J. Dhaka National Med. Coll. Hos. 2014; 20 (01): 3-8

Original article

Prevalence and Antimicrobial Susceptibility with Emergence of Multidrug Resistance of Salmonella SEROVARS Isolated from Blood in Bangladesh.

Nurun Nahar Mawla[1], Shahin Sultana[1], Professor Dr. Munir Hassan[2]

Department of Microbiology, [1]Delta Medical College, [2]Dhaka National Medical College, Dhaka, Bangladesh.

Abstract:

Enteric fever continues to remain a health problem as the causative organisms *Salmonella* has developed resistance to many of the antibiotics used. The aim of the present study was to evaluate frequency and antimicrobial susceptibility patterns with special reference to multidrug resistance (MDR) of *Salmonella typhi* and *Salmonella paratyphi A*, isolated from blood culture done in private hospitals, Dhaka, Bangladesh during November 2012 to January 2014. In total, 31 strains of *S. typhi* and 10 strains of *S. paratyphi A*, isolated and identified by standard microbiological procedures from 917 blood culture samples collected from suspected enteric fever cases, were tested for antimicrobial susceptibility by disc-diffusion method using 08 different antibiotics. All *S. typhi* and *paratyphi A* isolates were 90% sensitive to Ceftriaxone followed by Cefixime 84%. There were 35% *S. typhi* and 60% *S. paratyphi A* multidrug-resistant strains. Decreased susceptibility of *S. typhi* and *S. paratyphi A* was observed to Ciprofloxacin 48% and 80% respectively. All *Salmonella* isolates were resistant to Nalidixic acid (100%). This study concludes that precise information on antibiotic susceptibility pattern should be available to the clinician for optimal patients care. Ceftriaxone and Cefixime can be used as alternative anti-typhoidal treatment. Indiscriminate use of Ciprofloxacin should be strongly discouraged. Presence of MDR and quinolone resistance is concern and requires further study.

Key Words: *Salmonella* serovars, antibiotics susceptibility pattern, multidrug resistance, enteric fever

Introduction:

Enteric fever includes Typhoid and Paratyphoid fever, an acute systemic illness are endemic in tropic and subtropic, also in Bangladesh. According to recently revised global estimate, it causes 21.6 million illnesses every year, resulting in 216,500 deaths.[1] *Salmonella enteric* serovar *Typhi* and *Paratyphi A* are the predominant causative agents responsible for enteric fever in Bangladesh.[2]

Enteric fever has continued to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs. Antibiotic resistance among *S. Typhi* has been reported in India since 1960.[3] In late 1987, outbreaks of typhoid fever by a multidrug-resistant (MDR) strain of *S. Typhi*, resistant to three first-line antimicrobial agents namely ampicillin, chloramphenicol and cotrimoxazole[4] occurred in China, subsequently in India, Pakistan, Bangladesh, Vietnam and Africa

resulting in severe illness and high incidence of complications and mortality.[5-9] Though a study from Bangladesh reported a decrease in MDR isolates with no corresponding increase in sensitive strains,[10] ciprofloxacin and other fluoroquinolones become the antimicrobial drugs of choice for treatment of typhoid fever since 1990 in Bangladesh and other countries.[11] But the rapidly gained multidrug-resistance with simultaneous fluoroquinolone resistance of *Salmonella typhi* and *paratyphi A* have emerged as new challenges to the treatment of enteric fever.[12,13] Also isolates of *S. Typhi* with decreased susceptibility to ciprofloxacin have been found to be nalidixic acid-resistant, which require higher concentrations of ciprofloxacin for inhibition.[4,11,14] Various observations in sensitivity patterns reported for *S. Typhi* and *S. Paratyphi A* stress the significance of continuous monitoring of antimicrobial sensitivity patterns. This study was undertaken to know the frequency of *Salmonella serotypes* in blood samples in Bangladesh, their antimicrobial susceptibility patterns and emergence of multidrug resistance (MDR) *Salmonella* that will guide the physicians in choosing appropriate antibiotics for ensuring quick treatment of the infection and preventing antibiotic resistance.

Materials & Methods:

This retrospective study was conducted in two private hospitals, Dhaka, Bangladesh on patients attending for the treatment of fever for variable duration during November 2012 to January, 2014. Blood samples were drawn aseptically from patients, inoculated into blood culture bottles which were placed in BACTEC automated machine and sub-cultured onto Blood agar and Mac-Conkey agar.

The suspected non-lactose fermenting colonies were identified by standard microbiological procedures including colony morphology, Gram stain reaction and biochemical tests. The Salmonella isolates were confirmed by group and type specific Salmonella antisera (Denka Seiken, Japan).

The antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines[15] using Ampicillin, Chloramphenicol, Cotrimoxazole, Ciprofloxacin, Ceftriaxone, Cefixime, Azithromycin, and Nalidixic acid (Hi Media, India) (Table-1).

Table- 1: Antimicrobial agents used in the study

	Name of Antimicrobial agents used		
1	Ampicillin (Amp)	5	Ceftriaxone (CRO)
2	Chloramphenicol (C)	6	Cefixime (CFM)
3	Cotrimoxazole (Sxt)	7	Azithromycin (AZM)
4	Ciprofloxacin (Cip)	8	Nalidixic Acid (NA)

Data were analyzed by SPSS (Statistical Package for Social Science) program 11.5 version. A *P Value* ≤0.05 was considered significant for all comparisons.

Results:

Of the total 917 blood samples, 84 (9%, P≤0.003) were culture positive among which 49 (59%, P≤0.005) were different serotypes of *Salmonella* (Table-2)

Table-2: Prevalence of *Salmonella* and other bacterial isolates in blood samples

	(f)	% (n=917)	*Salmonella* % from organism isolates (n=84)
Total number of blood samples	917		
Number of organism isolates	84	9% (P≤0.003)	
Number of *Salmonella* Isolates	49	5% (P≤0.002)	59% (P≤0.005)

(f)=Frequency of distribution

Characterization and identification of the isolates revealed that *Salmonella* serovars had the highest frequency of occurrence with 49 (59%) isolates followed by *Escherichia coli* (25%), *Staphylococcus aureus* (12%), *Klebsiella* (2%), *Pseudomonas* (1%), and *Enterococcus* (1%) respectively (Table-3).

Table-3: Frequency of occurrence of bacterial isolates

Organisms isolates	Frequency (f)	%, (n=84)
Salmonella serovars	49	59
Escherichia coli	21	25
Staphylococcus aureus	10	12
Klebsiella	2	2
Pseudomonas	1	1
Enterococcus	1	1
Total	**84**	**100**

Isolates were from all age group, the mean age being 34 years. Out of 84 culture positive cases, the highest frequency of occurrence was found between the ages of 21-30 years while 31-40 years of age had the least frequency (Table-4)

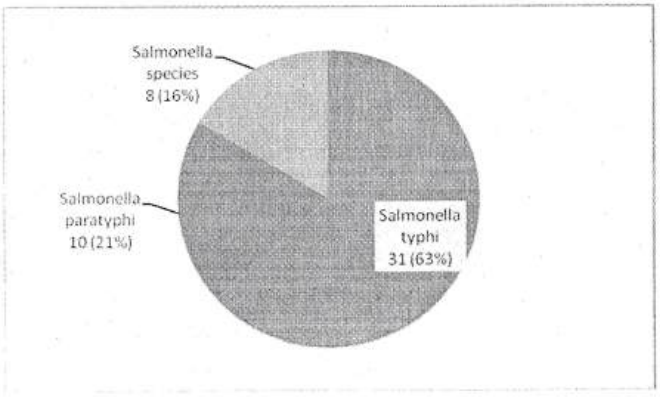
Table- 4: Distribution of age for culture positive cases

Age in yrs.	Total (f)	%, (n=84)
1-10	9	11
11-20	16	19
21-30	**28**	**33**
31-40	2	03
41-50	5	05
51-60	15	18
61-70	5	05
71-80	5	05
Total	**84**	**100**

(f)=Frequency of distribution

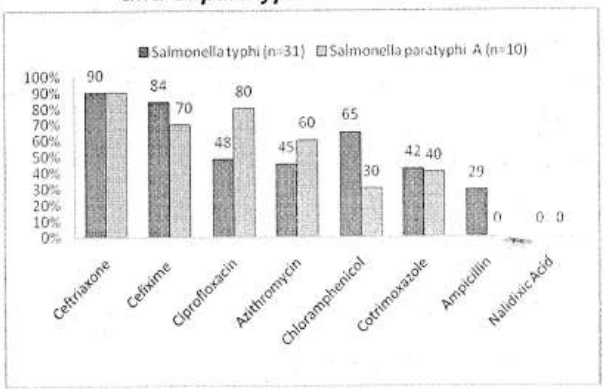
Among total isolated 49 *Salmonella* serotypes, *Salmonella typhi* was predominant (63%) followed by *Salmonella paratyphi A* (21%) and *Salmonella species* (16%) (Figure-1).

Figure-1: Pie chart showing the distribution of *Salmonella* serotypes



Salmonella typhi was found most susceptible towards Cefriaxone (90%), Cefixime (84%), and Chloramphenical (65%). They showed resistance to Nalidixic acid (100%) followed by Ampicillin (71%), Cotrimoxazole (58%), Azithromycin (55%), and Ciprofloxacin (52%). *Salmonella paratyphi A* showed high sensitivity to most tested antibiotics and least susceptable to Chlorampenical (70%) and Cotrimoxazole (60%). All *Salmonella paratyphi A* were found 100% resistant to both Ampicillin and Nalidixic acid (Figure-2, Table-5).

Figure-2: Antimirobials susceptibility pattern of *S. typhi*, and *S. paratyphi A*



We found that 35% *Salmonella typhi* and 60% *Salmonella paratyphi A* were multidrug resistant (MDR). This study also showed increase resistance of *Salmonella typhi* to Ciprofloxacin (52%), though *Salmonella paratyphi A* were still found 80% sensitive (Table-5, Figure-2).

Table-5: Percentage of antibiotics resistant pattern in *Salmonella Typhi* and *paratyphi A*

Antibiotics Screened	Antibiotics Resistant percentage: *Salmonella typhi* %, (n=31)*	*Salmonella paratyphi A* %, (n=10)*
Ampicillin	71	100
Cotrimoxazole	58	60
Chloramphenicol	35	70
Ciprofloxacin	52	20
Azithromycin	55	40
Cefixime	16	30
Ceftriaxone	10	10
Nalidixic Acid	100	100

*Values in parentheses are the number of isolates.

Discussion:

Irrationale use of antimicrobial agents in humans for treatment of enteric fever has lead to widespread resistance among *Salmonella* population in developing countries including Bangladesh.[1] Isolation of Salmonella species occurs throughout the year. This means that drinking water conditions and sanitation have not improved or a large number of carriers are present in the society.

Enteric fever is mainly caused by *Salmonella typhi* (*S. typhi*); *S. paratyphi A* has been reported less frequently. But the incidence of *S. paratyphi A* is on the rise.[16] In the present study, out of 49 *Salmonella* isolates, 31 (63%) were *S. typhi* and 10 (21%) were *S. paratyphi A*. This result has harmony with the results of Guha et al[17] and Tankhiwala et al.[18] This study also found that increase frequency of enteric fever occur between 21 and 30 years of age.

Prompt institution of appropriate antimicrobial therapy can reduce morbidity and mortality associated with the illness. Since 1948, chloramphenicol had been the mainstay of treatment of enteric fever until 1972 when chloramphenicol-resistant typhoid fever became a major problem.[19] Although initially susceptible to ampicillin and cotrimoxazole, *S. typhi* strains resistant to all the first-line anti-typhoidal drugs emerged in 1970s.[20] Contributory factors may be drug overuse, misuse and inappropriate prescribing practices by physicians.

MDR (i.e resistance to ampicillin, chloramphenicol and co-trimoxazole) typhoid fever epidemic started in Bangladesh in 1990, peaked in 1994, subsequently declined and re-emerged in 2001 and 2002.[10] The factor causing MDR is conjugative *R plasmid* that transfer *β-lactamase* gene, Chloramphenicol transferase gene and gene altering target of Cotrimoxazole from one *Salmonella* to another, leading to developing resistance against Ampicillin, Chloramphenicol and Cotrimoxazole respectively.[10] In our study, we found that 35% *S. typhi* and 60% *S. paratyphi A* isolates were MDR which is in accordance with recent reports from some regions.[18,21,22] Fortunately same MDR Salmonella strain failed to exhibit it's multidrug resistance property to different human population due to loss of *R-Plasmid*.[10]

With the emergence of MDR *S. typhi* and *S. paratyphi A*, Fluoroquinolones have become the treatment of choice for enteric fever.[20] Indeed these are the only effective oral drugs in this clinical situation.[23,24] In our study there was 48% and 80% sensitivity of *S. typhi* and *S. paratyphi A* to ciprofloxacine respectively. Unfortunately since 1993, *S. typhi* with decreased susceptibility to ciprofloxacin has been isolated.[9,25] Wide use of Ciprofloxacin in Bangladesh to treat many infections without prescription is likely to result in high prevalence of resistance, limiting it's utility.[26,27]

In 2000, *S. typhi* causing typhoid fever exhibited Nalidixic acid resistance with decreased Ciprofloxacin susceptibility was detected in Bangladesh.[28] All *Salmonella* isolates were 100% resistant to Nalidixic acid in our study also. Thus Nalidixic acid resistance is an indicator for predicting low-level resistance to Ciprofloxacin among *Salmonella*, S. typhi in particular.[22,29] Single-point mutation in quinolone resistance-determining region of the *gyrA* gene in *Salmonella* usually leads to simultaneous resistance against Nalidixic acid and decreased sensitivity to Ciprofloxacin.[30,31] Therefore, any isolate that shows resistance to Nalidixic acid should be reported as intermediately susceptible to Ciprofloxacin.[32]

In 2002, MDR *S. typhi* and *S. paratyphi A* isolates also exhibited decreases Ciprofloxacin susceptibility in Bangladesh[30] that make therapy of typhoid fever in Bangladesh even more complicated and leads to treatment failure typhoid cases with Ciprofloxacin. In such cases, Ceftriaxone, Cefixime or Azithromycin could

be considered as possible alternatives.[33] In our study, both *S. typhi* and *paratyphi A* were found 90% susceptible to ceftriaxone which was supported by Bhatia et al[34] and similar reports from Pakistan and Bangladesh.[35,36] High cost, need for parenteral administration and prolong course of therapy are the major limiting factors.

The sensitivity pattern of causative organisms must be studied before instituting appropriate therapy and physicians should advised against using these drugs empirically to prevent further emergence of drug resistance.

Reference:

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004; 82:346-53.
2. Jesudason MV, John TJ. Plasmid mediated multidrug resistance in *Salmonella typhi.* Ind J Med Res 1992; 95: 66-7.
3. Agarwal SC. Chloramphenicol resistance of Salmonella species in India, 1956-61.Bull Wld Hlth Orgn 1962; 17: 331-5.
4. Le TP, Hoffman SL. Typhoid fever. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases: principles, pathogens and practice. Philadelphia, PA: Livingstone, 1999:277-95.
5. Ahasan HN, Rafiqueuddin AKM, Chowdhury MAJ, Azhar MA, Ara M, Farazi MA. Complications of enteric fever encountered in medical wards. J Dhaka Med Coll 1993; 2: 32–33.
6. Wang F, Gu X, Zhang M, Tai T. Treatment of typhoid fever with ofloxacin. J Antimicrob Chemother 1989; 23: 785–788.
7. Jesudasan M, John TJ. Multiresistant Salmonella typhi in India. Lancet. 1990; 336: 252.
8. Rowe B, Ward LR, Threlfall EJ. Spread of multiresistant Salmonella typhi. Lancet 1990; 336:1065.
9. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med 2002;347: 1770-82.
10. Rahman M, Ahmad A, Shoma S (2002) Decline in epidemic of multidrug resistant Salmonella typhi is not associated with increased incidence of antibiotic susceptible strain in Bangladesh. Epidemiol Infect 129: 29-34.
11. Baliga S, Shenoy S Vidyalaxmi K, Pereira P. Ciprofloxacin- resistant Salmonella Typhi. Natl Med J India 1999; 12:138.
12. Zhang R, Eggleston K, Rotimi V, Zeckhaiser RJ. Antibiotic resistance as a global threat: Evidence from Curia, Kuwait and the United States. Globalization and Health. 2006; 2:1-14.
13. Dutta S, Sur D, Manna B, Sen B, Bhattacharya M, Bhattacharya SK,Wain J, Nair S, Clemens JD, Ochiai RL. Emergence of highly fluoroquinolone resistant Salmonella enterica serovar typhi in community-based fever surveillance from Kolkata, India. Int J Antimicrob Agents. 2008;31: 387-9.
14. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in Salmonella enterica serotype Typhi, United Kingdom. Emerg Infect Dis 2001;7: 448-50.
15. Performance Standards for Antimicrobial disk Susceptibility Tests. CLSI, Wayne, PA, USA, 2005. NCCLS document M7-A.
16. Versa G, Jaspal K, Chander J. An increase in enteric fever cases due to Salmonella paratyphi A in and around Chandigarh. Indian J Med Res. 2009; 129:95-8.
17. Guha S, Jalan BY, Dey S, Easow JM, Wilson G, Shivanandan PG. Salmonella bacteraemia in pokhara: emergence of antibiotic resistance. Nepal Med Coll J. 2005; 7:23-5.
18. Tankhiwale SS, Agrawal G, Jalgaonkar SV. An unusually high occurrence of Salmonella enterica serotype Paratyphi in-patients with enteric fever. Indian J Med Res. 2003; 117:10.
19. Raza S, Tamrakar R, Bhatt CP, Joshi SK. Antimicrobial Susceptibility Patterns of Salmonella typhi and Salmonella paratyphi A in a Tertiary Care Hospital. J Nepal Health Res Counc 2012 Sep; 10(22):214-7.
20. Basudha K, Sanjib KS, Shyamal KB, Narayan RB, Monorama D, Reba K. Antimicrobial Susceptibility Patterns of Salmonella enterica Serotype Typhi in Eastern Nepal. J Health Popul NUTR 2007 Mar;25(1):82-87

21. Nath G, Tikoo A, Manocha H, Tripathi AK, Gulati AK. (2003) Drug resistance in Salmonella typhi in North India with special reference to ciprofloxacin. J Antimicrobial Chemother 46: 145-53.
22. Rodrigues C, Shennai S, Mehta A. Enteric fever in Mumbai, India: the good news and the bad news. Clin Infect Dis. 2003; 36: 535.
23. Karamat KA, Butt T, Hannan A, et al. Problem of multi drug resistant typhoid fever in Rawalpindi/Islamabad. Pak Armed Forces Med J1996; 46:48-54.
24. Mirza SH, Beeching NJ, Hart CA. Prevalence and clinical features of multi-drug resistant Salmonella typhi infections in Baluchistan, Pakistan. Ann Trop Med Parasitol 1995; 89:515-19.
25. Chinh NT, Parry CM, Ly NT, et al. A randomized controlled comparison of azithromycin and ofloxacin for treatment of multidrug-resistant and nalidixic acid resistant enteric fever. Antimicrob Agents Chemother 2000;44:1855-59
26. Mandal S, Mandal DM, Pal NK. Antimicrobial resistance pattern of *Salmonella typhi* isolates in Kolkata, India during 1991–2001: a retrospective study. Jpn J Infect Dis 2002; 55: 58–59.
27. Rahman M, Mauff G, Levy J, et al. Detection of 4-qunolone resistance mutation in *gyrA* gene of Shigella dysenteriae type 1 by PCR. Antimicrob Agents Chemother 1994; 38: 2488–2491.
28. Haque SMZ, Haq JA, Rahman MM. Nalidixic acidresistant *Salmonella enterica serovar Typhi* with decreased susceptibility to ciprofloxacin caused treatment failures: a report from Bangladesh. Jpn J Infect Dis 2003; 56: 32–33.
29. Asna SM, Haq JA, Rahman, M. Nalidixic acid resistant *Salmonella* enterica serover *typhi* with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Jpn J Infect Dis. 2003; 56: 32-33.
30. Crump JA, Barrett TJ, Nelson JT, Angulo FJ. Reevaluating fluroquinolone breakpoints for *Salmonella enterica* serotype *Typhi* and for non Typhi salmonellae. Clin Infect Dis 2003; 37:75-81.
31. Hakanen A, Kotilainen P, Jalava J, Siitonen A, Huovinen P. Detection of decreased fluoroquinolone susceptibility in salmonellas and validation of nalidixic acid screening test. J Clin Microbiol 1999; 37:3572-7.
32. Harish BN, Madhulika U, Parija SC. Current Pattern in antimicrobial susceptibility of *Salmonella typhi* isolates in Pondichery. Ind J Med Res. 2004; 120: 111-4.
33. Parry CM. The treatment of multidrug-resistant and naldixic acid resistant typhoid fever in VieNam. Trans R Soc Trop Med Hyg 2004; 94: 413–422.
34. Bhatia JK, Mathur AD, Arora MM. Reemergence of Chloramphenicol sensitivity in enteric fever. Med J Armed Forces India. 2007;63:212-4.
35. Jamil B, Lashari I, Bhatti S, Hassan RS. Enteric fever in adult patients: clinical features, outcome and antibiotics susceptibility patterns. Infect Dis J Pakistan. 2004;13:68-72.
36. Sahs S, Talukdar SY, Islam M, Saha S. A highly ceftriaxone resistant *Salmonella typhi* in Bangladesh. Pediatr Infect Dis J. 1999; 18:387-90.