ORIGINAL ARTICLE

Antimicrobial Susceptibility Trend among Enteric Fever Isolates in a Tertiary Care Hospital of Coastal Karnataka, India

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Abstract:

Background: Enteric fever due to *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* A (*S. paratyphi* A) is one of the significant public health problems worldwide. *Aim and Objectives*: To determine the antibiotic susceptibility pattern of Enteric fever isolates to first-line drugs as well as other drugs such as ciprofloxacin, ceftriaxone, and azithromycin. Nalidixic acid as a surrogate marker was reviewed. *Material and Methods*: A total of 80 isolates were tested for antimicrobial susceptibility using Kirby Bauer disc diffusion method for ampicillin, chloramphenicol, co-trimoxazole, and azithromycin. Minimum Inhibitory Concentration (MIC) determination by E-test for ciprofloxacin and ceftriaxone were performed. *Results:* Out of the 80 *Salmonella* isolates, 64 (80%) were *S. typhi*, and 16 (20%) were *S. paratyphi* A. Susceptibility to ampicillin, co-trimoxazole, and chloramphenicol was 97.5%. Only two (2.5%) were MDR strain. Regarding ciprofloxacin and nalidixic acid, 78 (97.5%) were resistant. All the isolates were sensitive to ceftriaxone (100%). Six out of 80 (7.5%) strains of *S. typhi* showed resistance to azithromycin. *Conclusion:* Re-emergence of susceptibility to first-line drugs suggests the possibility of using these drugs again in treatment. MIC for ciprofloxacin and ceftriaxone should be done routinely. Nalidixic acid resistance cannot be considered as a reliable surrogate marker. The emergence of resistance for azithromycin is a cause of concern.

Keywords: Enteric Fever, Antibiotic Sensitivity Pattern, Multidrug Resistance, Minimum Inhibitory Concentration Determination

Introduction:

Enteric fever due to *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* A (*S. paratyphi* A) is one of the significant public health problems worldwide. These infections are mainly transmitted by the fecal-oral route in regions with poor hygiene and sanitation. The World Health Organization has estimated that annually, typhoid fever accounts for 21.7 million cases, and paratyphoid accounts for 5.4 million cases globally. The mainstay in the management of enteric fever is antibiotic therapy. The mortality rate can reach a 30% rate in untreated cases, which reduces to <1% with proper antibiotic treatment [1]. Even though there is a gradual decrease in the burden of typhoid fever, the emergence of drug resistance makes this infection still a severe threat to the developing nations. The development of antimicrobial resistance, mainly the multidrug resistance to Ampicillin, Chloramphenicol, and Co-trimoxazole (ACCo), has further complicated the treatment and management of enteric fever. The first antibiotic resistance was reported to chloramphenicol in England during the 1950s; however, the initial outbreak occurred in Mexico in 1972 [2]. In India, at the same time, the first outbreak of multidrug-resistant *S. typhi* was reported in Calicut [3].

The development of Multidrug-resistant *S. typhi* (MDRST) strains led to the use of fluoroquinolones [2]. The emergence of strains with Decreased Ciprofloxacin Susceptibility (DCS) has emerged in Asia. DCS is defined as ciprofloxacin MIC of 0.12 – 1 µg/mL [4]. Due to this continuous rise in Minimum Inhibitory Concentration (MIC) levels, there have been reports of treatment failure or prolonged defervescence time with ciprofloxacin [5]. Fluoroquinolone-resistant strains were identified by determining susceptibility to nalidixic acid. However, a consistent increase in the MIC levels of ciprofloxacin is now often associated with an increase in Nalidixic Acid Resistant *S. typhi* (NARST) strains due to mutations in the gyrA gene [4, 6].

With the emergence of decreased susceptibility and resistance to fluoroquinolones, third-generation cephalosporins are used as a drug of choice. *S. typhi* resistant to Third-generation Cephalosporins (3GC) like cefotaxime (2%) as well as cefepime (1%), though low at present (1%) is also emerging in India [6]. In uncomplicated cases, azithromycin has been used as an effective alternative. However, an increase in MICs has been reported in India [7].

This study's main aim was to determine the effect of revised CLSI breakpoints for ciprofloxacin susceptibility in typhoidal *Salmonellae*. Nalidixic Acid Resistance (NAR) as a marker for ciprofloxacin resistance was also reviewed. MIC for ceftriaxone studied because 3GC now used as a drug of choice in the management of severe typhoidal infections and due to the emergence of a delayed response to these antimicrobials, susceptibility pattern for azithromycin was observed.

Material and Methods:

A prospective time-bound study was carried out in our diagnostic laboratory of nine months (between September 2016 and May 2017). A total of 5432 blood samples were received for blood culture, both from inpatients and outpatients. Blood samples were added into BacT/ALERT FA Plus bottles ((Biomeriux, Inc. Durham, North California, and USA) and incubated aerobically using BACT/ALERT 3D system ((Biomeriux, Inc. Durham, North California and USA). Aliquot of positive samples were taken for Gram stain and sub-cultured by standard methods into Blood agar, Chocolate agar, and MacConkey's medium. After isolation, the growth of Gram-Negative bacteria was identified by either conventional methods or an automated Vitek 2 compact system. The serotype was confirmed by agglutination with polyvalent and group-specific antisera (Denka Seiken Co., Ltd. Japan) [8].

Minimum Inhibitory Concentration (MIC) and antibiotic susceptibility pattern were determined by Vitek 2 compact system (Biomeriux, Inc. Durham, North California and USA), and Modified Kirby-Bauer disk diffusion method respectively for ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg) and azithromycin (15 µg) and interpreted using Clinical and Laboratory Standards Institute (CLSI) 2017 Guidelines. Azithromycin was used only for *S. typhi* [9]. The MIC for ciprofloxacin and ceftriaxone were also determined exclusively by using E-test (HiMedia, Mumbai). For quality control, a standard strain of *E. coli* ATCC 25922 was included.

Results:

Out of the 5432 blood cultures, 811 samples (14.93%) yielded a positive result. Of the 811 positive blood cultures, 80 (9.86%) were positive for Salmonella. Of the 80 isolates, 64 (80%) were *S. typhi*, and 16 (20%) were *S. paratyphi* A. None of the strains was *S. paratyphi* B. Antibiotic susceptibility pattern among Salmonella isolates was depicted in Table 1. The percentage of *S. typhi* and *S. paratyphi* A isolates susceptible to various antibiotics were illustrated in Figs. 1 and 2, respectively. Only 2 (2.5%) isolates were resistant to chloramphenicol and co-trimoxazole. Seventy-eight (97.5%) was sensitive to ciprofloxacin and nalidixic acid. Six out of 64 (9.38%) isolates of *S. typhi* were resistant to azithromycin. Among all the isolates, only 2 (2.5%) were MDR strain. Two strains showed moderate resistance to ampicillin. Distribution of MIC range of ciprofloxacin and ceftriaxone for Salmonella isolates were depicted in Table 2 and 3, respectively. Of the 80 isolates of Salmonella, 22 strains (27.5%) had the ciprofloxacin MIC range of 2 µg/ml followed by 12 isolates (15%) with 3 µg/ml and 6 µg/ml respectively. Whereas 36 (45%) strains of Salmonella were with MIC of 0.094 µg/ml to ceftriaxone followed by 32 (40%) strains with the MIC of 0.125.

Table 1: Antibiotic Susceptibility Pattern among Salmonella Isolates

Antibiotics	Number of Isolates	Number of Resistant Isolates and Percentage
Ampicillin	80	02 (2.5)
Chloramphenicol	80	02 (2.5)
Co-trimoxazole	80	02 (2.5)
Ciprofloxacin	80	78 (97.5)
Nalidixic acid	80	78 (97.5)
Azithromycin	64	06 (9.37)
Ceftriaxone	80	0

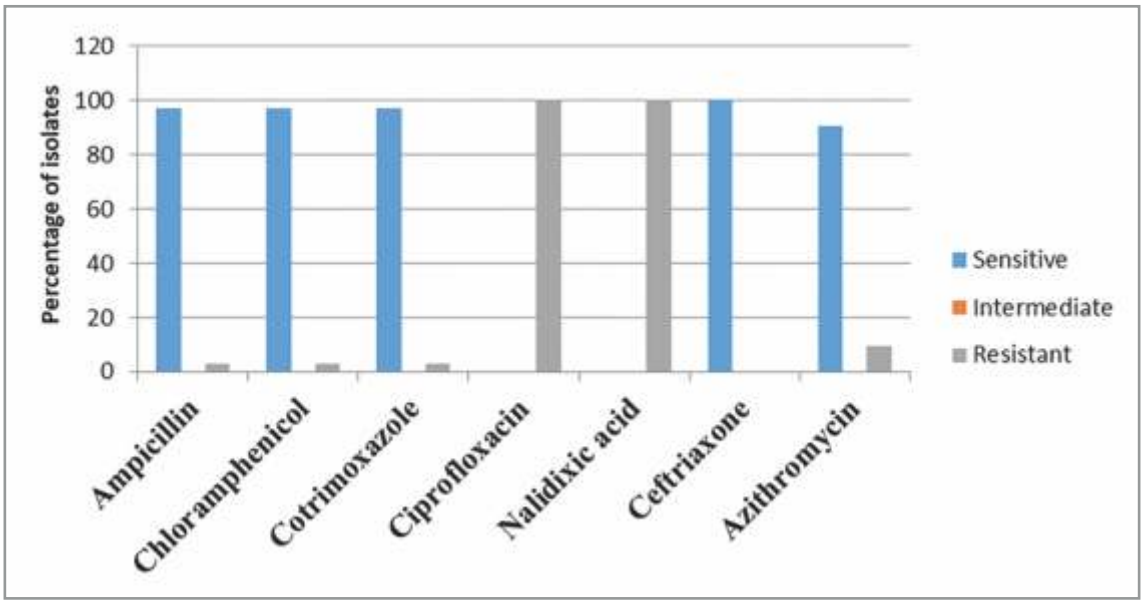

Fig. 1: Antibiotic Susceptibility of *Salmonella typhi*

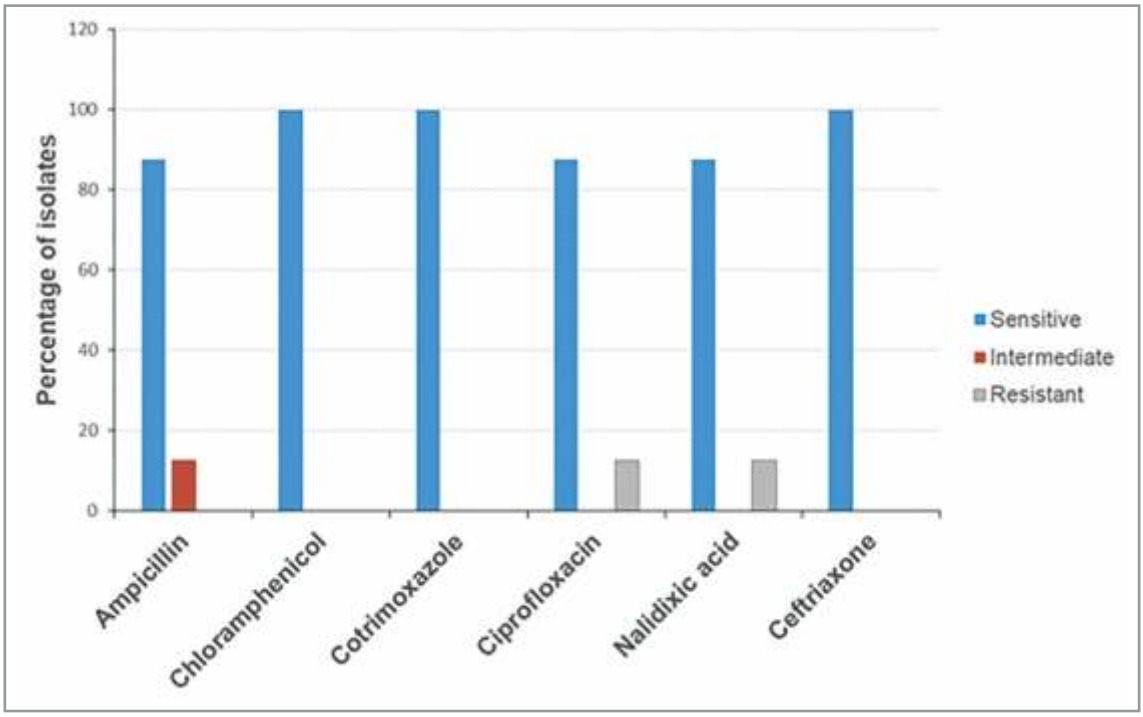

Fig. 2: Antibiotic Susceptibility of *Salmonella paratyphi A*

Table 2: Distribution of MIC Range of Ciprofloxacin for Salmonella Isolates

MIC µg/ml	*S. typhi*	*S. paratyphi A*	Percentage
⩽0.75	0	2	2.5
1	4	0	5
1.5	6	0	7.5
2	14	8	27.5
3	10	2	15
4	2	2	5
6	10	2	15
8	8	0	10
12	2	0	2.5
⩾32	8	0	10
Total	**64**	**16**	**100**

Table 3: Distribution of MIC Range of Ceftriaxone for Salmonella Isolates

MIC µg/ml	*S. typhi*	*S. paratyphi A*	Percentage
0.064	10	0	12.5
0.094	30	6	45
0.125	22	10	40
0.19	2	0	2.5
Total	**64**	**16**	**100**

Discussion:

Enteric fever is an infection with multisystem involvement caused by *S. typhi* and *S. paratyphi A, B,* and *C*. The disease is characterized by a prolonged incubation period, fever, and systemic bacterial dissemination. Enteric fever caused by resistant strains of Salmonella species is one of the primary concerns in India. The most common isolate in our study was found to be *S. typhi*, followed by *S. paratyphi* A. Our findings were found to be consistent with other previous studies [6, 10, 11]. The changing susceptibility trends in the case of enteric fever isolates is a dangerous condition where systematic reviews should be done to approach the problem efficiently. Reduced use of Ampicillin, Chloramphenicol, and Co-trimoxazole (ACCo) has led to the re-emergence of susceptibility to these first-line drugs [4, 7, 12]. A study was done in the same region also showed a decrease in MDR strains, with only 3 (3.29%) cases recorded [6].

Similarly, our study showed a significant decline of MDR strains being only 1 (1.25%) case. With the emergence of MDR Salmonella strains, fluoroquinolones become the drug of choice for empirical therapy [13]. However, due to single and multiple mutations at the gyrA gene and gyrB gene, resistance to ciprofloxacin had developed. GyrA mutations also conferred high-level resistance to the nonfluorinated quinolone nalidixic acid [14-15]. Most of the previous studies showed the presence of strains with decreased susceptibility to ciprofloxacin or high-level resistance to ciprofloxacin with or without resistance to nalidixic acid [6, 16, 17]. However, in the present study, all the *S. typhi* isolates were resistant to ciprofloxacin, whereas exactly one strain of *S. paratyphi* A was sensitive to ciprofloxacin. All the strains resistant to ciprofloxacin were also NARST.

In contrast to previous studies, none of the isolates showed resistance to nalidixic acid and decreased susceptibility to ciprofloxacin [18]. Resistance to ceftriaxone has been reported in several parts of the world. In one of the studies, a total of 7 strains out of 344 (2%) showed resistance to ceftriaxone [19-21]. However, all the 80 isolates in our study were susceptible to ceftriaxone. Even though there are a high cure rate and better efficacy profile associated with ceftriaxone treatment, the requirement of parenteral administration and high relapse rate narrowed down the therapeutic options. Therefore, newer drugs like azithromycin have been recommended [5]. This macrolide is recommended for empirical therapy due to its high intracellular concentration and good clinical response. However, there have been sporadic reports of the increase in MIC as well as resistance to azithromycin in several parts of the world [7, 10, 22-24]. The study's main limitation was that clinical outcomes were not determined, small sample size and short duration of the study.

Conclusion:

There is a re-emergence of susceptibility to the first-line drugs ACCo due to the reduced use of these drugs. Regarding ciprofloxacin, it is strongly recommended that the use of ciprofloxacin be limited, and nalidixic acid should not be used as a surrogate marker for the detection of ciprofloxacin resistance. MIC determination for ciprofloxacin is preferred if ciprofloxacin therapy is used. Ceftriaxone remains the drug of

choice in this region. Azithromycin is suggestive in the treatment of enteric fever, but the emergence of resistance to azithromycin should be monitored regularly.

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