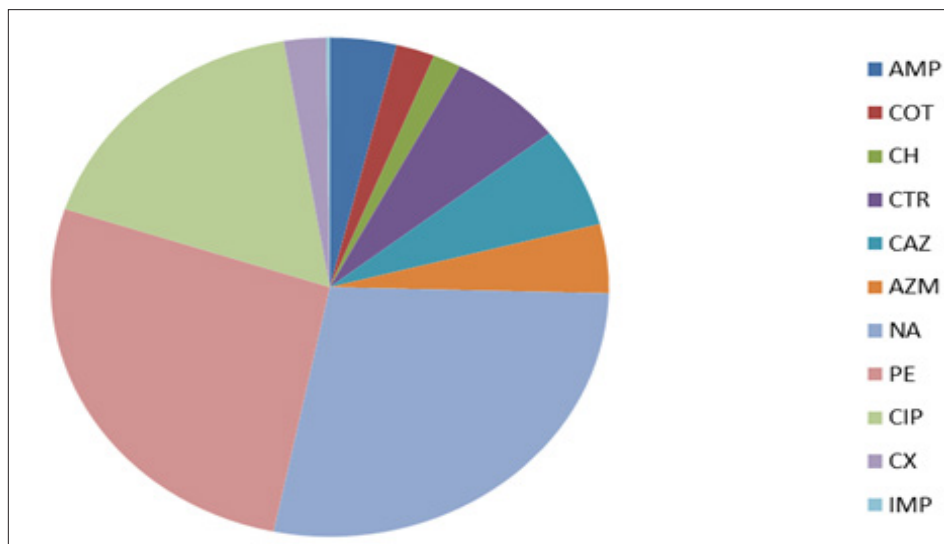


Figure 1. Resistance Pattern of *Salmonella Typhi* (N = 164)

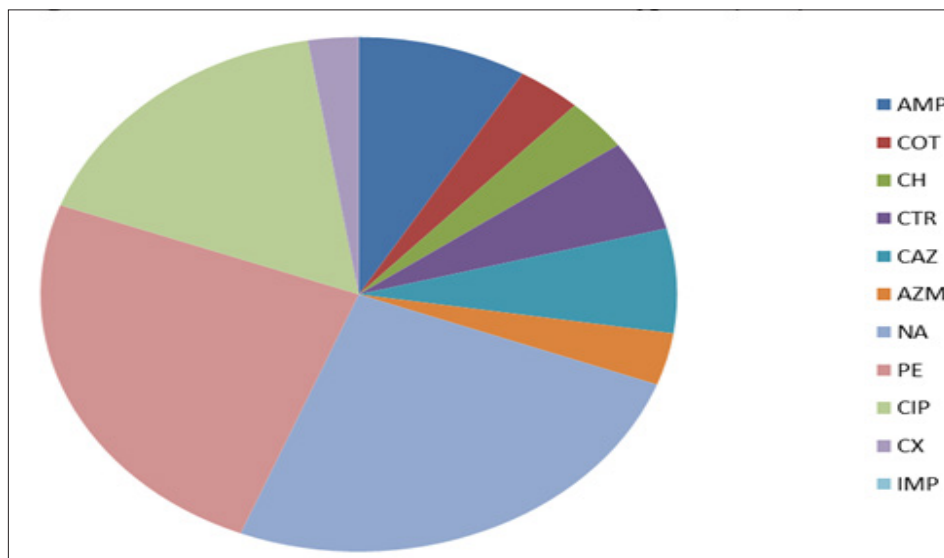


Figure 2. Resistance Pattern of *Salmonella Paratyphi A* (N = 64)

Table I. Distribution of MIC Trend for Ceftriaxone (CTR)

Salmonella Isolates	MIC Breakpoint ($\mu\text{g/ml}$)													
	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	
<i>S. typhi</i> (n = 164)	0	21	69	24	06	02	09	15	07	05	04	02	0	
<i>S. paratyphi</i> A (n = 64)	0	11	23	09	0	03	04	08	03	03	0	0	0	

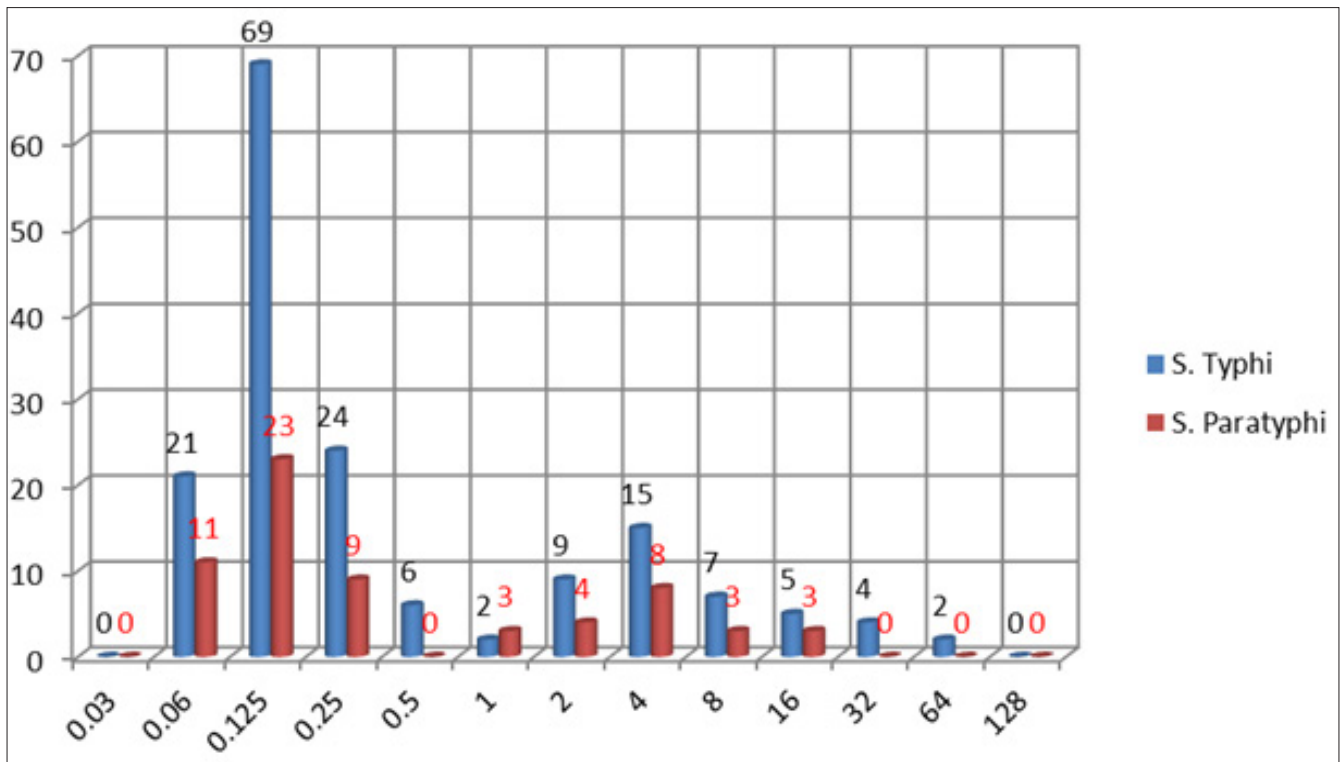


Figure 3. Distribution of MIC Trends for Ceftriaxone (CTR) among Salmonella Isolates

Discussion

In developing nations like India, Pakistan, Nepal, and African countries, enteric fever has become a significant problem. Enteric fever is more prevalent in underdeveloped nations than it is in developed nations, which may be because developed nations have better water treatment, good hygiene practices, and proper sanitation. A thorough understanding of the prevalence of the various *Salmonella* serovars and their resistance pattern is crucial for the prompt management of cases of enteric fever. A total of 228 *Salmonella* enterica species were isolated in the current study, out of which 164 isolates (71.92%) were *S. typhi* and 64 isolates (28.07%) were *S. paratyphi* A. The ratio of typhi and paratyphi is 2.56:1 in this study. Worldwide, the ratio of typhoid fever:paratyphoid fever is 4:1, but there are several reports of increased *S. paratyphi* A infection rate in India.⁹ The discontinuation of the trivalent TAB vaccination

and the introduction of the oral typhoid vaccines, Ty21a and Vi, which only provide protection against *S. typhi*, may be to blame for the rise of *S. paratyphi* A causing enteric fever. In the lack of a vaccination that is effective against *S. paratyphi* serotypes, travellers to the region should be concerned about a rise in the prevalence of paratyphoid fever.¹⁰

After fluoroquinolone resistance emerged, the National Guidelines for Antimicrobial Use state that ceftriaxone and azithromycin should be used to treat enteric fever. A study conducted by Kokare et al.² reported 12.5% resistance to ceftriaxone and the highest MIC to be more than 16 $\mu\text{g/ml}$. Another study from Karnataka by Narasanna et al.¹¹ found that third-generation cephalosporin significantly increased the MIC between 128 and 256 $\mu\text{g/ml}$, and 09 *S. typhi* isolates were resistant to third-generation due to the presence of blaCTX-M2 and blaCTXm-9 genes. In this

study, 20.12% of the *S. typhi* isolates and 21.87% of the *S. paratyphi* A isolates were resistant to ceftriaxone, with MIC₅₀ value of 0.125 µg/ml being the same in *S. typhi* as well as in *S. paratyphi* A and MIC₉₀ values being 0.8 µg/ml in *S. typhi* and 0.4 µg/ml in *S. paratyphi* A isolates. It is remarkably almost similar to Taneja et al.'s¹² work, which revealed ceftriaxone resistance to be 12.3% in *S. typhi* and 34.7% in *S. paratyphi* A. Complete ceftriaxone susceptibility was reported by Kumar and Jose et al.¹⁰ although the MIC for ceftriaxone was on the rise. A 0.06 µg/ml MIC was present in 69.7% of the isolates, while a 0.12 µg/ml MIC was present in 30.3% of the isolates. In their investigation, Behl et al.¹³ from Chandigarh in 2017 did not find any ceftriaxone-resistant strains. The MIC₅₀ and MIC₉₀ values for *S. typhi* and *S. paratyphi* A were determined to be 0.125 µg/ml and 0.25 µg/ml, respectively. The MIC values for ceftriaxone over the past 12 years have increased from 0.023 µg/ml to 0.064 µg/ml in case of MIC₅₀ and from 0.038 µg/ml to 0.19 µg/ml in MIC₉₀, indicating a creeping trend towards resistance, according to a study from AIIMS, New Delhi¹⁴ that found 100% susceptibility to ceftriaxone.

In this investigation, MIC data were interpreted in accordance with CLSI 2020 recommendations. The resistance data may change if European Committee for Antimicrobial Susceptibility Testing (EUCAST) is used. EUCAST states that the resistance breakpoint for ceftriaxone is ≥ 2 µg/ml, but CLSI states that it is ≥ 4 µg/ml.

Across the nation, there seems to be a wide range of ceftriaxone susceptibility. The length of therapy and the indiscriminate, improper dose may be to blame for the rise in ceftriaxone resistance. In order to ensure that the clinicians may administer the medications in the proper dose and duration, it is vital to record the antibiotic susceptibility along with MIC.

Pressuring ceftriaxone could lead to the establishment of XDR (extended drug resistance - MDR plus resistance to ceftriaxone and fluoroquinolones), which occurred in Pakistan in 2016.¹⁵

Conclusion

Our research revealed elevated MIC₅₀ and MIC₉₀ values for ceftriaxone and decreased susceptibility to ciprofloxacin, both of which are cause for worry. First-line antibiotic susceptibility has returned, and MDR strains of *S. typhi* have significantly decreased. To rationalise the treatment procedure and stop the evolution of resistance like XDR, antibiograms of *Salmonella* isolates must be continuously monitored and analysed. We further suggest that in order to ensure adequate therapy and enhance clinical outcomes of enteric fever, a precise determination of the MIC for ceftriaxone is required.

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