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Original Article

Antimicrobial susceptibility pattern of multidrug resistant typhoidal *Salmonella* isolates at Bangabandhu Sheikh Mujib Medical University

Rokshana Akhtar[1], Saifa Kismat[1], Fatima Afroz[1], Sanjida Khondakar Setu[1], Shaheda Anwar[1], Sharmeen Ahmed[1]

[1]Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Abstract

Typhoid fever is a major public health problem in the developing world caused by Salmonella Typhi and *Salmonella* Paratyphi A. Multidrug resistant (MDR) *Salmonella* has emerged as a cause of concern. This study was done to evaluate status in antimicrobial susceptibility pattern of *Salmonella* Typhi and *Salmonella* Paratyphi A from blood culture isolates in Bangabandhu Sheikh Mujib Medical University during the period of September 2018 to August 2019. Suspected *Salmonella* spp. was collected from blood culture samples and identification was done by conventional microbiological procedure. Antimicrobial susceptibility test was performed by Kirby Bauer disc diffusion method and minimum inhibitory concentration (MIC) of ciprofloxacin was determined by agar dilution method following 2018 guideline of CLSI. Of total 160 isolates studied, 98 (61.3%) were *S*. Typhi and 62 (38.7%) were *S*. Paratyphi A. Sensitivity to ampicillin, chloramphenicol and cotrimoxazole was 83.1%, 87.5% and 89.3% respectively. Majority of the *Salmonella* isolates were in ciprofloxacin intermediate susceptible category by MIC determination (83.8%). Most of all (95.6%) isolates were resistant by pefloxacin disc diffusion test. All the isolates were 100% sensitive to ceftriaxone, cefixime and cefepime; and sensitivity to azithromycin was 31.9%. Isolates showed a high degree of susceptibility to ampicillin, cotrimoxazole and chloramphenicol. Thus, these antibiotics may once again be useful for the management of typhoid fever.

Introduction

Salmonella Typhi resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole termed as multidrug-resistant *Salmonella* Typhi (MDRST) have emerged since 1989 in many developing countries especially Pakistan, India[1].

Decreased ciprofloxacin susceptibility (DCS) strains of the organism have now appeared in the Indian subcontinent and other region like Bangladesh[2,3]. However, the degree of resistance to commonly used antimicrobials like chloramphenicol, ampicillin and cotrimoxazole is not clear in the era of quinolone resistance[4,5,6]. Therefore this study was designed to document the change in the antibiotic susceptibility of *S*. Typhi and *S*. Paratyphi isolates obtained from blood culture samples.

Correspondence:
Dr. Rokshana Akhtar
Department of Microbiology and Immunology
Bangabandhu Sheikh Mujib Medical University, Dhaka.
Mobile: 01797-785891
E-mail: rokshanarpmc36@gmail.com

Materials and Methods

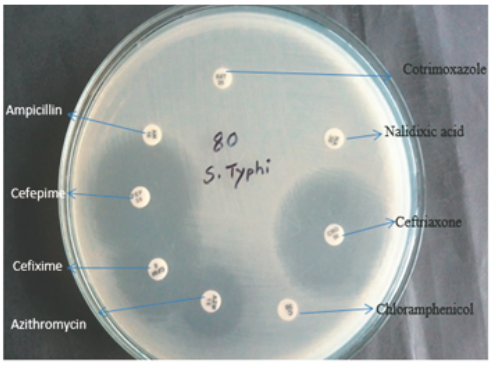
The cross-sectional study was carried out in the Department of Microbiology and immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), during the period of September, 2018 to July, 2019.

Collection and identification of organism

Suspected *Salmonella* spp. were collected from blood culture samples and subsequently identified by colony morphology and biochemical tests. All the *Salmonella* isolates were tested with *Salmonella* specific polyvalent 'O' antisera (A to S) (Mast group, UK) for slide agglutination test.

Antimicrobial susceptibility test

Antimicrobial susceptibility pattern were determined by using commercial antimicrobial disc (Bio Maxima, Poland): Ampicillin (10 µg), Chloramphenicol (30µg), Cotrimoxazole (1.25/23.75 µg), Pefloxacin (5µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Ceftriaxone (30 µg), Cefixime (5 µg), Cefepime (30 µg) and Azithromycin (15 µg), Zone of inhibition were interpreted as per recommendation of the Clinical Laboratory Standards Institute (CLSI 2018) by Kirby-Bauer disc diffusion method[7].



Photograph: Antimicrobial susceptibility tests of *Salmonella* Typhi (MDR)

Ciprofloxacin MIC

Minimum Inhibitory Concentration (MIC) of ciprofloxacin was done by Agar dilution method[8]. Ciprofloxacin MIC breakpoints define by CLSI 2018[9] [≤0.06µg/ml (susceptible), 0.12-0.5 µg/ml (intermediate), ≥1 µg/ml (resistant)] was followed for interpretation[2]. *Pseudomonas aeruginosa* ATCC27853 was used as the quality control strain.

Results

Table I showed antimicrobial sensitivity pattern of *Salmonella* isolates. All (160,100%) the isolates were sensitive to ceftriaxone, cefixime and cefepime. Sensitivity to ampicillin and cotrimoxazole and chloramphenicol were found to be 83.1% and 89.4%, 87.5% respectively. Azithromycin resistance rate was 68.1%. Nalidixic acid resistant *Salmonella* was found to be 93.8%. Out of 160 *Salmonella* isolates, 17(10.6%) isolates were found to be multidrug resistant (MDR), being simultaneously resistant to first line drugs like ampicillin, chloramphenicol and cotrimoxazole and 143 (89.4%) isolates were non-MDR.

Table 1: Antimicrobial susceptibility pattern of *Salmonella* isolates (n=160)

Antimicrobial agents	Sensitive n(%)	Resistant n(%)
Ampicillin	133(83.1)	27(16.9)
Cotrimoxazole	143(89.4)	17(10.6)
Nalidixic acid	10(6.2)	150(93.8)
Ciprofloxacin	7(4.4%)	14 (8.7)*
Pefloxacin	7(4.4%)	153(95.6)
Ceftriaxone	160(100.0)	0(0.0)
Cefixime	160(100.0)	0(0.0)
Cefepime	160(100.0)	0(0.0)
Azithromycin	51(31.9)	109(68.1)
Chloramphenicol	140(87.5)	20(12.5)

Note: Intermediate sensitive was considered as resistant as MIC of drugs were not evaluated. *139 (86.9%) Salmonella isolates were intermediate susceptible to ciprofloxacin, MDR=17 (10.6%), non-MDR=143 (89.4%).

Table 2 showed ciprofloxacin sensitivity pattern of *Salmonella* Typhi and *Salmonella* Paratyphi A by determining MIC breakpint. Only 05(3.1%) *Salmonella* isolates were sensitive, Most of isolates (134, 83.8%) were intermediate susceptible and 21 (13.1%) were resistant to ciprofloxacin. Out of 98 *Salmonella* Typhi, 5 (5.1%) were sensitive, most (77, 78.6%) were intermediate susceptible and 16 (16.3%) were resistant to ciprofloxacin. Among the 62 *Salmonella* Paratyphi A 55 (91.9%) were intermediate susceptible and 5 (8.1%) were resistant to ciprofloxacin. No *Salmonella* Paratyphi A was found sensitive to ciprofloxacin by determining MIC.

Table 2: Ciprofloxacin sensitivity pattern of *Salmonella* Typhi and *Salmonella* Paratyphi A by determining MIC Breakpoints

Name of species	Susceptible ≤0.06µg/ml n(%)	Intermediate (0.12-0.5µg/ml) n(%)	Resistant (≥1µg/ml) n(%)
Salmonella Typhi (n=98)	05(5.1)	77(78.6)	16(16.3)
Salmonella Paratyphi A (n=62)	0(0)	57(91.9)	5(8.1)
Total (n=160)	05(3.1)	134(83.8)	21(13.1)

Discussion:

In this study 10.6% *Salmonella* isolates were found to be multidrug resistant (MDR). In the several studies of Nepal reported nearly similar findings, they found 16.6%, 26.5% and 5.18% MDR *Salmonella* isolates respectively.[10,11,12]

The present study showed reduced susceptibility to ciprofloxacin is increasing (83.8%) and simultaneous decrease number of MDR *Salmonella* isolates (10.6%). These findings are most likely due to decreased prescribing of traditional first line antimicrobials and increasing reliance on ciprofloxacin as the first line treatment for typhoid fever. Reports from many studies showed the decrease

incidence of MDR *Salmonella* isolates with significantly increase in reduced susceptibility to fluoroquinolones[13,14,15]. In present study antimicrobial susceptibility pattern of *Salmonella* isolates showed all (160,100%) isolates were 100% susceptible to ceftriaxone, cefixime and cefepime. A Bangladeshi study[16] also reported 100% susceptibility to cefixime and ceftriaxone. A study in India was also reported 100% susceptibility to ceftriaxone[17].

In this study azithromycin resistance rate was 68.1%.The result of present study is not consistent with another study in Bangladesh where 88.4 % *Salmonella* isolates were susceptible to azithromycin[18]. A study in India reported 21% resistance to azithromycin[16].

In this study susceptibility to conventional first line drugs; ampicillin, chloramphenicol and cotrimoxazole were found to be 83.1%, 87.5% and 87.5% respectively. A Bangladeshi study reported 28.7%, 26.7% and 27.1% *Salmonella* isolates resistant to ampicillin, chloramphenicol and cotrimoxazole respectively[19].

Conclusion

This study indicates that there is a significant variation in resistance pattern among *S.*Typhi isolates to different antimicrobial agents with remarkable decline in the number of MDR isolates. There is an emergence of strains with intermediate susceptibility to ciprofloxacin. Therefore, the use of ciprofloxacin for treatment of typhoid fever needs routine surveillance to prevent further spread of these strains. Azithromycin resistance is also emerging. First line drugs like chloramphenicol, cotrimoxazole and ampicillin have re-emerged as a valuable oral alternative and ceftriaxone remains as viable parenteral option for treatment of typhoid fever.

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