ANTIMICROBIAL SUSCEPTIBILITY OF *Salmonella* SEROVARS ISOLATED FROM BLOOD

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ABSTRACT

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The prospective study was conducted in LabAid Hospital, Dhaka, Bangladesh on patient's attending for the treatment of suspected bacterium during January 2008 to May 2008. For optimal patients care and saving hospital cost, precise information on antibiotic susceptibility pattern should be available to the clinician. With the aim of giving a message to the clinicians about the susceptibility pattern of *Salmonella typhi* and *Salmonella paratyphi A*, blood culture was done for 50 suspected cases of enteric fever and 30 strains of *salmonella typhi* and 16 strains of *salmonella paratyphi A* were isolated and identified by specific antisera and with standard biochemical tests. Antimicrobials susceptibilities were determined by Kirby-Bauer disc diffusion method using 16 different routinely used antibiotics. Antibiotic susceptibility test demonstrated that 50% isolates of *S. typhi* and 83.33% isolates of *S. paratyphi A* were multidrug resistant. All of the isolates of *S. typhi* were sensitive (100%) to Aztreonam Amikacin and Gentamycin and all of the isolates of *S. paratyphi A* were sensitive (100%) to Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Gentamycin, Mecillinam. All of the isolates of *Salmonella typhi* and *Salmonella paratyphi A* were resistant to Nalidixic acid (100%). In addition isolates of *S. paratyphi A* were also resistant to Azithromycin, Netilmicin. Decreased susceptibility of *S. typhi* and *S. paratyphi A* was observed in case of ciprofloxacin 73.33% and 70% respectively. Our study concludes that appropriate data of antimicrobial susceptibility should available to all of the clinicians for prescribing an antibiotics, Aztreonam Amikacin ,Gentamycin and Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Gentamycin, Mecillinam should be the choice of antibiotics for the treatment of *S. typhi* and *S. paratyphi A* infections respectively.

Keywords: *Salmonella* serovars, antibiotics susceptibility pattern, multi-drug resistant

INTRODUCTION

Enteric fever such as typhoid are endemic in Bangladesh, where there is a high incidence in children (Saha *et al.,* 2001) and continues to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs. The causative organism *Salmonella typhi* has rapidly gained resistance to antibiotics like ampicillin, chloramphenicol and cortrimoxazole, and also to previously efficacious drugs like ciprofloxacin (Jesudason *et al*, 1992, Butt *et al*, 2003). The incidence of multidrug resistant *S. typhi* was reported to be as high as 60% while there are reports noting a decline (Sanghavi *et a., l*1999; Chaude *et al.,* 2002; Saha *et al.*, 2002). Resurgence of resistant strains has also been reported (Kumar *et al.*, 2002) a us-based study (Ackers *et al.*, 2000) noted an increase in the number of MDR strains and nalidixic acid resistant *S. typhi*, although overall, the isolates were sensitive to ciprofloxacin and cefriaxone. Another study from Bangladesh (Rahman *et al.*, 2002) reported a decrease in MDR isolates with no corresponding increase in sensitive strains. A decreased susceptibility in ciprofloxacin resistance has been recorded in UK (Threlfall *et al.*, 2001) as well as India (Baliga *et al*., 1999; Bhat *et al*., 1999; Jesudason *et al*., 1996). Given the variation, in the susceptibility patterns reported for *S. typhi*. It is important to constantly monitor it so as to provide suitable guidelines for treatment. Recent reports on current sensitivity patterns of *S. typhi* isolates in Pondicherry are lacking. This study was undertaken to assess the antimicrobial susceptibility of *Salmonella* enterica serovars to the routinely used antibiotics in Dhaka city, Bangladesh with the ultimate to give a message to the clinicians about the antibiotics susceptibility and resistant pattern of *Salmonella* enterica serovars to help them in choosing an appropriate antibiotics optimal patient's care, saving hospital waste and to prevent antibiotics resistant development.

MATERIALS AND METHODS

The prospective study was conducted in LabAid Hospital; Dhaka, Bangladesh on patient's attending for the treatment of suspected bacterium during January 2008 to May 2008. The clinical history and examination finding were recorded on the standard form before preceding the blood culture.

The evidential microbiological diagnosis of bacteraemia was made by isolation of bacteria from blood culture. Total 5ml blood sample was drawn aseptically from adult patients and inoculated in 45ml of Brain heart infusion (BHI) (Hi Media, India) broth containing 0.03% of SPS as anticoagulant. For children, 3 ml of blood was inoculated in 20ml of BHI so that blood to broth ratio 1:12 was maintained. The culture bottle was incubated for four days at

37^0C. All blood culture bottles were examined daily. If the bottle showed any visible sign of growth such as uniform turbidity with gas bubble formation, haemolysis of blood with greenish tinge or cottony ball formation. Subculture was done on blood agar and MacConkey agar plate (Hi Media, India). The plates were then ncubated at 37c for 24 hours. Before discarding the culture bottle, subculture was done after 96 hours of incubation.

The isolates were identified by adopting standard microbiological procedure which includes colony morphology, Gram stain reaction and biochemical reaction such as Catalase, Oxidase, citrate agar slant, Suphide indole Motility test, Methyl red test. Boges Proskauer test, Triple sugar Iron agar test and urease test. The isolate of Salmonella were further confirmed by abblutination with polyvalen O antisera A-S and individual H antisera (Denka Seiken, Japan).

Further antibiotics susceptibility test of all isolates were performed by NCCLS recommended Karby-Bauer disc diffusion method. The antibiotic incorporated plates were incubated at 37^0C and zone of inhibition around the antibiotic were measurd after 18hours and within 24 hours of incubation. The routinely used antibiotics, all from Hi Media, were Amoxicillin, Amoxyclave, Azithromycin, Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Cephalexin, Ciprofloxacin, Co-trimoxazole, Gentamycin, Mecillinam, Nalidixic acid, Netilmicin. The isolates were considered as multidrug resistant if they were resistant to at least two classes of antibiotics.

RESULTS AND DISCUSSION

A total of 46 isolates of *Salmonella spp* were obtained from 150 blood cultures. *Salmonella typhi* was predominant serotype, followed by *S. paratyphi A*. Results of Antimicrobial susceptibility pattern of *Salmonella paratyphi A* against antibiotics showed that isolates were sensitive to Amoxicillin (75%), Amoxyclave (75%), Aztreonam (100%), Amikacin (100%), Cefaclor (100%), Cefixime (100%), Ceftazidime (68.75%), Ceftriaxone (68.75%), Cephalexin (75%), Ciprofloxacin (50%), Co-trimoxazole (75%), Gentamycin (100%), Mecillinam (100%), Nalidixic acid (75%), Netilmicin (100%) and resistant to Amoxicillin (25%), Amoxyclave (25%), Azithromycin (100%), Ceftazidime (31.25%), Ceftriaxone (31.25%), Cephalexin (25%), Ciprofloxacin (50%), Co-trimoxazole (25%), Nalidixic acid (25%).

Table 1: Antibiotics susceptibility pattern of *Salmonellap paratyphi A*

Antibiotics used	Susceptible		Resistant		Total isolates
	Number	%	Number	%	
Amoxicillin (AML)	12	75	4	25	16
Amoxyclave(AMC)	12	75	4	25	16
Azithromycin(AZM)	0	0	16	100	16
Aztreonam(ATM)	16	100	0	0	16
Amikacin(AK)	16	100	0	0	16
Cefaclor(CEC)	16	100	0	0	16
Cefixime(CFM)	16	100	0	0	16
Ceftazidime(CAZ)	11	68.75	5	31.25	16
Ceftriaxone(CRO)	11	68.75	5	31.25	16
Cephalexin(CL)	12	75	4	25	16
Ciprofloxacin(CIP)	8	50	8	50	16
Co-trimoxazole(SXT)	12	75	4	25	16
Gentamycin(GN)	16	100	0	0	16
Mecillinam(MEL)	16	100	0	0	16
Nalidixic acid(NA)	12	75	4	25	16
Netilmicin(NET)	16	100	0	0	16

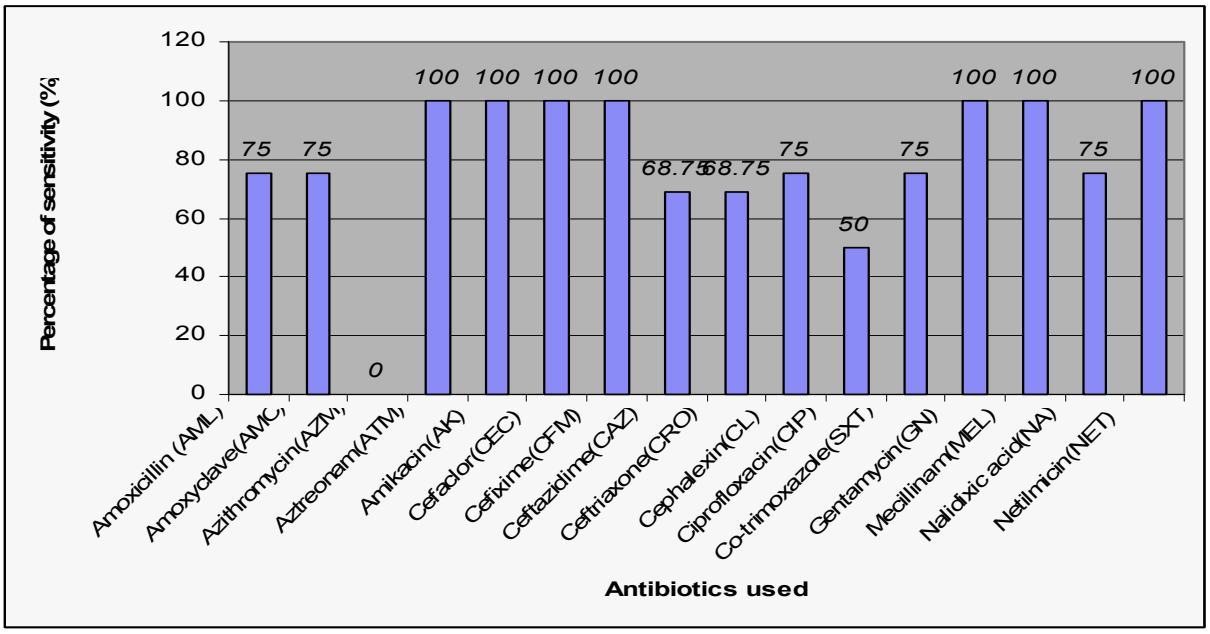

Figure 1. Antibiotics susceptibility pattern of *Salmonella paratyphi A*

Table 2: Antibiotics susceptibility pattern of *Salmonella typhi*

Antibiotics used	Susceptible		Resistant		Total isolates
	Number	%	Number	%	
Amoxicillin (AML)	18	60	12	40	30
Amoxyclave(AMC)	19	63.33	11	36.67	30
Azithromycin(AZM)	0	100	30	100	30
Aztreonam(ATM)	29	96.67	1	3.33	30
Amikacin(AK)	26	86.67	4	13.33	30
Cefaclor(CEC)	30	100	0	0	30
Cefixime(CFM)	23	76.67	7	23.33	30
Ceftazidime(CAZ)	30	100	0	0	30
Ceftriaxone(CRO)	30	100	0	0	30
Cephalexin(CL)	26	86.67	4	13.33	30
Ciprofloxacin(CIP)	20	66.67	10	33.33	30
Co-trimoxazole(SXT)	19	63.33	11	36.67	30
Gentamycin(GN)	30	100	0	0	30
Mecillinam(MEL)	29	96.67	1	3.33	30
Nalidixic acid(NA)	3	10	27	90	30
Netilmicin(NET)	30	100	0	0	30

Results of Antimicrobial susceptibility pattern of *Salmonella paratyphi A* against antibiotics showed that isolates were sensitive to Amoxicillin (60%), Amoxyclave (63.33%), Azithromycin (100%), Aztreonam (96.67%), Amikacin (86.67 %), Cefaclor (100 %), Cefixime (76.67 %), Ceftazidime (100%), Ceftriaxone (100%), Cephalexin (86.67%), Ciprofloxacin (66.67%), Co-trimoxazole (63.33%), Gentamycin (100%), Mecillinam (96.67%), Nalidixic acid (10%), Netilmicin (100%) and resistant to Amoxicillin (40%), Amoxyclave (36.67%), Azithromycin (100%), Aztreonam (3.33%), Amikacin (13.33%), Cefixime (13.33%), Cephalexin (13.33%), Ciprofloxacin (13.33%), Co-trimoxazole (3.33%), Nalidixic acid (90%).

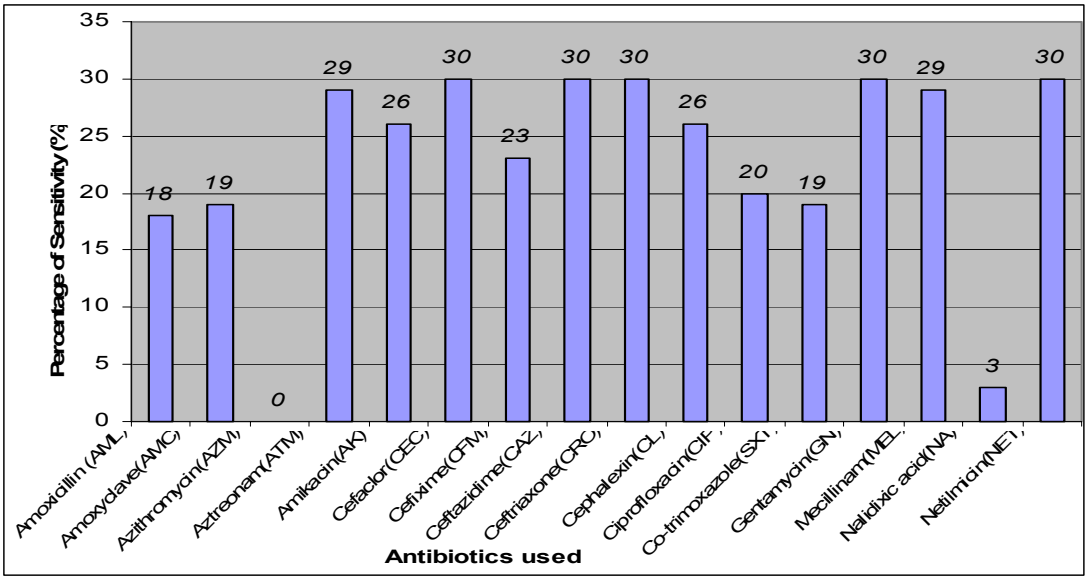

Figure 2. Antibiotics susceptibility pattern of *Salmonella typhi*

Antibiotic susceptibility of multidrug resistant *S. typhi* were Amoxicillin (40%), Amoxyclave (46.67%), Azithromycin (6.67%), Aztreonam (100%), Amikacin (100%), Cefaclor (86.67%), Cefixime (80%), Ceftazidime (86.67%), Ceftriaxone (73.33%), Cephalexin (93.33%), Ciprofloxacin (73.33%), Co-trimoxazole (26.67%), Gentamycin (100%), Mecillinam (86.67%), Netilmicin (6.67%).

Table 3: Antibiotics susceptibility of multi-drug resistant *S. typhi* and *S. paratyphi A*

Antibiotics used	*Salmonella typhi*		*Salmonella paratyphi A*	
	Susceptibility		Susceptibility	
	Number (%)	Total isolates	Number (%)	Total isolates
Amoxicillin (AML)	12(40%)	30	7(70%)	10
Amoxyclave(AMC)	14(46.67%)	30	7(70%)	10
Azithromycin(AZM)	2(6.67%)	30	0(0%)	10
Aztreonam(ATM)	30(100%)	30	10(100%)	10
Amikacin(AK)	30(100%)	30	10(100%)	10
Cefaclor(CEC)	26(86.67%)	30	10(100%)	10
Cefixime(CFM)	24(80%)	30	10(100%)	10
Ceftazidime(CAZ)	26(86.67%)	30	10(100%)	10
Ceftriaxone(CRO)	22(73.33%)	30	10(100%)	10
Cephalexin(CL)	28(93.33%)	30	9(90%)	10
Ciprofloxacin(CIP)	22(73.33%)	30	7(70%)	10
Co-trimoxazole(SXT)	8(26.67%)	30	7(70%)	10
Gentamycin(GN)	30 (100 %)	30	10(100%)	10
Mecillinam(MEL)	26(86.67%)	30	10(100%)	10
Nalidixic acid(NA)	0(0%)	30	0(0%)	10
Netilmicin(NET)	2(6.67%)	30	0(0%)	10

Again 50% isolates of *Salmonella paratyphi A* were multidrug resistant. Four were found to resistant to 2 antibiotics, two were found to resistant to 3 , two were resistant to 4 antibiotics, one was found to resist 5 antibiotics and one was also found to resistant to 6 antibiotics. Susceptibility of multi-drug resistant *S. typhi* against various antibiotics was Amoxicillin (70%), Amoxyclave (70%), Azithromycin (0%), Aztreonam (100%), Amikacin (100%), Cefaclor (100%), Cefixime (100%), Ceftazidime (100%), Ceftriaxone (100%), Cephalexin (90%), Ciprofloxacin (70%), Co-trimoxazole (70%), Gentamycin (100%). Again 83.33% isolates of *S. paratyphi A* were multidrug resistant. One was found to resistant to eight antibiotics, 4 were found to resistant to two antibiotics, 3 were found to resistant to four antibiotics, 2 were found to resistant to 6 antibiotics and another one was found to resistant to 3 antibiotics. All of the multi-drug resistant *S. paratyphi A* were sensitive to Mecillinam (100%), Amoxicillin (70%), Amoxyclave (70%), Aztreonam (100%), Amikacin (100%), Cefaclor (100%), Cefixime (100%), Ceftazidime (100%), Ceftriaxone (100%), Cephalexin (90%)), Ciprofloxacin (70%)), Co-trimoxazole (70%)), Gentamycin (100%), Mecillinam (100%).

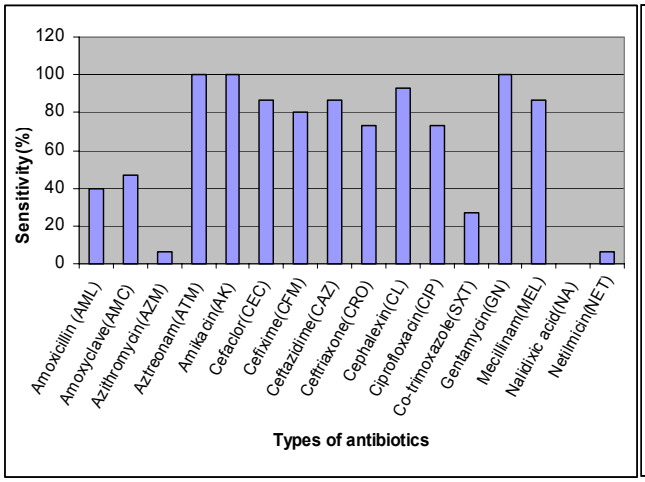

Figure 3. Susceptibility of MDR isolates of *Salmonella Typhi* to other antibiotics.

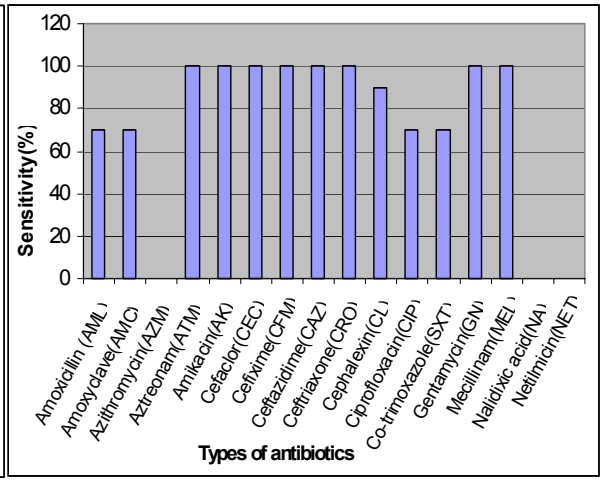

Figure 4. Susceptibility of MDR isolates of *Salmonella paratyphi A*

Enteric fever is major public health problem in our country, isolation of Salmonella spp occurs throughout the year in Bangladesh (Saha *et al.*, 2001). We found 92% of enteric fever is caused by *Salmonella spp*. This means that drinking water conditions and sanitation have not improved or a large number of carriers are present in the society. Isolation rates of *Salmonella spp*. have increased in recent years, particularly in the summer months (92% in April, 2008). Proper sanitation, public health education and vaccination are lone term preventive measures that would improve this situation.

Multidrug resistant *Salmonella typhi* is a major therapeutic concern for physicians in developing countries like Bangladesh. In our study we found that 50% isolates of *S. typhi* and 83.33% isolates of *S. paratyphi A* were multidrug resistant. This means that *Salmonella* infection is increasing in our country. Contributory factors may be drug overuse, misuse and inappropriate prescribing practices by physicians along with intrinsic microbiological plasmid-mediated factors. In the years 1982-89 the rate of multidrug resistant *salmonella* in India was below 15%, but it increased to 50% in 1990, to more than 70% in 1992 (Pillai *et al.*, 1993). Vikas *et al.* (2002) also found that multidrug resistnce in *S. typhi* to increase from 53.6% to 63.9%, while in *S. paratyphi A* it dropped from 68.8% to 27.7% from 1997 to 2001.

For improved treatments of enteric fever and multidrug resistant *S. typhi* in particular, attention has been focused on fluoroquinolones compounds and broad-spectrum cephalosporins because of their excellent properties. These highly active drug reduce the duration of treatment from the traditional 14 days that is necessary first line antibiotics. Wrong treatment regimens and reduced periods of hospitalization have obvious financial benefits, particularly in developing countries. They are also more likely to ensure compliance (Smith, 1994). In our study there was 73.33% and 70% sensitivity of *S. typhi* and *S. paratyphi A* to ciprofloxacin respectively while sensitivities to third generation cephalosporins- Cefaclor, Cefixime, Ceftazidime, Ceftriaxone were 100% , in case of *S. paratyphi A* and 86.67%, 80%, 86.67% and 73.33% were found repectively in case of *S. typhi*. This means that cephalosporins antibiotics is better than fluoroquinolones for the treatment of infection caused by *S. paratyphi A*. Results of susceptibility of *S. typhi* isolates showed that all them were sensitive to Aztreonam (100%) Amikacin (100%) and Gentamycin (100%). So clinicians should prescribe these antibiotics for the treatment of *S. typhi* infection.

However, fluoroquinolones have now been safely, used in typhoid and other types of systemic salmonellsis, as well as for other childhood life-threatening infections such as multidrug resistant *Pseudomonas* and drug resistant shigellosis (Hein, 1995; Schoad, 1992). In our study 32-40% of resistance was observed to third generation cephalosporins by *S.typhi* (Vikas *et al.,* 2002; Bhat, 1998). We also found that all of the isolates were resistant to Nalidixic acid (100%) with a decreased susceptibility to ciprofloxacin. This indicates that existence of Nalidixic acid resistant strains indicates the ciprofloxacin resistance to a certain extent.

Thus the sensitivity pattern of causative organisms must be studied before instituting appropriate therapy to prevent further emergence of drug resistance. Our study concludes that appropriate data of antimicrobial susceptibility should available to all of the clinicians for prescribing an antibiotics, Aztreonam Amikacin ,Gentamycin and Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Gentamycin, Mecillinam should be the choice of antibiotics for the treatment of S. typhi and S. paratyphi A infections respectively.

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