Bangladesh J Med Microbiol 2008; 02 (02): 22-26
Bangladesh Society of Medical Microbiologists

Original Article

Antibiotic Sensitivity Pattern of Salmonella Species Isolated by Blood Culture in Bangabandhu Sheikh Mujib Medical University

Ahmed Abu Saleh, Naser Ibne Sattar, Sharmeen Ahmed, Md. Ruhul Amin Miah

Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka

Abstract

Typhoid fever occurs in all parts of the world where water supplies and sanitation are sub-standard. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in the management of Typhoid fever cases. The aim of this study was to determine the antibiotic sensitivity pattern of Salmonella species isolated by blood culture. This was a retrospective study considering the period of January to December, 2007 at Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka. Blood samples for culture were collected by venepuncture, immediately inoculated into Brain Heart Infusion broth and incubated at 37°C. After 24 hours incubation, subcultures were done twice on Blood agar and MacConkey agar plates. Any growth on the subcultured plates were identified by standard microbiological techniques. All of the isolates were then subjected to antibiotic susceptibility test performed by disk diffusion method. A total 2,424 specimens of blood, collected from patients suspecting of fever, were cultured, out of which 218 (8.99%) were found positive for Salmonella species. *In vitro* sensitivity test showed that Cefixime was highly sensitive (99.41%) followed by Ceftriaxone (97.03%), Azithromycin (83.58%), Ciprofloxacin (67.47%), Cefuroxime (73.03%), ChloramphenicoI (72.62%), Co-trimoxazole (69.49%), Amoxicillin (64.33%) and Nalidixic acid (19.26%). The results call for nationwide surveillance programme to monitor microbial trends and antimicrobial susceptibility pattern of Salmonella species in Bangladesh.

Keywords: Typhoid fever, Salmonella species, Blood culture, Brain Heart Infusion broth, Antimicrobial susceptibility

Introduction

Typhoid fever is an acute generalized infection of the reticulondothelial system, intestinal lymphoid tissues and gall bladder, caused by *Salmonella enterica* subtype *typhi*. Typhoid fever, being a severe systemic illness is still an important public health problem in many developing countries, including Bangladesh. According to a recently revised global estimate, 21.6 million illnesses were found every year resulting in 216,500 deaths.[1]

Being resistance to a number of antibiotics, *S. typhi* has become a serious health problem. Strains of the organism resistant to Chloramphenicol and other recommended antibiotics have been identified in several parts of Asia and Africa.[2-4] Chloramphenicol was the drug of choice for Enteric fever since its introduction in 1948. But in 1970s, plasmid-mediated resistance to Chloramphenicol appeared and has been associated with outbreaks in Latin America and Asia.[5] With this emergence of Chloramphenicol-resistant strains, Ampicillin and Trimethoprim were considered suitable alternatives. Since 1989, however, with the emergence of multi-drug resistant *S. typhi* (MDRST) strains, which were no

✉ *Correspondence:*
Dr. Ahmed Abu Saleh
Associate Professor
Department of Microbiology and Immunology
Bangabandhu Sheikh Mujib Medical University
Shahbag, Dhaka
E-mail : aasaleh@gmail.com

longer susceptible to these antibiotics, three first-line antibiotics namely Amoxicillin, Co-trimoxazole and Nalidixic acid have been emerged.[6,7] In response to the emergence of MDRST, a number of studies have investigated the efficacies of newer compounds including expanded spectrum Cephalosporins and Fluoroquinolones. [6,7]

Adequate documentation of resistance profile of these organisms is also lacking in our country. Antibiotic resistance is supposed to be further accelerated due to irrational use of antibiotics, which is a very common phenomenon in Bangladesh. Typhoid fever, caused by MDRST, has become a significant cause of morbidity and mortality over recent years. These strains have also caused outbreaks throughout the world, especially in South America, Indian subcontinent, Africa, and South East Asia. [8,9]

The incidence of MDRST was reported very high up to 60%, although some reports were noting its decline.[10,11] With the emergence of MDRST, fluoroquinolones have gained importance for the treatment of Enteric fever in recent years. However, in 1997, the first major outbreak of Typhoid fever with strains resistant to Nalidixic acid and reduced fluoroquinolone susceptibility were reported.[12] These strains are now endemic in several countries in the Indian subcontinent. There are reports of prolonged defervescence after Ciprofloxacin therapy.[13,14] Strains isolated from these cases exhibited sensitivity to Ciprofloxacin in disk diffusion testing, but minimum inhibitory concentrations (MICs) for these strains were about 10-times higher than that of sensitive break-point. This reduced susceptibility is probably the cause of poor clinical response to treatment. Isolates of *S. typhi* with decreased susceptibility to Ciprofloxacin have been found to be resistant to Nalidixic acid as well. These Nalidixic acid resistant *S. typhi* (NARST) strains require higher concentrations of Ciprofloxacin for their inhibition.[9,13,15] A considerable variation has been noted in the antimicrobial susceptibility pattern among isolates of *S. typhi* as suggested in various studies conducted in different geographical locations.[16-18]

Knowledge of local antimicrobial resistance pattern is essential for practicing Physicians, clinical Microbiologists and public health officials, to guide empirical and pathogen-specific therapy. This work was, therefore, set out to investigate retrospectively, the antimicrobial resistance profile of Salmonella species against the most commonly prescribed antibiotics, isolated from clinical cases in Bangabandhu Sheikh Mujib Medical University.

Methods

A retrospective analysis of hospital laboratory records of blood culture-positive cases of Enteric fever, diagnosed between January to December, 2007 at Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital, was conducted. The Microbiology laboratory records regarding sensitivity data were collected for analysis.

At the Microbiology laboratory, blood samples for culture were obtained from patients who attended the BSMMU out patient department (OPD) with a history of fever of variable duration. Brain heart infusion (BHI) broth, which establishes the growth of all common pathogens causing bacteraemia/ septicaemia, was used as the culture medium.

Collection of blood, incubation, and subculture(s) onto Blood agar and MacConkey agar plates were done as per the standard methods.[19] Suspected non-lactose fermenting colonies were further processed. *Salmonella typhi* and *S. paratyphi* were identified by standard biochemical tests.

Antimicrobial susceptibility was determined by the Kirby Bauer disk diffusion method performed on Muller Hinton agar plates against Ampicillin (10 µg), Ceftriaxone (30 µg) Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Co-trimoxazole (25 µg), Cefuroxime sodium (30 µg), Cefixime (5 µg), Azithromycin (15 µg), and Nalidixic acid (30 µg) (Hi Media Laboratory Ltd., Mumbai, India).[20] The disk strength and zone size interpretation criteria were in accordance with the National Committee for Clinical Laboratory Standards (NCCLS).[20]

Results

In total, 170 strains of *S. typhi* and 48 strains of *S. paratyphi* A were isolated from 2,424 blood samples submitted to the Microbiology department of Bangabandhu Sheikh Mujib Medical University. (Table I)

Table I: Blood culture positivity and distribution of species of isolated Salmonella

Salmonella species	No of specimens showing growth
S. typhi	170 (77.98%)
S. paratyphi A	48 (22.02%)
Total	218 (100.0%)

Considering the antimicrobial susceptibility pattern of *S. typhi* of the 170 isolates, most (147, 86.5%) were resistant to Nalidixic acid, followed by Amoxicillin (100, 58.83%), Co-trimoxazole (86, 50.59%), Chloramphenicol (86, 50.54%). The highest (169, 98.82%) sensitivity with the lowest resistance (02, 1.2%) were demonstrated against Cefixime for the same isolates. (Table II)

Among 48 *S. paratyphi* A isolates, a high degree of resistance was also observed to Nalidixic acid (36, 75.0%) and all (48, 100%) isolates showed sensitivity to Cefixime. (Table II)

Table II: Antimicrobial susceptibility pattern of *S. typhi* and *S. paratyphi A*

Susceptibility results		No (%) of the isolates showing susceptibility against the antibiotics								
		AMX	CXT	CIP	NA	CRO	CHL	CFX	AZT	CFM
S. typhi	Sensitive	70 (41.2)	84 (49.4)	109 (64.1)	23 (13.5)	167 (98.2)	84 (49.4)	135 (79.4)	139 (81.8)	169 (98.8)
	Resistant	100 (58.8)	86 (50.6)	61 (35.9)	147 (86.5)	03 (1.8)	86 (50.6)	35 (20.6)	31 (18.2)	02 (1.2)
S. paratyphi A	Sensitive	42 (87.5)	43 (89.6)	34 (70.8)	12 (25.0)	46 (95.8)	46 (95.8)	32 (66.7)	41 (85.4)	48 (100.0)
	Resistant	06 (12.5)	05 (10.4)	14 (29.2)	36 (75.0)	02 (4.2)	02 (4.2)	16 (33.3)	7 (14.6)	00

Figures in parentheses represent percentages

AMX=Amoxicillin, CXT=Co-trimoxazole, CIP=Ciprofloxacin, NA= Nalidixic acid, CRO=Ceftriaxone, CHL=Chloramphenicol, CFX=Cefuroxime, AZT=Azithromycin, CFM=Cefixime

Discussion

Species of Salmonella are highly adapted human-specific-pathogens that have evolved remarkable mechanisms to persist in hosts and to ensure survival and transmission. Enteric fever is a major public health problem in our country. Proper sanitation, public health education and vaccination are the long-term preventive measures that could improve this situation. The emergence of antibiotic-resistant-strains of bacteria is closely linked to the irrational use of antibiotics in treating infections.

Since 1948, Chloramphenicol had been the mainstay of treatment of Enteric fever until 1972 when Chloramphenicol-resistant Typhoid fever became a major problem. Outbreaks of Typhoid fever occurred frequently in Mexico, India, Viet Nam, Thailand, Korea, and Peru.[21,22] Although initially susceptible to Ampicillin and Co-trimoxazole, *S. typhi* strains were simultaneously resistant to all first-line anti-typhoidal drugs emerged in the 1970s. Since then, these multi-drug-resistant (MDR) strains have spread to Mexico, India, and other regions in an epidemic form and have rapidly emerged worldwide.[21,22]

With the emergence of MDR *S. typhi*, Quinolones particularly Fluoroquinolones has been widely used and recommended as an alternative drug for Typhoid fever when the first-line drug was no longer in use. Fluoroquinolones, available since the 1980s, have good *in vitro* susceptibility and *in vivo* efficacy against Salmonellae, including *S. typhi*. Nalidixic acid, the prototype and the first member of the Quinolone group, is now seldom used due to the emergence of resistant serotypes of Salmonellae. It has also been observed that Nalidixic acid-resistant *S. typhi* has decreased susceptibility to Fluoroquinolones. In the present study, about 81% isolates of *S. typhi* were Nalidixic acid-resistant, and almost all appeared susceptible to Ciprofloxacin in disk-diffusion testing. This finding is similar to the reports from various series, suggesting that these strains were endemic in several countries of the Indian subcontinent. [21-25]

All the isolates in the present study, including Nalidixic acid resistant *S. typhi* (NARST) strains, were susceptible to Ceftriaxone. This finding may be important for considering the use of this antibiotic for treating infection with multi-drug resistant (MDR, including resistance to Ciprofloxacin) *S. typhi* in this region. This study shows that majority of Enteric fever cases identified in BSMMU hospital were caused by *S. typhi* (77.98%), follwed by *S. paratyphi* A (22.02%).

Over the recent years, Azithromycin has also been used as the option for the treatment of MDR and Quinolone-resistant strains of *S. typhi*.[21,26] The present study showed that majority (>80%) of the isolates were sensitive to Azithromycin. However, its high cost and limited availability in endemic areas are again the major disadvantages.

Activity of antimicrobials on the causative agents of Enteric fever appears to be changing over the past decade. From Nalidixic acid to Ciprofloxacin, development of resistance has steadily progressed. Although not a major problem at present, there are sporadic reports of Ceftriaxone-resistant *S. typhi*.[27] Moreover, detection of integrons in isolates of *S. typhi* from Asia has suggested that this organism has the potential for the acquisition of new resistant genes.[28,29]

The demonstration of a high rate of resistance among the

isolates against Ampicillin, Amoxicillin, Chloramphenicol and Co-trimoxazole is a cause for concern. This is simply an indication that in the near future, these antibiotics which are the first-line treatment for Enteric fever, can no longer be used for the treatment of Typhoid fever in this part of the world. Other works on *S. typhi* also reported a high percentage of resistance against Penicillin, Ampicillin, Cloxacillin, Erythromycin, Tetracycline, Chloramphenicol, Fluoroquinolones, Macrolides and Co-trimoxazole from other geographical areas.[30,31] Concerns have been raised about the emergence of effectively untreatable Typhoid fever in the developing nations if the appropriate control measures are not taken on time.[21,26] The prudent use of antibiotics and effective hospital infection-control practice may play a crucial role in preventing the emergence and spread of resistant organisms.

Antimicrobial susceptibility pattern of Salmonella species shows that Cefixime or Ceftriaxone has the highest sensitivity and Nalidixic acid has the highest resistance among both of the isolates of *S. typhi* and *S. paratyphi* A. The data from this investigation suggests that antimicrobial resistance among *S. typhi* is common and significant. Results of this study also have important implications for practicing physicians with regard to empirical antibiotic selection. The results also have important implications for authorities involved in hospital formulary decision and in the development of policies regarding antibiotic utilization, infection control and public healthcare. The judicious use of antibiotics by health workers and efforts to control procurement and use of antibiotics officially in the locality will probably help to limit the increasing rates of drug resistance in pathogens. Results of the present study call for further epidemiological studies to determine whether such isolates exist in the community and on a larger scale, for the implementation of a regional and nationwide surveillance system to monitor antimicrobial resistance trends.

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[Conflict of interest: None declared]