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RESEARCH ARTICLE

Isolation of *Salmonella* in bloodstream infections and antibiogram in a tertiary care setting in Dhaka, Bangladesh

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ABSTRACT

BACKGROUND: The antibiotic resistance pattern of *Salmonella* is ever changing over time.

OBJECTIVE: This study is a retrospective analysis of the rate of isolation of *Salmonella* Typhi and *Salmonella* Paratyphi A from blood culture and their antibiotic resistance pattern.

METHODS: Blood cultures submitted in Uttara Adhunik Medical College Hospital from July 2013 to June 2014 were analyzed. Isolated *Salmonella* sp were identified, and antimicrobial susceptibility testing was carried out by a standard disc diffusion method.

RESULTS: Among 314 isolated *Salmonella* sp, 229 (72.93%) were *Salmonella enterica* serovar Typhi and 85 (27.07%) were *Salmonella enterica* serovar Paratyphi A. Average prevalence rate of *Salmonella* in blood was 11.41%. In this study, 101 (32.17%) of *Salmonella* sp were isolated from hospitalized patients, while the remaining 213 (67.83%) were isolated from outpatients. Among culture-positive cases, the highest 109 (34.71%) is in age group 11–20 years and males are more susceptible to *Salmonella* septicemia than females. Analysis showed that most of the isolated *S.* Typhi and *S.* Paratyphi A were resistant to nalidixic acid, azithromycin, and ciprofloxacin. All *S.* Typhi and *S.* Paratyphi A were sensitive to ceftriaxone (100%).

CONCLUSION: This study showed that there was a gradual increase in the prevalence of *S.* Paratyphi A and decline in the resistance of *Salmonella* to first-line antibiotics but very high prevalence of nalidixic acid-resistant *S.* Typhi and *S.* Paratyphi A in Bangladesh.

KEYWORDS

Salmonella, blood culture, antibiotic sensitivity pattern

INTRODUCTION

Typhoid fever is an important public health problem in Bangladesh. *Salmonella enterica serovar* Typhi and *S. enterica* serovar Paratyphi are common etiological agents of typhoid fever. With the emergence of resistance to ampicillin, chloramphenicol, and cotrimoxazole, first choice of empiric treatment for typhoid fever has changed to ciprofloxacin and ceftriaxone.[1–3] But there are reports of emergence of ciprofloxacin-resistant *Salmonella* from Bangladesh and other countries.[4,5] Resistance to ceftriaxone has also been reported from Bangladesh in 1999.[6] Recently, there is emergence of *Salmonella* strains that are ciprofloxacin sensitive but nalidixic acid resistant. Treatment of patients with these strains with the usual dose of ciprofloxacin resulted in poor clinical outcome and treatment failure.[7–10] All these reports suggest that fluoroquinolones should no longer be used as the first-line therapy in population where nalidixic acid resistance is common among isolates

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of *S.* Typhi. Moreover, recent studies indicate that the proportion of *S.* Paratyphi A is increasing over the years in the subcontinent.[11,12] Therefore, it is important to know the species of *Salmonella* that are prevalent in Bangladesh and their antibiotic susceptibility patterns.

Blood is sterile body fluid, and its sterility is maintained by various antimicrobial substances present in it. The presence of microorganisms in the circulating blood is a threat to every organ. Various infections at different primary anatomical sites such as genitourinary tract, respiratory tract, surgical sites and abscesses often result in bloodstream infection (BSI), and fever is the commonest presentation. BSIs are significant cause of morbidity and mortality worldwide.[1] Approximately 200,000 cases of bacteremia and fungemia occur annually with mortality rates ranging from 20 to 50%.[2] It has been estimated that, in the United States, 2 million patients every year acquire infections during their hospital stay; approximately 350,000 (10–20%) of these infections involve the bloodstream and 90,000 (4.5%) are fatal.[2,3] In a study from Nepal, Gram-negative bacteria were found to be predominant cause of BSIs. *Salmonella* spp, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were the common etiological agents of BSIs.[4] BSIs can be healthcare associated or community associated. BSI is defined as healthcare associated if it occurred more than 48 hours after hospital admission or was associated with the presence of an indwelling medical device or it occurred within 30 days of a surgical procedure (where the BSI was related to the surgical site infection). BSI is defined as community associated if it is manifested within 48 hours after hospital admission unless an organism with a long incubation period was isolated.[1] An increasing resistance among pathogens is worrisome for the clinicians to start empirical therapy especially in the developing countries like Bangladesh, where enough laboratory data are not available on regular basis for monitoring and formulating antibiotic policies. Isolation of the pathogen by blood culture, identification, and antibiotic susceptibility pattern of the isolate are important steps in the diagnosis and management of the BSI. Rapid clinical diagnosis and early empirical antibiotic therapy can significantly reduce the mortality rate in BSI. Selection of antibiotic for empirical therapy by a clinician requires adequate knowledge about the etiological agents and their antimicrobial susceptibility patterns in various geographical areas. In developing countries like Bangladesh, many of the infections including BSIs are poorly diagnosed because of limited diagnostic resources. Febrile illness is one of the most common complaints among patients for medical attention in our hospital, but information regarding frequency of specific infections is limited. Therefore, we conducted this study, which was mainly focused to determine various bacterial agents and to get the currents updates of antimicrobial resistance pattern in a tertiary care centre of Bangladesh. Results of this study would be helpful for clinician to start early empirical therapy for the management of BSI and also in minimizing the spread of antimicrobial resistance among the blood pathogens.

METHODS

Retrospective type of study was done in Microbiology Laboratory at Uttara Adhunik Medical College Hospital from July 2013 to June 2014. All the suspected cases of bacteremia and septicemia from both outpatient and inpatient were included. Of total, 2751 blood samples were processed.

Data on sociodemographic variables such as age, sex, blood culture results, antibiotic-resistant pattern were collected manually by using a pre-prepared data abstraction format from the Department of Microbiology and from the registration book in which laboratory findings after investigation of patient's blood were recorded.

Blood samples were collected aseptically from patients for routine blood culture before administering any antibiotic. The vein puncture site was disinfected with 70% alcohol and 2% tincture iodine before collecting approximately 5 ml of blood for culture. For blood culture, 5 ml of blood was inoculated into 50 ml of Brain Heart Infusion broth (Bectec 9050 automated blood culture system). Subculture was done on Blood, MacConkey's, and Chocolate agar media, and finally, biochemical test was done to classify bacteria in triple sugar iron, indole, citrate, and urea agar. *Salmonella* antisera and motility were for Gram negative bacteria following standard procedures. Susceptibility testing was performed on Mueller-Hinton agar (Oxoid, Hampshire, UK) using agar disc diffusion technique of Kirby-Bauer method as recommended by the National Committee for Clinical Laboratory Standards.[13] The broths were kept till 7 days and then discarded after blind subculture.

The antimicrobials for disc diffusion testing were obtained from Oxoid in the following concentrations:

Antimicrobials	Concentration level (µg)	Antimicrobials	Concentration level (µg)
Trimethoprim–sulfamethoxazole	25	Amoxicillin	10
Cefuroxime	30	Azithromycin	30
Nalidixic acid	30	Ceftriaxone	30
Levofloxacin	05	Ciprofloxacin	05

Data analysis

Statistical analysis was done using *z*-test.

RESULTS

Of 2751 blood specimens sent for culture to the microbiology laboratory during the period of July 2013 to June 2014, *Salmonella* was isolated in 314 (11.41%) blood specimens (Figure 1, Table 1).

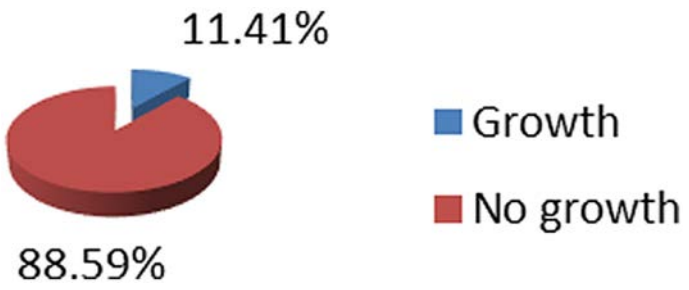

Figure 1 Distribution of growth of *Salmonella* among all patients.

In this study, among the isolated *Salmonella* sp, 213 (67.83%) were from outpatient departments, while the remaining 101 (32.17%) were isolated from hospitalized patients. However, there was a significant association between outpatient and inpatient blood culture. The *Z*-score is –8.9386. The *p* value is 0. The result is significant at $p < 0.05$ (Figure 2).

Table 1 Distribution of departments with growth, positive cases

Name of departments	Frequency (*N*)	%
Outpatient departments (*N* = 1632 patients)		
Medicine	202	64.33
Gynaecology	06	01.91
Surgery	05	01.59
Total	213	67.83
Inpatient departments (*N* = 1119 patients)		
Medicine	99	31.53
Gynaecology	02	0.64
Surgery	00	00
Total	101	32.17

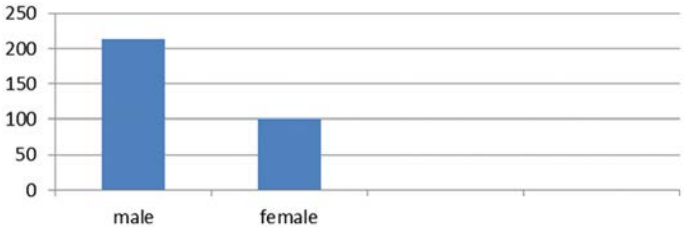

Figure 2 Gender variation regarding growth positive case.

The gender distribution of positive samples was found to be 213 (67.83%) males and 101(32.17%) females. The *Z*-score is –8.9386. The *p* value is 0. The result is significant at $p < 0.05$ (Table 2).

Table 2 Distribution of growth, positive cases by age group

Age (years)	No. isolates (*N* = 314)	%
≤10	57	18.15
11–20	109	34.71
21–30	93	29.62
31–40	27	08.60
41–50	10	03.18
51–60	08	02.55
≥61	10	03.18

Among culture-positive cases, the highest 109 (34.71%) is in age group 11–20 years and lowest 10 (03.18%) is in ≥61 years. The *Z*-score is –12.8344. The *p* value is 0. The result is significant at $p < 0.05$ (Table 3).

Among *Salmonella*, most common are *Salmonella* Typhi (229 [72.93%]) followed by *S.* Paratyphi A (85 [27.07%]). The Z-score is 11.4925. The *p* value is 0. The result is significant at $p < 0.05$.

Table 3 Types of *Salmonella* isolated in blood culture.

Types of organisms isolated	No. isolates	%
Salmonella Typhi	229	72.93
Salmonella Paratyphi A	85	27.07
Total	314	100

Table 4 shows a relation between the types of isolated organisms and age. Highest numbers of *Salmonella* Typhi were isolated in the age group 11–20 years and lowest in ≥60 years.

But in case of *S.* Paratyphi A, highest rate of isolation is in the age group 21–30 years and lowest in 51–60 years. There was a significant association of *Salmonella*-related BSI between these two groups. The *Z*-score is 2.202. The *p* value is 0.0278. The result is significant at $p < 0.05$ (Table 5).

Table 4 Types of bacteria isolated according to the age group.

Age (years)	Types of isolates	
	Salmonella Typhi	*Salmonella* Paratyphi A
≤10	37	10
11–20	97	31
21–30	69	25
31–40	16	13
41–50	05	02
51–60	04	00
≥61	01	04
Total	2ç29	85

Table 5 Commonly used antimicrobial resistance status.

Antimicrobials tested	*Salmonella* Typhi (N = 229), n (%)	*Salmonella* Paratyphi A (N = 85), n (%)
Ceftriaxone	0 (0)	0 (0)
Cefuroxime	2 (0.87)	0 (0)
Levofloxacin	02 (0.87)	11 (12.94)
Ciprofloxacin	4 (1.75)	33 (38.82)
Nalidixic acid	204 (89.08)	73 (85.88)
Co-trimoxazole	80 (34.93)	9 (10.59)
Amoxicillin	95 (41.48)	6 (07.06)
Azithromycin	108 (47.16)	63 (74.12)

Among the isolated organisms, *Salmonella* Typhi *and S.* Paratyphi A are highly resistant to nalidixic acid. The *Z*-score is 16.7282. The *p* value is 0. The result is significant at $p < 0.05$. *Salmonella* Typhi *and S.* Paratyphi A show low resistance in case of ceftriaxone and cefuroxime.

DISCUSSION

Typhoid fever is endemic in Bangladesh.[14] An overall low prevalence (6.5%) of *Salmonella* bacteremia among patients in Ghana[15] is comparable with our study. This study was conducted to determine the prevalence of *Salmonella* sp and their antimicrobial susceptibility patterns, which were very important to administer appropriate antimicrobial therapy.

This study shows that males are mainly affected because males of this age group usually work outside their homes and may eat hawked food that is liable to contamination. Also females aged 11–20 years had a higher typhoid antibody than their male counterparts. Among the total isolated *Salmonella*, *S.* Typhi was about 72.93%, whereas *S.* Paratyphi A was 27.07%. This study revealed that there was a gradual emergence of *S.* Paratyphi A during the period as a cause of paratyphoid fever. Similar emergence of *S.* Paratyphi A in the subcontinent has been reported, but a study in Nepal contradicts where the isolation rate of *S.* Paratyphi *A* is 64.4% followed by *S.* Typhi with 35.6%.[11,12] This does not have any implications for the therapy, but it has implications for vaccination strategies, as the current typhoid vaccines do not protect against paratyphoid fever. With the emergence of *Salmonella* strains resistant to the first-line drugs, quinolones and third-generation cephalosporin (ceftriaxone and cefixime) are extensively being used in the treatment of typhoid fever during last decade. Although the susceptibility of the organisms to the third-generation cephalosporin was generally good in this study, the high cost of this group of drugs precludes their use as a first choice in the treatment of septicemia. Ciprofloxacin is being used as the first choice of empiric treatment for typhoid in Bangladesh in last several years. But this study revealed that about 89.08% of *S.* Typhi and 85.88% of *S.* Paratyphi A were resistant to nalidixic acid. The treatment for typhoid fever with ciprofloxacin due to nalidixic acid-resistant *Salmonella* sp is less effective as there were reports of treatment failures from Bangladesh and other countries.[7–10] It was due to higher minimum inhibitory concentration of ciprofloxacin of these isolates. Nalidixic acid-resistant *S.* Typhi had been reported to have higher minimum inhibitory concentration of ciprofloxacin compared to susceptible strains.[10] So, it has been recommended that quinolones should not be used as the first-line therapy in population like Bangladesh where nalidixic acid resistance is common among isolates of *Salmonella*. Thus, this study indicates that the isolation of *S.* Paratyphi A is increasing over the years, and the resistance to antibiotics among *S.* Typhi and *S.* Paratyphi A is increasing in our country. Nalidixic acid-resistant organisms should be screened routinely to detect organisms with reduced susceptibility to ciprofloxacin to avoid treatment failure with quinolone therapy. Amoxicillin and cotrimoxazole are less resistant to *S.* Paratyphi A, but azithromycin is highly resistant to *S.* Typhi and S. Paratyphi A in disc diffusion method. The number of *S.* Typhi simultaneously resistant to all three first-line drugs, namely ampicillin, chloramphenicol, and cotrimoxazole, declined toward 2007 and continued through 2009. It might be due to the loss of unstable resistant gene resulting from the removal of selection pressure of these antibiotics.[16] Re-emergence of the sensitivity to these drugs was also reflected from the individual upward trend of sensitivity pattern through recent years.[17] Thus, this study indicates that the isolation of *S.* Paratyphi A is increasing over the years, and the resistance to antibiotics among *S.* Typhi and *S.* Paratyphi A is increasing in our country.

CONCLUSION

As the degree of the antibiotic resistance rate for the bloodstream pathogens is alarming, it is mandatory to monitor the susceptibility of these isolates to avoid the inappropriate use of antibiotics in hospitals. It is obviously clear that this purpose can really be achieved only by maintaining a close correlation between the physicians and the laboratory.

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