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Original Article

Determination of minimum inhibitory concentration of Azithromycin, 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. The antibiotics currently available are the quinolones, third-generation cephalosporins and azithromycin . 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). Ciprofloxacin resistant (1oo%) *Salmonella* strains were sensitive to ofloxacin and ceftriaxone showing MICs of 0.0078-2 g /ml and 0.0156-2g /ml respectively. *Salmonella* strains (98%) had MIC values 1-32 g /ml for azithromycin. These results indicate that ofloxacin and ceftriaxone may be convenient alternative antimicrobial agents for *Salmonella* isolates.

Keywords*: Salmonella*, Minimum inhibitory concentration, Multi drug resistant *Salmonella*

Introduction

Enteric fever is caused by *Salmonella typhi* & *Salmonella paratyphi* A.B.C. Infection caused by ingestion of organisms in contaminated food or water or from contaminated hand [1]. From 1948 to 1970s chloramphenicol was the drug of choice in developed countries and its use resulted in a reduction in mortality rates from 10% to 2% [2]. Chloramphenicol resistant *Salmonella* strains first reported in Britain, in 1950 and in India in 1972. Gradually, resistance to multiple antibiotics developed. The first major epidemic of multidrug-resistant (MDR) *Salmonella typhi* (isolates resistant to ampicillin, chloramphenicol and cotrimoxazole) was reported in 1972 in Mexico [3]. In the last two decades, the worldwide emergence of multi-drug resistant strains of *Salmonella* has led to virtual withdrawal of chloramphenicol & 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[4].

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Where fluoroquinolones, such as ciprofloxacin and ofloxacin, have become widely used, isolates of *Salmonella typhi* and *paratyphi* with reduced susceptibility to fluoroquinolones have become common [5]. The prevalence of resistance *of Salmonella typhi* to ciprofloxacin is very high also in Bangladesh . The injudicious administration and rampant use of quinolones in Bangladesh probably contributed to the high prevalence of reduced susceptibility (>88%) and the emergence of very high level or complete resistance (>4 g/ml) of isolates of *Salmonella typhi* to ciprofloxacin[6]. Furthermore, the recent report of an isolate of *Salmonella typhi* from Bangladesh with high level resistance to ceftriaxone means that , untreatable typhoid may become a reality. There is a need for alternative antimicrobial agents to treat such MDR infections[7] . In the present study, minimum inhibitory concentration of ciprofloxacin, ofloxacin, ceftriaxone and Azithromycin for *Salmonella enterica serovar typhi* was determined by agar dilution method.

The present study was aimed to compare the MICs of Azithromycin, Ofloxacin Ceftriaxone and Ciprofloxacin to find out the therapeutic alternative available for the treatment of enteric fever.

Methodology

This cross-sectional study was carried out in the department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, during the period from January 2008 to December 2008.

One hundred *Salmonella* species were collected from Microbiology & Immunology Laboratory of BSMMU. The isolated strains were identified by biochemical tests and preserved in nutrient agar slant at 2 to 8 degree Celsius.

Sensitivity of the isolates to the antimicrobial agents was done by agar dilution method in Mueller-Hinton agar media to determine the minimum inhibitory concentrations (MICs). The antimicrobial agents used were obtained from dry powder of known potencies.

Preparation of Antimicrobial Solutions: Powder of the antimicrobial agents were dissolved into appropriate solvents, as per instruction of the manufacturer. Stock solutions were prepared and sterilized by membrane filtration. Antimicrobial agents were diluted from stock solutions in distilled water to make a series of two-fold dilutions of intermediate concentrations, that were 10 times higher than the required final concentration in the agar medium.

Preparation of stock solution: For preparation of stock solution of antimicrobial agent 2000 ug powder of antimicrobial agent was added to the per ml of distilled water (Lalitha, 2007

Preparation of the agar dilution plates: Mueller-Hinton agar base after dissolving into distilled water was autoclaved at 121^0C for 15 minutes. After cooling to 50^0C in a water bath antimicrobial solution was added. For preparing 5 petridish (90 mm diameter) 90ml media and 10 ml antimicrobial solution was taken in a flask. Then the antibiotic containing medium was plated quickly (20ml for each petridish). After solidification, the plates were stored in sealed plastic bags at 4-8^0C.

Preparation of the Inocula: A bacterial suspension was made in sterile nutrient broth by colonies from a pure culture and the turbidity adjusted to 0.5 Mc Farland standard to make a concentration of about 10^7CFU/ml.

Inoculation: Using sterile microtips, 1µl of the diluted suspension containing 10^4CFU was inoculated on to the appropriate MIC plates.

Incubation: The inoculated plates were incubated at 37^0C for 18-24 hours.

Rusults

A total of 100 *Salmonella* strains were studied, of which 72(72.0%) were *Salmonella typhi*, 28(28.0%) were S. *Paratyphi* A and none was S. Paratyphi B . Table I shows the MIC value of ciprofloxacin among 100 *Salmonella* strains. Highest number of strains 36(36.0%) had MIC 2µg/ml followed by 31 (31.0%) strains with MIC 4 µg/ml, 11(11.0%) with MIC 0.05µg/ml, and 9(9%) with MIC 1 µg/ml.

Table-I: MIC value of ciprofloxacin among the *Salmonella* strains (n=100)

Sl	MIC value (µg/ml)	No. of strain
1	0.0625	0 (0.0%)
2	0.125	0 (0.0%)
3	0.25	4(4.0%)
4	0.5	11(11.0%)
5	1	9(9.0%)
6	2	36(36.0%)
7	4	31(31.0%)
8	8	9(9.0%)
9	16	0(0.0%)
	Total	100(100%)

Table II shows the MIC value of ofloxacin among 100 *Salmonella* strains, Highest number of strains 23(23.0%) had MIC 0.125 µg/ml followed by 16(16%) strains with MIC 0.25 µg/ml, 14(14.0%) with MIC 0.5 µg/ml, 10(10.0%) with MIC 0.0625 µg/ml, 10(10.0%) with MIC 0.03125 µg/ml, 8(8.0%) with MIC 0.0156 µg/ml and 8(8%) strains with MIC 0.0078 µg/ml and 7(7.0%) with MIC 2µg/ml regarding MIC breakpoint in terms of sensitivity 100% strains were sensitive

Table-II: MIC value of ofloxacin among the *Salmonella* strains (n=100)

Sl	**MIC value (µg/ml)**	**No. of isolates**
1	0.004	0 (0.0%)
2	0.0078	8 (8.0%)
3	0.0156	8 (8.0%)
4	0.03125	10 (10.0%)
5	0.0625	10 (10.0%)
6	0.125	23 (23.0%)
7	0.25	16 (16.0%)
8	0.5	14 (14.0%)
9	1	7 (7.0%)
10	2	4 (4.0%)
11	4	0 (0.0%)
12	8	0 (0.0%)
	Total	100 (100%).

Table III shows the MIC value of ceftriaxone among 100 *Salmonella* strains. Highest number of strains 38(38.0%) had MIC 0.125µg/ml, followed by 28(28.0%) strains with MIC 0.0625 µg/ml, 20(20.0%) strains with MIC 0.03125 µg/ml, 7(7.0%) strains with MIC 0.25µg/ml. Regarding MIC breakpoint in terms of sensitivity 100% strains were sensitive to ceftriaxone .

Table- III: MIC value of ceftriaxone among the *Salmonella* strains (n=100)

Sl	MIC value (µg/ml)	No. of isolates
1	0.004	0 (0.0%)
2	0.0078	0 (0.0%)
3	0.0156	4 (4.0%)
4	0.03125	20 (20.0%)
5	0.0625	28 (28.0%)
6	0.125	38 (38.0%)
7	0.25	7 (7.0%)
8	0.5	0 (0.0%)
9	1	2 (2.0%)
10	2	1 (1.0%)
11	4	0 (0.0%)
	Total	100 (100%)

Table IV shows MIC value of Azithromycin among 100 *Salmonella* strains. Highest numbers of strains 36(36.0%) had MIC 8µg/ml, followed by 28(28.0%) strains with MIC 4 µg/ml, 19(19.0%) strains with MIC 2 µg/ml, 9(9.0%) strains with MIC 16µg/ml, 2 strains with MIC 32 µg/ml and 2 strains with MIC 64 µg/ml. Azithromycin has no definitive breakpoints for *Salmonella* isolates so either resistant or sensitive figure had not been given.

Table-IV: MIC value of azithromycin among the *Salmonella* strains (n=100)

Sl	MIC value (µg/ml)	No. of isolates
1	0.0625	0 (0.0%)
2	0.125	0 (0.0%)
3	0.25	0 (0.0%)
4	0.5	0 (0.0%)
5	1	4 (4.0%)
6	2	19 (19.0%)
7	4	28 (28.0%)
8	8	36 (36.0%)
9	16	9 (9.0%)
10	32	2 (2.0%)
11	64	2 (2.0%)
	Total	100 (100%)

Table V shows the sensitivity pattern of ofloxacin, ceftriaxone and azithromycin by MIC method among 40 ciprofloxacin resistant *Salmonella* species. All 40(100.0%) ciprofloxacin resistant *Salmonella* strains were sensitive to ofloxacin and ceftriaxone. All 36 (100.0%) ciprofloxacin intermediate sensitive strains and all 24 ciprofloxacin sensitive *Salmonella* strains were also sensitive to ofloxacin and ceftriaxone.

Table-V: Sensitivity pattern of ofloxacin, ceftriaxone and Azithromycin by MIC method among ciprofloxacin resistant *Salmonella* species (n=40)

Antimicrobial Agents	No. of isolates	
	Resistant	Sensitive
Ofloxacin	0(0.0%)	40 (100.0%)
Ceftriaxone	0(0.0%)	40 (100.0%)
Azithromycin	No defin itiv e break points for Salmonella isolates	

Discussion

The present study was carried out among 100 *Salmonella* strains (72 *Salmonella typhi* and 28 *Salmonella paratyphi* A) to determine the MICs of four drugs and to find out the antimicrobial susceptibility pattern of *Salmonella* strains.

In the present study, the MIC of ciprofloxacin showed that the highest number of strains 36 (36%) had MIC 2 g/ml and 9 (9%) showed highest MIC value 8g/ ml indicate high level resistance to ciprofloxacin. MIC range 8 to > 32 g/ml indicate high level resistance to ciprofloxacin[8] . Regarding susceptibility of the ciprofloxacin in terms of MIC value, 40% of the *Salmonella* isolates were resistant (MIC value 4 g/ml), 36% were intermediate sensitive (MIC value 1 to 4 g/ml) and 24% were sensitive (MIC value 1g/ml). Chowta et al. In India 18.1% *Salmonella* isolates were resistant to ciprofloxacin [9] . Increase resistance to ciprofloxacin in this study might be due to widespread indiscriminate use, their oral route of administration, easy avilability and affordibility of ciprofloxacin[10]. In the present study intermediate susceptibility of the *Salmonella* isolates to ciprofloxacin was 36% (MIC 1 g/ml). Similar findings were reported by in India where intermediate susceptibility of the *Salmonella* isolates to ciprofloxacin was 39.96 % (MIC 1 g/ml) [11].

In the present study, 100% *Salmonella* strains were sensitive to ofloxacin (2 g/ml). Similar findings were observed where all *Salmonella* strains were sensitive to oflaxacin (MICs of 0.5 to 1g/ml) [5] .

In India treatment failure occurred with ofloxacin therapy if infection occurred with *Salmonella* isolates with ofloxacin MICs 0.5 µg/ml .The isolates among those with ofloxacin MICs of 0.075 g/ml responded to the ofloxacin therapy [12] . It was also reported that most of the *Salmonella* isolates in their study showed ofloxacin resistance due to MICs that were 0.5g/ml of ofloxacin [13] .

The present study showed that, 100% ciprofloxacin resistant *Salmonella* strains were sensitive to ofloxacin. Another study showed that variation in result between first and second generation quinolones, 96.2% *Salmonella* 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 [10] . It was also found that all the isolates of *Salmonella* were susceptible to ofloxacin while two isolates were detected to be resistant to ciprofloxacin [14] .

In this study 100% *Salmonella* strians were found sensitive to ceftriaxone with MIC 2 g/ml. Similar results were also found that, 100% *Salmonella* strains were ceftriaxone sensitive with MIC value 0.06 to 0.5g/ml . In this study 100% ciprofloxacin resistent strains were sensitive to ceftriaxone [14, 15] .

An interesting feature was observed by various other studies conducted worldwide that, there is lack of correlation between the results of disc diffusion and MIC methods [15 ,16] . These variations could be due to fewer *Salmonella* strains being tested with MIC and through random selection of the isolates. This latter hypothesis is supported by the fact that when used both method for sensitivity testing there was 90% correlation between the two methods[15]. MIC value of azithromycin either sensitive or resistant can not be detected because azithromycin has no definite breakpoint for *Salmonella* isolates[10]. There was as yet no data on the breakpoint of azithromycin for enteric fever which was found the MIC range to be 4-16 g/ml [17]. Present study showed that most *Salmonella* strains MIC value were 4 to 8 g/ml which is consistent to the result of another study where the MICs values of most *Salmonella* were 4-8 g/ml [18].

In this study, we have determined the minimum inhibitory concentration of azithromycin , ofloxacin and ceftriaxone. It was found that all the ciprofloxacin resistant and sensitive isolates are sensitive to these drugs. So , it can be considered that these drugs are better alternatives for treating enteric fever.

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