Antibiotic Resistance Pattern of Salmonella Typhi Isolated from Blood Culture

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Typhoid fever is a very common disease that can affect anyone at any age. Where the infections rate is higher in man than women. This study aimed to ascertain the current situation of typhoid fever and drug resistance determination caused by S. typhi in different age groups of patients. A retrospective data analysis of culture results of S. typhi was performed. This study determined the antibiotic susceptibility of 129 isolates of S. typhi from blood culture of 552 patients. Blood samples were aseptically collected in sterile containers and then cultured, susceptibility to 8 antibiotics was tested by disc diffusion technique. Among 129 cultured confirmed patients 53% were males and 47% patients were females. It states that males were more vulnerable to typhoid fever than female (P <0.001). The onset of typhoid fever was high at the pediatric age group (upto 15 years) 37.23%. Studies revealed that the most resistance rate for S. typhi from blood culture were ampicillin, cotrimoxazole and chloramphenicol. Whereas no isolate was resistant to ceftriaxone, ceftazidime and meropenem.

[Shaheed Syed Nazrul Islam Med Col J 2017 Jul; 2 (2):59-63]

Key words: Typhoid fever, Blood culture, Antibiotic resistance, S. typhi.

Introduction

Water borne diseases represent a serious threat to public health, affecting many people and resulting in considerable economic consequences in many part of the world. Typhoid fever is endemic in many developing countries particularly in Indian subcontinent including Bangladesh.[1,2] Current estimates from the World Health Organization (WHO) suggest that there are 16.6 million cases of typhoid fever with 6,00,000 deaths annually.[3] Rapid and sensitive laboratory methods for diagnosis of typhoid fever are essential for prompt and effective therapy. Today due to its changing modes of presentation, as well as the development of multidrug resistance, typhoid fever is becoming increasingly difficult to diagnose and treat. Improved standards of public health have resulted in a marked decline in the incidence of typhoid fever in developed countries.[4] The emergence of strains of Salmonella typhi resistant to multiple antibiotics poses a serious problem. An increasing frequency of antibiotic resistance has been reported from all parts of the world, but more so from the developing countries.[5] Multidrug resistance S. typhi (MDRST) is reserved for strains resistant to all three first line anti-typhoidal antimicrobial agents, namely ampicillin, chloramphenicol and cotrimoxazole.[6] The emergence of MDRST with resistance to fluoroquinolones and third generation cephalosporins is severely limit the possibilities for effective treatment of human infections. Infection with Salmonella typhi, the causative organism of this disease, requires effective antimicrobial chemotherapy in order to reduce mortality.[7]

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Unlike most developed countries, Bangladesh does not have a nation wide typhoidal Salmonella surveillance program to monitor multidrug resistant Salmonella in the human population. There are a few published studies with limited data on the prevalence and characterization of Salmonella isolates from clinical samples like blood. The objective of the present study was to determine the antimicrobial resistance profiles of Salmonella in clinical samples in Bangladesh. These updated baseline data are needed to establish an integrated and unified antimicrobial resistance surveillance system.

Method

This is a retrospective study of the antibiotic resistance profile of 129 positive blood culture, sample from 552 blood samples over a period of 15 months (From December, 2015 to February 2017) at 250 Bed General Hospital, Kishoreganj, Bangladesh. Patients were identified and data were extracted using the hospital information and support system.

Laboratory Techniques

In brief, using a sterile syringe and needle, about 8-10 ml of blood from each adult study subject and about 3-5 ml blood from each young child was collected. Then 5-7ml from adults and 2-3 ml of blood from children was dispensed into the culture medium bottle, containing 45 ml of tryptic Soy broth (OXOID, England) mixed with the broth and then incubated at 37^0C. Then the colonies were plated on MacConkey agar to establish a pure culture.[8] One growth was obtained on the solid medium typical colony was picked up and inoculated into peptone water as well as into MacConkey agar. The tubes were then incubated at 37^0C for 24 hours. The peptone broth culture was used for typical biochemical tests (Kligler Iron Agar, Motility Indole Urease and Citrate media) and identification of S. enterica serovar typhi using API 20E system (Biome'srieux, Inc., Hazelwood, MO).[9]

Antibiogram

Bacterial susceptibility to antimicrobial agents was determined in vitro by using agar disc diffusion method following CLSI guideline.[10] The following antibiotic discs were used: ampicillin (10 µgm), ciprofloxacin (5 µgm), cotrimoxazole (25 µgm), ceftriaxone (30 µgm), azithromycin (15 µgm), chloramphenicol (30 µgm), ceftazidime (30 µgm) and meropenem (10 µgm). After incubation at 37^0C for 24 hours, the diameter of inhibition zone was measured by digital slide calipers and interpreted as "Sensitive", "Intermediate" or "Resistant" according to CLSI standard interpretative charts.

Statistical Analysis

The data were analyzed using SPSS version 16 statistical software for Confidence Interval (CI) and P value. Statistical significance was defined when P value is <0.05. The confidence interval was set at 95% level of significance for all the proportions.

Results

A total of 129 typhoid fever caused by Salmonella typhi were confirmed on blood culture of 552 patients. Among then 53% patients were males (292) and 47% were females (260); male to female ratio is 1.12:1 (Table – I)

Table I: Sex distribution of the patients (N-552)

Sex	No. of patients	Ratio
Male	292	1.12
Female	260	1

Out of 552 suspected cases of typhoid fever blood culture positive for S. Typhi were 129 (23%) and remaining 423 (77%) were negative (Fig 1.).

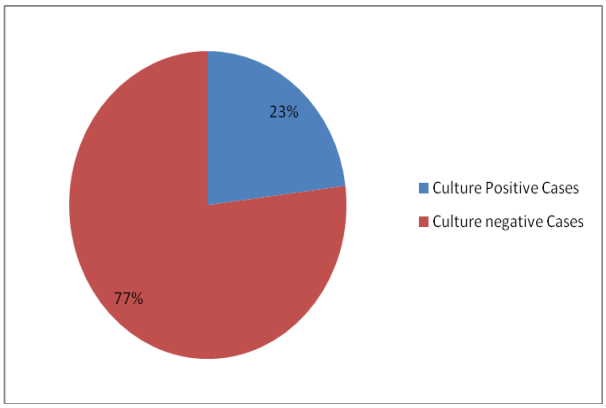

Fig I. Rate of isolation of S.typhi in blood culture

Study population were divided into two different age groups viz., upto 15 years of age were catagorized as pediatric group and above 15 years as adult group. The rate of isolation of S. typhi was noted higher (37.23%) among pediatric group when compared to adult group (18.48%), which is shown in TableII.

Table II: Rate of siolation of s.typhi in relation to age group

Age groups	No. of suspected cases	No. of isolates
Pediatric group (Upto 15 years)	130 (23.6%)	51 (37.23%)
Adult group (Above 15 years)	422 (76.4%)	78 (18.48%)
Total	552	129

Antimicrobial susceptibility pattern of 129 isolates of S.typhi show that no isolate was resistant to ceftriaxone, ceftazidime and meropenem. Ciprofloxacin and azithromycin were resistant to 28 (21.7%), 44 (34.1%) respectively, whereas first line anti-typhoid drugs ampicillin, cotrimoxazole and chloramphenicol were resistant to 97 (75.2%), 102 (79.1%) and 107 (82.95%) respectively.

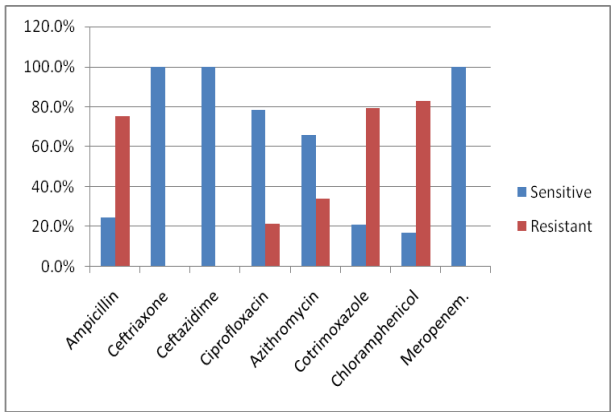

Fig 2. Antimicrobial susceptibility pattern of S. typhi (N-129)

Discussion

Typhoid fever is still a significant public health problem in may developing countries. It is a dreaded disease because of its long course and associated complications if not detected and treated early. There are reports of changing clinical features in typhoid fever caused by drug resistant S. typhi leading to difficulty in clinical diagnosis.[11,12] The occurrence of antibiotic resistance in lower-middle income countries take place due to the poor access to doctors. It eventually encourages the unacceptable practice of selling antibiotics over the country.[13] A relation between antibiotics use and its resistance in clinical isolates had been proven in many isolates. Even though, a direct quantitavtive relationship between the amount of antibiotic used and the frequency of resistance is still lacking.[14] Typhoid fever is endemic in Bangladesh, where there is a high incidence in children.[15] The emergence of MDR S. typhi isolates in the early 1990s, particularly from the Indian sub-continent, prompted the suggestion that ceftriaxone, ceftazidime and ciprofloxacin should be the drug of choice for empirical treatment of typhoid fever.[16, 17, 18] Initially, reduced use of ampicillin, cotrimoxazole and chloranphenicol was associate with a decreased prevalance of MDR strains, but

more recently, continued dependance on ciprofloxacin for the empirical treatment of typhoid fever in Bangladesh and elsewhere has led to the emergence of resistance of S. typhi to this drug.[19, 20] In the present study difference in antibiotic sensitibity against S. typhi were observed. Most isolates were resistant to ampicillin, cotrimoxazole and chloramphenicol. Although previous studies showed the growing resistance of ciprofloxacin,[21] but we found both ciprofloxacin resistant and susceptible samples. 78.3% of studied samples were susceptible to ciprofloxacin. On the other hand 21.7% of the samples were resistant to it. In our study we found that, 79.08% isolates of S. typhi are multidrug resistant. This shows alarming increase of multidrug resistance of this organism in Bangladesh supported by a number of previous studies.[22,23] Similar pattern of increase in multidrug resistance has been observed in the last 30 years in India.[24] Contributory factors may be drug over use, misuse and inappropriate prescribing practices by physicians along with intrinsic microbiological plasmid-mediated factors.

Finally, it can be concluded that the present study demonstrates that S. typhi isolated have shown various trends in isolation rate and resistances to various classes of antibiotics. In addition multidrug resistant S. typhi can be expected to cause serious treatment problems. As the antibiotic resistance is an important factor, blood culture and antibiotics sensitivity test should be routinely performed in all suspected patients, depending on the antibiotic susceptibility, therapy should be designed of an antibacterial agent with the narrowest spectrum, least cost and few adverse effects.

Conclusion

It is quite alarming to note that almost all of the isolates included in this study were found resistant to multiple drugs. Antibiotic resistance has been emerged as a major problem in the management of hospitalized patents and adds considerably to health care cost. Our research has been confined to a particular region of Bangladesh. Larger samples sized from other regions of Bangladesh can lead to more significant results. Therefore it is increasingly important to study this resistant S. typhi, including mechanism of resistance, in order to develop effective future drugs.

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