An Epidemiological Study of Antibiotic Resistance of *Salmonella typhi* and *Salmonella paratyphi* - A from Clinical Samples in Dhaka City

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Typhoid fever is a serious bacterial disease caused by *Salmonella typhi* or *Salmonella paratyphi.*
Study Design: The objectives of this study is to determine the prevalence and antibiotic resistance pattern of *Salmonella typhi* and *Salmonella paratyphi* isolated from adults and children presenting with symptoms at different hospital in Dhaka from 1st September-31st December 2013.
Materials and Methods: The antibiotic susceptibility of 106 clinical isolates collected from blood samples was identified. Susceptibility pattern was tested by Kirby-Bauer disc diffusion method using 8 regularly used antibiotics. The patients were categorized in different 9 age groups. Data analysis was performed by using Statistical Package for Social Science (SPSS version 10.0).
Results: A total of 1178 blood samples were analyzed in this study. Among which 106 showed

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positive result, 9% patients were ill, the causative agents-Salmonella typhi was found to be most prevalent (73.58%) followed by Salmonella paratyphi (26.42%). Result of the susceptibility test showed that the isolates were resistant to antibiotics; Nalidixic Acid and Azithromycin (79.49%) and 62(79.49%) for S. typhi isolates, 27(96.43%) and 27(96.43%) for S. paratyphi isolates respectively.
Conclusion: Our study revealed high prevalence of and (100%) of MDR Salmonella isolates. This is a serious health problem in the region under study and should be prevented by some effective measure such as eating safely prepared foods and get vaccinated (Ty21a) are recommendable for the treatment of typhoid fever in this community.

1. INTRODUCTION

Typhoid fever is an epidemic disease in Bangladesh, Indian subcontinent; South and Central America and Africa [1]. Despite the use of newly developed antibacterial drugs, enteric fever such as typhoid and paratyphoid caused by multidrug-resistant bacterial strains are one of major health problems in Bangladesh, especially for the children [2]. On a global scale, at least 16–20 million cases of typhoid fever occur annually, resulting in approximately 600,000 deaths, found in all ages, with the highest incidence in children [3].

Given such a specificity for humans, S. typhi might be younger than the split between humans and higher primates several million years ago and possibly even as young as the expansion of anatomical modern humans from Africa approximately 50,000–100,000 years ago [4]. Preliminary results from recent studies conducted in Bangladesh by the International Centre for Diarrheal Disease Research, Bangladesh revealed an incidence of approximately 2000 per 100 000 people per year [5].

Typhoid fever also has a very high social and economic impact because of the hospitalization of patients with acute disease and the complications and loss of income attributable to the duration of the clinical illness [6].

Since all the signs and symptoms of typhoid fever are nonspecific, a definitive diagnosis of the disease depend on the clinical presentation alone is very difficult. Therefore, laboratory-based investigations are essential for supporting the diagnosis of typhoid fever. The "gold standard" for diagnosis of typhoid fever is the isolation of S. typhi from appropriate samples including blood, bone marrow aspirates, stool, urine and rose spots [7].

A study in Bangladesh reported a gradual change in resistance to ampicillin and cotrimoxazole among S. typhi populations [7]. In the same study, the rate of resistance to cotrimoxazole, ampicillin and chloramphenicol decreased from 59.6 to 5.6% of the organisms over a 3 years period [8]. For this reason, antimicrobial susceptibility test is essential to see the changing trend of antibiogram of circulating strains in the community. Therefore, we performed the present study to investigate the resistance of S. typhi and S. paratyphi samples to commonly used antibiotics in different private hospitals in Bangladesh and to determine the prevalence of typhoid fever among different age groups and sexes.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Salmonella

This hospital-based cross sectional study was performed in Microbiology research laboratory, Primeasia University, Dhaka, Bangladesh. All samples were collected from OPD (Out patients department) and IPD (In patients department) patients of hospital having clinical symptoms of microbial infection both sexes and different age groups. Blood samples were collected from different hospital in Dhaka, during 1st September-31st December 2013. With Bactec technique, 5-10 ml blood for adults and 1-4 ml for children were taken and then put into a special bottle (septum of the blood culture bottle) and put in the Bactec machine for incubation at 37° for. The bacterial growth was detected during the incubation: Whenever the bacteria grow, both the machine and the computer will indicate the growth by alarm message on the computer screen [9].

The isolates were identified by adopting standard microbiological procedure which includes gram stain reaction and biochemical [8]. All isolates were sub cultured on Xylose-lysinedeoxycholate (XLD) agar (OXOID, UK), MacConkey agar and Salmonella-Shigella agar (SS agar) plates (OXOID, UK) for 24 hrs at 37℃ to identify the

specific colonies. Suspected colonies were examined biochemically using Oxidase, TSI, simmon citrate agar, MIU, (MERCK Company Supplier, Germany) [10].

2.2 Antibiotic Susceptibility of Salmonella Isolates

Muller Agar plates were inoculated with 0.5 Mc Farland standards of the isolates; antibiotic disks were placed and incubated at 37℃ for 24 hrs. Zone of inhibition diameters were measured and interpreted according to the guidelines of the Clinical Laboratory Standard Institute [11]. Resistant isolates to at least one member of three different antimicrobial groups are considered as MDR [12]. The antibiotic

susceptibility was performed by disc diffusion method measurement of zone of inhibition using the following antimicrobial discs [13,14].

3. RESULTS

During the period, total of 1178 typhoid suspected patients were enrolled, covering 722 males and 456 females. Among them, bacterial growth was positive for samples 106(9%) typhoid fever patients. Single colony were isolated from each of the 106 samples, while *Salmonella typhi* and *Salmonella paratyphi* were isolated from 78(73.58%) and 28(26.42%) of these samples. 67 male and 39 female blood specimens showed the growth of *Salmonella typhi* and *Salmonella paratyphi*.

Table 1. Disc diffusion method measurement of zone of inhibition of the following antimicrobial discs

Antibiotics	Resistance zone (mm)	Intermediate (mm)	Sensitive zone (mm)
Cotrimoxazole 30 µg	≤10	14-16	≥16
Nalidixic acid 30 µg	≤13	14-16	≥ 19
Ciprofloxacin 5 µg	≤15	16-20	≥21
Ceftriaxone 30 µg	≤13	16-20	≥21
Amoxicillin 20/10 µg	≤13	14-16	≥18
Azithromycin 15 µg	≤13	14-17	≥18
Cefixime 5 µg	≤15	14-17	≥19
Levofloxacin 5 µg	≤13	14-16	≥17

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Table 2. The age distribution of the study subject, the mean age of the individual was 25.86± (SD=±2.54)

Positive studied patients (n = 106)						
Age group (yrs)	n	Male	Female	(%)	Mean	±SD
0-10	25	15	10	23.6%	7.44	± 2.55
11-20	32	20	12	30.18	16.31	±2.44
21-30	36	30	6	33.96	25.86	± 2.54
31-40	10	2	8	9.43	36.9	± 2.07
41-50	1	0	1	0.94	0.98	0
51-60	1	0	1	0.94	1.23	0

Table 3. Degree of susceptibility of *Salmonella typhi* and *Salmonella paratyphi* to antibiotics

Antibiotics	*Salmonella typhi (n=78)*				*Salmonella paratyphi (n=28)*			
	S	%	R	%	S	%	R	%
Ceftriaxone	78	100	0	0	28	100	0	0
Ciprofloxacin	78	100	0	0	28	100	0	0
Cefixime	78	100	0	0	28	100	0	0
Levofloxacin	77	98.72	1	1.28	28	100	0	0
Amoxicillin	66	84.62	12	15.38	26	92.86	2	7.14
Cotrimoxazole	64	82.05	14	17.95	26	92.86	2	7.14
Azithromycin	16	20.51	62	79.49	1	3.57	27	96.43
Nalidixic Acid	2	2.56	76	97.44	1	3.57	27	96.43

Here S= Sensitive, R= Resistance, %= Percentage

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Table 3 showed that, resistance rate of *S. typhi* and *S. paratyphi* to Ciprofloxacin, Ceftriazone, Cefixime and Levofloxacin all by (0%) except *S. typhi* which showed resistance rate to Levofloxacin by (1.28%). *S. typhi* was resistant to Nalidixic Acid (79.49%) and Azithromycin 62(79.49%) while *S. paratyphi* resistance rate to Nalidixic acid 27(96.43%) and Azithromycin 27(96.43%).

4. DISCUSSION

These studies provide valuable data and monitor the status of antimicrobial resistance among typhoid patients to improve efficient empirical treatment. Increasing antimicrobial resistance has been documented globally [15]. A relation between antibiotic use and its resistance in clinical isolates had been proven in many studies. Direct quantitative relationship between the amount of antibiotic used and the frequency of resistance is still lacking [16,17]. In this study, the most affected age group for typhoid fever was 21-30yrs (33.96%) love to eat outside food and in spite of having knowledge about hygiene they can't maintain properly. greater exposure to contaminated food [18,19,20].

5. CONCLUSION

In this study, the most affected age group for typhoid fever was 21-30 (33.96%). At the end of the study the objectives we carried out were: To identify *Salmonella typhi* and *Salmonella paratyphi* by placing the blood samples collected from the patients. Improvement to determine the prevalence of typhoid fever among different ages and sexes. Determination of the antibiotic resistance pattern of *Salmonella typhi* and *Salmonella paratyphi*. Typhoid can easily be prevented by some effective measures eat only safely prepared foods, avoid raw meat, eggs, vegetables and fruits, drink clean water and get vaccinated (Ty21a). *S. typhi* and *S. paratyphi* showed highest sensitivity to Ceftriaxone, Ciprofloxacin, Cefixime & Levofloxacin that are the drugs of choice for the treatment of typhoid fever.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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