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Prevalence of Ciprofloxacin Resistance and Emergence of Ceftriaxone Resistance among *Salmonella Enterica Serovar Typhi* and *S. Enterica Serovar Paratyphi A* Isolates, From the Indoor and Outdoor Patients of a Tertiary Care Hospital of North India

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Abstract **Original Research Article**

Multidrug-resistance is a major problem in the treatment of enteric fever caused by Salmonella enterica serovar Typhi/Paratyphi and is prevalent in parts of Asia and Africa. Reduced susceptibility/resistance to fluoroquinolones is highly prevalent, and sporadic cases of resistance to third-generation cephalosporins has also been reported in literature. The present study was undertaken to evaluate the magnitude of fllouroquinolone resistance and emergence of Ceftriaxone resistance among *Salmonella enterica serovar Typhi/ Paratyphi A* isolates, from a tertiary care hospital of North India. A total of 537 *S. enterica* isolates were obtained; 359 isolates (66.8%) were identified as *Salmonella enterica serovar Typhi* and 178 (33.2%) were identified as *Salmonella enterica serovar Paratyphi A*. Apprximately, 59% and 100% of the *S. Typhi and S. Paratyphi A* isolates, respectively were resistant to ciprofloxacin. Interpretation and conclusions: The incidence of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A isolates with resistance or decreased flouroquinolone susceptibility is very high. 1.7% of S typhi and 1.1% of S paratyhi A isolates were found resistant to ceftriaxone with an MIC of ≥64 µg/ml. The emergence of ceftriaxone resistant *S. enterica* strains needs prompt action in terms of control of the disease and further spread of the resistant strains.

Keywords: Salmonela enterica serovar typhi/paratyphi, Ciprofloxacin, ceftriaxone., MDR.

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INTRODUCTION

Typhoid fever, also known as enteric fever, caused primarily by *Salmonella enterica* serotype *typhi* and, to a lesser extent, *S enterica* serotypes *paratyphi* A, B, and C. Enteric fever, is a potentially fatal multisystemic illness. It is an important public health problem of the underdeveloped countries including India and is caused by the ingestion of contaminated food or water. The disease is highly endemic with a morbidity of 1000-20000/million population. Antibiotic are the backbone of management of enteric fever, which can successfully reduce the mortality [1-3]. However, emergence of S. Typhi and Paratyphi strains resistant to first line antibiotics used for the treatment of enteric fever further accentuate the magnitude of problem. The first line antibiotics for the treatment of typhoid included chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole till 1980s. However, increasing number of reports on - resistance to these first line drugs against S. Typhi isolates from India and other subcontinents, along with reports of treatment failure [4-7] resulted in the use of fluoroquinolones and third generation cephalosporins as alternatives for treatment of drug resistant S. Typhi cases [8]. Increased use of fluoroquinolones such as ciprofloxacin for treatment has resulted in the emergence of strains resistant or with reduced susceptibility to this particular antibiotic [9, 10]. Based on the current revised MIC breakpoints of ciprofloxacin by CLSI, it is important to have a clarity regarding incidence/prevalence of resistance, especially to ciprofloxacin. Since the emergence and spread of fluoroquinolone-resistant S.Typh/Partyphi isolates, third generation cephalosporin such as ceftriaxone is extensively used as empirical treatment of choice for typhoid fever [2]. However there are sporadic reports of reduced susceptibility or resistance to ceftriaxone for S typhi and paratyphi isolates [11, 12]. The present study was undertaken to evaluate the magnitude of fllouroquinolone resistance and emergence of Ceftriaxone resistance among Salmonella enterica

serovar Typhi and S. enterica serovar Paratyphi A isolates, from a tertiary care hospital of North India.

MATERIALS AND METHODS

A retrospective study was carried out on the Salmonella enterica isolate, which were obtained from blood samples of the patients suspected of enteric fever during January 2019–August 2019. All the blood specimens were collected taking all aseptic precautions and were processed as per standard procedure (Ref), using automated systems BACTEC 9240, BD, India/ Bac-T-Alert, Biomerieux, USA Bottles--).As soon as the bottle flagged positive subculture was done on blood agar and MacConkey agar plates. The isolates were identified using VITEK 2 GNB ID cards and their antimicrobial susceptibility testing was performed using VITEK 2 AST-N281 cards on fully automated Vitek-2 system (BioMérieux, USA). All the Salmonella enterica isolates were further confirmed by serotyping. The Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines were used to interpret the susceptibility profile of S. Typhi and S. Paratyphi A.

Table-1: Minimum inhibitory Concentration (MIC)/µg/ml breakpoints as per Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines

	Minimum inhibitory Concentration (MIC)/µg/ml		
Antimicrobial	**Sensitive**	**Intermediate**	**Resistant**
Nalidixic Acid	≤16	-	≥32
Ciprofloxacin	≤0.0625	0.125-0.5	≥1
Chloramphenicol	≤8	16	≥32
Cotrimoxazole	≤40	-	≥80
Ampicillin	≤8	16	≥32
Ceftriaxone	≤8	16-32	≥64
Cefuroxime	≤8	16	≥32

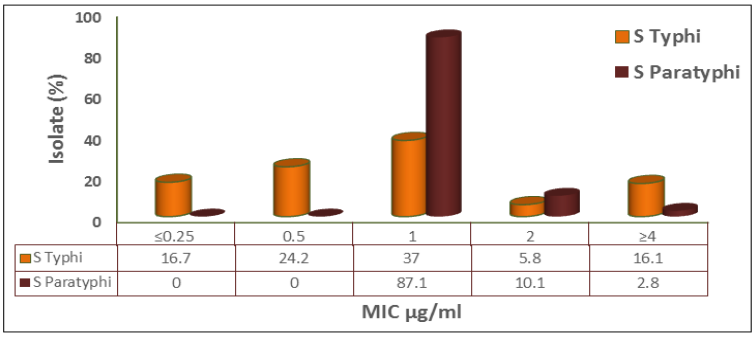
RESULTS

A total of 537 *S. enterica* isolates were obtained; 359 isolates (66.8%) were identified as *Salmonella enterica serovar Typhi*, and 178 (33.2%) were identified as *Salmonella enterica serovar Paratyphi A*. Using the recommended breakpoints (CLSI), as mentioned in Table-1, it was analyzed that 59.1% and 100% of the S. *Typhi* and *S. Paratyphi A* isolates, respectively were resistant to ciprofloxacin (Table-2) with 16.1% and 2.8% of the *S. Typhi* and *S. Paratyphi A* isolates, respectively with an MIC of >4 µg/m.L In addition 40.9% of *S. Typhi* isolates showed reduced susceptibility to ciprofloxacin with an MIC ranging between of 0.25– 0.5 µg/mL (Graph-1).

Table-3 depicts the distribution of *Salmonella enterica serovar Typhi/ Paratyphi A* isolates according to their susceptibility towards Ceftriaxone indicating emergence of resistance to ceftriaxone for *S. Typhi* and *S. Paratyphi A* isolates. A total of 10 isolates of *Salmonella enterica* were isolated showing resistance or reduced susceptibility towards ceftriaxone. Six isolates of *S. Typhi* and two isolates of *S. Paratyhi A* found to have very high MIC of >64 µg/ml , whereas two isolates of *S. ParatyphiA* showed reduced susceptibility to ceftriaxone with an MIC of 32 µg/ml. The antibiotic susceptibility profile of ceftriaxone resistant isolates towards other antimicrobials is shown in Table-4. These isolates were found resistant to other drugs including ciprofloxacin, cefuroxime, cotrimoxazole, nalidixic acid and ampicillin. However all these isolates were found susceptible to chloramphenicol and azithromycin.

Table-2: Distribution of S. Typhi (n=359) & S. Paratyphi A (n=178) isolates according to their susceptibility towards ciprofloxacin

Susceptibility to Ciprofloxacin	S. Typhi - Number (%)	S. Paratyphi A- Number (%)
Intermediate (≤0.25-0.5 µg/ml)	147 (40.9%)	0 (0%)
Resistant (≥ 1 µg/ml)	212 (59.1%)	178 (100%)



Graph-1: Percent distribution of S.Typhi (n=359) and S. Paratyphi A (n=178) isolates according to MIC (µg/ml) of ciprofloxacin

Table-3: Distribution of S. Typhi (n=359) & S. Paratyphi A (n=178) isolates according to their susceptibility towards Ceftriaxone

Susceptibility to Ceftriaxone	S. Typhi - Number (%)	S. Paratyphi A- Number (%)
Susceptible (≤8 µg/ml)	353 (98.3%)	174 (97.7%)
Intermediate (16-32)	0 (0%)	2 (1.1%)
Resistant (≥ 64 µg/ml)	6 (1.7%)	2 (1.1%)

Table-4: Susceptibility pattern (MIC#/Susceptibility) of Styphi and paratyhhi isolates with reduced susceptibility/resistance to ceftriaxone

	Nalidixic Acid	Ciprofloxacin	Cotrimoxazole	Ampicillin	Ceftriaxone	Cefuroxime	Azithromycin *	Chloramphenicol*
S.Typhi								
1	>=32/R	1/R	<=20/S	4/S	>=64/R	>=64/R	S	S
2	>=32/R	1/R	<=20/S	>=32/R	>=64/R	>=64/R	S	S
3	>=32/R	1/R	<=20/S	8/S	>=64/R	>=64/R	S	S
4	>=32/R	0.5/I	<=20/S	>=32/R	>=64/R	>=64/R	S	S
5	>=32/R	2/R	>=320/R	>=32/R	>=64/R	>=64/R	S	S
6	>=32/R	>=4/R	>=320/R	>=32/R	>=64/R	>=64/R	S	S
S.Paratyphi								
1	>=32/R	>=4/R	<=20	>=32/R	>=64/R	>=64/R	S	S
2	>=32/R	1/R	<=20	4/S	32/I	>=64/R	S	S
3	>=32/R	>=4/R	>=320/R	>=32/R	>=64/R	>=64/R	S	S
4	>=32//R	>=4/R	80/R	16/I	32/I	16/I	S	S

- Minimum inhibitory concentration/µg/ml; * - Azithromycin and Chloramphenicol were not in Vitek Panel, hence tested manually using Kirby Bauer Method.

DISCUSSION

Typhoid fever remains a public health concern especially in developing countries where availability of clean potable water to a large part of the population is a matter of concern. The problem is further aggravated by the growing antimicrobial resistance and diminishing antimicrobial armamentarium for the treatment of enteric fever caused by multidrug resistant (MDR) *Salmonella enterica* serovar *Typhi/ Paratyphi* and poses a major challenge in management of the disease. Multidrug resistant salmonella is defined as the organisms resistant to first line antibiotics used for the treatment of enteric fever i.e. chlromphenicol, ampicillin and cotrimoxazole [2].

Fluoroquinolones have become the first line drug for the treatment of enteric fever with the emergence of MDR *S. enterica* serovar *Typhi/Paratyphi* isolates and has proven to be effective for the treatment of typhoid fever caused by MDR strain [8, 13]. With loads of reports from the various parts of the world, observing *S. enterica* serovar *Typhi/Paratyphi* isolates with decreased ciprofloxacin susceptibility (MIC, ≥0.125 µg/ml) and subsequently development of resistance have become the matter of concern for the entire world (4-10, 14-16). A report from the united kingdom had shown an increase in incidence of *S. enterica serovar Typhi* isolates with decreased ciprofloxacin susceptibility from 0.9% to 33% in a span of just 8 years [14**]**. Japan has observed similar increase in percentage of *S. enterica* serovar Typhi isolates with reduced ciprofloxacin susceptibility from 10% to 31.8% within 2 years *i.e.* from 1997-1999. The emergence and spread of these resistant organisms have also been reported from many other countries as well. Further, several failures of clinical treatment of typhoid patients with ciprofloxacin and other fluoroquinolones was a matter of concern [14-16]. A previous study carried out on Salmonella isolates during the year 2017, from our lab had also reported, that the large number of *S. enterica serovar Typhi* and *S. enterica serovar Paratyphi A* isolates not only showed decreased susceptibility towards ciprofloxaxin but a major chunk of isolates were resistant(67.3% and 97.6% respectively), with very high MIC values. Ciprofloxacin resistant was observed in [11]. However, in the present study, it was analyzed that 59.1% and 100% of the *S. Typhi* and *S. Paratyphi A* isolates, respectively were resistant to ciprofloxacin, with 16.1% and 2.8% of the *S. Typhi* and *S. Paratyphi A* isolates, respectively with an MIC of >4 µg/ml. In addition remaining isolates of *S. Typhi* isolates showed reduced susceptibility to ciprofloxacin with an MIC ranging between of ≤0.25–0.5 µg/mL as the AST-GN281 card does not differentiation between susceptible and intermediate susceptible isolates of Salmonella. As per CLSI recommended susceptible breakpoint for ciprofloxacin is ≤0.0625 µg/mL. However few of the S. Typhi isolates categorized as intermediately susceptible, might be actually susceptible to ciprofloxacin.

Since the emergence and spread of fluoroquinolone-nonsusceptible *S.enterica*, the empirical treatment of choice for typhoid fever has been a third generation cephalosporin such as ceftriaxone/cefotaxime (parenteral) or cefixime (oral). Ceftriaxone resistance, although previously uncommon in *S. Typhi*, is associated with the acquisition of an

extended-spectrum -lactamase (ESBL) gene. However emergence of ceftriaxone resistance, though a very small proportion (0.08%) has been reported in one of the study from Karachi during the year 2010-11 [12]. Thereupon, since 2016 there are quite a few reports of emergence of ceftriaxone resistance among *Salmonella enterica* isolates. These strains were found simultaneously resistance to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and other third-generation cephalosporins and has been labeled as extensively drug-resistant (XDR) [17-21]. In the present study, we observed that 1.7% of S typhi and 1.1% of S paratyhi A have very high MIC of >64 µg/ml, whereas another 1.1% S paratyphiA had reduced susceptibility to ceftriaxone with an MIC of 32 µg/ml. However, these isolates were susceptible to chloramphenicol and azithromycin, but resistant to other drugs including ciprofloxacin, cefuroxime, cotrimoxazole, nalidixic acid and ampicillin. These XDR isolates may harbored an extended-spectrum β-lactamase (ESBL) gene that mediates resistance to ceftriaxone [22].

CONCLUSION

A large proportion of *S. Typhi/Paratyphi* isolates showed resistance or reduced susceptibility to ciprofloxacin. In addition resistance for third generation cephalosporin is emerging and this may pose a serious challenge to the clinician for the treatment of enteric fever in future. Luckily the ceftriaxone resistant isolates obtained in the present study are susceptible to chloramphenicol and azithromycin, unlike XDR *S. Typhi* reported from other countries. The emergence of XDR *S. Typhi* calls for an urgent action before it becomes very difficult to treat typhoid with existing armamentarium of drugs.

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