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Suvarney Datta
Department of Microbiology,
Primeasia University 9 Banani,
Dhaka-1213, Bangladesh

Prevalence and Antibiotic Susceptibility Patterns of Bloodstream *Salmonella* Infections in a Tertiary Care Hospital, Dhaka

¹Hafsa Afroz, ^{1,2}Md. Manjur Hossain, ³Md. Fakruddin, ^{1,4}Md. Afzal Hossain, ¹Zahed Uddin Mahmood Khan and ¹Suvarney Datta

Blood stream *Salmonella* infections range from self-limiting infections to life-threatening sepsis causing significant mortality and morbidity worldwide and require rapid and aggressive anti-microbial treatment. The antibiotic resistance pattern of *Salmonella* is ever changing over time. Rational and correct use of antibiotics requires understanding of the pathogen and drug resistance patterns in a community. This study was conducted to determine the status of bloodstream *Salmonella* infection and their antibiotic susceptibility patterns in a tertiary care hospital at Dhaka, Bangladesh. Six hundred and fifty six blood samples collected from clinically diagnosed enteric fever patients from Dhaka Medical college Hospital, Dhaka during January 2012 to December 2012 were processed. *Salmonella enterica* serovar *typhi* and *paratyphi* isolates were identified by standard microbiological and biochemical procedures. Ninety four isolates of *Salmonella typhi* and 59 isolates of *S. paratyphi* were isolated. Average prevalence rate of *Salmonella* in blood was 24.8%. Young, neonates and elderly persons are more prone to *Salmonella* infection and males are more susceptible to *Salmonella* septicemia than females. Most of the isolates *Salmonella* spp. were Multi-drug Resistance (MDR) and showed high resistance against cefixime, ceftriaxone, cefipime, ciprofloxacin, chloramphenicol and meropenem. Nalidixic acid was found to be effective against them. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and usage according to the standard antimicrobial susceptibility testing may help to decrease or prevent the emergence of resistance and incidence of blood stream infections.

Key words: *Salmonella*, blood stream infections, antibiotics, susceptibility

¹Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh

²Bhaluka Upazila Health Complex, Bhaluka, Mymensingh, Bangladesh

³Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

⁴Marks Medical College, Mirpur-14, Dhaka, Bangladesh

INTRODUCTION

Salmonella typhi has long been recognized to cause typhoid fever in various parts of the world. Although the incidence of typhoid fever is falling in developed country, the disease is still endemic in many parts of the world including Bangladesh (Hoque *et al.*, 1992; Bhattacharya and Das, 2000; Coovadia *et al.*, 1992; Gautam *et al.*, 2002). Typhoid fever is usually associated with bacteremia and inflammatory destruction of intestine and other organs and is fatal to adults, children and immunocompromised persons (Afroj *et al.*, 2011; Islam *et al.*, 2007).

Emergence of multi-antibiotic resistant *Salmonella* spp., especially fluoroquinolones and third-generation cephalosporin resistant *Salmonella* spp., has been reported worldwide and is considered as a serious problem due to limitations of the effective treatment of human infections (Albert *et al.*, 1991; Saha and Saha, 1994). Therefore, now it is a prime concern to consider new, effective and alternative choice of drug in forthcoming days to combat against typhoid caused by *S. enterica* serovar Typhi and Paratyphi resistant to traditional antibiotics like ciprofloxacin (Jesudason *et al.*, 1996; Rahman *et al.*, 2005).

Appearance of Multi drug resistance *Salmonella typhi* resulted in a pressing need to test newer antimicrobials, develop their dose regimen for the treatment of typhoid fever (Khan and Hoque, 1992). And to find the mechanism of acquiring resistance in these multidrug resistance *Salmonella typhi* isolates (Aker *et al.*, 2012). Knowledge of the prevalent species of *Salmonella* in a country and their antibiotic susceptibility pattern is therefore important to determine any preventive strategy (Shahriar and Kabir, 2010).

The present study aimed to assess prevalence of bloodstream *Salmonella* infections in patients with typhoid fever and to determine the antibiotic susceptibility patterns of the isolates.

MATERIALS AND METHODS

Duration and place of study: This study was conducted on 656 samples of blood collected from same number of patients over a period of one year (January to December, 2012). Blood samples were collected from patients suspected of suffering from septicemia and typhoid fever admitted to Dhaka Medical College Hospital. The age of patients included in this study ranged from 02 to 61 years. The population under study included both male and female patients. With the permission of the hospital

authority and institutional ethical review committee, informed consent was obtained from each of the participating subjects.

Collection of blood samples: The skin of venipuncture site of the patient was first cleaned with 95% alcohol. Ten ml of blood was drawn into a 10 mL disposable pyrogen free syringe. Five milliliter of blood was inoculated into blood culture bottle containing 45 mL of brain-heart infusion broth. These bottles were immediately transported to Laboratory (Saha *et al.*, 1999).

Isolation of *Salmonella typhi* and *Salmonella paratyphi*: Blood cultures bottles were incubated aerobically at 37°C for one week. These bottles were examined daily throughout the week of incubation. When growth appeared on any of the medium, a gram stain film was made. If gram negative bacilli were detected, the culture from blood culture bottle was inoculated onto a blood agar, XLD agar and MacConkey's agar plate (Saha and Saha, 1994).

Biochemical identification of *Salmonella typhi*: The API 20E was used for biomedical identification of *Salmonella typhi* and *Salmonella paratyphi*. The system consists of a plastic strip with 20 microtubes containing dehydrated substrate of ortho-nitrophenyl-galactopyranoside. The incubation box was prepared by putting 5 mL of sterile water into the honey combed wells of the tray. This was done to create a humid chamber. The strip was then place in the tray. A single isolated colony of suspected *Salmonella* was picked up from MacConkey's plate. It was emulsified in sterile saline to achieve homogenous bacterial suspension. With a sterile pipette, the tubes and cupules of citrate, VP and gelatin were filled with bacterial suspension. The remaining tubes and cupules were filled with bacterial suspension. Anaerobic environment was created in the tests ADH, LDC, ODC, URE and H₂S by overlaying mineral oil. The box was closed and incubated at 37°C for 24 h. A MacConkeys agar plate was inoculated with the same homogenous bacterial suspension to check the purity of the bacterial suspension and incubated at 37°C for 24 h.

Serological identification of *Salmonella typhi* and *Salmonella paratyphi*: Anti-*Salmonella* agglutinating sera contain *Salmonella typhi*, *Salmonella paratyphi* A, B, C and Vi from BioMerieux Laboratory was used. Kauffmann and White Scheme was followed for serological confirmation of *Salmonella typhi* and *Salmonella paratyphi*. One drop of agglutinating sera

was placed on a clean glass slide. One colony of the test strain of was picked up with a loop from MacConkey's agar plate. This bacterial culture and agglutinating serum was mixed slowly with a sterile stick. When fully mixed the slide was rotated for 5-10 sec. The agglutination was watched by naked eye. Positive and negative controls were tested in the similar way on the same slide (Morshed *et al.*, 1986).

Antibiotic susceptibility testing: Twenty two strains were tested for antibiotic resistance by the standard agar disc diffusion technique (Bauer *et al.*, 1966) on Mueller Hinton agar using commercial discs (Oxoid, UK). The following antibiotics with the disc strength in parentheses were used: Ciprofloxacin (Cip, 5 µg), Cephalexin (Cep, 30 µg), Ceftriaxone (Cef, 30 µg), Cotrimoxazol (Cot, 25 µg), Ampicillin (Amp, 10 µg), Erythromycin (Ery, 15 µg) and Nalidixic acid (Nal, 30 µg). A control strain of *E. coli* ATCC 25922 was included in each plate. Antimicrobial breakpoints and interpretation were taken from the CLSI standards (Clinical and Laboratory Standards Institute, 2006), formerly NCCLS).

RESULTS

Isolation of *Salmonella* spp.: A total of 153 *Salmonella* were isolated from the blood samples. Isolation was based on the colony characteristics on MacConkey agar and XLD agar. They produced colorless colonies on MacConkey agar as they does not ferment lactose and black centered red colonies on XLD agar.

Biochemical and serological identification of *Salmonella typhi* and *Salmonella paratyphi*: API 20E was used for biochemical identification and differentiation of

Salmonella typhi and *Salmonella paratyphi*. All the isolates showed negative results in ONPG, ADH, Citrate, urease, tryptophane deaminase, Indole, Voges proscauer, Gelatine, inositol, saccharose and amylose test and showed positive results in LDC, ODC, H₂S, Glucose, mannose, sorbose, rhamnose, melibiose and arabinose test. Upon serology, 94 isolates agglutinates with *Salmonella typhi* polyvalent antisera and 59 isolates agglutinates with *Salmonella paratyphi* polyvalent antisera, confirming their identity.

Rate of *Salmonella typhi* and *Salmonella paratyphi* isolated from blood cultures: The month wise percent prevalence of *Salmonella* spp. (*Salmonella typhi* and *Salmonella paratyphi*) isolated from blood cultures were as follows,- In January it was 40%, February 25%, March 27%, April 24%, May 25%, June 19%, July 28 %, August 20%, September 17%, October 15% November 23% and December 35% (Fig. 1). The typhoid and para typhoid fever was prevalent throughout the year. Average prevalence rate of *Salmonella* in blood was 24.8%. Peak prevalence was in the month of January 2012, it was about 40%. The results show that a rise in typhoid fever was observed during the months of June to August, This was expected because of the rains in the country, un-hygienic conditions prevailed and this resulted in the increase in the percentage of typhoid fever.

Month wise prevalence of *Salmonella typhi* and *Salmonella paratyphi* is shown in Fig. 2. Highest number of *Salmonella* was isolated in July, 2012 of which 20 were *Salmonella typhi* and 9 were *Salmonella paratyphi*.

Age distribution of bloodstream *Salmonella* positive patients showed that young child and neonates and elderly persons are more susceptible to *Salmonella* infection in blood. It is likely that their immune system is

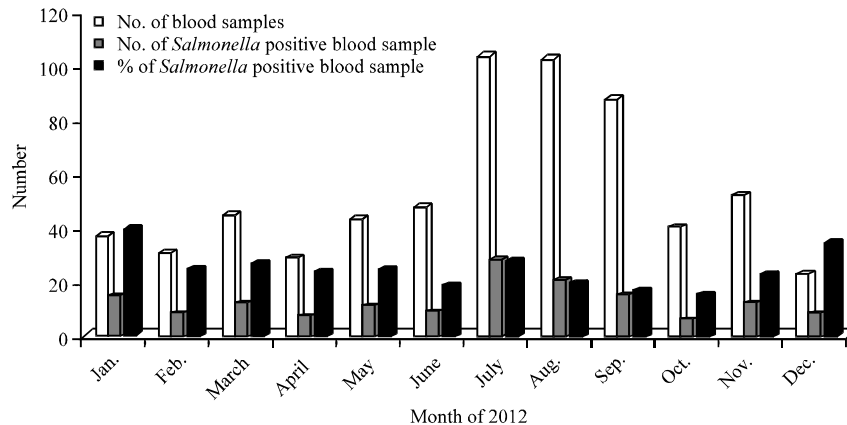


Fig. 1: Month wise distribution pattern of *Salmonella* spp. in blood

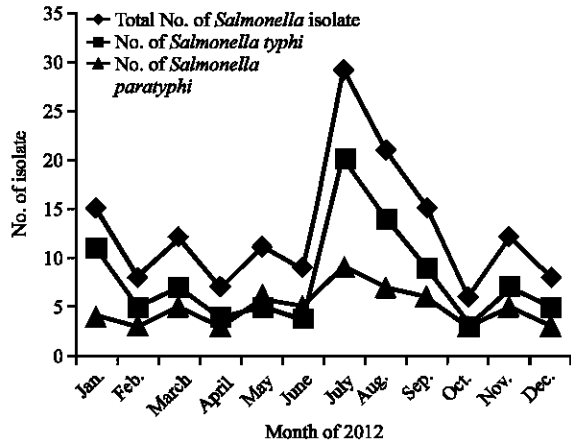


Fig. 2: Month wise prevalence of *Salmonella typhi* and *Salmonella paratyphi*

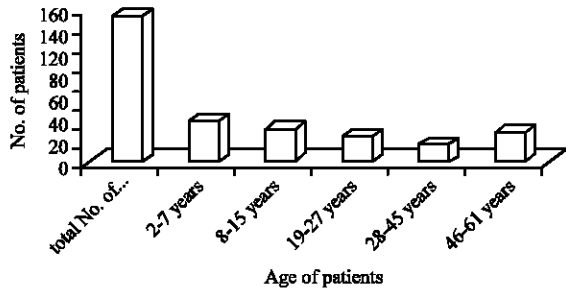


Fig. 3: Age distribution of bloodstream *Salmonella* positive patients

weaker than healthy adults and thus they are prone to septicemia. Patients of 16-45 years are less susceptible to *Salmonella* infections (Fig. 3).

Sex distribution of *Salmonella* infected patients indicate that males are more susceptible to infection than female. Of 153 *Salmonella* positive patients, 91 were male and 62 were female. Of 94 *Salmonella typhi* infected patient, 59 were male and 35 were female and of 59 *Salmonella paratyphi* infected patients, 32 were male and 27 were female (Fig. 4).

Sensitivity/resistance of *Salmonella* isolates to antimicrobial compounds: All the 94 isolates of *Salmonella typhi* and 59 isolate of *Salmonella paratyphi* were tested against nine antimicrobial agents, namely Amoxicillin, Chloramphenicol, Trimethoprim-sulfamethoxazole, Cefotaxime, Cefipime, Ceftriaxone, Nalidixic acid, Ciprofloxacin and Meropenem. All the mentioned drugs are the drug of choice for the treatment of typhoid fever.

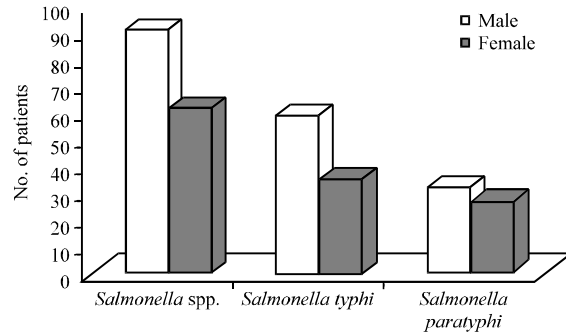


Fig. 4: Sex distribution of *Salmonella* positive patients

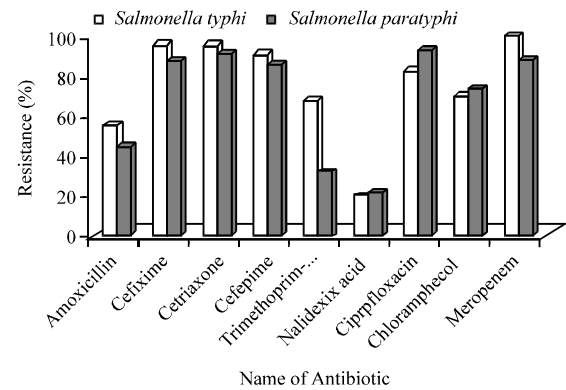


Fig. 5: Antibiotic resistance pattern of isolated *Salmonella typhi* and *Salmonella paratyphi*

Among the 94 *Salmonella typhi* isolate, more than 90% isolates were resistant to cefixime, ceftriaxone, meropenem and cefipime- all of which are third generation cephalosporin antibiotic. Resistance to ciprofloxacin was 82% and to chloramphenicol was 70% and to Trimethoprim-sulfamethoxazole was 68%. Only Nalidixic acid was found to be effective as only 20% of the *Salmonella typhi* isolates were resistant to nalidixic acid. Among 59 *Salmonella paratyphi* isolate, more than 85% isolates were resistant to cefixime, ceftriaxone, cefipime, ciprofloxacin and Meropenem. Moderate resistance was found against amoxicillin (44%) and Trimethoprim-Sulfamethoxazole (31%). Nalidixic acid was found most effective against *Salmonella paratyphi*. Antibiotic resistance pattern of *Salmonella typhi* and *Salmonella paratyphi* is shown in Fig. 5.

DISCUSSION

Typhoid fever caused by *Salmonella typhi* and *Salmonella paratyphi* is one of the most common infections in Bangladesh (Rahman *et al.*, 2002). Strains of

Salmonella typhi and *Salmonella paratyphi* resistant to commonly used antibiotics such as chloramphenicol, Amoxicillin, Trimethoprim-sulfamethoxazole, Ciprofloxacin are emerging in many parts of the world including Bangladesh (Alam *et al.*, 2010). Therefore, isolation of *Salmonella* from typhoid patient samples such as blood and assessment of antibiotic susceptibility pattern of those isolates is necessary to select drug of choice to treat such infections (Datta *et al.*, 1981).

This study was carried out to have an idea of the antibiotic susceptibility pattern of *Salmonella* spp. (*Salmonella typhi* and *Salmonella paratyphi*) isolated from typhoid patient's blood. Blood samples were collected from patients admitted with typhoid fever during January to December, 2012. A total of 656 blood samples have been collected. Out of 656 samples of blood cultures 153 turned out to be positive for *Salmonella* spp. which is about 23% of the total samples. Though most of the patients have symptoms for typhoid, the reason for the low *Salmonella* positive blood samples may be due to antibiotic medication during blood collection.

Salmonella spp. isolated from patients were identified by biochemical tests using API20E reaction system and all the isolates produced typical reactions of *Salmonella*. The isolates were further confirmed and identified to serotype level by serotyping with polyvalent antisera against *S. typhi* and *S. paratyphi*. *Salmonella typhi* (94) has been found to be more prevalent than *S. paratyphi* (59). *Salmonella* infection in blood was registered throughout the year but highest prevalence was found in December-January.

Neonates and young (age 2-15) and elderly persons (age 46-60) were more susceptible to *Salmonella* infection may be due to their weaker immune system. Healthy adults are less susceptible to bloodstream infection of *Salmonella*. Males were more infected than females and the same pattern was observed in case of *Salmonella* spp., *Salmonella typhi* and *Salmonella paratyphi* infection.

A high percentage of the isolates showed resistance to first line (amoxicillin, chloramphenicol, Trimethoprim-Sulfamethoxazole) and second line drugs (cefotaxime, cefipime, ceftriaxone, ciprofloxacin). Only Nalidixic acid showed to be effective against these isolates.

Several previous studies reported bloodstream *Salmonella* spp. in patients from Bangladesh. In a study by Alam *et al.* (2010), 66% patients were *Salmonella* spp. positive and one third of the isolated *Salmonella typhi* were multi drug resistance. The isolates were most resistant to amoxicillin, cotrimoxazole and chloramphenicol.

In another study by Shadia *et al.* (2011), among 385 isolated *Salmonella* sp., 304 (79%) were *Salmonella enterica* serovar Typhi and 81 (21%) were *Salmonella enterica* serovar Paratyphi. About 40% of the *Salmonella typhi* isolates were resistant to ampicillin, chloramphenicol and co-trimoxazole compared to only 1.8% *S. paratyphi*. All *S. typhi* and Paratyphi A were sensitive to ceftriaxone.

Chowdhury and Anwar (2010) isolated 12 MDR *Salmonella* isolates from hospital waste those were resistant to ciprofloxacin, ampicillin, amoxicillin and penicillin. Presence of such MDR *Salmonella* in hospital waste indicates bloodstream infection of patients with *Salmonella*. In another study, 943 *Salmonella typhi* were isolated from patients with typhoid fever and more than 50% isolates were resistant to ampicillin, cotrimoxazole and chloramphenicol and more than 90% isolates were resistant to nalidixic acid though resistance to ciprofloxacin was very low (Mahmud *et al.*, 2010). Similar results were also found in the study of Sarker *et al.* (2010) where resistance was higher to ampicillin, cotrimoxazole and chloramphenicol and all isolates were sensitive to ceftriaxon and ceftazidim.

Our study reports lower prevalence rate than the above mentioned studies, may be due to lower sample size. But isolates of the present study pose higher resistance compared to the reported cases from Bangladesh.

This study contradicts with the study of Mahmud *et al.* (2010) in terms of sensitivity of *Salmonella typhi* and *S. paratyphi* to nalidixic acid as our isolates were sensitive to nalidixic acid compared to high resistance reported by Mahmud *et al.* (2010).

This study is in conjunction of the previous studied warns us about increasing prevalence of bloodstream *Salmonella* infection in a wide group of patients and their increased resistance to the first line and second line drugs of choice.

Overuse and availability of antimicrobial is a common practice in this part of the world. The selective pressure of un-restricted antimicrobial usage has probably contributed to the genesis of resistant *Salmonella typhi* (Mills-Robertson *et al.*, 2002). In Dhaka antimicrobials are available from chemist shops without legal prescriptions. This encourages the patients to buy antimicrobials from the counter and use them without consultation with the doctor. By doing so, these patients often wrong antimicrobial drug which help in selection of resistance in bacteria rather than curing the patient (Saha and Saha, 1994). This self-medication is often taken for wrong

duration which further helps in selection of resistant bacteria against these antimicrobial. Very often antimicrobials are prescribed without determining the causative organism or its susceptibility to antimicrobial. When antimicrobials are prescribed for infections due to viruses or resistant bacteria, this also results in the increased resistance among bacteria including *Salmonella typhi* and *Salmonella paratyphi*.

CONCLUSION

This study provided much needed information and alarms us to the increasing prevalence of multi-antibiotic resistant *Salmonella typhi* and *Salmonella paratyphi* causing blood stream infections in Bangladesh. The rise in antibiotic resistance in blood isolates emphasizes the importance of sound hospital infection control, rational prescribing policies and the need for new antimicrobial drugs and vaccines. Our results seem helpful in providing useful guidelines for choosing an effective antibiotic in cases of septicaemia. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic recycling may help to decrease or prevent the emergence of resistance.

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