Study of Bloodstream Salmonella Infection and Antibiotic Susceptibility Patterns of *Salmonella Enterica* Serover Typhi and Paratyphi in A 1000 Bedded Tertiary Care Hospital in Dhaka, Bangladesh

*Naser MJ,[1] Alam SS,[2] Rahman MF,[3] Shamsuzzaman SM[4]

Abstract

Background: Enteric fever caused by the human-adapted pathogens *Salmonella enterica* serovars Typhi (*S.* Typhi) and Paratyphi A (*S*. Paratyphi A), B, and C remains a major health problem in Bangladesh. Knowledge of the diagnostic value of different test and antibiotic susceptibility is crucial in determining which drug to use.

Objectives: To assess the antibiotic resistance against *Salmonella enterica* serovars Typhi and Paratyphi A , B, and C.

Methodology: A cross-sectional study was done to observe the culture and sensitivity of 293 blood samples among all clinically suspected enteric fever patients attended outpatient department of medicine and in the department of Microbiology for blood culture and antibiotic susceptibility patterns in Dhaka Medical College Hospital, between July, 2016 to June, 2017.

Result: Among 48 isolated organisms, 25 (52.08%) were *S.* Typhi, 4 (8.33%) were *S.* Paratyphi A and 19 (39.58%) were other organisms. Regarding antibiotic susceptibility pattern, 65.52% were sensitive to ampicillin, 68.97% to chloramphenicol, 79.31% to sulphamethoxazole/trimethoprim, 82.76% to ciprofloxacin, 75.86% to levofloxacin, 86.21% to ceftazidime, 72.41% to azithromycin, 82.76% to amoxycillin/clavulanic acid and 89.66% to piperacillin/tazobactam. All of the *Salmonella* were sensitive to cefixime, ceftriaxone, cefepime, imipenem and resistant to nalidixic acid.

Conclusion: *Salmonella enterica* serovars were relatively highly resistant to nalidixic acid, ampicillin, chloramphenicol, sulphamethoxazole/trimethoprim, ciprofloxacin, levofloxacin, ceftazidime, azithromycin, amoxycillin/clavulanic acid and highly sensitive to piperacillin/tazobactam, cefixime, ceftriaxone, cefepime, imipenem.

[Shaheed Syed Nazrul Islam Med Col J 2021, Jul; 6 (2):141-150]

Keywords: *Salmonella enterica, S.* Typhi, *S*. Paratyphi, Enteric fever, Antibiotic susceptibility.

Introduction

Enteric fever is a systemic disease characterized by fever and abdominal pain and caused by dissemination of *Salmonella enterica* serovers Typhi (*S*. Typhi) and *Salmonella enterica* serovers Paratyphi (*S*. Paratyphi A, B and C). The disease was initially called typhoid fever because of its clinical similarity to typhus.[1] During 2000 *S*. Typhi caused 2,16,50,974 illness and 2,16,510 deaths and *S*. Paratyphi caused 54,12,744 illness.[2] Typhoid and paratyphoid fever were included in the Global Burden of Disease 2010 (GBD 2010) project, when they were together estimated to account for 12.2 million disability-adjusted life years and 190,200 deaths.[3,4] More than 90% of typhoid fever cases are estimated to occur in Asia.[5]

1. **Dr. Mohammad Jamal Abdel Naser, Assistant Professor, Department of Microbiology, Shaheed Syed Nazrul Islam Medical College, Kishoregonj. drnaserdhaka@gmail.com*
2. *Dr. S. K. Saiful Alam, Assistant Professor, Department of Microbiology, Shaheed Tajuddin Ahmad Medical College, Gazipur.*
3. *Dr. Md. Faizur Rahman, Lecturer, Department of Microbiology, Dhaka Medical College, Dhaka.*
4. *Prof. S. M. Shamsuzzaman, Professor and Head of the Department of Microbiology, Dhaka Medical College, Dhaka.*

**For correspondence*

The infection is transmitted by ingestion of food or water contaminated with faeces.[6] As Bangladesh is a overcrowded densely populated developing country and its sanitary conditions remain poor, it is also a major health problem in this country. Reliable data from which to estimate the burden of disease in these areas are difficult to obtain, since many hospitals lack facilities for blood culture and up to 90 percent of patients with typhoid are treated as outpatients.[7] The challenges of reliably diagnosing typhoid fever have led to varying estimates of the disease burden and epidemiology in Asia.[5] The definitive diagnosis of typhoid fever depends on the isolation of *Salmonella* from blood, bone marrow or a specific anatomical lesion. The presence of clinical symptoms characteristic of typhoid fever or the detection of a specific antibody response is suggestive of typhoid fever but not definitive. Blood culture is the mainstay of the diagnosis of this disease.[6] To treat these infections, fluoroquinolones (FQ) and third- generation cephalosporins have been considered as first-line drugs, owing to the resistance to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole that appeared during the 1980s.[8,9] Nalidixic acid resistant *Salmonella* Typhi with decreased susceptibility to ciprofloxacin is now endemic in India and neighboring countries.[10,11] Failure to treat an infection properly leads to prolong illness, thus increasing the chance of developing a carrier state in which persons are contagious and able to spread the resistant strain to others.[10,12] We conducted blood culture surveillance in Dhaka Medical College and Hospital to estimate the incidence and determine the antimicrobial susceptibility patterns of *S.* Typhi and *S.* Paratyphi.

Methods

This Cross sectional study was carried out in the department of Microbiology, Dhaka Medical College (DMC) from 1st July, 2016 to 30th June, 2017. Blood samples were collected from 293 clinically suspected enteric fever patients attended outpatient department of medicine and in the department of Microbiology for blood culture and antibiotic susceptibility patterns in Dhaka Medical College Hospital. Patients or legal guardians of the patients who did not give consent and Patients who took antibiotics were excluded from the study. Data regarding age, sex, and occupation were collected by using predesigned data collection sheet. The protocol was approved by the Research Review Committee (RRC) of Microbiology department of DMC followed by Ethical Review Committee (ERC) of Dhaka Medical College. Informed written consent was obtained from each patient before sample collection and professional secrecy was maintained about the diagnosis.

Blood samples were collected in a sterile rounded blood culture bottle of 100 ml capacity, fitted with a rubber cap, containing Trypticase soya broth (TSB) with sodium polyanethole sulphonate (SPS). The optimum ratio of the volume of blood to traditional culture broth was 1:10. For patient group, school children and adults, 7 ml venous blood were collected (5 ml for blood culture and 2 ml for Widal test) and for patient group, toddlers and preschool childrens 4 ml (2 ml for blood culture and 2 ml for Widal test) were collected by a single venepuncture by a sterile disposable syringe.[6] The bottles containing blood were sent to laboratory promptly and incubated at 37°C in aerobic condition. After introducing of blood in blood culture bottle for culture, the remaining blood was collected in a sterile test tube for separation of serum for Widal test.

In the Laboratory, blood culture bottles were incubated at 37°C in aerobic condition. The bottles were examined by naked eye for

growth once on 1st, 2nd, 3rd and 7th day and checked for turbidity, gas formation, and other evidence of growth.[6,13,14] For day-1, day-2 and day-3, only bottles showing sign of positive growth were subcultured on blood agar media and MacConkey agar media plates. On day-7 all bottles were subcultured on blood agar media and MacConkey agar media plates before being discarded as negative.[6] In case of positive growth the smear was prepared with gram stain. If gram negative bacilli were detected, biochemical identification was done using Triple sugar iron agar media, Simmons` citrate agar media, motility-indole-urease agar media and oxidase test reagent and final identification was done by using *Salmonella* specific antisera.[6,15] Liquid stable antisera for the determination of O, H and Vi antigens for the serological identification of *S.* Typhi, *S.* Paratyphi A and *S.* Paratyphi B (Mast™ Diagnostic, UK) were used.

The Widal test was performed by slide agglutination test against O and H antigen. It was considered positive when a titre agaist O antigen is equal or more than 1:160.[16,17,18] All the strains of *Salmonella* isolates were tested for antimicrobial susceptibility by Kirby-Bauer modified disc-diffusion technique.[19] and the zone of inhibition was interpreted according to CLSI 2015.[20] Antibiotic discs were obtained from commercial source (Oxoid ltd, UK). Antimicrobial agents ampicillin (10 µg), chloramphenicol(30µg), sulphamethoxazole/ trimethoprim ((25µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ceftazidime (30 µg), cefixime (5 µg), ceftriaxone (30 µg), cefepime (30 µg), azithromycin (15 µg), amoxycillin / clavulanic Acid (30 µg), piperacillin / tazobactam (110µg), imipenem (10 µg) were used for antibiotic susceptibility test. *Escherichia coli* ATCC 25922 was used as control strain to assess the performance of the method.[15]

Result

Among 293 patients 89 (30.38%) were in the age group of 15 to 25 years, followed by 74 (25.26%) were in 25 to 35 years, 65 (22.18%) were in 35 to 40 years and the mean age of study population was 29.18 ± 5.

Table I: Distribution of study population according to age (N=293)

Age group (years)	Frequency	Percentage
5-15	35	11.95
15-25	89	30.38
25-35	74	25.26
35-45	65	22.18
45-55	20	6.82
55-65	10	3.41
Total	293	100.00

Note: Mean±SD=29.18±5

Table II: Distribution of study population according to blood culture (N=293)

Blood culture	Frequency	Percentage
Positive	48	16.38
Negative	245	83.62
Total	293	100.00

Among 48 isolated organisms, 25 (52.08%) were *S.* Typhi, 4 (8.33%) were *S.* Paratyphi A and 19(39.58%) were other organisms.

Table III: Organisms isolated from blood culture positive cases (N=48)

Organisms	Number	Percentage
S. Typhi	25	52.08
S. Paratyphi A	4	8.33
Other organisms	19	39.58
Total	48	100.00

Among 293 enteric fever patients, 86 (29.35%) had significant titre against *S.* Typhi, 25 (8.53%) had significant titre against *S*. Paratyphi A for O antigen and 13 (4.44%) had significant titre against *S.* Typhi, 3 (1.02%) had significant titre against *S*. Paratyphi A for both O and H antigen.

Table IV: Distribution of semi quantitative slide agglutination titration of Widal test of enteric fever patients (N=293)

Organisms	O antigen		O and H antigen	Total n (%)
	Significant Titre≥1:160 n (%)	Non significant or Titre<1:160 n (%)	Significant Titre≥1:160 n (%)	
S. Typhi	86 (29.35)	194 (66.21)	13 (4.44)	293(100.00)
S. Paratyphi A	25 (8.53)	265 (90.45)	3 (1.02)	293(100.00)
S. Paratyphi B	0 (0.0)	293 (100.0)	0 (0.0)	293(100.00)
S. Paratyphi C	0 (0.0)	293 (100.0)	0 (0.0)	293(100.00)

Among 29 blood culture positive patients, 18 (62.07%) had significant titre and among 264 blood culture negative patients, 109 (41.29%) had significant titre in Widal test. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Widal test were 62.07%, 58.71%, 14.17%, 93.37%, 59.04%, respectively.

Table V: Comparison of Widal titre with blood culture

Widal titre	Blood culture		Total n (%)	P value
	Positive n (%)	Negative n (%)		
Significant titre	18 (62.07)	109 (41.29)	127 (43.34)	P < 0.05
Non significant titre	11 (37.93)	155 (58.71)	166 (56.66)	
Total	29 (100.00)	264 (100.00)	293 (100.00)	

Table VI: Validity parameters of Widal test

Parameters	Value	95% Confidence Interval
Sensitivity	62.07%	56.50 to 67.64
Specificity	58.71%	53.07 to 64.35
Positive predictive value	14.17%	10.17 to 18.17
Negative predictive value	93.37%	90.52 to 96.22
Accuracy	59.04%	56.17 to 61.91

Regarding antibiotic susceptibility pattern, 65.52% were sensitive to ampicillin, 68.97% to chloramphenicol, 79.31% to sulphamethoxazole/trimethoprim, 82.76% to ciprofloxacin, 75.86% to levofloxacin, 86.21% to ceftazidime, 72.41% to azithromycin, 82.76% to amoxycillin/clavulanic acid and 89.66% were sensitive to piperacillin/tazobactam. All of the *Salmonella* were sensitive to cefixime, ceftriaxone, cefepime, imipenem, and 100% were resistant to nalidixic acid.

Table VII: Antibiotic susceptibility pattern of *Salmonella* isolated from enteric fever patients (N =29)

Antimicrobial agents	Total N=29	
	S n (%)	R n (%)
Ampicillin	19 (65.52)	10 (34.48)
Chloramphenicol	20 (68.97)	9 (31.03)
Sulphamethoxazole/Trimethoprim	23 (79.31)	6 (20.69)
Nalidixic Acid	0 (0.00)	29 (100.0)
Ciprofloxacin	24 (82.76)	5 (17.24)
Levofloxacin	22 (75.86)	7(24.14)
Ceftazidime	25 (86.21)	4 (13.79)
Cefixime	29 (100.0)	0 (0.00)
Ceftriaxone	29 (100.0)	0 (0.00)
Cefepime	29 (100.0)	0 (0.00)
Azithromycin	21 (72.41)	8 (27.59)
Amoxycillin /Clavulanic Acid	24 (82.76)	5 (17.24)
Piperacillin /Tazobactam	26 (89.66)	3 (10.34)
Imipenem	29 (100.0)	0 (0.00)

Discussion

In this study, there were total 293 enteric fever patients. Among them, 30.38% were in the age group of 15 to 25 years, followed by 25.26% were in 25 to 35 years, 22.18% were in 35 to 45 years, 11.95% were in 5 to 15 years, 6.82% were in 45 to 55 years and 3.41% were in the 55 to 65 years age group. The mean age of study population was 29.18 (±5). Jahan (2015) reported that mean age 29.75 (±16.59) and mentioned that the young adult groups are most commonly affected by enteric fever, which justifies the present study.[21]

In the present study, among 293 enteric fever cases, total 48 (16.38%) were culture positive cases and 29 (9.90%) were positive for *Salmonella* spp. which was confirmed by biochemical test and specific antisera and 19 (6.48%) were other organisms. Among 29 culture positive *Salmonella* spp. 25 (86.21%)

were *S.* Typhi and 4 (13.79%) were *S.* Paratyphi A. The isolation rate of *Salmonella* spp. by Suman *et al*. (2015) was 9.29%.[22] Akter *et al*. (2016) reported that 9.60% were culture positive for *Salmonella* spp., among which 77.68% were *S.* Typhi and 22.32% were *S.* Paratyphi A.[23] Saha (2017) reported that *S.* Typhi and *S.* Paratyphi ratio was 4:1.[24] The findings of these studies were consistent with the present study. Jahan (2015) reported that 55.5% were culture positive for *Salmonella* spp.[21] In the study of Jahan (2015) *Salmonella* spp. were not confirmed by specific antisera, but in the present study *Salmonella* spp. were confirmed by specific antisera, that might be the reason of higher isolation rate in the study Jahan (2015).
The distribution of Widal test titre and blood culture results, among the study population were recorded in this study. Widal test titre against 'O' antigen (≥1:160) was indicated active infection.[16,17] Among 293 enteric fever patients,127 (43.34%) had Widal test significant titre. Begum *et al.* (2013) reported that 41% patients had Widal test significant titre.[25] The findings of this study were consistent with the present study.

Out of 293 study subjects 99 (33.79%) had Widal test significant titre for *S.* Typhi and 28 (9.56%) had Widal test significant titre for *S.* Paratyphi A. Munir *et al*. (2015) reported that 25.70% and Andualem *et al*. (2014) reported that 32.6% had Widal test significant titre for *S.* Typhi.[26,27] Chowdhury *et al*. (2015) reported that 12% were Positive for *S.* Paratyphi A.[17] The findings of this study were consistent with the present study. No significant elevation of BO or CO titre was found in this study. Chowdhury *et al*. (2015) from Bangladesh reported that no significant elevation of BO was found in their study.[17]

The relationship between blood culture results and Widal test titre was recorded. Among 29 (9.90%, out of 293 suspected patients) culture positive *Salmonella* cases, 18 (62.07%) had Widal test significant titre and 11 (37.93%) had Widal test non significant titre. Anagha *et al*. (2012) reported that among blood culture positive cases, 63.16% had Widal test significant titre.[28] Garg (2017) reported that among blood culture positive cases 54.55% had Widal test significant titre.[29] The findings of these studies were consistent with the present study. Yadav *et al*. (2015) reported that among culture positive cases 45% had Widal test significant titre.[30] The lower rate of significant titre of Widal test in the study of Yadav *et al*. (2015) might be due to the fact of age group. In this study the age group was 5 to 65 years but in the study of Yadav *et al*. (2015) the age group was 6 months to 12 years. Choo *et al*. (1993) mentioned that falsely elevated Widal test titres appear to be more common in adults than children.[31] In this study, the false positive result of Widal test was 109 (41.29). False positive results of Widal test could be due to high prevalence of *Salmonella* antibodies in the local healthy population. In a country with poor sanitation exposure to *Salmonella* occurs repeatedly by contaminated food and water resulting in detectable titre by Widal test in the serum of even healthy persons. Individual host immune responses do play a role and may get stimulated in febrile conditions caused by other infectious agents. This memory response can cause false positive results of Widal test in previously sensitized persons.[32] Chowdhury *et al.* (2015) reported that the rate of incidence of false negative results for Widal test among the bacteriological proven cases was very high, 43.3%.[17] Present study also revealed that some patients of enteric fever with positive blood culture had no significant elevation of titres of O or H antibodies (37.93%). Although these patients might have antibodies at a lower titre, they might be had a negative Widal test titre throughout the course of their illness. This lack of antibody response among patients

with blood culture-positive enteric fever might be attributed to undefined host or bacterial factors or prior antibiotic treatment or late appearance of antibody titre.[17] In the study Jahan (2015), it was 40.5% and in the study Parvin (2012), it was 31.03%.[21,33] The findings of these studies were consistent with the present study.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of Widal test were 62.07%, 58.71%, 14.17%, 93.37%, 59.04% respectively. Andualem *et al*. (2014) mentioned that a negative result of Widal test would have a good predictive value of the disease (NPV=94.59%) but positive result would have a very low predictive value (PPV=12.32%) for enteric fever and low sensitivity for Widal test may be related to the data collection time. [27] In the current study Widal test was performed just at attended at the OPD or attended in the microbiology department in the hospital.

In the present study, antibiogram was done with commonly used antibiotics as well as third and fourth generation cephalosporin. Regarding antibiotic susceptibility pattern, out of 29 *Salmonella* confirmed cases, 65.52% strains were sensitive to ampicillin. Ali and Sultana (2016) reported that 62.5% were sensitive to ampicillin which is consistent with the present study.[34] In contrast to this study, Chiou *et al*. (2014) reported that 31.6%.[35] In this study, Chloramphenicol was sensitive to 68.97% cases. Jahan (2015) reported that 73.8% were sensitive to chloramphenicol.[21] In contrast to this study Hasan *et al*. (2011) reported that 37.50%.[36] Sulphamethoxazole/trimethoprim was sensitive to 79.31% cases**.** Nahar *et al*. (2016) reported that 83.75% and Jahan (2015) reported that 73.80% were sensitive to sulphamethoxazole/trimethoprim.[10,21] The findings of these studies are consistent with the present study. In contrast to this study Garg *et al*. (2013) from India reported that 97.6% were sensitive to sulphamethoxazole/trimethoprim.[29] In this study the sensitivity to ciprofloxacin and levofloxacin was 82.76% and 75.86% respectively and all (100%) were nalidixic acid resistant *Salmonella*. Hasan *et al*. (2011) reported that 81.25% were sensitive to ciprofloxacillin.[36] In contrast to this study Nahar *et al*. (2016) reported that 95.07% and Ali and Sultana, (2016) reported that 98.6% were sensitive to ciprofloxacillin.[10,34] Sharma *et al*. (2017) reported that 82.53% were sensitive to levofloxacin which is consistent with the present study.[37] Hasan *et al*. (2011) reported that 100% were resistant to nalidixic acid.[36] These findings are consistent with the present study. In contrast to this study, Nahar *et al*., (2016) reported that 12.32% were sensitive to nalidixic acid.[10]

Of the third generation cephalosporin and fourth generation cephalosporin, susceptibility pattern of ceftazidime, Cefixime, ceftriaxone and cefepime was done. In this study, among 29 *Salmonella* spp., 86.21% were sensitive to ceftazidime and 100% were sensitive to Cefixime, ceftriaxone and cefepime. Similar to this study, Nahar *et al*. (2016) reported that 86.20% *Salmonella* were sensitive to ceftazidime and 100% were sensitive to ceftriaxone,[10] Ali and sultana (2016) reported that 99.4% *Salmonella* were sensitive to cefixime,[34] Hawaldar *et al*. (2016) reported that 98.73% and 78.12% were sensitive to ceftriaxone and cefepime respectively.[38]

Among 29 *Salmonella* spp., 72.41% were sensitive to azithromycin. Nahar *et al*. (2016) reported that 71.42% *Salmonella* were sensitive to azithromycin which is consistent with this study.[10] In contrast to this study, Jahan (2015) reported that 88.10% *Salmonella* were sensitive to azithromycin. [21]

Among 29 *Salmonella* spp., 82.76% were sensitive to amoxycillin/clavulanic acid. Nahar et al. (2016) reported that 90.47% and Jahan (2015) reported that 92.81% *Salmonella* were sensitive to amoxicillin/clavulanic acid.[10,21] In contrast to this study, Narain and Gupta (2015) reported that 98.50% *Salmonella* were sensitive to amoxycillin/clavulanic acid. [39]

Among 29 *Salmonella* 89.66% were sensitive to piperacillin/tazobactam. Hawaldar *et al*. (2016) reported that 93.75% *Salmonella* were sensitive to piperacillin/tazobactam which is consistent with this study.[38]

Among 29 *Salmonella* spp., 100% were sensitive to imipenem. Chiou *et al*. (2014) reported that 100% *Salmonella* were sensitive to imipenam.[35] These are consistent with the present study.

Conclusion

In this study *Salmonella* Typhi and *Salmonella* Paratyphi A were identified and *S.* Typhi was predominant among the enteric fever patients in DMCH. The blood culture is the gold standard test for enteric fever diagnosis. The Widal test is an easy, inexpensive, and relatively noninvasive test that can be of diagnostic value in situations where blood cultures are not available or feasible. But the results must be interpreted cautiously, as negative results do not exclude typhoid fever and positive results do not always go in favor. 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. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic recycling may help to decrease or prevent the emergence of resistance.

References

1. Kasper DL, Fauci AS, Hauser SL, Jameson JL *et al*. Harrison`s principles of Internal Meducine, Vol. 2, 19th eds. Mc Grill Hill Education, USA, 2015: pp. 1050
2. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ, 2004; 82:346–53. [PubMed: 15298225].
3. Murray CJL, Vos T, Lozano R, Naghavi M *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. Lancet, 2012; 380: 2197-223.
4. Lozano R, Naghavi M, Foreman K, Lim S *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. Lancet, 2012; 380: 2095-128.
5. Naheed A, Ram KP, Brooks WA, Hosain MA, Parsons MB *et al*. Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. Int J Infect Dis, 2010; 14: 93-99.
6. World Health Organization (WHO). Background Document: The Diagnosis, Treatment and Prevention of Typhoid Fever. 2003. (visited on10-Jan-18).
7. Parry CM, Hein TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med, 2002; 347 (22): 1770-1782.
8. Baucheron S, Monchaux I, Hello S, *et al*. Lack of efflux mediated quinolone resistance in *Salmonella enteric* serovars Typhi and ParatyphiA. Front. Microbiol, 2014; 5(12):1-6 doi:10.3389/fmicb. 2014.00012
9. Hassing RJ, Menezes GA, Pelt WV, Petit PL, Genderen PJV, Goessens WHF. Analysis of mechanisms involved in reduced susceptibility to ciprofloxacin in Salmonella enterica serotypes Typhi and Paratyphi A isolates from travellers to

Southeast Asia. Int J Antimicrob Agents, 2011; 37: 240–43.
10. Nahar SS, Rashid A, Chowdhury SS, Begum A, Akter N, Khatun K, Shamsuzzaman SM. Pattern of antimicrobial susceptibility for Salmonella typhi in Dhaka medical college hospital from 2013-2014. J Dhaka Med Coll. 2016; 25 (2): 115-18.
11. Ray P, Sharma J, *et al*. Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in *Salmonella enterica* serovar Typhi. Indian J Med Res, 124, 2006; 124: 105-8.
12. Singhal L, Gupta PK, Kale P, Gautam V, Ray P. Trends in antimicrobial susceptibility of *Salmonella* Typhi from North India (2001-2012). Indian J Med Microbiol. 2014; 32: 149-52.
13. Collee JG, Marr W. Specimen collection, culture containers and media. In: Collee JG, Frased AG, Marmion BP, Simmons A, (editors). Mackie and McCartney Practical Medical Microbiology, 14th eds. Churchil. Livingstone USA, 1996: 95-112.
14. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Frased AG, Marmion BP, Simmons A, (editors). Mackie and McCartney Practical Medical Microbiology, 14th eds. Churchil. Livingstone USA, 1996: 113-130.
15. Cheesbrough M. Microbiological test. Cheesbrough M (editors). District laboratory practice in tropical countries, 2nd eds. Cambridge University press UK, 2000:178-195.
16. Khatun K, Muazzam N, Shamsuzzaman SM, Kamal AHMM *et al*. Quality assessment of widal test in microbiology laboratories at primary and secondary level before and after implementation of standard operating procedure (SOP): A comparative study. J Dhaka Med Coll. 2014; 23(2): 227-233.
17. Chowdhury MAJ, Shumy F, Anam AM, Chowdhury MK. Current status of typhoid fever: a review. Bangladesh Med J. 2014; 43 (2): 106-10.
18. Old D.C. Salmonella. In: Collee, J.G., Fraster, A.G., Marmion, B.P., Simons, A. (Eds). Mackie and McCartney practical medical microbiology, 14th edn. Churchil Livingstone, New York. Pp. 385- 402.
19. Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. American journal of clinical pathology,1966; 45: 493-96.
20. Clinical and Laboratory Standards Institute (CLSI) (2015). Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement tests. Approved standard, CLSI document M100-S25. CLSI, Wayne, PA.
21. Jahan N. Diagnosis of typhoid fever by detecting flagellin gene (*fliC*) in urine. [M.Phil. Microbiology thesis] Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh; Bangabandhu Sheikh Mujib Medical University. 2015.
22. Suman MA, Siddique MA, Shamsuzzaman SM, Khandakar A, Khondaker FA. Akter Sumi SA, Jahan R. Detection Of Mutated *gyrA* Gene From Nalidixic Acid Resistant *Salmonella* Typhi And Paratyphi *A* Isolated From Enteric Fever Patients In ATertiary Care Hospital Of Bangladesh. Bangladesh J Med Microbiol, 2016; 10 (1): 3-7.
23. Akter T, Hossain MJ, Khan MS et al. Prevalanc and antimicrobial susceptibility pattern of *Salmonella* spp. Isolated from clinical samples of Bangladesh. Am J Pharm Health Res, 2016; 4(3): 101-11
24. Saha SK. *Salmonella* Typhi and Paratyphi in Bangladesh and their antimicrobial resistance-SEAP Data. 10th international Conference on Typhoid and Invasive Salmonellosis, Kampala, 2017.

25. Begum Z, Ahsan MM, Begum A.Value of the single Widal test in the Diagnosis of Typhoid fever. CBMJ, 2013; 2(1): 34-38.
26. Munir T, Lodhi M, Ali S *et al*. Early diagnosis of typhoid by PCR For FliC-d gene of *Salmonella* Typhi in patients taking antibiotics. JCPSP, 2015; 25(9): 662-6.
27. Andualem G, Abebe T, Kebede K. A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Research Notes, 2014; 7: 653. doi:10.1186/1756-0500-7-653
28. Anagha K, Deepika B, Shahriar R *et al*.The easy and early diagnosis of typhoid fever. JCDR, 2012; 6(2): 198-199.
29. Garg N. A comparative study of Widal Test and typhidot in rapid diagnosis of typhoid fever. Int J Med Res Prof, 2017; 3(2): 88-92.
30. Yadav K, Yadav SK, and Parihar G. A comparative study of typhidot and widal test for rapid diagnosis of typhoid fever. Int. J. Curr. Microbiol. App. Sci, 2015; 4(5): 34 -38.
31. Choo KE, Razif AR *et al*. Usefulness of the Widal test in diagnosing childhood typhoid fever in endemic areas. J. Paediatr. Child Health, 1993; 29: 36-39.
32. Verma D, Kishore S, Siddique ME Comparative evaluation of various tests for diagnosis of concurrent malaria and typhoid fever in a tertiary care hospital of northern India. JCDR, 2014; 8(5): DC41-DC44. DOI: 10.7860/JCDR/2014/7745.4403
33. Parvin S. Diagnosis of typhoid fever by blood culture and detection of antibody in lymphocyte (ALS) by ELISA and dot blot assay in DMCH.[M. Phil. Microbiology thesis] Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh; Dhaka University. 2012.
34. Ali MK and Sultana S. Antimicrobial sensitivity patterns of Salmonella Typhi in children. Bangladesh J Med Sci, 2016; 15(3): 416-18.
35. Chiou C, Lauderdale T, Phung DC *et al*. Antimicrobial resistance in *Salmonella enterica* Serovar Typhi isolates from Bangladesh, Indonesia, Taiwan, and Vietnam. Antimicrob Agents Chemother, 2014; 58(11): 6501-7.
36. Hasan B, Nahar SG, Akter L, Saleh AA. Antimicrobial sensitivity pattern of *Salmonella* Typhi isolated from blood culture in a referral hospital.Bangladesh J Med Microbiol, 2011; 05 (01): 16-20.
37. Sharma P, Dahiya S *et al*. Pefloxacin as a surrogate marker for quinolone susceptibility in *Salmonella enterica* serovars Typhi & Paratyphi A in India. Indian J Med Res, 2017; pp 687-92.DOI: 10.4103/ijmr.IJMR_494_16
38. Hawlader R, Sodani S, Bhilware H. Antibiotic Sensitivity Pattern of Salmonella Typhi in a Stand Alone Lab in Central Madhya Pradesh. Indian J Microbiol Res, 2016; 3(1): 31-36.
39. Narain U and Gupta R. Emergence of resistance in community-acquired enteric fever. Indian Pediatrics, 2015; 52: 709.