SENSITIVITY PATTERN OF AZITHRYMYCIN, OFLOXACIN AND CEFTRIAXONE IN CIPROFLOXACIN RESISTANT SALMONELLA CAUSING ENTERIC FEVER

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Abstract

The therapeutic alternatives available for use against ciprofloxacin resistant enteric fever isolates in an endemic area are limited. A cross sectional study was carried out in the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January to December 2008, to see the sensitivity pattern of azithrymycin, ofloxacin and ceftriaxone in ciprofloxacin resistant salmonella causing enteric fever. In this study, the MICs of various drugs were determined for 100 enteric fever isolates (72 Salmonella enterica serovar Typhi and 28 Salmonella enterica serovar paratyphi A). By agar dilution method, 40% Salmonella strains were ciprofloxacin resistant showing MIC values of (4-8 mg/ml), 36% strains were intermediate sensitive with MIC values of 1 to 4 mg/ml and 24% strains were sensitive to ciprofloxacin showing MIC values of 0.125 to 1 mg/ml. All ciprofloxacin- resistant isolates were sensitive to ofloxacin (inhibitory zone diamater 16-32mm), ceftriaxone (inhibitory zone diameter 21mm), 66.66 % isolates were sensitive to azithromycin. These results indicate that ofloxacin and ceflriaxone may be convenient alternative antimicrobial agents for Salmonella isolates.

Key words: *Enteric fever,* Sensitivity *pattern, Ciprofloxacin resistant salmonella*

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Introduction

Enteric fever caused by Salmonella Typhi & Salmonella Paratyphi A.B.C .[1] Enteric fever due to infection with Salmonella enterica serovar Typhi or Salmonella enterica serovar Paratyphi A is estimated to cause more than 27 million infections each year worldwide with 216000 deaths.[2] After extensive outbreaks of typhiod fever occurred in Mexico and India in the early & mid 1970s, in which epidemic strains were resistant to chloramphenicol, the efficacy of this antimicrobial was in doubt. Alternative drugs for typhoid fever are ampicillin and trimethoprim. However, following outbreaks in the Indian subcontinent, the Arabian Gulf, the Philippines and South Africa in the late 1980s and early 1990s, in which causative strains were resistant to ampicillin and trimithoprim in addition to chloramphenicol, the efficacy of these antimicrobials has also been impaired.[3] In the last two decades, the worldwide emergence of multi-drug resistant strains of *Salmonella* has led to virtual withdrawal of chloramphenicol and its replacement with fluoroquinolones and third generation cephalosporins. Clinical treatment failures after the administration of ciprofloxacin and other fluoroquinolones to patient with typhoid fever attributable to these strains have been reported. The emergence of complete resistant to ciprofloxacin in S. Typhi or S. Paratyphi A would severely limit the choice of antimicrobial therapy for treating enteric fever.[4] Where fluoroquinolones, such as ciprofloxacin and ofloxacin, have become widely used, isolates of *Salmonella* enterica serovar Typhi and serovar Paratyphi A with reduced susceptibility to fluoroquinolones have become common.[2] The most common method of testing for resistance

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to ciprofloxacin in clinical laboratories is by disc diffusion, using discs with concentration of ciprofloxacin ranging from 1 to 5 mg/L.[5]

However, isolates of Salmonella enterica serovars Typhi and Paratyphi A with reduced susceptibility to fluoroquinolones (as indicated in the laboratory by resistance to nalidixic acid) have now appeared in the Indian subcontinent, Vietnam and Tajikistan and treatment failures with fluoroquinolones have also been reported. Isolates of serovar Typhi in the United Kingdom had reduced susceptibility to ciprofloxacin.[6] In order to effectively monitor the changing trends in the level of antimicrobial for testing the susceptibility of Salmonella Typhi strains to selected antibiotics should be used routinely in large hospital settings in more geographical areas. [7] A national-guide line on the proper usage of antibiotics is required for urgent implementation in Bangladesh.[8] In the present study, sensitivity pattern of this four drugs including nalidixic acid was determined by disc diffusion method.

Methods

This cross sectional study was carried out in the department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January to December 2008. 100 samples of isolated Salmonella were collected from Microbiology & Immunology Laboratory of BSMMU, Dhaka. Isolated *Salmonella* was collected from Microbiology & Immunology laboratory of BSMMU which was subsequently subcultured and confirmed by biochemical test. Specimens were preserved at 2-8° C in nutrient agar slant. Antimicrobial sensitivity test was done for all Salmonella isolates with five antimicrobial agents (Ciprofloxacin, Ofloxacin, Ceftriaxone, Azithromycin & Nalidixic acid) using Mueller-Hinton agar medium. The sensitivity test includes disc diffusion method to find out inhibitory zone diameters to the disc of recommended strength

Disc diffusion method of antimicrobial sensitivity test:

Disc of the antimcrobial agents used in different strengths. Mueller-Hinton agar medium was used for disc diffusion test. A suspension of each isolate was made in sterile nutrient broth. At least three to five well-isolated colonies of the same morphological type was selected from an agar plate culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4-5 ml of sterile nutrient broth. The turbidity of the actively growing broth culture was adjusted with that of the 0.5 McFarland standard, which is equivalent to 10^7 CFU/ml. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. The disc were placed over the inoculated surface about 15-20 mm away from the edge of the petridish and 24mm from center to center to prevent overlapping of zones of inhibition. In this method, a maximum of 5 discs were placed into a 90mm plate. After placing the antimicrobial disc, the inoculated plates were incubated at 37^0C for 18-24 hours. Then the diameters of the zones of inhibition were measured in millimeter. The interpretive criteria to evaluate the susceptibility of the isolates were done according to Cultural Laboratory Standard Institute (CLSI) guideline.

Results

A total of 100 Salmonella strains were studied, of which 72 (72%) were Salmonella Typhi, 28 (28%) were S. Paratyphi A and none was S. Paratyphi B. Sensitivity pattern of ciprofloxacin, ofloxacin, ceftriaxone azithromycin and nalidixic acid among salmonella species by disc diffusion method was given in (Table-I). 75 (75%) sensitive, 22 (22%) intermediately sensitive and 3 (3%) salmonella strains were resistant to ciprofloxacin. 100 (100%) strains were sensitive to ofloxacin. 94 (94%) strains were sensitive and 6 (6%) strains were intermediately sensitive to ceftriaxone. 71 (71%) strains were sensitive and 29 (29%) strains were intermediately sensitive to azithromycin. Again, 90 (90%) strains were resistant and 10 (10%) were sensitive to nalidixic acid. Table-II shows that out of 90 nalidixic acid resistant strains of Salmonella, 66 (73.33%) were sensitive, 21 (23.33%) were intermediate sensitive and 3 (3.33%) salmonella strains were resistant to

ciprofloxacin. Among 10 nalidixic acid sensitive strain, 8 (80%) were sensitive and 2 (20%) were resistant to ciprofloxacin. Sensitivity pattern of ofloxacin, ceftriaxone and azithromycin among three ciprofloxacin resistant salmonella species by disc diffusion method was shown in (Table-III, IV & V). All 3 ciprofloxacin resistant strains were sensitive to ofloxacin and ceftriaxone. 2 (66.66%) salmonella strains were sensitive and 1 (33.33%) strain was intermediate sensitive to azithromycin. All 22 ciprofloxacin intermediate sensitive salmonella strains were sensitive to ofloxacin, 21 (95.45%) strains were sensitive and 1 (4.55%) strains intermediate sensitive to ceftriaxone. 11 (50%) strains were sensitive and 11 (50%) salmonella strains were intermediate sensitive to azithromycin. All 75 ciprofloxacin sensitive salmonella strains were sensitive to ofloxacin. 70 (93.33%) strains were sensitive and 5 (6.66%) strains were intermediate sensitive to ceftriaxone. 57 (76%) strains were sensitive and 18 (24%) strains were intermediate sensitive to azithromycin.

Table-I

Sensitivity pattern of ciprofloxacin, ofloxacin, ceftriaxone, azithromycin and nalidixic acid among Salmonella species by disc diffusion method (n=100)

Name of antimicrobial agents	No. of isolates		
	Resistant	Intermediate sensitivity	Sensitive
Ciprofloxacin	3(3.0%)	22(22.0%)	75(75.0%)
Ofloxacin	0 (0.0%)	0 (0.0%)	100 (100%)
Ceftriaxone	0 (0.0%)	6 (6.0%)	94 (94.0%)
Azithromycin	0 (0.0%)	29(29.0%)	71 (71.0%)
Nalidixic acid	90(90.0%)	0(0.0%)	10(10.0%)

Table- II

Sensitivity pattern of ciprofloxacin in relation to sensitivity pattern of nalidixic acid by disc diffusion test (n=100)

Nalidixic acid	Ciprofloxacin		
	No. of resistant strain	No. of intermediate sensitive strain	No. of sensitive strain
Resistant strain (n=90)	3 (3.3%)	21 (23.33%)	66 (73.33%)
Sensitive strain (n=10)	0	2 (20.0%)	8 (80.0%)

Table-III

Sensitivity pattern of ofloxacin, ceftriaxone and azithromycin among ciprofloxacin resistant Salmonella species by disc diffusion method (n=3)

Antimicrobial Agents	No. of isolates		
	Resistant	Intermediate sensitivity	Sensitive
Ofloxacin	0 (0.0%)	0 (0.0%)	3 (100.0%)
Ceftriaxone	0 (0.0%)	0 (0.0%)	3 (100.0%)
Azithromycin	0 (0.0%)	1(33.33%)	2 (66.66)

Table-IV

Sensitivity pattern of ofloxacin, ceftriaxone and Azithromycin among ciprofloxacin intermediate susceptibility Salmonella species by disc diffusion method (n=22)

Antimicrobial Agents	No. of isolates Resistant	Intermediate sensitivity	Sensitive
Ofloxacin	0 (0.0%)	0 (0.0%)	22 (100%)
Ceftriaxone	0 (0.0%)	1 (4.54%)	21 (95.45%
Azithromycin	0 (0.0%)	11 (50%)	11 (50%)

Table-V

Sensitivity pattern of ofloxacin, ceftriaxone and Azithromycin among ciprofloxacin sensitive Salmonella species by disc diffusion method (n=75)

Antimicrobial Agents	No. of isolates Resistant	Intermediate sensitivity	Sensitive
Ofloxacin	0 (0.0%)	0 (0.0%)	75 (100%)
Ceftriaxone	0 (0.0%)	5 (6.66%)	70 (93.33%)
Azithromycin	0 (0.0%)	18 (24%)	57 (76%)

Discussion

In the present study, by disc diffusion method showed that only 3 % salomenlla isolates were resistant to ciprofloxacin, 22% were intermediate sensitive and 75% were sensitive to ciprofloxacin. The results were in consistent with the study of Gautam et al. (2004)[9] who showed that 79 % salmonella strains were sensitive to ciprofloxacin by disc diffusion method. A study by Chowta and Chowta (2005)[10] showed that there was in vitro sensitive to ciprofloxacin, but the patient did not respond to the drug therapy. These findings suggest that sensitivity of salmonella to ciprofloxacin in vivo gradually decreasing. A strain with decreased sensitive to ciprofloxacin was reported from the Indian subcontinent and south east and central Asia. Disc diffusion testing revealed that these isolates were resistant to nalidixic acid but sensitive to ciprofloxacin, according to the current CLSI interpretive criteria.[11]

In the present study, 90% salmonella strains were resistant and 10% were sensitive to nalidixic acid by disc diffusion method. The resistant rate to nalidixic acid in this study was significantly higher than that of Chinh et al. (2000)[6] in Vietnam, who reported only 53% of isolates were resistant to nalidixic acid. Mendal (2004)[12] from Kolkata reported 100% salmonella strains were resistant to nalidixic acid, indicating the reduced susceptibility to ciprofloxacin. In present study, 73.33% of nalidixic acid resistant salmonella strains were sensitive, 23.33% were intermediate sensitive and 3.33% were resistant to ciprofloxacin. Among nalidixic acid sensitive salmonella strains, 80% strains were sensitive and 20% were resistant to ciprofloxacin. Therefore, nalidixic acid resistance determined by the disc diffusion method could be an indication of decreased susceptibility to ciprofloxacin.[13] The presence of nalildixic acid resistance has been suggested as a laboratory marker of isolates with reduced susceptibility to fluoroquinolones and indicates that invasive infection may fail to respond to fluoroquinolone therapy.[14] 100% salmonella strains were sensitive to ofloxacin with zone diameter ³16 mm in this study. This finding correlates with Mandal et al. (2003)[12], who found 99.04% salmonella strains were sensitive to ofloxacin by disc diffusion method with zone diameter ³16 mm. It also correlates with Choudhari and Bansal (1997)[15] and Kumar et al. (2001)[16], who found to most of the strains

were ofloxacin sensitive by disc diffusion method.

Several previous studies had observed clinical failure in response to ciprofloxacin therapy due to infection with nalidixic acid resistant salmonella isolates and many studies had considered as nalidixic acid sensitivity test as surrogate marker for decreased sensitivity to ciprofloxacin among Salmonella enterica serovar Typhi.[17,18] Wain et al. (1997)[19] reported similar observation regarding ofloxacin sensitivity to Salmonella enterica serovar Typhi that means treatment failure occured to ofloxacin therapy if infection occured with nalidixic acid resistant Salmoella isolates. The present study showed that, 100% ciprofloxacin resistant salmonella strains were sensitive to ofloxacin. A study done by Capoor et al. (2006)[20] showed that variation in result between first and second generation quinolones, 96.2% of strains resistant for ofloxacin and 92.3% for levofloxacin. These observations indicate that fluoroquinolones should be tested individually and the ciprofloxacin not represent this group adequately Kumar et al. (2002).[16] Disc diffusion method of sensitivity testing of salmonella to ceftriaxone showed that 94% strains were ceftriaxone sensitive. This result is not consistent with the result of Gautom et al. (2002)[8] , who showed 88% S. Typhi were sensitive to ceftriaxone by disc diffusion method. Azithromycin in the present study showed that 29% of salmonella isolates were intermediate sensitive to azithromycin with zone diameter 14-17 mm with MIC 2-64 mg/ml and 71% of isolates were sensitive to azithromycin by disc diffusion method had zone diamerter ³18 mm. In enteric fever, the role of azithromycin needs to be appreciated, as it is highly effective in removing intracellular salmonella, defervescence is rapid, gastrointestinal carriage is eradicated and it represents a potential alternative in pediatric populations where quinolones are contraindicated.[21]

Conclusion

This study has been revealed that most of the *Salmonella typhi* isolates which are resistant to nalidixic acid will also be resistant to ciprofloxacin. However, they may show in vitro susceptibility. For this reason, it is essential to find out a therapeutic alternative against this resistant organism.

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