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RESEARCH ARTICLE

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STUDIES ON ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF SALMONELLA ISOLATES FROM CHENNAI, INDIA

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ABSTRACT

Typhoid fever is a major health problem as the causative agent *Salmonella enterica serovar typhi* and *paratyphi A* has developed resistance to many antimicrobial agents. The study was undertaken to find out the antimicrobial susceptibility pattern of *Salmonella* isolates. We have studied 176 *Salmonella* isolates - 172 blood isolates, 3 stools isolates and one isolate from bone marrow. Antimicrobial susceptibility testing was done by disc diffusion method and MIC by agar dilution method. Out of 176 *Salmonella* isolates, 133 were *S. typhi*, 41 were *S.paratyphi A* and 2 were *S.typhimurium.* 98.5% of *S.typhi* and 92.7% of *S.paratyphi A* were resistant to nalidixic acid by MIC studies. 95.5% *S.typhi* and 97.6% of *S.paratyphi A* were found to have reduced susceptibility (0.125-0.5µg/ml) by MIC studies as reported earlier. As per CLSI standard MIC value of ≤ 1µg/ml is taken as sensitive, ≥ 4µg/ml is taken as resistance for *Salmonella* isolates.

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KEYWORDS

Antimicrobial susceptibility-Salmonella, Typhoid fever, Nalidixic acid, MIC values.

INTRODUCTION

Enteric fever is a global public health problem and is endemic in many developing countries, including India. It has a mortality rate of 30% if not treated properly though appropriate treatment reduces the mortality rate to as low as 0.5% [1].

Until the mid 1980's the first line drugs, ampicillin, chloramphenicol and co-trimoxazole (ACCo) were used as standard treatment for enteric fever. Simultaneous resistance to three or more different groups of antimicrobial drugs is defined as MDR *Salmonella* [2]. MDR *Salmonella typhi* has appeared throughout the world, especially in South America, the Indian sub continent, Africa and South East Asia [3]. In India, *Salmonella typhi* drug resistance has been reported since 1960 following the first outbreak of MDR *Salmonella typhi* in Calicut [4, 5]. MDR *Salmonella typhi* is still common in many areas, although in some regions highly sensitive strains have re-emerged.

In India the emergence of MDR Salmonella enterica isolates led to the use of fluoroquinolones (ciprofloxacin and ofloxacin) as the first line drugs for its treatment. Fluoroquinolones have revealed higher sensitivity in invitro and effective clinical outcome against *Salmonella* species [6]. Recently strains with low level resistance (MIC 0.125- 0.5µg/ml) started appearing which later shows increased resistance towards fluoroquinolones [7, 8.]

The emergence of increased resistance to ciprofloxacin in *Salmonella typhi* or *Salmonella paratyphi A* would severely limit the choice of antimicrobial therapy for enteric fever. Recent reports of infections due to highly resistance strains of *Salmonella paratyphi A* against fluoroquinolones cause concern in the management of enteric fever [9, 10, 11].

The first major outbreak of typhoid fever with resistant strains to nalidixic acid was reported from different places [12]. Nalidixic-acid-resistant strains exhibiting reduced susceptibility towards ciprofloxacin (MICs 0.125–1 mg/L) have become endemic in several geographical areas of the Indian subcontinent and have also been reported in US, UK and in other developed countries, thus Salmonella emerged as resistant pathogen to conventionally used antimicrobial drugs [13,14,15].

There are resistance strains showing intermediate MIC values. These isolates showed reduced susceptibility to ciprofloxacin (0.125—0.5µg/ml), inspite of resistance to nalidixic acid. Resistance to nalidixic acid is a useful marker of this subpopulation of strains. This low-level resistance turns out to be clinically relevant but may not be reported by microbiology laboratories [15, 16, 17]. These isolates have been variously described as nalidixic acid-resistant with low-level ciprofloxacin resistance, or quinolone-resistant (as distinct from fluoroquinolone resistance) [18].

In addition resistance was also reported for cephalosporins due to ESBL production from different places [19]. Due to variation in the susceptibility patterns of *Salmonella* species from different geographical areas, it is important to constantly monitor its susceptibility pattern so as to provide suitable guidelines for successful treatment. The present study was undertaken to find out recent changes in the susceptibility pattern of *Salmonella* isolates from Chennai, South India.

MATERIALS & METHODS

A total of 176 isolates of Salmonella were collected during October 2007- December 2009 from a tertiary care hospital and other clinical laboratories in Chennai. All the strains

were isolated and identified biochemically using standard procedures [20] and confirmed by slide agglutination test using specific antisera procured from King Institute of Preventive Medicine, Chennai, India. All the isolates were stored in brain heart infusion broth with 15% glycerol at -20°C untill further use.

Antibiotic susceptibility testing:

Antimicrobial susceptibility testing of the *Salmonella* isolates to various routinely used antibiotics was determined by disc diffusion technique on Muller Hinton agar using commercially available discs following CLSI guidelines The panel of antimicrobials were included, ciprofloxacin (5 µg), norfloxacin (10µg), nalidixic acid (30µg), ampicillin (10µg), chloramphenicol (30µg), co-trimoxazole (1.25/23.75µg), tetracycline (30µg), ceftriaxone (30µg), cefotaxime (30µg) and gentamicin (10µg). MIC against ampicillin, nalidixic acid, ciprofloxacin, cefotaxime and ceftriaxone was determined by agar dilution method following CLSI guidelines 2008.[21]

RESULTS

We have obtained 133 *Salmonella typhi,* 41 *Salmonella paratyphi A* and 2 *Salmonella typhimurium* in the present study. 172/176 isolates from blood, 3/176 isolates from stool and 1/176 was from bone marrow.

Out of 133 *Salmonella typhi*, 131 were blood isolates, one was stool isolate and one was bone marrow isolate and in case of *Salmonella paratyphi A*, 40 were blood isolates and 1 was stool isolate and out of 2/176 *Salmonella typhimurium* 1 isolate each was obtained from blood and stool respectively.

The antimicrobial susceptibility pattern of *Salmonella typhi* against various antibiotics is shown in Table-1.

Table 1
Antimicrobial susceptibility pattern of Salmonella enterica serovar typhi- Disc diffusion technique (n=133).

Antimicrobial agents	Sensitive (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	125(94)	7(5.25)	1(0.75)
Norfloxacin	123(92.5)	10(7.5)	0
Nalidixic acid	5(3.75)	1(0.75)	127(95.5)
Ceftriaxone	131(98.5)	0	2(1.5)
Cefotaxime	105(79)	23(17.25)	5(3.75)
Ampicillin	90(67.5)	7(5)	36(27.5)
Chloramphenicol	130(97.5)	0	3(2.5)
Co-trimoxazole	130(97.5)	0	3(2.5)
Tetracycline	124(93.2)	7(5.2)	2(1.5)
Gentamicin	130(97.7)	1(0.75)	2(1.5)

Increased sensitivity is reported for chloramphenicol (97.5), co-trimaxazole (97.5), ciprofloxacin (**93.5**%), tetracycline (**93.2**%) followed by ampicillin (**67.5%)**. Nalidixic acid showed only 3.75% sensitivity by disc diffusion however ceftriaxone showed **98.5**% sensitivity.

The antibiotic susceptibility pattern of *Salmonella paratyphi A* against various antibiotics is shown in Table-2 Chloramphenicol (**95%**), co-trimaxazole (**95%**), ciprofloxacin (87.5%), ceftriaxone (**95.5**%), and tetracycline (**92.5%)**.

Table 2

Antimicrobial susceptibility pattern of Salmonella enterica serovar paratyphi A- Disc diffusion technique (n=41)

Antimicrobial agents	Sensitive (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	36 (88)	5 (12)	0
Norfloxacin	35 (85.5)	5 (12)	1 (2.5)
Nalidixic acid	3 (7.5)	0	38 (92.5)
Ceftriaxone	39 (95)	1 (5.5)	1 (5.5)
Cefotaxime	37 (90.2)	2 (4.9)	2 (4.9)
Ampicillin	16 (39)	5 (12.5)	20 (48.5)
Chloramphenicol	39 (95)	0	2 (5)
Co-trimoxazole	39 (95)	0	2 (5)
Tetracycline	38 (92.5)	3 (7.5)	0
Gentamicin	41 (100)	0	0

The ACCo-T group of drugs showed better sensitivity to *Salmonella typhi* and *Salmonella paratyphi A* probably due to increased use of fluoroquinolone and cephalosporins. We have observed 95.5% of nalidixic acid resistance to *Salmonella typhi* and only 2.5% of MDRST was observed in our study.MIC values of ampicillin, nalidixic acid, ciprofloxacin, cefotaxime and ceftriaxone against *Salmonella typhi* and *Salmonella paratyphi A* isolates are shown in Tables-3&4.

Table 3

MIC values of Salmonella enterica serovar typhi against different antimicrobial agents. (n=133)

S. No.	Drugs Concentrations (µg/ml)	Ampicillin	Nalidixic acid	Ciprofloxacin	Ceftriaxone	Cefotaxime
1	256	-	104	-	-	-
2	128	-	14	-	-	-
3	64	0	7	-	-	-
4	32	4	7	0	-	-
5	16	1	0	0	-	-
6	8	1	0	0	-	-
7	4	25	1	0	-	-
8	2	95	0	0	-	-
9	1	7	0	28	0	0
10	0.5	-	0	25	32	36
11	0.25	-	-	58	62	79
12	0.125	-	-	16	30	12
13	0.0625	-	-	6	9	6
14	0.0312	-		-	0	0

Nalidixic acid showed 95.5% resistance by disc diffusion and 98.5 % resistance by MIC studies. The MIC ranges of nalidixic acid are: 104 isolates (256 µg/ml), 14 isolates (128 µg/ml), 7 isolates (64 µg/ml) and 7 isolates (32µg/ml). 5/133 isolates were sensitive by disc diffusion. Out of which 4 of them were found to be resistant by MIC with a range of 32µg/ml for 2 strains, 128 µg/ml and 256 µg/ml for each 1 strain respectively. In our study 1/133 isolate of *Salmonella typhi* was found to be NSST by disc diffusion and MIC studies.

When we attempted to correlate the results obtained by us , with ciprofloxacin the following observations were made. Better correlation was obtained between disc diffusion and MIC of nalidixic acid in our study. However, though resistance was observed by both disc diffusion and MIC, different range of MIC's were obtained (32 µg/ml-256 µg/ml) for nalidixic acid by agar dilution method.

Table-3 shows the MIC values of ampicillin, nalidixic acid, ciprofloxacin, cefotaxime, and ceftriaxone. 125/133 *Salmonella typhi* isolates of our study showed sensitivity to ciprofloxacin by disc diffusion, while MIC values revealed different reduced susceptibility ranges. Only 6 isolates were found to be sensitive by both disc diffusion and MIC (0.0625µg/ml) and 119 strains showed sensitivity to ciprofloxacin by disc diffusion and reduced susceptibility by MIC as given below: 14 strains - 0.125µg/ml, 55 strains - 0. 25µg/ml, 23 strains - 0.5 µg/ml and 27 strains 1 µg/ml.

All the seven intermediate sensitive isolates towards ciprofloxacin by disc diffusion showed a reduced susceptibility range, though not resistant with MIC values.

We have not observed high level ciprofloxacin resistance to *Salmonella typhi* and *Salmonella paratyphi A* isolates.

In general we found that *Salmonella paratyphi A* isolates exhibited better sensitivity to the routinely used antibiotics by disc diffusion except nalidixic acid which showed only 7.5% of sensitivity by disc diffusion. 4.5% of the *Salmonella paratyphi A* strains were found to be MDR in nature.

38/41 isolates of *Salmonella paratyphi A* were resistant to nalidixic acid by MIC with a range of 256 µg/ml (35 isolates) and 128 µg/ml (3 isolates) except 3 strains which showed sensitivity with a MIC values of 16 µg/ml. (Table – 4)

Salmonella paratyphi A obtained in our study were found to be sensitive to ciprofloxacin. However they had a MIC range of 0.5-0.125 µg/ml which was considered as reduced susceptibility as reported by many studies.[14] 40/41isolates of *Salmonella paratyphi A* showed reduced susceptibility to ciprofloxacin as follows: 25 isolates showed 0.5 µg/ml, 14 isolates showed 0.25 µg/ml, one isolate showed 0.125 µg/ml and with disc diffusion results revealed these isolates as sensitive and intermediate resistant. Only one isolate was (0.0625 µg/ml) sensitive by both MIC and disc diffusion methods.

Interestingly 131/133 isolates of *Salmonella typhi* were sensitive to Ceftriaxone by disc diffusion and MIC (with range of 0.25µg/ml to 0.125 µg/ml). The 2 isolates resistant by disc diffusion also turned out to be sensitive by MIC.

Though few strains showed resistance in disc diffusion all the strains were found to be sensitive by MIC with a range of 0.5- 0.063 µg/ml for cefotaxime. These observations once again emphasize the need to do MIC routinely in the laboratories. The two *Salmonella typhimurium* obtained in our study showed sensitivity to all the tested drugs and MIC_{50} and MIC_{90} could not be determined for the two *Salmonella typhimurium.* But MIC_{50} and MIC_{90} were determined for *Salmonella typhi* and *Salmonella paratyphi A,* which are given in Table -5

Table 5
MIC $_{50}$ and MIC $_{90}$ For Salmonella typhi and paratyphi A (n=176)

Antibiotics	MIC $_{50}$	MIC $_{90}$	MIC Ranges in CLSI standard
Ciprofloxacin	0.25	1	≤1 - ≥4
Nalidixic acid	256	256	≤16 - ≥32
Ceftriaxone	0.25	0.5	≤8 - ≥64
Cephotaxime	0.25	0.5	≤8 - ≥64
Ampicillin	2	4	≤8 - ≥32

DISCUSSION

The emergence of MDR *S.typhi* and *S.paratyphi A* strains in Asia in the late 1980 and early 1990s led to the increased use of fluoroquinolones for treating enteric fever. However, during the last decade treatment failures with ciprofloxacin have been increasingly reported. These failures have been associated with infection with *S. typhi* and *Paratyphi A* strains that are resistance to nalidixic acid and exhibiting decreased susceptibility to ciprofloxacin [15, 22, 23].Strains that are already resistant to nalidixic acid may require fewer exposures to fluoroquinolones to develop high level resistance to ciprofloxacin, than the strains that are fully ciprofloxacin susceptible[24].

Reduced susceptibility to ceftriaxone and ciprofloxacin has been reported previously for *Salmonella typhi* and *Salmonella paratyphi A* in Kuwait [25].

The current study suggests that ciprofloxacin usage should be re-considered in treating *Salmonella* infections due to reduced susceptibility, MIC range 0.063-1 µg/ml. The resistance to first line antimicrobials (ACCo-T) appears to be declining in *Salmonella typhi* and *Salmonella paratyphi A.* Based on our experience of in vitro susceptibility pattern, ACCo-T may be considered in the empiric therapy of enteric fever. If susceptibility shows otherwise, the therapy can be switched over to ceftriaxone. We observed that strains were completely sensitive to ceftriaxone and cefotaxime by MIC studies.

While the first line antimicrobials may still have a role to play in the treatment of typhoid fever, ceftriaxone remains as the effective drug against ciprofloxacin resistant *Salmonella typhi* and *paratyphi A*. Hence the use of ciprofloxacin in the empirical therapy should be discouraged. Considering the rapid and increased emergence of high-level ciprofloxacin resistance, attempts should be made to explore the possibility of alternate antimicrobial therapy for successful treatment of enteric fever.

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