TYPHOID FEVER: NARROWING THERAPEUTIC OPTIONS IN INDIA

Malini R Capoor[1], Deepthi Nair[1], Azra S Hasan[1], Pushpa Aggarwal[1] and B Gupta[2]

Departments of [1]Microbiology, [2]Medicine, Vardhman Mahaveer Medical College and Safdarjung Hospital, New Delhi, India

Abstract. Typhoid fever remains an important public health problem in India. One thousand four hundred fifty-eight blood cultures were screened, 178 grew out *Salmonella enterica* serovar Typhi. On agar dilution minimum inhibitory concentration (MIC) testing, 0.6% of the isolates were resistant to ciprofloxacin, 2% to cefotaxime and 1% to cefepime. Nalidixic acid resistance was observed in 51% isolates, of which 98.9% had decreased susceptibility (MIC> or= 0.125-4 μg/ml) to ciprofloxacin. One strain of nalidixic acid sensitive *S.* Typhi (NASST) also had a decreased MIC (0.125 μg/ml) to ciprofloxacin. Resistance to third and fourth generation cephalosporins is emerging in India and will gain significance in the coming decade. The molecular basis of resistance to cephalosporins and ciprofloxacin resistance in NASST strains need to be further evaluated for *S.* Typhi.

INTRODUCTION

Typhoid fever is estimated to occur in 16 million people causing 600,000 deaths worldwide annually. It is endemic to and known to cause epidemics in tropical and subtropical countries. Sporadic cases of typhoid fever are seen in travelers returning to developed countries from these regions (Rowe *et al*, 1997). Worldwide, cases and deaths due to typhoid fever have decreased following improvements in sanitation, vaccinations and effective antimicrobial therapy. It is rampant in lesser developed countries due to a paucity of the above and the emergence of multi-drug resistance (MDR) (Mirza *et al,* 2000). The indiscriminate use of antimicrobials in humans and animals has led to an emergence of multidrug resistant *S*. Typhi (MDRST) in India (Nath *et al*, 2000; Gautam *et al*, 2002).

In 1989, outbreaks due to the plasmid, R type ACCoSuTTm of H_1 incompatibility group were reported worldwide (Therlfall *et al*, 1992). This led to the use of quinolones as the first line of therapy in the 1990s. Subsequently, nalidixic acid resistant *S.* Typhi (NARST) with decreased susceptibility to ciprofloxacin with therapeutic failure emerged (Aarestrup *et al*, 2003; Crump *et al*, 2003). Currently, cefixime, an oral third generation cephalosporin, has replaced ciprofloxacin in out-patient therapy of quinolone resistant typhoid fever in India. Resistance to third generation cephalosporins has been documented, which is interon associated (Ploy *et al*, 2003). This grim scenario leaves very limited therapeutic alternatives for the treatment of typhoid fever.

MATERIAL AND METHODS

The present study was conducted in a tertiary-care hospital over a period of 14 months. Two hundred fifty-nine isolates of *Salmonella enterica* serovar Typhi (*S.* Typhi) were recovered from a total of 1,458 blood cultures from medical and pediatric in-patients with suspected enteric fever. Blood cultures were collected prior to initiation of antibiotic

Correspondence: Dr D Nair, D-II/2201, Vasant kunj, New Delhi-110070, India.
E-mail:deepthinair2@gmail.com

therapy using aseptic precautions and processed per standard microbiological protocols (Old, 1996). Of 259 isolates of *S.* Typhi, 178 were randomly selected for the study. The biochemical reactions and serotyping was performed to confirm all isolates. Antimicrobial screening of the isolates was done by the disc diffusion method of Kirby Bauer on Mueller Hinton agar using NCCLS, 2004. The antimicrobials used were chloramphenicol (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), nalidixic acid (30 μg), ampicillin (10 μg), ciprofloxacin (5 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefixime (5 μg) and cefepime (30 μg). Screening for the presence of extended spectrum beta lactamase (ESBL) production was done by the double disc diffusion method using cefotaxime (30 μg), ceftriaxone (30 μg) and cefoperazone (75 μg) disc along with an Augmentin (20/10 μg) disc. The MIC for ciprofloxacin (178 isolates), cefotaxime (100 isolates) and cefepime (100 isolates) was done by agar dilution method, following NCCLS, 2004. The control strain used was *E. coli* ATCC 25922.

RESULTS

The majority of *S.* Typhi recovered were from male patients (59.6%) and the median age of patients was 24 years. Fifty-seven point nine percent of isolates were from patients in the Medicine Department, of these 26% required hospitalization. Summer and monsoon months (April to September) showed the maximum rate of isolation (61%).

The disc diffusion method revealed the following resistance patterns: ampicillin 57 (32%), chloramphenicol 73 (41.4%), trimethoprim-sulfamethoxazole 57 (32.1%), cefotaxime 1 (0.6%), ceftriaxone 1 (0.6%), cefixime 0 (0%), cefepime 0 (0%), ciprofloxacin 1 (0.6%) and nalidixic acid 91 (51%). Multi-drug resistance (ACCO) was seen in 32% of strains. ESBL production was not found in any of the *S.* Typhi samples.

The agar dilution MIC testing of *S.* Typhi to ciprofloxacin, cefotaxime and cefepime is recorded in Table 1. The MIC 90 for ciprofloxacin was 0.125 μg/ml, cefotaxime and cefepime were 0.06 μg/ml each. Decreased susceptibility to ciprofloxacin was observed in 98.9% of NARST strains (MIC ≥0.125-4 μg/ml). Nalidixic acid susceptible *S.* Typhi (NASST) had an MIC ranging from < 0.0313-0.063 μg/ml for ciprofloxacin, with 1 (0.01%) strain having an MIC of 0.125 μg/ml.

DISCUSSION

In the present study, multi-drug resistance (ACCO) was observed in 32% of strains. Currently, the incidence of MDRST varies from 25-55% in India (Gautam *et al*, 2002). Some studies have reported higher rates (65%) from abroad (Kariuki *et al*, 2000). After 2000, a re-emergence of sensitivity to the classical first-line agents has been observed due to their restricted use in the "ciprofloxacin era" of the 1990s. There has been a concomitant decrease in susceptibility to ciprofloxacin and nalidixic acid in this region (Mandal *et al*, 2004; Renuka *et al*, 2004).

In this current study, the incidence of NARST was 51%. Other workers from India have reported an incidence varying from 47% (Renuka *et al*, 2004) to 100% (Kadhiravaran *et al*, 2005). In developed countries nalidixic acid resistance has been reported less frequently (0-17%) (Kariuki *et al*, 2000; Hirose *et al*, 2001). In a previous study (Nair *et al*, 2003) from this hospital on archived strains from 1990-2000, the occurrence of NARST was 22%, with a single strain showing resistance to ciprofloxacin (MIC= 4 μg/ml). In the current study, a single strain (0.6%) of *S.* Typhi was found resistant to ciprofloxacin at 4 μg/ml. A report from the Salmonella Reference Center in India also showed similar findings with 0.56% of *S.* Typhi being resistant and 39.96% with an intermediate level of MIC to ciprofloxacin (Mehta *et al*, 2001). Most of the NARST (98.9%) had

Table 1
Agar dilution MIC to ciprofloxacin, cefotaxime and cefepime.

MIC range (μg/ml)	Antimicrobial (no. tested)		
	Ciprofloxacin (178)	Cefotaxime (100)	Cefepime (100)
<0.0313	9	-	-
0.063	78	97	99
0.125	75	0	0
0.250	11	0	0
0.5	2	0	0
1	2	0	0
2	0	0	0
4	1	1	0
8	0	0	0
16	0	0	0
32	0	0	0
64	-	1	1
128	-	1	0
Total	178	100	100

NCCLS (2004) interpretive criteria for *S.* Typhi: sensitive, intermediately sensitive and resistant strains, respectively for ciprofloxacin: ≤1 μg/ml, 2 μg/ml, ≥4 μg/ml; cefotaxime: ≤8 μg/ml, 16-32 μg/ml, ≥64 μg/ml; Cefepime: ≤8μg/ml, =16 μg/ml, ≥32 μg/ml.

a decreased susceptibility to ciprofloxacin (MIC ≥0.125 μg/ml) and one was resistant to ciprofloxacin (MIC=4μg/ml). Several researchers have corroborated this finding in other countries (Aarestrup *et al*, 2003; Crump *et al*, 2003) and in India (Kadhiravaran *et al,* 2005).The use of the NCCLS breakpoint for resistance to ciprofloxacin, by *Salmonella* Typhi at ≥4 μg/ml is being challenged and considered to obscure the true occurrence of resistance. There have been several recommendations that the MIC cut-off should be reduced to ≥0.125 μg/ml for redefining resistance (Aarestrup *et al*, 2003; Crump *et al*, 2003).

The molecular basis for decreased ciprofloxacin susceptibility is attributed to point mutations in the Quinolone Resistance Determining Region (QRDR) of *gyr* gene (Wain *et al*, 1997; Threlfall *et al*, 1999). In this study, an interesting finding was the occurrence of a single isolate (0.01%) of NASST with decreased susceptibility to ciprofloxacin (0.125 μg/ml). In a comprehensive review by Crump *et al* (2003), this phenomenon has been reported. These strains need to be genotyped to detect additional methods of resistance which could evolve to play an important role in the future. A single study from India sequenced the NASST strains with a higher MIC (0.002-0.125 μg/ml) (Renuka *et al*, 2004) and they could not detect any of the usual gyrA mutations.

Resistance to third generation cephalosporins by disc diffusion has been documented in India, ranging from 0-11% (Gautam *et al*, 2002; Kadhiravaran *et al*, 2005).The MIC 90 for cefotaxime was very low at 0.06 μg/ml, Previous studies have also observed similar findings (Hirose *et al*, 2001; Mandal *et al*,

2004). They have not detected any resistance to cephalosporins of the third generation using MIC methods. In the current study, 2 strains of *S.* Typhi were resistant to cefotaxime (MIC=64, 128 μg/ml, respectively). However, a single published report from the United States has reported ceftriaxone resistance in 0.1 - 0.4% of their isolates (Marano *et al*, 1999). In our study, only a single strain of *S.* Typhi was resistant to cefepime (64 μg/ml) and to the best of our knowledge there has been no previous published report on fourth generation cephalosporins activity towards *S.* Typhi.

In India, with a large reservoir of NARST strains which are converting to quinolone resistance and a few NASST strains showing increasing ciprofloxacin MIC, treatment failure with quinolones is now the norm. First line antimicrobials, which had been shelved for almost two decades, need to be revisited. The third and fourth generation cephalosporins represent treatment alternatives, although resistance is gradually increasing to these drugs. Understanding the molecular basis of NASST resistance to quinolones and the emerging cephalosporin resistance would be useful for the therapy and molecular epidemiology of *S.* Typhi in the future.

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