Antimicrobial Susceptibility Pattern of Typhoidal *Salmonella* Species in Tertiary Hospitals of Dhaka City

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ABSTRACT

Enteric fever caused by *Salmonella enterica* remains an unresolved public health problem and due to the prevalence of multidrug resistant (MDR) strains for the last two decades, the first line antimicrobials were discontinued and currently the second line antimicrobials are used. A total of 325 blood samples from clinically suspected enteric fever patients were collected during the study period of July, 2014 to June, 2015. After identification of organisms, antimicrobial susceptibility tests were done by disc diffusion method and MIC by broth microdilution method. Rate of isolation of organisms were 19.38%, among them *Salmonella* Typhi were 85.71% and *Salmonella* Paratyphi A were 14.29%. About 74.07%, 77.78% and 72.22% strains of *Salmonella* Typhi and 88.89%, 77.78% and 77.78% strains of *Salmonella* Paratyphi A were sensitive to chloramphenicol, ampicillin and cotrimoxazole respectively. By disc diffusion method, 94.44% *Salmonella* Typhi strains and by broth microdilution method, 98.15% strains of *Salmonella* Typhi were sensitive to ceftriaxone. All the strains of *Salmonella* Paratyphi A were sensitive to ceftriaxone by both disc diffusion and broth microdilution method. In case of *Salmonella* Typhi, by disc diffusion method, 88.88% strains and by broth microdilution method, 92.59% strains were sensitive to cefotaxime. In case of *Salmonella* Paratyphi A, by both disc diffusion and broth microdilution method, 88.89% strains were sensitive to cefotaxime by each method. About 81.48% strains of *Salmonella* Typhi and 88.89% strains of *Salmonella* Paratyphi A were sensitive to cefixime by disc diffusion method. By disc diffusion method, 70.37% strains of *Salmonella* Typhi and by broth microdilution method, 72.22% *Salmonella* Typhi strains were intermediate sensitive to ciprofloxacin. By disc diffusion method, 66.67% strains of *Salmonella* Paratyphi A and by broth microdilution method, 77.78% strains of *Salmonella* Paratyphi A were intermediate sensitive to ciprofloxacin. All the strains of *Salmonella* Typhi and Paratyphi A were resistant to azithromycin by disc diffusion method but 87.04% strains of *Salmonella* Typhi and 77.78% strains of *Salmonella* Paratyphi A were sensitive by broth microdilution method. To determine antimicrobial susceptibility, broth microdilution method along with disc diffusion method should be done especially in case of azithromycin. Treatment of enteric fever with the first line antimicrobials should be reconsidered after testing their susceptibility pattern.

Keywords: Enteric fever, broth microdilution method, disc diffusion method, antimicrobial susceptibility pattern, *Salmonella* Typhi

INTRODUCTION

Enteric fever remains an important public health problem in many developing countries[1]. *Salmonella* Typhi causes typhoid fever, while *Salmonella* Paratyphi and *Salmonella* Choleraesuis are the major serotypes and *Salmonella* Typhimurium is a minor serotype which causes non-typhoidal salmonellosis in humans[2]. Current estimation shows that worldwide total number of typhoid fever episodes was 13.5 million in 2010[3].

In Bangladesh, the incidence of typhoid fever was 2.0 episodes/1,000 persons/year, with a higher incidence in children aged less than 5 years (10.5/1,000 persons/year) than in older persons (0.9/1,000 persons/year). The incidence of paratyphoid fever was 0.4/1,000 persons/year without variation by age group[4]. Bangladesh, India and Pakistan together account for about 85% of the world's typhoid cases[5].

Diagnosis of enteric fever depends on the isolation of *Salmonella*, most commonly by blood culture method[6]. Blood culture is the gold standard diagnostic method for diagnosis of enteric fever[7,8]. Identification of bacteria is important in confirming the clinical diagnosis of typhoid and will contribute to the effective management and treatment of typhoid cases[9]. Antibiotics have for long been the standard treatment for typhoid fever and antibiotics brought case fatality rates down from over 25% to around 1%[5].

Antibiotic therapy must be guided by *in vitro* sensitivity testing[10]. To determine *in vitro* antimicrobial susceptibility, the most commonly used methods include disc diffusion and minimum inhibitory concentration (MIC) which is determined by broth dilution, agar dilution, rapid automated instrument based methods and also by E-test[11].

One of the greatest advantages of the disc diffusion test is convenience and user friendliness because results are generally accurate and most commonly encountered bacteria are reliably tested. The disc diffusion test is still among the most commonly used method for antimicrobial susceptibility testing and results are determined by zone of inhibition. Broth dilution testing is divided into two categories: microdilution and macrodilution[12]. Both microdilution and macrodilution procedures generate quantitative result (the MIC), but the advantages of microdilution susceptibility procedure include the economy of reagents and space due to miniaturization of the test[13]. The MIC obtained using a dilution test can detect the concentration level of resistance and may help physicians[14].

Thus minimum inhibitory concentrations (MICs) are considered the gold standard for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing[15].

First line antibiotics most readily available for the treatment of typhoid fever are chloramphenicol, ampicillin and cotrimoxazole[16,17,18]. Typhoid fever caused by *Salmonella* Typhi strains which are resistant to all the three first line recommended drugs for treatment are defined as the multidrug resistant (MDR) strains[19,20]. The first line antimicrobials are cost effective and oral forms of chloramphenicol, ampicillin and cotrimoxazole are available[21,22].

Typhoid fever is endemic in tropics and subtropics including Bangladesh and India due to substandard personal hygiene and poor sanitation[23,24]. To complicate matters further, in the last two decades, multidrug resistant (MDR) *Salmonella* Typhi have emerged and spread worldwide, resulting in huge health care costs and high rates of morbidity and mortality[19,25].

Second line drugs for the treatment of typhoid fever include ceftriaxone, cefotaxime, cefixime, ciprofloxacin and azithromycin[22]. Among these drugs, ceftriaxone and cefotaxime are given by intravenous route, and their disadvantages include high costs and prolonged defervescence time[26]. Ceftriaxone, cefotaxime and

cefixime are effective for treatment of MDR enteric fever including fluoroquinolone resistant strains[27]. In case of cefixime, ciprofloxacin and azithromycin, oral forms are available[22]. Azithromycin, a member of the macrolide class of antibiotics possesses many characteristics for effective and convenient treatment of typhoid fever[28].

Considering the above facts this study was done to isolate typhoidal *Salmonella* species from blood among clinically suspected enteric fever cases and also finds out the antimicrobial susceptibility pattern of typhoidal *Salmonella* species by disc diffusion and broth microdilution method in some hospitals in Dhaka, Bangladesh.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Microbiology, Sir Salimullah Medical College, Dhaka over a period of one year from July, 2014 to June, 2015. Ethical clearance was obtained from Instituitional Ethics Committee (IEC) of SSMC (Memo no 2015/94, Dated 13.7.15) before performing the study. A total 325 blood samples were collected from clinically suspected enteric fever patients attending the out-patient Department of Medicine and Pediatric unit of Sir Salimullah Medical College and Mitford Hospital and also from Bangladesh Medical College and Hospital.

Isolation and identification of organisms:

Blood samples were collected from each patient into a conventional blood culture bottle following standard procedure[29,30]. Tryptone soya broth was used for conventional blood culture. Subcultures were done on MacConkey agar and blood agar media and incubated aerobically at 37ºC for 24 hours and extended up to 7 th day in culture negative cases[29]. Colonies were identified by observing colony morphology, Gram staining characteristics and relevant biochemical tests such as inoculation into KIA media, oxidase test, indole test, motility test, urease test and citrate utilization test[31,32]. All the media were procured from Oxoid Ltd, UK.

Antimicrobial susceptibility test:

After identification all the *Salmonella* Typhi and *Salmonella* Paratyphi A strains were tested for antimicrobial susceptibility testing by disc diffusion method following modified Kirby-Bauer technique and broth microdilution method to determine MIC[33,34]. Susceptibility patterns were determined following CLSI guidelines and BSAC[35,36]. The antimicrobial discs were procured from Oxoid Ltd, UK. First line antimicrobial drugs were ampicillin, chloramphenicol, cotrimoxazole and second line antimicrobial drugs were cefixime, cefotaxime, ceftriaxone, ciprofloxacin and azithromycin[10,27,37]. MIC of first line drugs and in case of second line drugs all except cefixime were not performed because powder or liquid forms were not available.

Quality Control:

Reference strains of *Escherichia coli* (ATCC 25922) was used as a control reference strains for identification and drug susceptibility testing. Quality control for media was done by randomly taking the prepared culture media and incubating over night to see for any growth.

RESULTS

A total of 325 blood samples were collected from clinically suspected enteric fever cases in the study period of one year of which, 63 (19.38%) cases showed growth and the remaining 262 (80.62%) cases showed no growth. Among these 63 isolates, 54 (85.71%) isolates were *Salmonella* Typhi and 9 (14.29%) isolates were

Salmonella Paratyphi A.

Table – I shows susceptibility pattern of *Salmonella* Typhi and Paratyphi A to first line antimicrobial agents by disc diffusion method. Among 54 isolates of *Salmonella* Typhi, 40 (74.07%), 42 (77.78%) and 39 (72.22%) strains were sensitive to chloramphenicol, ampicillin and cotrimoxazole respectively. Among the 9 isolates of *Salmonella* Paratyphi A, 8 (88.89%) strains were sensitive to chloramphenicol and in case of both ampicillin and cotrimoxazole 7 (77.78%) strains were sensitive individually.

Table – II shows susceptibility pattern of *Salmonella* Typhi and Paratyphi A to second line antimicrobial agents by disc diffusion method. Among 54 isolates of *Salmonella* Typhi, 51 (94.44%), 48 (88.88%) and 44 (81.48%) strains were sensitive to ceftriaxone, cefotaxime and cefixime respectively. In case of ciprofloxacin, 38 (70.37%) strains of *Salmonella* Typhi were intermediate sensitive and 16 (29.63%) strains were resistant. All the strains of *Salmonella* Typhi were resistant to azithromycin. All the 9 isolates of *Salmonella* Paratyphi A were sensitive to ceftriaxone. In case of both cefotaxime and cefixime, 8 (88.89%) strains were sensitive to each of them. In case of ciprofloxacin, 6 (66.67%) strains of *Salmonella* Paratyphi A were intermediate sensitive and 3 (33.33%) strains were resistant. All the strains of *Salmonella* Paratyphi A were resistant to azithromycin.

Table – III shows MIC breakpoint of ceftriaxone, cefotaxime, ciprofloxacin and azithromycin for *Salmonella* species. Among 54 isolates of *Salmonella* Typhi, 53 (98.15%), 50 (92.59%) and 47 (87.04%) strains were sensitive to ceftriaxone, cefotaxime and azithromycin respectively. In case of ciprofloxacin, 39 (72.22%) strains were intermediate sensitive and 13 (24.08%) strains were resistant by broth microdilution method. Among 9 isolates of *Salmonella* Paratyphi A, all the strains were sensitive to ceftriaxone and 8 (88.89%) and 7 (77.78%) strains were sensitive to cefotaxime and azithromycin. However, in case of ciprofloxacin 7 (77.78%) strains showed intermediate sensitivity by broth microdilution method.

Table – IV shows comparison between the susceptibility patterns of *Salmonella* Typhi determined by broth microdilution method (MIC) and disc diffusion method. In case of ceftriaxone, cefotaxime and azithromycin 53 (98.15%), 50 (92.59%), and 47 (87.04%) strains were sensitive, 39 (72.22%) strains were intermediate sensitive to ciprofloxacin, 13 (24.08%) and 7 (12.96%) strains were resistant to ciprofloxacin and azithromycin by broth microdilution method. Fifty one (94.44%) and 48 (88.88%) strains were sensitive to ceftriaxone and cefotaxime, 38 (70.37%) strains were intermediate sensitive to ciprofloxacin, 16 (29.63%) and 54 (100%) strains were resistant to ciprofloxacin and azithromycin by disc diffusion method.

Table – V shows comparison between the susceptibility patterns of *Salmonella* Paratyphi A determined by broth microdilution method (MIC) and disc diffusion method. In case of ceftriaxone, cefotaxime and azithromycin, 9 (100%), 8 (88.89%) and 7 (77.78%) strains were sensitive, 7 (77.78%) strains were intermediate sensitive to ciprofloxacin, 2 (22.22%) strains were resistant to both ciprofloxacin and azithromycin individually by broth microdilution method. 9 (100%) and 8 (88.89%) strains were sensitive to ceftriaxone and cefotaxime, 6 (66.67%) strains were intermediate sensitive to ciprofloxacin, 3 (33.33%) and 9 (100%) strains were resistant to ciprofloxacin and azithromycin by disc diffusion method.

Table – I: Susceptibility pattern of *Salmonella* Typhi and Paratyphi A to first line antimicrobial agents by disc diffusion method (n=63)

Antimicrobial agents	***Salmonella* Typhi (n=54)**			***Salmonella* Paratyphi A (n=9)**		
	S	**I**	**R**	**S**	**I**	**R**
Chloramphenicol	40 (74.07%)	0 (0%)	14 (25.93%)	8 (88.89%)	0 (0%)	1 (11.11%)
Ampicillin	42 (77.78%)	0 (0%)	12 (22.22%)	7 (77.78%)	0 (0%)	2 (22.22%)

Cotrimoxazole	39 (72.22%)	0 (0%)	15 (27.78%)	7 (77.78%)	0 (0%)	2 (22.22%)

Note: S = Sensitive, I = Intermediate, R = Resistant

Zone diameter was measured according to CLSI guideline, 2014

Table – II: Susceptibility pattern of *Salmonella* Typhi and Paratyphi A to second line antimicrobial agents by disc diffusion method (n=63)

Antimicrobial agents	***Salmonella* Typhi (n=54)**			***Salmonella* Paratyphi A (n=9)**		
	S	**I**	**R**	**S**	**I**	**R**
Ceftriaxone	51 (94.44%)	3 (5.56%)	0 (0%)	9 (100%)	0 (0%)	0 (0%)
Cefotaxime	48 (88.88%)	3 (5.56%)	3 (5.56%)	8 (88.89%)	1 (11.11%)	0 (0%)
Cefixime	44 (81.48%)	2 (3.70%)	8 (14.82%)	8 (88.89%)	0 (0%)	1 (11.11%)
Ciprofloxacin	0 (0%)	38 (70.37%)	16 (29.63%)	0 (0%)	6 (66.67%)	3 (33.33%)
Azithromycin	0 (0%)	–	54 (100%)	0 (0%)	–	9 (100%)

Note: Zone diameter was measured according to CLSI guideline, 2014

In case of azithromycin, according to BSAC, 2015 only sensitive and resistant zone diameter was given

Table – III: MIC breakpoint of ceftriaxone, cefotaxime, ciprofloxacin and azithromycin for *Salmonella* species (n=63)

Antimicrobial agents	***Salmonella* Typhi (n=54)**			***Salmonella* Paratyphi A (n=9)**		
	S	**I**	**R**	**S**	**I**	**R**
Ceftriaxone	53 (98.15%)	1 (1.85%)	0 (0%)	9 (100%)	0 (0%)	0 (0%)
Cefotaxime	50 (92.59%)	1 (1.85%)	3 (5.56%)	8 (88.89%)	1 (11.11%)	0 (0%)
Ciprofloxacin	2 (3.70%)	39 (72.22%)	13 (24.08%)	0 (0%)	7 (77.78%)	2 (22.22%)
Azithromycin	47 (87.04%)	–	7 (12.96%)	7 (77.78%)	–	2 (22.22%)

Note: MIC break point was measured according to CLSI guideline, 2014

In case of azithromycin, according to BSAC, 2015 only sensitive and resistant MIC break point was given

Table – IV: Comparison between the susceptibility patterns of *Salmonella* Typhi determined by broth microdilution method (MIC) and disc diffusion method (n=54)

Name of antimicrobial agents	**Methods of susceptibility**					
	Broth dilution method (MIC)	**Disc diffusion method**	**Broth dilution method (MIC)**	**Disc diffusion method**	**Broth dilution method (MIC)**	**Disc diffusion method**
	S	**S**	**I**	**I**	**R**	**R**
Ceftriaxone	53 (98.15%)	51 (94.44%)	1 (1.85%)	3 (5.56%)	0 (0%)	0 (0%)
Cefotaxime	50 (92.59%)	48 (88.88%)	1 (1.85%)	3 (5.56%)	3 (5.56%)	3 (5.56%)
Ciprofloxacin	2 (3.70%)	0 (0%)	39 (72.22%)	38 (70.37%)	13 (24.08%)	16 (29.63%)

Azithromycin	47 (87.04%)	0 (0%)	–	–	7 (12.96%)	54 (100%)

Note: Kappa test showed moderate agreement in ceftriaxone, substantial agreement in cefotaxime and ciprofloxacin, whereas poor agreement in azithromycin

Table – V: Comparison between the susceptibility patterns of *Salmonella* Paratyphi A determined by broth microdilution method (MIC) and disc diffusion method (n=9)

Name of antimicrobial agents	**Methods of susceptibility**					
	Broth dilution method (MIC)	**Disc diffusion method**	**Broth dilution method (MIC)**	**Disc diffusion method**	**Broth dilution method (MIC)**	**Disc diffusion method**
	S	**S**	**I**	**I**	**R**	**R**
Ceftriaxone	9 (100%)	9 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cefotaxime	8 (88.89%)	8 (88.89%)	1 (11.11%)	1 (11.11%)	0 (0%)	0 (0%)
Ciprofloxacin	0 (0%)	0 (0%)	7 (77.78%)	6 (66.67%)	2 (22.22%)	3 (33.33%)
Azithromycin	7 (77.78%)	0 (0%)	–	–	2 (22.22%)	9 (100%)

Note: Percentage agreement of ceftriaxone was 100%. Kappa test showed almost perfect agreement in cefotaxime, substantial agreement in ciprofloxacin, whereas poor agreement in azithromycin

DISCUSSION

Re-emergence of susceptibility to conventional first line drugs were strongly observed in this study, which supports the possibility of using these drugs in the treatment of enteric fever. To determine antimicrobial susceptibility for certain antibiotics along with disc diffusion method, minimum inhibitory concentration must be determined for proper clinical management and prevention of therapeutic failure[13].

In present study, out of 325 clinically suspected enteric fever cases, 63 (19.38%) *Salmonella* species were isolated from conventional blood culture method. This finding correlated with the findings reported in various studies done in Bangladesh by Sultana, Khan and in India by Saha and his associates[38,39,40]. The widespread availability and use of antibiotics in the community makes it frequently difficult to isolate the organisms from blood culture[41].

In the current study, predominant isolates were *Salmonella* Typhi which were 54 (85.71%) and similar to the findings of Gupta et al[42]. In Bangladesh, Shadia et al., in her study observed 79% isolates were *Salmonella* Typhi[43]. In this study, 9 (14.29%) *Salmonella* Paratyphi A were found and this finding correlated with a study done by Gupta et al[42]. In Bangladesh Shadia et al., in her study found 21% isolates were *Salmonella* Paratyphi A which did not correlate with the present study[43]. The reason may be due to the increase use of *Salmonella* Typhi vaccines in the general population which presumably lead to a decline in enteric fever cases due to *Salmonella* Typhi[44].

In this study, the sensitivity pattern to these first line drugs (chloramphenicol, ampicillin and cotrimoxazole) was detected by disc diffusion method because injectable forms of these drugs were not available in Bangladesh during the study period. 40 (74.07%), 42 (77.78%) and 39 (72.22%) isolates of *Salmonella* Typhi were sensitive to chloramphenicol, ampicillin and cotrimoxazole respectively by disc diffusion

method (Table – I). This finding correlated with a study done by Nagshetty et al., in India[45]. In another study, done in Bangladesh by Sultana showed 73.9% and 69.6% strains were sensitive to chloramphenicol and cotrimoxazole respectively[38]. Makanjuola et al., from Nigeria reported less than 50% sensitivity to all the previously mentioned first line drugs in case of *Salmonella* Typhi which did not correlate with the present study. This may be due to differences in study population and study pattern[18]. In case of *Salmonella* Paratyphi A, 8 (88.89%), 7 (77.78%) and 7 (77.78%) isolates showed sensitivity to chloramphenicol, ampicillin and cotrimoxazole respectively by disc diffusion method. Studies done by Kumar et al., and Chand et al., observed higher sensitivity to these drugs than the current study because in those area conventional first line drugs have been restricted for therapeutic use for almost two decades due to the development of MDR strains[46,47].

With the emergence of resistance to ampicillin, chloramphenicol and cotrimoxazole, first choice of empiric treatment of typhoid fever has changed to ceftriaxone and ciprofloxacin[43]. Third generation cephalosporins have gained importance for the treatment of enteric fever because of their pharmacodynamic properties and the very low prevalence of resistance to these agents[48]. In current study, out of 54 *Salmonella* Typhi, 51 (94.44%) isolates were sensitive to ceftriaxone by disc diffusion method (Table – II). The sensitivity pattern was similar to the findings done by Raza et al., and Muthu et al[49,50]. Monica et al., in her study observed 68.75% strains were sensitive to ceftriaxone by disc diffusion method which did not correlate with the present study[21]. *Salmonella* Typhi obtained in this study by broth microdilution method were found to be 53 (98.15%) sensitive to ceftriaxone (Table – III). A study done by Muthu et al., in India found 100% strains were sensitive to ceftriaxone by agar dilution method[50]. Similarly Kawser et al., in Bangladesh also reported 100% strains were sensitive to ceftriaxone by agar dilution method[51]. In this study, to campare between disc diffusion and broth microdilution method statistical analysis was done by Kappa test which showed moderate agreement between these two methods in ceftriaxone for *Salmonella* Typhi strains (Table – IV). The reason may be due to lesser sensitivity was observed in disc diffusion method in comparison to broth microdilution method because in case of disc diffusion method zone sizes are affected by the media, incubation condition and growth rates of organisms being tested[13]. In this study, 9 (100%) *Salmonella* Paratyphi A isolates were sensitive to ceftriaxone by disc diffusion method (Table – II), which was similar to the findings done by Raza et al[49]. Current study also showed all the strains of *Salmonella* Paratyphi A were sensitive to ceftriaxone by broth microdilution method (Table – III) which correlated with another study done by Kawser et al., in Bangladesh and the method was agar dilution[51]. In the present study, in case of *Salmonella* Paratyphi A percentage agreement of ceftriaxone was 100% (Table – V).

In current study, out of 54 *Salmonella* Typhi, 48 (88.88%) strains were sensitive to cefotaxime detected by disc diffusion method (Table – II) and Mushtaq in his study also reported parallel findings of sensitivity[52]. All the isolates of *Salmonella* Typhi were sensitive to cefotaxime by disc diffusion method which were found in various studies done by Gupta et al., Kumar et al., and Pokharel et al[42,46,53]. *Salmonella* Typhi obtained in this study by broth microdilution method were found to be 50 (92.59%) sensitive to cefotaxime (Table – III). In India, Muthu et al., and Capoor et al., reported all the strains of *Salmonella* Typhi were cefotaxime sensitive by agar dilution method[50,54]. In present study, there was substantial agreement showed between these two methods (Table – IV). In case of *Salmonella* Paratyphi A out of 9 isolates, 8 (88.89%) were sensitive to cefotaxime by disc diffusion method (Table – II). Pokharel et al., found 76% strains were sensitive and all the strains were reported sensitive by Gupta et al., and also by Kumar et al., and the method was disc diffusion[42,46,53]. By broth microdilution method in current study 8 (88.89%) of *Salmonella* Paratyphi A strains were sensitive to cefotaxime (Table – III). All the strains were reported sensitive to cefotaxime by Muthu et al., and Capoor et al., and the method was agar dilution[50,54]. In present study, in case of *Salmonella* Paratyphi A there was almost perfect agreement showed between these two methods (Table – V).

For the treatment of typhoid fever in comparison to other antibiotics, cefotaxime and ceftriaxone are much

more widely used. Despite the fact, in current study, the resistance pattern of cefotaxime is higher than ceftriaxone. Similar observations were found in studies done by Mushtaq, Muthu et al., and Khoharo and Memon[50,52,55]. The increasing drug resistance results from the exploitation of drug used by chemist, quacks and paramedics.

In present study, out of 54 *Salmonella* Typhi strains, 44 (81.48%) were sensitive and 8 (14.82%) were resistant to cefixime by disc diffusion method (Table – II). In a review article by Chowdhury et al., in BSMMU, it was reported that 78.8% strains were sensitive to cefixime which correlated with the present study[56]. Afzal et al., who found 70% strains were sensitive to cefixime[57]. Another study in India done by Monica et al., found 56.25% strains were sensitive to cefixime which did not correlate with the present study[21].The availability of cefixime in oral form may be responsible for the increasing resistance pattern by *Salmonella* Typhi strains. Of 9 *Salmonella* Paratyphi A, 8 (88.89%) were sensitive to cefixime by disc diffusion method (Table – II) in current study. Jain and Chugh in India reported total 2.5% isolates of *Salmonella* Paratyphi A were resistant to cefixime[58].

In this study, 38 (70.37%) *Salmonella* Typhi strains were intermediate sensitive to ciprofloxacin by disc diffusion method (Table – II) which correlated with the study of Afzal et al[57]. The resistance pattern of our study correlated with the study done by Chowdhury et al[56]. In contrast Kumar et al., in their study reported higher sensitivity of *Salmonella* Typhi strains to ciprofloxacin[46]. In current study, by broth microdilution method 39 (72.22%) isolates showed intermediate sensitivity and 13 (24.08%) isolates were resistance to ciprofloxacin (Table – III) which were opposite to the findings observed by a study done by Kawser[59]. In present study, there was substantial agreement showed between these two methods (Table – IV). In current study, in case of *Salmonella* Paratyphi A, 6 (66.67%) were intermediate sensitive and 3 (33.33%) were resistant to ciprofloxacin by disc diffusion method (Table – II) which correlated with another study done by Agrawal et al[60]. The finding of current study was different from the study done by Muthu et al., and Chand et al[47,50]. By broth microdilution method 7 (77.78%) isolates showed intermediate sensitivity and 2 (22.22%) were resistant to ciprofloxacin (Table – III) which were different from observation by Kawser in Bangladesh[59]. In present study, to compare between disc diffusion and broth microdilution method statistical analysis was done by Kappa test which observed substantial agreement between these two methods in case of ciprofloxacin in *Salmonella* Paratyphi A strains (Table – V). The reason may be due to lesser sensitivity was observed in disc diffusion method in comparison to broth microdilution method because in case of disc diffusion method zone sizes are affected by the media, incubation condition and growth rates of organisms being tested[13].

Most of the studies showed that azithromycin is highly effective in uncomplicated typhoid fever. Very few studies have been carried out in Bangladesh to see the effectiveness and sensitivity of azithromycin with uncomplicated typhoid fever[61]. Due to lack of breakpoint concentrations in CLSI guidelines, *in vitro* interpretation for *Salmonella* to azithromycin has often been difficult[62]. In this study, all the isolates of *Salmonella* Typhi and *Salmonella* Paratyphi A were resistant to azithromycin by disc diffusion method (Table – II) which was similar to another study done by Islam et al., and contradictory to Patel et al[63,64]. Islam et al., found 18% *Salmonella* isolates were azithromycin resistant by disc diffusion method; out of these 77.8% showed clinical improvement with azithromycin[61]. More than 90% clinical cure rates in patients treated with azithromycin was found in a study done by Frenck et al[65]. As azithromycin has no definite breakpoint for *Salmonella* isolates according to CLSI, 2014; BSAC, 2015 guideline was followed in this study. In current study, in case of *Salmonella* Typhi, 47 (87.04%) and in *Salmonella* Paratyphi A 7 (77.78%) strains were sensitive to azithromycin by broth microdilution method (Table – III). Susceptibility patterns of azithromycin by disc diffusion and broth microdilution method did not correlate with each other in the present study (Table – IV & V). Statistical analysis by Kappa test to compare between disc diffusion and broth microdilution method in case of both *Salmonella* Typhi and *Salmonella* Paratyphi A showed poor agreement between these two methods (Table – IV & V) and this might be due to lack of breakpoint

concentrations for azithromycin in various international guidelines because *in vitro* interpretation has often been difficult for *Salmonella*[62].

Currently, third generation cephalosporins or fluoroquinolones are the only real options available for enteric fever infections, yet increasing reports of resistance with these agents mean that azithromycin may itself emerge as a crucial drug in the future treatment and control of enteric fever[66].

CONCLUSION

Sensitivity of *Salmonella* Typhi and Paratyphi A to the first line antimicrobials were more than 70%, indicating returning of sensitivity pattern to first line antimicrobials. All the strains of *Salmonella* Typhi and Paratyphi A were resistant to azithromycin by disc diffusion method, whereas approximately 87% strains of *Salmonella* Typhi and 78% strains of *Salmonella* Paratyphi A exhibited sensitivity to it by broth microdilution method. So if possible along with disc diffusion method broth microdilution method should be done to determine the antimicrobial susceptibility. The very high resistance to azithromycin may likely be due to its overt-use by clinicians, so its use in the treatment of salmonellosis should be suspended, while reverting to the first line antibiotics which in vitro efficacy is outstanding from this study.

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