In vitro activity of azithromycin, newer quinolones and cephalosporins in ciprofloxacin-resistant *Salmonella* causing enteric fever

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The therapeutic alternatives available for use against ciprofloxacin-resistant enteric fever isolates in an endemic area are limited. The antibiotics currently available are the quinolones, third-generation cephalosporins and conventional first-line drugs. In this study, the MICs of various newer drugs were determined for 31 ciprofloxacin-resistant enteric fever isolates (26 *Salmonella enterica* serovar Typhi and 5 *S. enterica* serovar Paratyphi A). MICs for ciprofloxacin, ofloxacin, gatifloxacin, levofloxacin, cefotaxime, cefixime, cefepime and azithromycin were determined using Etest strips and the agar dilution method. By Etest, all of the ciprofloxacin-resistant isolates had ciprofloxacin MICs $\geq 32 \mu\text{g ml}^{-1}$. *S. Typhi* showed MIC₉₀ values of 0.50, 0.25 and 0.38 $\mu\text{g ml}^{-1}$ for cefixime, cefotaxime and cefepime, respectively. For the cephalosporins, a negligible difference in MIC₉₀ and MIC₅₀ values for *S. Typhi* and *S. Paratyphi A* was observed. A single isolate of *S. Typhi* showed a high azithromycin MIC of 64 $\mu\text{g ml}^{-1}$. The MIC₉₀ value for azithromycin in *S. Typhi* and *S. Paratyphi* was 24 $\mu\text{g ml}^{-1}$. Gatifloxacin demonstrated lower resistance (80.8 %) compared with the other quinolones (92–100 %) in *S. Typhi*. The rise in MIC levels of these antimicrobials is a matter for serious concern.

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INTRODUCTION

In India, ciprofloxacin has been the treatment of choice for enteric fever for over a decade. Unfortunately, after its introduction there was a decrease in the susceptibility to this drug, with consequent therapeutic failure (Capoor *et al.*, 2006; Cooke *et al.*, 2006; Kownhar *et al.*, 2007; Renuka *et al.*, 2005; Saha *et al.*, 2006). This was detected in the laboratory as nalidixic acid resistance. It was followed by the widespread occurrence of isolates with decreased susceptibility to ciprofloxacin (indicated by nalidixic acid resistance, with ciprofloxacin MICs of 0.125–1.0 $\mu\text{g ml}^{-1}$), associated with an impaired response to ciprofloxacin treatment, and the subsequent emergence of highly resistant isolates (ciprofloxacin MICs $>1.0 \mu\text{g ml}^{-1}$). High-level ciprofloxacin-resistant enteric fever has evolved in Asian countries, including India (Capoor *et al.*, 2006; Cooke *et al.*, 2006; Gaiind *et al.*, 2006; Kownhar *et al.*, 2007; Pokharel *et al.*, 2006; Renuka *et al.*, 2005; Saha *et al.*, 2006). Resistance is also emerging to extended-spectrum cephalosporins (Capoor *et al.* 2006; Pokharel *et al.*, 2006; Saha *et al.*, 1999). These alternative regimens have several disadvantages such as expense, the intravenous route of administration required and prolonged defervescence time (Saha *et al.*, 2006).

Azithromycin, a broad-spectrum azilide, has prolonged intracellular concentrations and a prolonged half-life. A large number of experimental and clinical trials carried out in the 1990s support its efficacy in traditional multidrug-resistant (ampicillin, chloramphenicol, co-trimoxazole and tetracycline) enteric fever (Butler *et al.*, 1999; Girgis *et al.*, 1999; Gordillo *et al.*, 1993). However, studies reporting the MICs of azithromycin and newer quinolones in the current scenario of ciprofloxacin-resistant enteric fever are scarce (Frenck *et al.*, 2004; Parry, 2004; Parry *et al.*, 2007). The aim of this study was to determine the *in vitro* MIC patterns of various therapeutic alternatives available for the treatment of enteric fever in an endemic region reporting a recent increase in ciprofloxacin resistance.

METHODS

Quinolone-resistant *Salmonella* isolates were recovered from patients with enteric fever admitted to a 1570-bed tertiary care centre at Vardhman Mahavir Medical College and Safdarjung Hospital and Majeedia Hospital, in New Delhi, India, from December 2004 to December 2006. Of 384 isolates of *Salmonella enterica* serovar Typhi (*S. Typhi*) and *S. enterica* serovar Paratyphi A (*S. Paratyphi A*), 31 (8.1 %) demonstrated ciprofloxacin (5 μg) resistance on screening using the Kirby–Bauer disc diffusion method. These were confirmed

by biochemical reactions and serotyping with specific antisera using polyclonal, monovalent O, H and A antisera (Central Research Institute, Kasauli, India). They were also tested by the agar dilution method for their nalidixic acid MICs. The isolates were subjected to Etest strip (AB Biodisk) and agar dilution MIC testing to ciprofloxacin, cefotaxime and ceftazidime (Sigma) on cation-adjusted Mueller–Hinton agar (Difco). In addition, the MICs for ofloxacin, gatifloxacin, levofloxacin, cefotaxime, cefixime, ceftazidime and azithromycin were determined using the Etest strip method. The results were interpreted following Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). The breakpoint MIC levels for azithromycin have not been determined for isolates of *S. Typhi* and *S. Paratyphi A* (CLSI, 2006). In a previous *in vitro* study, azithromycin had an MIC range of 4–16 $\mu\text{g ml}^{-1}$ against *S. Typhi* (Girgis *et al.*, 1999).

RESULTS AND DISCUSSION

Of the 31 resistant isolates, 26 were *S. Typhi* and 5 were *S. Paratyphi A*. Tables 1 and 2 show the MICs of ciprofloxacin-resistant *S. Typhi* and *S. Paratyphi A*, respectively, to ciprofloxacin, cefotaxime and ceftazidime as determined by agar dilution. Tables 3 and 4 show the MICs of the newer antimicrobials tested against the 31 ciprofloxacin-resistant *S. Typhi* and *S. Paratyphi A* isolates, respectively, as determined using Etest strips. All of the isolates had ciprofloxacin MICs $\geq 32 \mu\text{g ml}^{-1}$ by the Etest

Table 1. Agar dilution MICs of ciprofloxacin-resistant *S. Typhi* for ciprofloxacin, cefotaxime and ceftazidime

The CLSI (2006) interpretive criteria for sensitive, intermediate and resistant strains, respectively, are: ciprofloxacin (CIP), ≤ 1 , 2 and $\geq 4 \mu\text{g ml}^{-1}$; cefotaxime (CTX), ≤ 8 , 16–32 and $\geq 64 \mu\text{g ml}^{-1}$; ceftazidime (CEP), ≤ 8 , 16 and $\geq 32 \mu\text{g ml}^{-1}$.

MIC value/resistance	No. of isolates for each antimicrobial		
	CIP	CTX	CEP
MIC ($\mu\text{g ml}^{-1}$)			
≤ 0.125	0	8	2
0.25	0	15	22
0.5	0	0	2
1	0	2	–
2	1	1	–
4	0	–	–
8	5	–	–
16	1	–	–
32	2	–	–
64	10	–	–
128	1	–	–
256	1	–	–
≥ 512	5	–	–
Total	26	26	26
MIC ₉₀ ($\mu\text{g ml}^{-1}$)	512	0.25	0.25
MIC ₅₀ ($\mu\text{g ml}^{-1}$)	64	0.25	0.25
MIC range ($\mu\text{g ml}^{-1}$)	2– ≥ 512	0.125–2	0.125–0.25
Resistance (%)	100	0	0

Table 2. Agar dilution MICs of ciprofloxacin-resistant *S. Paratyphi A* for ciprofloxacin, cefotaxime and ceftazidime

For CLSI interpretive criteria see Table 1.

MIC value/resistance	No. of isolates for each antimicrobial		
	CIP	CTX	CEP
MIC ($\mu\text{g ml}^{-1}$)			
≤ 0.125	0	3	3
0.25	0	2	2
0.5	0	0	–
1	0	0	–
2	1	0	–
4	0	–	–
8	0	–	–
16	0	–	–
32	2	–	–
64	1	–	–
128	0	–	–
256	0	–	–
≥ 512	1	–	–
Total	5	5	5
MIC ₉₀ ($\mu\text{g ml}^{-1}$)	64	0.25	0.25
MIC ₅₀ ($\mu\text{g ml}^{-1}$)	32	≤ 0.125	≤ 0.125
MIC range ($\mu\text{g ml}^{-1}$)	2– ≥ 512	≤ 0.125 –0.25	≤ 0.125 –0.25
Resistance (%)	100	0	0

strip test and had nalidixic acid MICs $\geq 256 \mu\text{g ml}^{-1}$ by the agar dilution method (results not shown). By agar dilution, five *S. Typhi* isolates and one *S. Paratyphi A* isolate showed MICs $\geq 512 \mu\text{g ml}^{-1}$ (Tables 1 and 2). Gatifloxacin resistance was seen in 80.8 and 80 % of *S. Typhi* and *S. Paratyphi A* isolates, respectively. *S. Typhi* showed MIC₉₀ values of 0.50, 0.25 and 0.38 $\mu\text{g ml}^{-1}$ for cefixime, cefotaxime and ceftazidime, respectively, by Etest strip test. The MIC₅₀ values of these agents were 0.19, 0.125 and 0.25 $\mu\text{g ml}^{-1}$. For the cephalosporins tested, the difference in MIC₉₀ and MIC₅₀ for *S. Typhi* and *S. Paratyphi A* was minimal. A single isolate of *S. Typhi* showed a high azithromycin MIC (64 $\mu\text{g ml}^{-1}$), and the MIC₉₀ value for azithromycin was 24 $\mu\text{g ml}^{-1}$ for both *S. Typhi* and *S. Paratyphi A*.

There was a discrepancy in the MICs observed in the Etest strip and agar dilution tests, which has been reported previously (Capoor *et al.* 2006). The first- and second-generation quinolones had varying results. Gatifloxacin (80.8 % resistance) demonstrated better *in vitro* activity compared with other quinolones (96.2 % resistance for ofloxacin and 92.3 % for levofloxacin) in *S. Typhi*. This finding was consistent with the results of a study from Nepal (Pokharel *et al.*, 2006). These observations indicate that fluoroquinolones should be tested individually and that ciprofloxacin does not represent this group adequately. The ‘target-specific’ action of quinolones was originally studied in *Streptococcus pneumoniae* where

Table 3. MICs of ciprofloxacin-resistant *S. Typhi* by the Etest strip method

The CLSI (2006) interpretive criteria for sensitive, intermediate and resistant strains, respectively, are: ofloxacin (OFX) and levofloxacin (LVX), ≤ 1 , 2 and ≥ 4 $\mu\text{g ml}^{-1}$; gatifloxacin (GA), ≤ 2 , 4 and ≥ 8 $\mu\text{g ml}^{-1}$; cefixime (CFX), ≤ 1 , 2 and ≥ 4 $\mu\text{g ml}^{-1}$; cefotaxime (CTX), ≤ 8 , 16–32 and ≥ 64 $\mu\text{g ml}^{-1}$; cefepime (CPM), ≤ 8 , 16 and ≥ 32 $\mu\text{g ml}^{-1}$. Azithromycin (AZ) MIC breakpoints have not been defined.

MIC value/resistance	No. of isolates for each antimicrobial						
	OFX	LVX	GA	AZ	CFX	CTX	CPM
MIC ($\mu\text{g ml}^{-1}$)							
≤ 0.06125	0	0	0	1	2	5	0
0.125	0	0	1	0	8	8	0
0.19	0	0	0	0	5	7	4
0.250	0	0	0	0	4	3	18
0.38	0	0	0	0	3	0	2
0.50	0	0	0	0	1	1	2
0.75	1	1	2	0	0	0	–
1	0	0	2	0	3	0	–
1.5	0	0	0	0	–	1	0
2	1	1	0	1	–	1	0
4	0	0	2	1	–	–	–
6	1	1	1	0	–	–	–
8	0	0	0	5	–	–	–
12	0	3	0	4	–	–	–
16	0	3	3	10	–	–	–
24	0	0	7	1	–	–	–
32	23	17	8	2	–	–	–
64	–	–	–	1	–	–	–
Total	26	26	26	26	26	26	26
MIC ₉₀ ($\mu\text{g ml}^{-1}$)	32	32	32	24	0.50	0.25	0.38
MIC ₅₀ ($\mu\text{g ml}^{-1}$)	32	32	24	16	0.19	0.125	0.250
MIC range ($\mu\text{g ml}^{-1}$)	0.75–32	0.75–32	0.125–32	<0.0612–64	<0.0612–1	<0.0612–2	0.19–0.5
Resistance (%)	96.2	92.3	80.8	–*	0	0	0

*A resistance figure for azithromycin has not been given as no definite breakpoints have been set for *Salmonella* isolates. A single strain with a high MIC (64 $\mu\text{g ml}^{-1}$) was observed.

quinolones have different binding-target affinities (Richardson *et al.*, 2001). Disparity in the MIC levels of quinolones has been attributed to differences in the additional fluoro group and other substitutions in their chemical structure. There are no similar studies in *Salmonella* spp.

Amongst the cephalosporins tested against *S. Typhi* and *S. Paratyphi* A, cefotaxime had the lowest MIC₅₀ and MIC₉₀ levels. In a previous study, we showed that cefepime also had a high activity (Capoor *et al.*, 2006). Cefixime is widely used in India due to its oral route of administration. This could be the reason for the rising MIC levels of third- and fourth-generation cephalosporins (Capoor *et al.*, 2006; Saha *et al.*, 1999), and their overuse can induce strains with extended-spectrum β -lactamases (Pokharel *et al.*, 2006).

Only one isolate of *S. Typhi* showed a high azithromycin MIC (64 $\mu\text{g ml}^{-1}$) and the MIC₉₀ was 24 $\mu\text{g ml}^{-1}$ azithromycin. This was slightly higher than previous reports, which have found the MIC range to be 4–16 $\mu\text{g ml}^{-1}$ (Girgis *et al.*,

1999; Butler *et al.*, 1999). There are as yet no data on the break points of azithromycin for enteric fever (CLSI, 2006). Thus, molecular analysis of such strains with higher MICs is warranted. The MIC₉₀ value observed by Butler *et al.* (1999) was higher for *S. Paratyphi* A compared with *S. Typhi*. A single isolate of *S. Typhi* with an MIC of ≥ 32 $\mu\text{g ml}^{-1}$ was detected as early as 1999. In our study, the MIC₉₀ values for *S. Typhi* and *S. Paratyphi* A were 24 $\mu\text{g ml}^{-1}$. In enteric fever, the role of azithromycin needs to be appreciated, as it is highly effective in removing intracellular salmonellae, defervescence is rapid, gastrointestinal carriage is eradicated and it represents a potential alternative in paediatric populations where quinolones are contraindicated (Girgis *et al.*, 1999). The higher clinical and bacteriological cure rate is attributable to the >100-fold intracellular concentrations of azithromycin in macrophages compared with serum (Butler *et al.*, 1999; Frenck *et al.*, 2004; Girgis *et al.*, 1999; Gordillo *et al.*, 1993; Parry, 2004; Parry *et al.*, 2007). Thus, there is speculation that intracellular MICs may not be represented fully by the

Table 4. MICs of ciprofloxacin-resistant *S. Paratyphi* by the Etest strip method

For CLSI interpretive criteria see Table 3.

MIC value/resistance	No. of isolates for each antimicrobial						
	OFX	LVX	GA	AZ	CFX	CTX	CPM
MIC ($\mu\text{g ml}^{-1}$)							
≤ 0.06125	0	0	0	0	0	1	0
0.125	0	0	0	0	3	2	0
0.19	0	0	0	0	0	1	3
0.250	0	0	0	0	0	0	2
0.38	0	0	0	0	1	0	0
0.50	0	0	0	0	0	1	0
0.75	0	0	1	0	0	–	–
1	0	0	0	1	1	–	–
1.5	0	0	0	0	–	–	–
2	2	1	0	1	–	–	–
4	0	1	1	0	–	–	–
6	0	0	0	0	–	–	–
8	0	0	0	0	–	–	–
12	0	0	0	0	–	–	–
16	0	0	0	1	–	–	–
24	0	0	1	1	–	–	–
32	3	3	2	1	–	–	–
64	–	–	–	–	–	–	–
Total	5	5	5	5	5	5	5
MIC ₉₀ ($\mu\text{g ml}^{-1}$)	32	32	32	24	0.38	0.19	0.25
MIC ₅₀ ($\mu\text{g ml}^{-1}$)	2	32	24	4	0.125	0.125	0.19
MIC range ($\mu\text{g ml}^{-1}$)	2–32	2–32	0.75–32	1–32	0.125–1	<0.061–0.5	0.19–0.250
Resistance (%)	80	100	80	–*	0	0	0

*The resistance figure for azithromycin has not been given as no definite breakpoints have been set for *Salmonella* isolates. A single strain with a high MIC (64 $\mu\text{g ml}^{-1}$) was observed.

currently available *in vitro* MIC testing methods and therefore such testing should be coupled with therapeutic trials. As this was a retrospective study, the therapeutic efficacy of this drug was not determined. Due to its negligible relapse rate and faecal carriage, and its favourable outpatient compliance, azithromycin could become the preferred drug of choice over ceftriaxone, ofloxacin and chloramphenicol.

The indiscriminate use in patients of the existing therapeutic options for enteric fever, and a concomitant rise in MIC levels demonstrated for these antimicrobials for *S. Typhi* and *S. Paratyphi* A in the current study and in prior studies (Frenck *et al.*, 2004; Gordillo *et al.*, 1993; Kownhar *et al.*, 2007; Parry, 2004) is a serious concern. The effects of some of the newer drugs, such as tigecycline and carbapenems, against salmonellae have yet to be elucidated by *in vitro* and *in vivo* trials. The presence of fluoroquinolone resistance warrants a review of the current therapy and initiation of the search for a new effective and affordable drug. Meanwhile, azithromycin and other available antimicrobials will require large-scale, randomized clinical trials to establish their population efficacy.

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