Case Report

A case report and review of the literature: Ciprofloxacin resistant *Salmonella enterica* serovar Typhi in India

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Abstract

Background: Enteric fever is a major public health problem in India. The current treatment of choice is the fluoroquinolones.

Methods: The minimum inhibitory concentration (MIC) of ciprofloxacin was determined by E-test, HIMEDIA HiComb MIC test and agar dilution.

Results: An isolate of *Salmonella enterica* serovar Typhi (*S.* Typhi) from a case of enteric fever gave a ciprofloxacin MIC of 64µg/ml.

Conclusions: To our knowledge there have been no reports of such high-level resistance to ciprofloxacin in *S.* Typhi from southern India. HIMEDIA HiComb MIC test method is an alternative to the E-test. Ciprofloxacin resistant typhoid fever responds to treatment with ceftriaxone.

Key Words: Enteric fever, *Salmonella* Typhi, drug resistance.

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Introduction

Enteric fever is a global major public health problem. Almost 80% of the cases and deaths are in Asia and the rest occur mostly in Africa and Latin America [1]. Enteric fever is endemic in many developing countries, including India and, if not treated appropriately, has a mortality rate of 30%. Appropriate treatment reduces the mortality rate to as low as 0.5% [2].

Specific treatment of enteric fever used to be chloramphenicol, trimethoprim-sulfamethoxazole or ampicillin. The causative organism, *Salmonella enterica* serovar Typhi (*S.* Typhi) has rapidly gained resistance to these antibiotics [3,4] and so fluoroquinolones, such as ciprofloxacin, became the drug of choice for the treatment of enteric fever [5,6]. The incidence of multidrug resistant (MDR) *S.* Typhi was as high as 60% but then declined in Pune (1999), Nagpur (2001) and Calcutta (2000) [7,8,9]. The resurgence of resistant strains in Ludhiana in 2002, however, is of concern [10]. A US-based study of imported strains [11] noted an increase in the number of MDR and nalidixic acid resistant *S.* Typhi globally (NARST), although all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh [12] there has been a reported decrease in MDR isolates with no corresponding increase in sensitive strains. For ciprofloxacin there has been an increase in MIC in strains imported into the UK [13], in Bangladesh [14], as well as in India [15,16,17,18]. The exact mechanism of resistance is not fully understood but various studies have found that a single mutation in the *gyrA* gene is sufficient to confer resistance to nalidixic acid and reduced susceptibility to fluoroquinolones. Turner *et al*. observed that one of the single amino acid substitutions in GyrA is sufficient for resistance to the quinolones nalidixic acid and cinoxacin, but resistance to the fluoroquinolones (gatifloxacin, ofloxacin, ciprofloxacin, enrofloxacin and moxifloxacin) requires two substitutions in GyrA and one in ParC, confirming that these three point mutations (Ser-83→Phe or Tyr and Asp-87→Asn in GyrA with Glu-84→Lys in ParC) combined are sufficient to confer fluoroquinolone resistance in *S.* Typhi. The high levels of resistance observed for these triple point mutants indicate that the amino

acid substitutions protect GyrA and ParC from relatively high concentrations of fluoroquinolones [19]. A study on *Salmonella enterica* serovar Paratyphi A (*S.* Paratyphi A) revealed that high-level resistance to ciprofloxacin is also associated with double mutations in the DNA gyrase subunit *gyrA* (Ser83→Phe and Asp87→Gly) and a mutation in topoisomerase IV subunit *parC* (Ser80→Arg) [20]. Given the variation in the susceptibility patterns reported for *S.* Typhi, it is important to constantly monitor it to provide suitable guidelines for treatment.

There have been several reports of therapeutic failure of ciprofloxacin in patients with enteric fever [21,22]. All these strains were interpreted as ciprofloxacin susceptible by the standard antibiotic susceptibility tests done in a clinical laboratory using the CLSI (formerly NCCLS) guidelines [23]. There was a an increase in the MIC of ciprofloxacin compared to nalidixic acid sensitive isolates, although the values were below the breakpoint of resistance [17,24]. *S.* Paratyphi A resistant to ciprofloxacin with a MIC value of 8µg/ml and 32µg/ml has been reported from our centre [25,26]. Here we report the isolation of a strain of *S.* Typhi showing high-level resistance to ciprofloxacin.

Case Report

An isolate of *S.* Typhi from a case of enteric fever was found to have a ciprofloxacin MIC of 64µg/ml. The strain was isolated from the blood sample of a 19-year-old male presenting with enteric fever at Government General Hospital, Pondicherry, India, in September 2007. The antimicrobial susceptibility was determined by the disc diffusion method as per the CLSI guidelines. The isolate was found to be resistant to ciprofloxacin (did not exhibit zone of inhibition), intermediate susceptible to ampicillin and susceptible to chloramphenicol, cotrimoxazole and ceftriaxone. The MIC of ciprofloxacin as determined by the E-test method (AB-Biodisk, Sweden) was found to be >32µg/ml, the agar dilution method and the HIMEDIA HiComb MIC test (HIMEDIA, Mumbai, India) revealed MIC of 64µg/ml. This exceeds the limit set for resistance by CLSI by a large margin, which is 4 µg/ml [27]. The E-test, HIMEDIA HiComb MIC test and agar dilution method MICs show good concordance and interpretative agreement. E test is simple, easy to perform, and a reliable method. However, its cost and limited availability in India may restrict its use. The reference agar dilution method can be used reliably in routine laboratory susceptibility testing. HIMEDIA HiComb MIC test method is an alternative to the E-test and it was used since the MIC against ciprofloxacin was crossing the upper gradient limit of concentration, 32µg/ml on the E-test strips. The MIC of ofloxacin and gatifloxacin as determined by the E-test method (AB Biodisk) was found to be >32µg/ml and 8µg/ml respectively.

A regimen of ciprofloxacin (500 mg orally twice daily for 10 days) was initiated. Since the patient did not respond to ciprofloxacin the treatment was switched over to ceftriaxone (1gm intravenously every 12 hours) and responded within 3 days. The patient was discharged after 10 days on ceftriaxone with no relapse on follow-up.

Discussion

The strain in this study was found to be sensitive to most antibiotics except quinolones and was intermediately susceptible to ampicillin. This is unusual, but can be explained by the fact that resistance to other antibiotics is plasmid-mediated, and is independent of resistance to fluoroquinolones, which is chromosomally mediated. It is possible that the development of resistance to ciprofloxacin is due to exposure to the drug at concentrations near the MIC.

This highly resistant ciprofloxacin *S.* Typhi strain is still susceptible to most of the first-line antibiotics, and this is different from the other cases reported in India where *S.* Typhi strains are resistant to first-line antibiotics as well as fluoroquinolones [28,29,30,31].

In India, by the mid 1990s, reports began to appear of treatment failures with ciprofloxacin [32], followed by reports from 2001 onwards of rising MICs (reduced susceptibility) of ciprofloxacin for *S.* Typhi isolates [17]. In 2008 there are reports of high-level ciprofloxacin resistant *Salmonella enterica* from many centres in India, including ours (Table 1) [25,26,28,33-36]. This is the first report of a high-level ciprofloxacin resistant *S.* Typhi, 64µg/ml, from South India.

In developing countries such as India, ciprofloxacin continues to be the mainstay in the treatment of enteric fever as it is orally effective and economical. The emergence of *S.* Typhi highly resistant to ciprofloxacin is a cause for worry for

both clinicians and microbiologists as well as for patients. Though fluoroquinolone resistance is chromosomally mediated, selective pressures exerted by the overuse of these drugs may see such isolates becoming more common in the future. Of interest, though, is the possibility of turning to an older drug such as co-trimoxazole for treatment, in case of susceptible isolates.

Table 1. Studies reporting high-level ciprofloxacin resistance in *Salmonella enterica* serovar Typhi and Paratyphi A in India.

Serovar	No. of isolates	Year and place of isolation	MIC of ciprofloxacin (µg/ml)	References
S. Typhi	3	2001-2003; North India	≥32	[34]
S. Paratyphi A	2	2001-2003; North India	8	[34]
S. Paratyphi A	1	2004; South India	8	[25]
S. Typhi	2	2004; North India	16	[28]
S. Paratyphi A	4	2004-2005; South India	8-32	[26]
S. Typhi	1	2005; North India	16	[33]
S. Typhi, *S.* Paratyphi A	28	2005-2006; North India	8-≥512	[35]*
S. Typhi	22	2005-2006; North India	16->32	[36]

*This report doesn't clearly indicate MIC of *S.* Typhi and *S.* Paratyphi A individually.

Third-generation cephalosporins, particularly ceftriaxone, have remained as the viable alternative in the treatment of typhoid fever in this country and elsewhere. In this case report the particular strain involved was also sensitive to ceftriaxone but this may not remain the case. Already there are reports of extended spectrum beta-lactamase (ESBL)-mediated cephalosporin resistance in non-typhoidal *Salmonellae* from many centres including ours [37,38]. It is only a matter of time before these genes encoding ESBLs will cross over to the typhoidal *Salmonellae* and cause resistance to ceftriaxone. Indeed this is already being reported from Pakistan and Bangladesh [39,40].

The emergence of *S.* Typhi highly resistant to ciprofloxacin further emphasizes the importance of the prudent use of antibiotics.

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