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Original Article

Microbiology Section

Antibiotic Resistance among Enteric Fever Pathogens in a Tertiary Care Centre

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ABSTRACT

Introduction: Enteric fever, a public health problem endemic in India involves multiple systems and is caused by Salmonella enterica, sub-species enterica serovar typhi and serovars paratyphi A, B and C. Fluoroquinolones and third-generation cephalosporins are first-line drugs used in treatment, which has led to increased MIC of Ciprofloxacin causing therapeutic failure. Hence, finding out the isolates with decreased susceptibility to Ciprofloxacin is important for a good treatment response and favourable clinical outcome.

Aim: To study the antibiotic resistance pattern of enteric fever pathogens with special reference to quinolones. To study the isolation rate of typhoidal *S. typhi* and *S. paratyphi* among different age groups, to determine their antimicrobial susceptibility pattern & note MIC of Ciprofloxacin.

Materials and Methods: A retrospective study was done in Microbiology Lab of KMC Hospital, Mangalore during December 2014 to December 2015. Ninety one Blood culture samples with growth of *S.* typhi & *S.* paratyphi were processed .The antibiotic susceptibility was done by Modified Kirby Bauer disk diffusion. MIC of ciprofloxacin was tested using Automated Vitek 2 system.

Results: Of 1279 positive blood cultures, isolation rate of enteric fever pathogens was 91 (7.11%). Majority of the isolates were *S.* typhi (69) and *S.* paratyphi A were 22 in number. 74 (81.31%) isolates were from 13-38 years. Antibiotic resistance pattern: Ampicillin (3.29%), Chloramphenicol (2.19%), Ceftriaxone (0%), Ciprofloxacin (2.19%), Nalidixic acid (85.71%), Cotrimoxazole (3.29%). Isolates with MIC of Ciprofloxacin 0.12 to 1 µg/ml were 81 (89%). Nalidixic acid was resistant in 78 (85.7%). Three isolates (3.29%) were MDR. The most susceptible antibiotic was ceftriaxone while most resistant was nalidixic acid.

Conclusion: The increasing numbers of enteric fever pathogens with decreased ciprofloxacin susceptibility is a cause of concern. There is re-emergence of sensitivity to the first line drugs.

Keywords: Quinolones, Screening, Susceptibility

INTRODUCTION

Enteric fever is an infection with multisystem involvement caused by *Salmonella enterica*, subspecies enterica serovar typhi and serovars paratyphi A, B & C. 16.6 million cases of enteric fever occurs each year accounting for 600,000 deaths in developing countries, according to WHO [1].The infection is characterized by a prolonged incubation period, fever, and systemic bacterial dissemination. The main mode of transmission is ingestion of food or water contaminated with faeces harbouring *S.* typhi. Typhoid is endemic in developing countries especially in India [2] with annual incidence rate estimated to be greater than 900 per 100,000 populations.

Prompt antibiotic treatment is effective. Third-generation cephalosporins and Fluoroquinolones (FQ) are first-line drugs owing to the Multidrug resistance (MDR) that started during the 18th century (1980s) [3].

Multi drug resistant strains (resistant to chloramphenicol, ampicillin and co-trimoxazole) of *Salmonella enterica* have emerged all over the world in the last 20 years [3]. The increased use of fluoroquinolones has increased the Minimum Inhibitory concentration (MIC) of ciprofloxacin causing therapeutic failure of the drug [4].

Isolates of *S. enterica*, with decreased ciprofloxacin susceptibility (DCS) have already emerged in India (69%) in 1996 and subsequently in other parts of the world [4]. Strains of Salmonella with DCS are isolated with ciprofloxacin, Minimum Inhibitory Concentration (MIC)s of 0.12–1.0 µg/mL. DCS with high-level resistance to the non-fluorinated quinolone, nalidixic acid (NAL) is due to mutations in gyrA gene [5].

There are reports of NAL-susceptible isolates with DCS, because of resistance mechanisms outside the gyrA gene [5]. To circumvent this problem, Clinical and Laboratory

Standards Institute (CLSI) approved a ≤0.06 ug/mL susceptibility breakpoint for *S*. typhi for ciprofloxacin [6].

Nalidixic acid screening has a specificity of 98.4% and sensitivity of 92.9% for *Salmonella* typhi in endemic areas [7]. Thus, disk diffusion for NAL and Ciprofloxacin is done for isolates with CIP MICs ≤1 µg/mL to detect NALR DCS phenotype and for NAL-susceptible isolates with DCS (PMQR) CIP DD zone diameter is interpreted according to the latest guidelines. The present study is taken up to study the rate of isolation of typhoidal *Salmonella* typhi and *S*. paratyphi among the different age group patients and to note the antimicrobial susceptibility pattern of *Salmonella enterica* serovar typhi (*S*. typhi) and *S*. paratyphi obtained from blood culture with special reference to fluoroquinolones.

MATERIALS AND METHODS

A retrospective study was carried out at Microbiology Laboratory of KMC Jyothi Hospital, Ambedkar Circle, Mangalore for a duration of 1 year (December 2014-December 2015). Out of the total 1279 samples, 91 blood culture samples of patients of all age groups having fever for 4-7 days, received during the year with the growth of *Salmonella enterica* serovar typhi (*S*. typhi) and *S*. paratyphi were included in this study. Both males and females were included in the study. There were 66 males and 25 females among the total 91 positive samples. The samples which showed growth of organisms other than salmonella were excluded from the study.

For all 1279 isolates received during the whole year, blood culture was done by the BacT ALERT microbial detection system (Biomeriux, Inc. Durham, North California and USA). Positive samples were sub cultured on blood agar, chocolate agar, and MacConkey agar and incubated over night at 370C. Growth of gram negative bacteria was identified by conventional methods /automated Vitek 2 Compact system. The identification of the isolates of *Salmonella enterica* serovar typhi (*S*. typhi) and *S*. paratyphi was confirmed by agglutination with polyvalent and group specific antisera [8, 9]. The antibiotic susceptibility of the isolates of *Salmonella enterica* serovar typhi (*S*. typhi) and *S*. paratyphi was done by modified Kirby Bauer disk diffusion method according to CLSI guidelines on Muller- Hinton agar plates using Ampicillin (10µg), Ceftriaxone (30µg) , Chloramphenicol (30µg),Nalidixic acid (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25µg) and Ofloxacin (5µg) (Hi Media Laboratory Ltd., Mumbai, India). The results were interpreted using CLSI guidelines 2012 [10,11]. The antibiogram was noted.

For all the isolates, MIC of ciprofloxacin was tested using the Automated Vitek 2 system (Bio-Mérieux, Co., Ltd.) to detect the DCS phenotype.

The sensitivity and specificity,PPV,NPV of the Nalidixic acid assay as a surrogate marker of DCS was calculated. The antibiotic treatment given was recorded from the case records. Ethical committee clearance: The study has been approved by the Institutional Ethics Committee.

RESULTS

Of the total 1279 blood cultures in BacT ALERT, 598 blood cultures were positive. Isolation rate of enteric fever pathogens was 91 (7.11%).Among these, *S*. typhi were isolated more in number i.e. 69 compared to *S*.paratyphi A which were 22 in number. Maximum isolation rate was reported in the month of September (18 isolates) and a pattern of male preponderance was observed. Among all the isolates, 3 were MDR. The season wise, age wise distribution, antibiotic resistance pattern, MIC of ciprofloxacin is shown in the [Table/Fig-1-4] respectively.

In 78 Nalidixic acid Resistant strains 26 strains had MIC of 1 µg/ml (ciprofloxacin resistant), 52 strains had MIC of 0.5 µg/ml (DCS), none were sensitive to Ciprofloxacin.

Time period	Total no. of Salmonella isolates	*S*. typhi	*S*. paratyphi A
December 2014	3	2	1
January 2015	7	5	2
February 2015	7	7	0
March 2015	5	3	2
April 2015	5	5	0
May 2015	11	11	0
June 2015	7	5	2
July 2015	7	3	4
August 2015	6	3	3
September 2015	18	16	2
October 2015	8	6	2
November 2015	10	6	4
December 2015	3	2	1

[Table/Fig-1]: *Salmonella* isolates among enteric fever patients from December 2014-December 2015.

Age group (years)	Total Enteric fever pathogens
0-12	2
13-25	40
26-38	34
39-51	7
52-64	3
65-77	4
78-90	1

[Table/Fig-2]: Age wise distribution of *Salmonella* isolates.

Antimicrobial agents	Resistant isolates Number/ Percentage
Ampicillin	3 (3.29%)
Chloramphenicol	2 (2.19%),
Ceftriaxone	0 (0%)
Ciprofloxacin	2 (2.19%),
Nalidixic Acid	78 (85.71%),
Cotrimoxazole	3 (3.29%).

[Table/Fig-3]: Antibiotic resistance pattern of *Salmonella* isolates: 3 isolates (3.29%) were MDR.

MIC (µg/ml)	No. of isolates (%)
≤0.25	11 (12.08%)
0.5	54 (59.34%)
1-4	26 (28.57%)

[Table/Fig-4]: MIC values of ciprofloxacin.

In 13 Nalidixic acid susceptible pathogens, 2 isolates were DCS (0.5 µg/ml), 11 had MIC of </0.25 µg/ml for ciprofloxacin and none were resistant to ciprofloxacin. The antibiotic treatment given was ceftriaxone.

DISCUSSION

Typhoid and paratyphoid fever is endemic in this region. The rate of isolation of enteric fever pathogens was 7.1% in patients presenting with 4-5 days of fever. The most susceptible antibiotic was ceftriaxone and the resistance was more to cotrimoxazole among the enteric fever pathogens. Nalidixic acid screening test to detect quinolone resistance had a sensitivity of 100% and specificity of 84%. The worrisome fact is emergence of isolates of *S.* typhi with reduced susceptibility to quinolones. DCS are associated with treatment failures and increased morbidity in patients on ciprofloxacin [11].

Previous studies have reported rates from 2.3% to 23.1% [12,13]. *Salmonella* typhi was more commonly isolated compared to *Salmonella* paratyphi, a finding consistent with other previous studies [14].

Enteric fever cases were predominantly reported from males coming to 66 cases (72.52%). Salmonella isolates were obtained in the highest number from the age group 13-38 years. The explanation could be due to more frequent outdoor activities in this population. Our findings are consistent with Mathura KC et al.,[15].

The changing trend in the antibiogram of the enteric fever pathogens is a challenge to the clinicians. With the emerging resistance to the first line drugs (Ampicillin, Chloramphenicol, Cotimoxazole), quinolones were the main stay of treatment from 1990's. With the increasing use of quinolones, strains with reduced susceptibility to ciprofloxacin ((0.125-1 µg/ml) have emerged, leading to treatment failures [16].

The sensitivity, specificity, PPV and NPV of Nalidixic acid screen test to predict quinolone resistance are 100%, 84%, 92% and 84%. Thus, Nalidixic acid screening test can be used as a surrogate marker of quinolone resistance.

The interesting fact noted, out of the 26 Ciprofloxacin, only 2 were picked up the Kirby Bauer disk diffusion method. Kirby-Bauer disc diffusion assay using currently recommended breakpoints to ciprofloxacin may not be a reliable method.

A study done in the same region, of the 71 strains of *Salmonella* typhi, 15 were sensitive to both ciprofloxacin and NA. 39 strains with NA resistance were sensitive to Ciprofloxacin with decreased susceptibility. MDR was 9%, close to the finding in our study [17]. A study done in a tertiary care centre to compare the trends in antibiotic resistance patterns of enteric fever isolates for 3 consecutive years showed complete susceptibility to chloramphenicol, cefotaxime, ceftriaxone, and azithromycin in 2009 and 2010, with an increase in resistance to Nalidixic acid (100%) and ciprofloxacin (13.6%)[18]. Of the 50 isolates of *Salmonella enterica* serovar typhi and paratyphi A studied, the antibiotic sensitivity pattern was chloramphenicol (86%), ampicillin (84%), cotrimoxazole (88%). Quinolones (98%) and cephalosporins(100%) in study conducted in South India [19]. The main limitation of the study was the exact MIC was not calculated using E test.

CONCLUSION

There is re-emergence of sensitivity to the traditional drugs like ampicillin, chloramphenicol, cotrimoxazole. With the widespread use of quinolones in treatment, there is emergence of strains with DCS. Ciprofloxacin Disc diffusion is not a reliable method. The best method is determination of MIC to quinolones. But in resource poor countries, Nalidixic acid screening can be used to predict the low level quinolone resistance, with 100% sensitivity and 84% specificity .To combat that, cephalosporins are being used. Time has come to reconsider bringing back the traditional drugs or use other alternatives like azithromycin.

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