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# High-Level Resistance to Ciprofloxacin and Rising MIC to Ceftriaxone and Azithromycin among Enteric Fever Isolates from a Tertiary Care Center, Puducherry, India

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**A B S T R A C T**

Enteric fever is an important public health problem in India and accounts for high morbidity and mortality. Specific antibiotic therapy is the mainstay of management of enteric fever. Emergence of multi drug resistant *Salmonella* isolates has led to the need of continuous monitoring of susceptibility pattern to these agents. Hence this study was conducted to detect minimum inhibitory concentrations of ciprofloxacin, ceftriaxone and azithromycin among enteric fever isolates and to formulate an antibiotic policy. A prospective study was conducted for three years duration among enteric fever isolates. Susceptibility was determined phenotypically by disk diffusion testing and MIC by E-strip testing. Total of 62 *Salmonella* isolates from blood samples of enteric fever patients were studied. *Salmonella paratyphi* A isolates outnumbered *Salmonella typhi* isolates. Ciprofloxacin resistance was observed among 16 (25.8%) isolates with MIC of ≥1 µg/mL. MIC estimation was better predictor of resistance *in vivo* as compared to disk diffusion testing. We observed rising MIC to ceftriaxone and azithromycin. As MIC is superior to disk diffusion testing in predicting the clinical outcome, susceptibility reporting should depend on MIC results, if facilities are available to prevent treatment failures.

## Introduction

Enteric fever is an important public health problem in India with *Salmonella enterica* var *typhi* (*S. typhi*) and *Salmonella enterica* var *paratyphi* A (*S. paratyphi* A) being the leading causative agents (Singhal *et al.*, 2014). These human restricted pathogens pose great threat in regions with poor standards of hygiene and sanitation and they account for high morbidity and mortality both among pediatric and adult populations (Adabara *et al.*, 2012). Specific antibiotic therapy is the mainstay of management of enteric fever; if left untreated then mortality can be as high as 30%, and with appropriate treatment mortality rate reduces to as low as 0.5% (Menezes *et al.*, 2012).

In the past few decades, there have been changes documented by various researchers across India, in both epidemiology and drug resistance pattern of enteric fever isolates. Firstly, there has been an increasing trend of *S. paratyphi* A over the last decade in India (Gupta *et al.*, 2009; Singhal *et al.*, 2014).

Secondly, there has been a noted re-emergence of susceptibility to first-line antibiotics, such as ampicillin, co-trimoxazole and chloramphenicol; which were earlier used for the treatment of enteric fever cases before the era of multi drug resistant (MDR) *Salmonella* (Choudhary *et al.,* 2013; Gupta *et al.,* 2009; Raveendran *et al.,* 2008). Thirdly, emergence of reduced susceptibility to ciprofloxacin and slow rise of minimum inhibitory concentrations to 3rd generation cephalosporins and azithromycin, which are used as current front line drugs in the treatment of enteric fever (Capoor *et al.,* 2007; Garg*et al.,* 2013; Srirangaraj *et al.,* 2014). However, recent reports of decreased susceptibility to these agents have led to the prospect of re-emergence of untreatable enteric fever and an increasing global burden and hence, continual monitoring of drug resistance is imperative (Choudhary *et al.,* 2013; Kawser *et al.,* 2013).

Considering wide variation in the susceptibility pattern of various strains of *Salmonella* among different geographical areas, with high endemicity, it is mandatory to test antimicrobial susceptibility before instituting definite therapy (Menezes *et al.,* 2012; Singhal *et al.,* 2014). Therefore, this study was undertaken to detect antimicrobial susceptibility profile and minimum inhibitory concentrations of ciprofloxacin, ceftriaxone and azithromycin among enteric fever isolates and thereby formulating an antibiotic policy for management of drug resistant enteric fever cases in our tertiary care hospital.

## Materials and Methods

A prospective study was carried out after obtaining ethics clearance from Institute Ethics Committee (RC No- RC/14/104), at department of Microbiology, Pondicherry Institute of Medical Sciences, Pondicherry for a duration of three years from June 2013 to May 2016. A total of sixty two clinical isolates of *Salmonella* from clinically suspected enteric fever patients were included. Identification of salmonellae isolates was performed using standard biochemical tests and isolates were confirmed by serotyping with specific *Salmonella* polyvalent antisera O and with O9 or O2 antisera (Koneman's Color Atlas and Textbook of Diagnostic, 2007).

Antimicrobial susceptibility testing was performed by using Kirby- Bauer disk diffusion method by using0.5 McFarland bacterial suspension on Mueller-Hinton agar. Commercially available disks (Hi-media Laboratories, Mumbai, India) of ampicillin (10 µg), chloramphenicol (30 µg), co- trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg) and azithromycin (15µg) were tested.

Minimum inhibitory concentrations (MICs) of ciprofloxacin, ceftriaxone and azithromycin for all *Salmonella* isolates were determined by Epsilometer strip test method (HiMedia, Mumbai, India).

The antimicrobial agent concentration, at which edge of the inhibition ellipse intersects the side of the E-strip, was taken as MIC value. *Escherichia coli* ATCC 25922 was used as control for both the disc diffusion and MIC testing.

Results of disk diffusion as well as MIC breakpoints were interpreted as per CLSI2015 guidelines except for azithromycin (as its interpretation is not given in this document). As CLSI 2017 gives interpretative criteria for azithromycin disc diffusion testing and azithromycin MIC breakpoints only for *S. typhi* isolates, same was used for interpretation of azithromycin results for *S. typhi* isolates and also for *S. paratyphi* A isolates (CLSI 2017).

## Results and Discussion

A total of 62 *Salmonella* isolates from blood samples of clinically suspected enteric fever patients were studied, of which28 isolates (45.2%) were *Salmonella typhi* and 34 isolates (54.8%) were *Salmonella paratyphi* A. Majority of patients were males 42 (67.7%) with a male-to-female ratio of 2.1:1. Age of the patients ranged from 3 to 67 years, with majority of the patients (40%) belonged to the age group of 21-30 years followed by 31-40 years (29%).

By disc diffusion testing, all 62 *Salmonella* isolates were uniformly susceptible to ampicillin, co-trimoxazole, chloramphenicol, ceftriaxone and azithromycin; whereas susceptibility to ciprofloxacin was variable and is shown in table 1.

MIC value for ciprofloxacin by E-strip test, ranged from 0.047µg/mL to >32µg/mL among 62 *Salmonella* isolates. Highest number of isolates 15 (24.2%) had MIC 0.5µg/mL, followed by 11 (17.7%) isolates with MIC 0.25 µg/mL. Six (11.5%) isolates (5 *Salmonella typhi* and 1 *S. paratyphi* A) had the highest MIC of>32µg/mL, and totally 16 (25.8%) isolates (7 *Salmonella typhi* and 9 *S. paratyphi* A) had MIC in the resistance zone with MIC of ≥1 µg/mL. Figure 1 shows the MIC of ciprofloxacin distribution among of 62 *Salmonella* isolates. Figure 2 shows *S. typhi* isolate with MIC >32µg/mL.

MIC value for ceftriaxone by E-strip test, ranged from 0.012µg/mL to 1.5µg/mL among 62 *Salmonella* isolates and is shown in figure 3. Highest number of isolates 19 (30.6%) had MIC of 0.25µg/mL, followed by 13 (21%) isolates with MIC of 0.19 µg/mL. Five (8.1%) isolates had the MIC in the intermediate susceptible zone (1 to 1.5µg/mL), and the remaining all 57 (91.9%) isolates were uniformly susceptible to ceftriaxone (MIC ≤1µg/mL).

Figure 4 shows *Salmonella paratyphi* A with MIC 1µg/mL to ciprofloxacin (intermediate susceptible) and 1.5µg/mL to ceftriaxone (intermediate susceptible).

MIC value for azithromycin ranged from 3 µg/mL to 24µg/mL among 62 *Salmonella* isolates and is shown in figure 5. Highest number of isolates 24 (38.7%) had MIC of 12µg/mL, followed by 18 (29.03%) isolates with MIC 8µg/mL. Three (4.9%) isolates had the highest MIC in the intermediate susceptible zone (24µg/mL), and the remaining 59 (95.1%) isolates were uniformly susceptible to azithromycin (MIC ≤16µg/mL). Figure 6 shows two isolates of *Salmonella paratyphi* A with MIC 24µg/mL to azithromycin (intermediate susceptible).

**Table.1** Distribution of antimicrobial susceptibility pattern of *Salmonella* isolates to ciprofloxacin (both disk diffusion and MIC). Total number of isolates 62

| Ciprofloxacin | Interpretation | *S.*Typhi (%) | *S.*Paratyphi A (%) | Total (%) |
|---|---|---|---|---|
| **Disk diffusion (zone size in mm)** | Sensitive | 2 (3.2%) | 1 (1.6%) | 3 (4.8%) |
| | Intermediate | 14 (22.6%) | 30 (48.4%) | 44 (71%) |
| | Resistant | 12 (19.4%) | 3 (4.8%) | 15 (24.2%) |
| **MIC by E-strip (in µg/mL)** | Sensitive | 4 (6.5%) | 1 (1.6%) | 5 (8.1%) |
| | Intermediate | 11 (17.7%) | 30 (48.4%) | 41 (66.1%) |
| | Resistant | 13 (21%) | 3 (4.8%) | 16 (25.8%) |

**Fig.1** MIC value of ciprofloxacin among the 62 *Salmonella* isolates from enteric fever cases (in µg/mL)

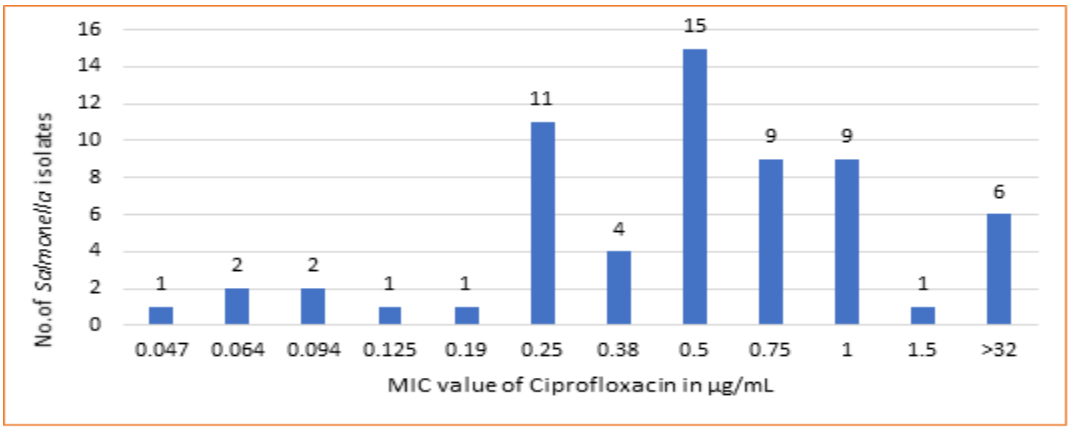



**Fig.2** *S. typhi* isolate with MIC >32µg/mL to ciprofloxacin showing high level resistance (no inhibition ellipse seen)

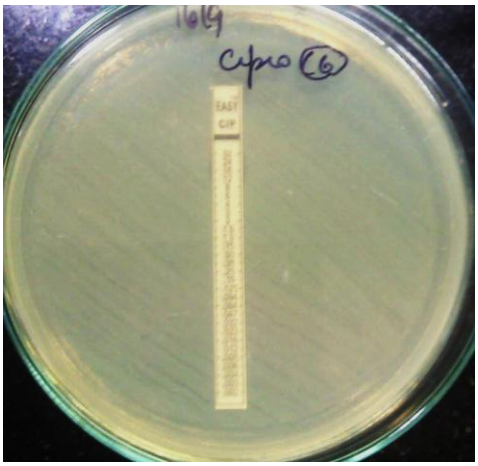


**Fig.3** Distribution of MIC of ceftriaxone among the 62 *Salmonella* isolates

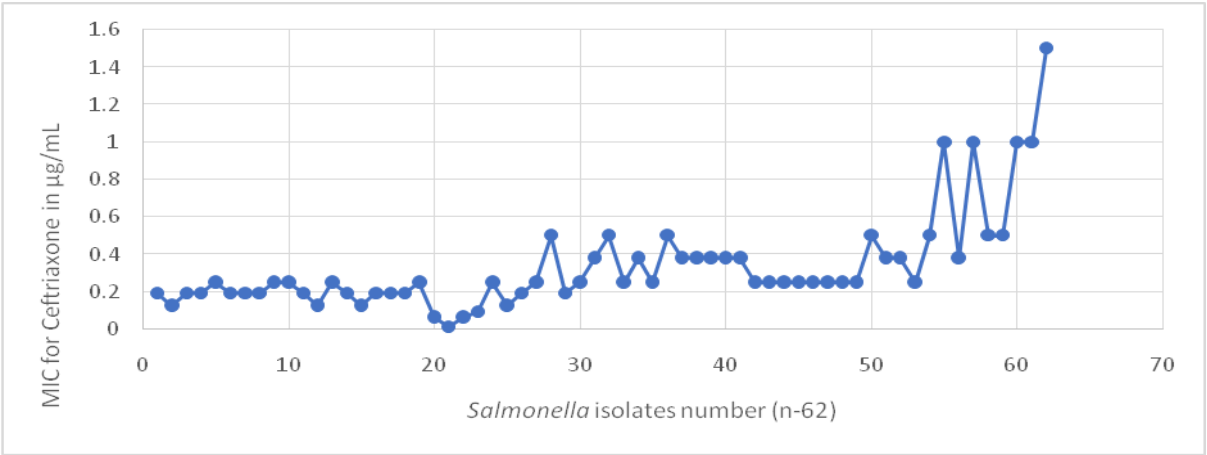


**Fig.4** *Salmonella paratyphi* A isolate with MIC 1µg/mL to ciprofloxacin (intermediate susceptible) and 1.5µg/mL to ceftriaxone (intermediate susceptible)

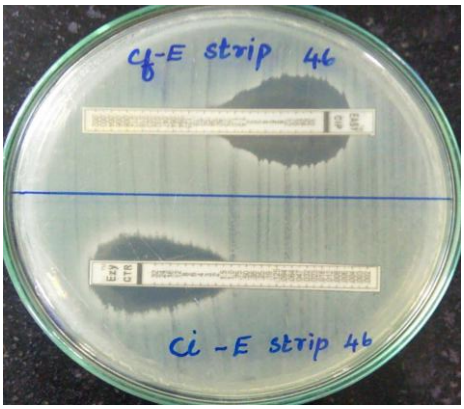



**Fig.5** MIC value of azithromycin among the 62 *Salmonella* isolates (in µg/mL)

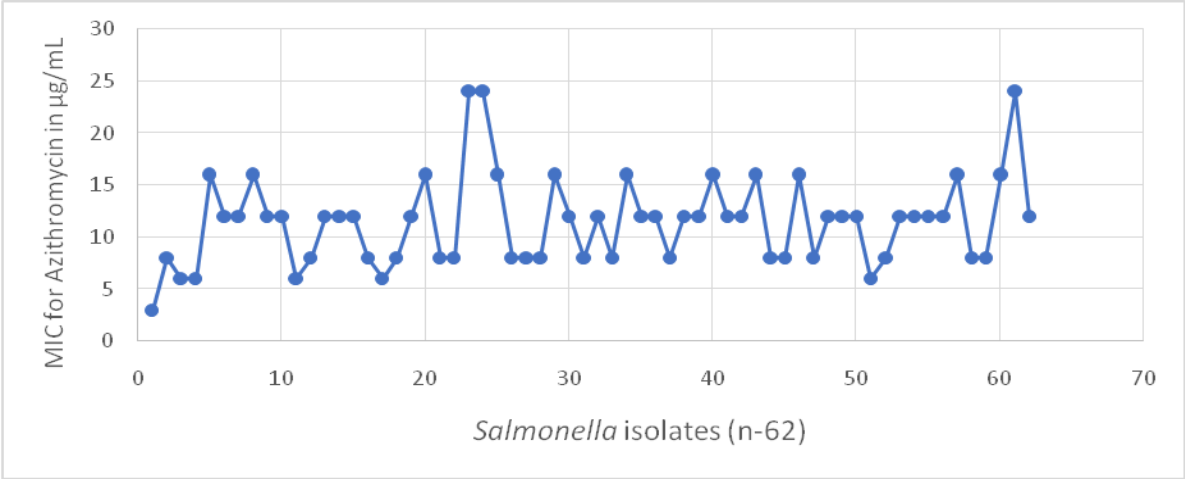
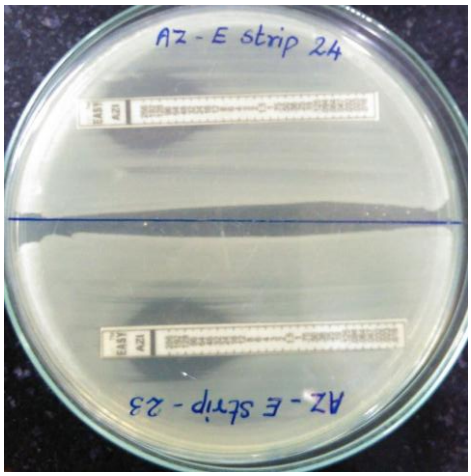



**Fig.6** Two *Salmonella paratyphi* A isolates with MIC 24µg/mL to azithromycin (intermediate susceptible)



The MICs of the control strain ATTC *Escherichia coli* 25922 was 0.008µg/mL for ciprofloxacin, 0.25µg/mL for ceftriaxone, and 1.5 µg/mL for azithromycin.

For timely management of the enteric fever cases adequate knowledge about prevalence of the various serovars of *Salmonella* and their susceptibility patterns is of extreme importance. In our study, *Salmonella paratyphi* A outnumbered 34(54.8%) the *Salmonella typhi* 28(45.2%) isolates. Similar reports are observed in other studies across India, such as in a study from Chandigarh showed an increase of 40.6% isolation of *Salmonella paratyphi* A from January 2003 to April 2007 (Bharmoria and BehariVaish, 2016; Gupta *et al.,* 2009). Recently, a multi-center surveillance study carried out by the Indian Network for Surveillance of Antimicrobial Resistance Group also showed the similar trend (Joshi, 2012). Probable reason for this observation could be explained as current vaccines offer protection against typhoid fever only. So *Salmonella typhi* infections are getting controlled and *Salmonella paratyphi* A is emerging (Bharmoria and BehariVaish, 2016).

Antimicrobial susceptibility reports serve as a guide to clinicians for selecting most appropriate therapeutic agent. In the present study, all 62 isolates were uniformly susceptible to ampicillin, chloramphenicol, co-trimoxazole, azithromycin and ceftriaxone by disc diffusion testing; whereas 15 (24.2%) isolates were resistant and 44 (71%) isolates were intermediate susceptible to ciprofloxacin by disk diffusion testing and 16 (25.8%) isolates were resistant and 41 (66.1%) isolates were intermediate susceptible to ciprofloxacin by MIC method. No isolate exhibited multidrug resistance.

Increased use of second line anti-salmonellae drugs and the discontinuation of use of the conventional first line antimicrobial agents for the treatment of the enteric fever for last two decades, probably have led to the re-emergence of susceptibility to first line antibiotics. As plasmids are responsible for determining resistance to these first line antibiotics; loss of these resistance determining plasmids, may be the reason for the re-emergence of the sensitive strains (Chand *et al.,* 2014; Shrestha *et al.,* 2016).

In our study, discrepancy was observed for susceptibility for ciprofloxacin reports by disk diffusion and MIC method. MIC is the better predictor for drug efficacy in vivo. Also, an increase in MIC, which was not detected by disc diffusion testing is also documented by several researchers (Choudhary *et al.,* 2013; Garg *et al.,* 2013). Hence relying on disk diffusion test results solely may lead to delayed clinical response and serious complications (Srirangaraj, 2014).

All though no resistance was recorded to ceftriaxone or azithromycin among all 62 *Salmonella* isolates, we observed rising MIC to both these antimicrobial agents. Continuous monitoring of an increasing trend in MIC, is vital for early detection of emergence of drug resistance to these antibiotics (Thompson *et al.,* 2017). According to our study results ceftriaxone and azithromycin can be still continued to be used as an effective therapeutic option for culture proven enteric fever cases.

Due to the rapid emergence widespread resistance to ciprofloxacin, prescription of the first-line drugs like ampicillin, cotrimoxazole, and chloramphenicol could be considered as treatment option for enteric fever cases in our hospital setting. This reinforces the potential need for antimicrobial recycling, wherein antibiotics that have a markedly reduced effect may be withdrawn from clinical use for a considerable period, so that they may regain

their efficacy. However ceftriaxone and azithromycin can be still used as an effective therapeutic option for enteric fever cases in our tertiary care center; but continual monitoring of MIC and susceptibility reporting based on MIC should be routinely done, were facilities are available than doing only disk diffusion testing for enteric fever isolates to avoid treatment failures.

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