Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 3
ISSN: 2321–3647(online)

Prevalence and Antimicrobial Susceptibility Pattern of *Salmonella spp.* Isolated from Clinical Samples of Bangladesh

Tanzina Akter*[1], Mohammad Jakir Hossain[1], Md Sumon Khan[1], Sohana Al Sanjee[1], Kaniz Fatema[1], Suvamoy Datta[1].
1. Department of Microbiology, Primeasia University, HBR Tower 9, Banani, Dhaka-1213, Bangladesh.

ABSTRACT

Typhoid fever (Enteric fever) is a serious life-threatening illness caused by mostly *Salmonella Typhi* and also by *Salmonella Paratyphi* to a lesser extent. This study was carried out to isolate, identify *Salmonella spp.* from the blood samples of patients who were suspected of suffering from typhoid fever and to determine the antibiotic susceptibility pattern of the selected *Salmonella spp. Salmonella spp.* was detected in 122 of 1167 samples. Among which 87 (77.68%) were *Salmonella Typhi* and 25 (22.32%) were *Salmonella Paratyphi.* The most susceptible age group for *Salmonella spp.* is 21-30 years. Males (67.86%) are mostly infected by *Salmonella spp.* than females (32.14%). *Salmonella Typhi* (77.68%) was found as the predominant etiological agent of typhoid fever followed by *Salmonella Paratyphi* (22.32%) in our study. *Salmonella spp.* were resistant against Nalidixic acid, Co-trimoxazole and Pipercillin but sensitive against Ceftriaxone, Imipenem, Meropenem, Amikacin, Cefixime, Ceftazidime, Gentamicin, Cefotaxime. From this study, it can be concluded that these third generation antibiotics still can be used as effective drugs for the treatment of typhoid fever.

Keywords: Typhoid fever, *Salmonella spp.*, Antibiotics, Multi-drug resistance.

*Corresponding Author Email: tanzinaakterju@gmail.com
Received 01 March 2016, Accepted 08 March 2016

Please cite this article as: Akter T *et al.*, Prevalence and Antimicrobial Susceptibility Pattern of Salmonella spp. Isolated from Clinical Samples of Bangladesh. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Typhoid fever is a fatal multi-systemic illness caused by *Salmonella Typhi* and *Salmonella Paratyphi* with a contagious incidence of as much as 50%[1]. Globally, the World Health Organization (WHO) has estimated that 21.7 million cases of typhoid fever occur annually but in Southeast Asia alone it is 110/100,000 persons per year [2,3]. This disease poses a serious problem in developing countries because of unhygienic sanitary conditions and lack of or inadequate potable water [4, 5]. It has been documented that food sources such as eggs, poultry and meat products comprise major risk of transmission of enteric fever to humans. However, typhoid fever can largely be passed via fecal-oral route through contact with active, convalescent cases or chronic carriers[6]. So use of antimicrobials is necessary for the eradication of the disease causing microorganisms, *Salmonella spp.* But inadequate and irrational use of antibiotics, incompletion of the dose and negligence of the rules of administration of antibiotics has led to the emergence of antibiotic resistant microorganisms which is a great global threat now. It is alarming that prevalence of *Salmonella Typhi* and *Salmonella Paratyphi* is increasing in developing countries[7].There have also been several reports of the plasmid mediated resistance of multidrug resistant *Salmonella spp.* to some commonly prescribed antibiotics such as Ampicillin, Chloramphenicol, Co-trimoxazole in different countries of the world[7,8]. The resistance of *Salmonella spp.* to Amoxicillin, Fluoroquinolone, Chloramphenicol and Co-trimoxazole has become a new challenge for the treatment of typhoid fever [8, 9]. Hence, it is necessary to study the prevalence and susceptibility patterns of *Salmonella spp.* to conventional antibiotics for understanding antibiotics susceptibility profile of the isolates which will ultimately help to treat and prevent *Salmonella* infection [10].

So this study was carried out to investigate the prevalence of *Salmonella Typhi* and *Salmonella Paratyphi* in typhoid fever depending on age and sex and also to determine antibiogram profile against some commonly prescribed antibiotics.

MATERIALS AND METHOD

Study Design

1167 blood samples were collected from the clinically suspected patients of a tertiary care hospital of Dhaka city of Bangladesh. This study was carried out from June, 2015 to December, 2015.

Chemicals and Media

Pure and analytical grade chemicals purchased from BDH Chemicals Ltd, England; Merck, Germany; and Sigma Chemical Co. Ltd., USA were used throughout the study including media preparation. All the media and media ingredients such as beef extract, peptone that are used throughout the study were from Scharlau, Spain.

The culture media used in this study are- Brain Heart Infusion Broth (BHIB), Xylose Lysine Deoxycholate (XLD), MacConkey Agar, Mueller-Hinton Agar (MHA), Simmon's Citrate Medium, Deep Glucose Agar Medium, Trypton Broth, Methyl Red (MR)- Voges Proskeur (VP) medium, Motility medium, Urease broth etc.

Sample Collection and Processing

5-10 ml of blood were collected from adult patients whereas 1 - 3 ml of blood from children in special bottles. Then, the bottles were put in the Bactec machine where it was incubated at 37˚C and agitated continuously. The growth was indicated by alarm message on the computer screen. Then those positive samples were sub-cultured on selective media for isolation & identification of *Salmonella spp.*

Isolation and Identification of *Salmonella spp.*

Blood samples were collected in sterile BHIB under aseptic precautions. After overnight incubation at 37°C, subcultures were made on solidified sterilized XLD agar plate and MacConkey agar plates to check for growth. Typical *Salmonella spp*. produce red colonies with black centers on XLD agar whereas on MacConkey agar *Salmonella spp.* produce off white colonies. The selected colonies were then identified as *Salmonella spp.* by microscopic and standard biochemical reactions viz. TSI (Triple Sugar Iron), Urease test, MR-VP test, Oxidase test, Citrate utilization test, gas production following the taxonomic guides of ''Bergey's Manual of Determinative Bacteriology'', 8th ed.[11.]

Antibiotic Susceptibility Test

Antibiotic resistance pattern of the isolates were performed by disc diffusion method[12] according to Clinical Laboratory and Standards Institute (CLSI) recommendations. Seventeen antibiotics were used for this purpose *viz.* Azithromycin (15µg), Cefotaxime (30 µg), Ceftazadime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (30 µg), Cotrimoxazole (1.25/23.75 µg), Gentamicin (10 µg), Cefixime (5 µg), Imipenem (10µg), Meropenem (10 µg), Levofloxacin (5 µg), Amikacin (30 µg), Netilmicin (30 µg), Tobramycin (10 µg), Pipercillin (10 µg), Nitrofurantoin (30 µg) and Nalidixic acid (30 µg). To perform the test, the inoculum was adjusted to the 0.5 McFarland standard and antibiotics discs were placed on the heavily seeded inoculum on the solidified Mueller-Hinton Agar plate and incubated at 37°C for 24 hours. The

zone of inhibition was recorded in millimeter (mm). Isolates were considered as sensitive or resistant on the basis of zone of inhibition following the criteria of CLSI guidelines.

RESULTS AND DISCUSSION

About 1167 samples were collected from patients attending hospitals suspected with enteric fever. Among them, 112 samples showed positive for *Salmonella spp.* Here, only 9.6% patients were infected with *Salmonella spp.* while the other 90.4% were non-infected. Although most of the patients have typhoid fever, the lower number of positive samples may be due to the antibiotic administration during blood collection. The organisms were identified by microscopic, cultural and biochemical properties which are presented in Table 1. Among them, 87 (77.68%) isolates were identified as *Salmonella Typhi* and the other 25 (22.32%) were as *Salmonella Paratyphi.*

Table 1: Cultural, microscopic and biochemical properties of *Salmonella spp.*

Features	*Salmonella Typhi*	*Salmonella Paratyphi*
Colony on XLD agar	Red color with black centre	Red color with black centre
Colony on MacConkey agar	Off white color	Off white color
Gram staining	Gram negative, rod Shaped, Pink Color	Gram negative, rod Shaped, pink Color
VP (Voges Proskeur)	Negative	Negative
Indole	Negative	Negative
Motility	Positive	Positive
H_2S production	Positive	Negative
Gas production	Negative	Positive
Oxidase	Negative	Negative
Citrate utilization	Negative	Negative
Catalase	Positive	Positive
Urease	Negative	Negative

To study the prevalence of *Salmonella* spp. depending on age, 9 groups were formed from newborn to 90 years. The results are shown in Figure-1. The highest incidence of enteric fever took place in the age group of 21-30 years. This may be because of their spontaneous activities, consumption of unhygienic food and water in outdoors[13].This result was similar with the studies conducted in Iraq [14] and [15]. On the other hand, little cases were observed in adults (≥40 year age group) due to their boosting of immunity [16] not predisposing themselves in contaminated areas unlike youth[17]. Furthermore, the emergence of little or no infection in the other age group may be related with the less water contact activities which reduce the risk of infection. Another inference is the possibility of developed immunity by the older individuals who might have contracted the disease in their young age.

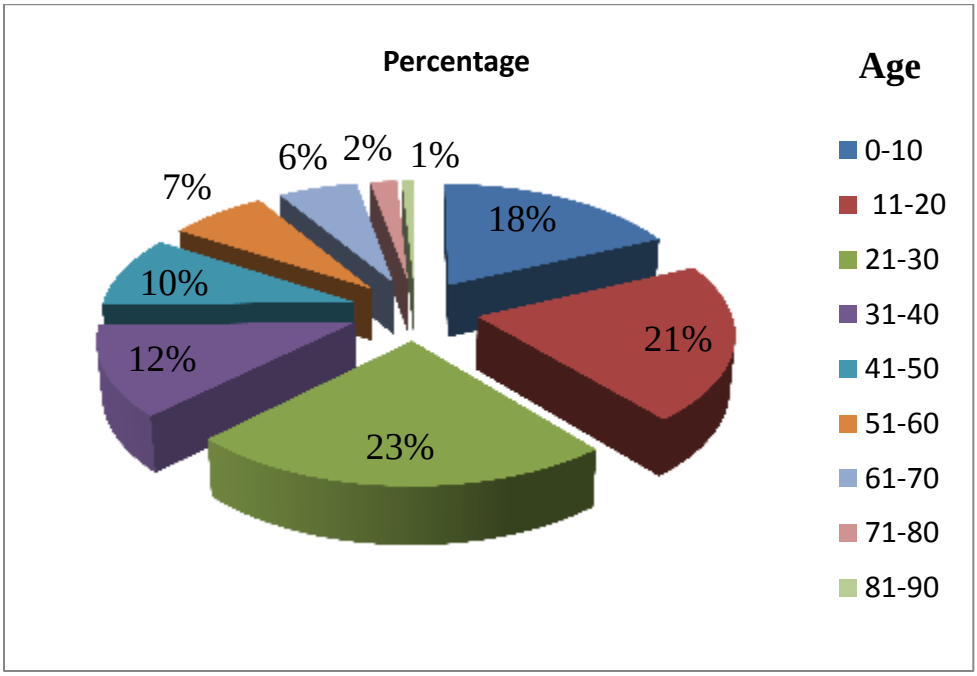

Figure 1: Prevalence of *Salmonella spp.* according to age group.

Males (67.86%) are affected more frequently than females (32.14%) by *Salmonella spp.* (Figure 2) because of their more outdoor activities, unconsciousness in unhygienic food consumption and more exposure to farming, water contact activities, contaminated environment and sharing of public toilet[18]. On the other hand, the outdoor eating, drinking and defecating are less commonly observed in females which may be because of cultural and religious courtesies. They are also less likely to be involved in farming, water contact activities and contaminated environment.

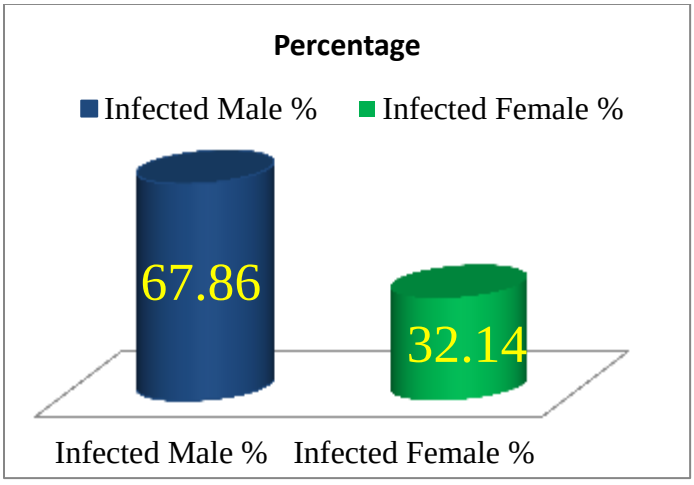

Figure 2: Prevalence of *Salmonella spp*. according to sex.

In the present study, it was found that *Salmonella Typhi* was the predominant cause of enteric fever. This may be because of the ingestion of contaminated food or water from contaminated hands. Among the 112 isolates, *Salmonella Typhi* was found 77.68% causing infection in male

(60.92%) whereas 22.32% of *Salmonella Paratyphi* was responsible for infection in 76% of males (Table 2). As *Salmonella Typhi* is mainly water-borne pathogen, this organism is mainly transmitted by consumption and use of unhygienic water. On the other hand, less infection with *Salmonella Paratyphi* (mainly food-borne) was reported in the present study. This may be because of the washing of food stuffs properly or cooking before consumption which reduces *Salmonella Paratyphi* in food staffs[18, 19].

Table 2: Prevalence of etiological agents of typhoid fever patients according to sex

Etiological Agents	No. of isolates	%	Infected males	%	Infected females	%
Salmonella Typhi	87	77.68%	53	60.92%	34	39.08%
Salmonella Paratyphi	25	22.32%	19	76%	06	24%

Emergence of multidrug resistance is a worldwide threat. The prevalence of resistance in *Salmonella* infection is increasing, which varies according to geographical and region allocation. Therefore, it is important to know the species of *Salmonella* that are prevalent in a country and the antibiogram profile of that prevalent *Salmonella spp*. In the present study, the selected isolates were examined for their susceptibility to common antibiotics by disc diffusion method[12]. The results were shown in Figure 3 and Figure 4.

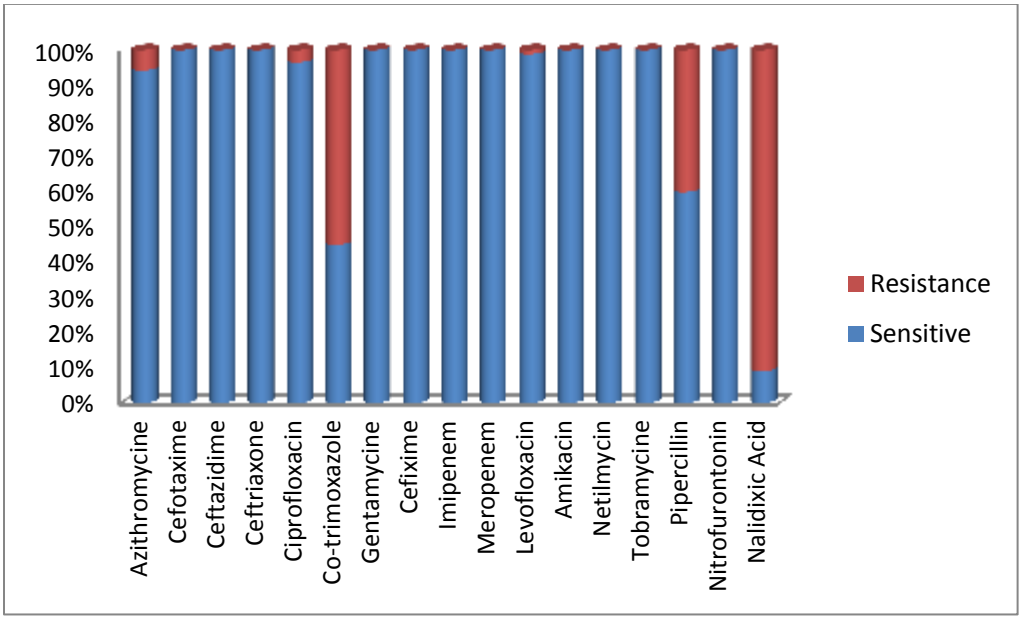

Figure 3: Antibiotic susceptibility pattern of *Salmonella Typhi.*

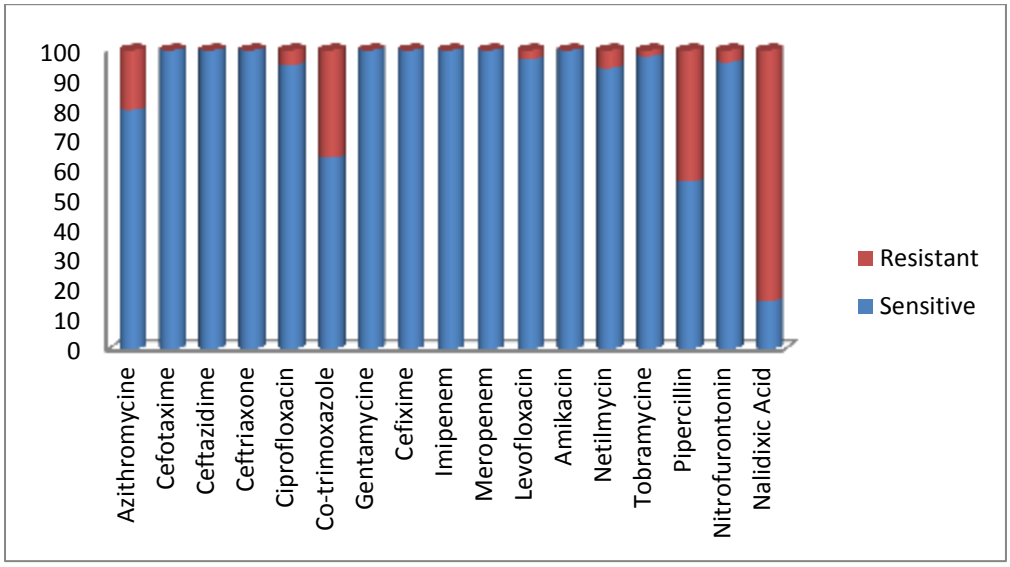

Figure 4: Antibiotic Susceptibility pattern of *Salmonella Paratyphi.*

In the present study, the sensitivity towards the third generation antibiotics of Cephalosporin group viz. Ceftriaxone, Cefixime, Ceftazidime and Cefotaxime was 100% for both *Salmonella Typhi* and *Salmonella Paratyphi*. This finding was in accordance with the study conducted in Pakistan[20]. So these drugs are still working effectively against the *Salmonella* infection, however some resistance problems, ranging from 5% to 25% have been encountered recently.

The better sensitivity pattern was showed by Aminoglycoside group viz. Gentamicin (100%), Amikacin (100%), Netilmicin (100%) and Tobramycin (100%) which was close enough to the findings of a study in India where sensitivity was noted as 70-90% [21].

The sensitivity towards Azithromycin for *Salmonella Typhi* and *Salmonella Paratyphi* was 94.25% and 80% respectively but lower sensitivity of Azithromycin has been reported in India[22]. This may be because of misuse of the antibiotic due to its oral route of administration and broad-spectrum antimicrobial activity with minimal side effects[23].

Salmonella Typhi also showed sensitivity to Co-trimoxazole (44.83%) and Pipercillin (59.77%) whereas 64% and 56% of *Salmonella Paratyphi* were sensitivity to those antibiotics respectively. These findings also meet with the studies conducted in Bangladesh [24] and Tajikistan[25] which contradicted with study conducted in Nepal[26].

In our study, Nalidixic acid was used as the first generation antibiotic of Quinolone group, Ciprofloxacin and Levofloxacin as representative of second and third generation antibiotic of the corresponding group. The maximum sensitivity was showed by Levofloxacin (98.85% for *Salmonella Typhi* and 100% for *Salmonella Paratyphi*) followed by Ciprofloxacin (96.55%) and Nalidixic acid (9%). This may be due to the continuous dependence on the antibiotics previously

which might exert selective pressure on the organisms to become resistant to that antibiotic. Similar results were also found in a study of Bangladesh[27].

CONCLUSION

The etiological agent of typhoid fever, *Salmonella spp.* is mainly transmitted by consumption of contaminated food or water. So maintenance of the proper hygiene, adequate cooking and refrigeration and minimization of the outdoor activities can reduce the prevalence of *Salmonella spp.* Emergence of multi-drug resistant *Salmonella spp.* is now a great challenge for the physicians to treat the illness. But in the present study, it was found that the third generation antibiotics are still working against *Salmonella spp.* So, appropriate guidelines should be followed to prescribe these antibiotics for avoiding multi-drug resistance problem.

REFERENCE

1. Cheesbrough M. Medical Laboratory Manuals for Tropical Countries.1st Edition. University Press Cambridge.58-59, 224-273.
2. Harish BN, Menezes GA. Antimicrobial resistance in typhoidal salmonellae. Indian J Med Microbiol 2011; 29:223–9.
3. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ 2004; 82:346-53.
4. Anita S, Indrayan AK, Guleria BS, Gupta CP .Antimicrobial Activity of Dye of Caesalpinia sappan (patang/Brazilwood). Indian J. Microbiol 2002; 42:359-360.
5. Doughari JH. A comparative study on effects of crude extracts of some local medicinal plants and some selected antibiotics on *Salmonella typhi pp* [M. Sc. Thesis]. Adamawa State: Federal University of Technology; 2005.
6. Kayser F. Medical Microbiology Bacteriol 2005; 283-89.
7. Ahmed D. Multidrug resistant *Salmonella enterica* serovar *typhi* isolates with high level resistance to Ciprofloxacin in Dhaka, Bangladesh. Antimicrob Agents Chemother 2006; 50: 3516-3517.
8. 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.
9. 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;31:387-389.

10. Alawode AO. Antibiotics and Drug Resistance Problem; A Matter of Public Concern. IPAN News. 2003; 4(4):9.
11. Buchanan RE and Gibbons N E. Bergey's Manual of Determinative Bacteriology", 8th ed. The Williams and Wilkins Company, Baltimore, 1974.
12. Bauer RW, Kirby MDK, Sherris JC and Turck M. Antibiotic susceptibility testing by standard single disc diffusion method. American J Clinical Pathol 1966; 45: 493-496.
13. Mangal HN. Prevalence of enteric fever Jaipur. Ind J of Med Res 1999; 55:219-223.
14. Saleh MAD. Comparison of widal agglutination test with ELISA typhi test for serological diagnosis of typhoid fever in some Iraq patients in Diyala governorate. 2013; 1:59-64.
15. A-Abdulla, Z. and Abdullah E. Comparison of The Widal Test And A Modified 2-mercaptonethanol Widal Test For The Diagnosis of Enteric Fevers. J Bahrain Med Society 2007; 19(1).
16. Parry CM. Epidemiologalcal and clinical aspects of human typhoid fever: Insalnonelly Infection:Clinicas, ed . Mastroen: P, Maskell D. Cambridge University 2005; Press .1- 1.
17. Mashi BH. Typhoid fever: a case study of an endemic disorder in Katsina metropolis. Biol Environ Sci J Tropics. 2013; 5: 27-30.
18. Abdullahi LO and Abdulkareem S. Bacteriological quality of some ready to eat vegetables as retailed and consumed in Sabongari, Zaria, Nigeria. Bayero J Pur Appl Sci. 2010; 3: 173-175.
19. Aarestrup FM, Lertworapreecha M, Evans MC, Bangtrakulnonth A, Chalermchaikit T, Hendriksen RS and Wagener HC. Antibiotic susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. J Antibiot Chemother 2003; 52: 715–718.
20. Abdullah FE, Haider F, Fatima K, Irfan S and Iqbal MS. Of Salmonellae Isolates. J College Phys Surg Pak 2012, *22*:147-150.
21. Gautam V, Gupta NK, Chaudhary U and Arora DR. Sensitivity pattern of *Salmonella* serotypes in Northern India. Braz J of Infect Dis. 2002;6:281-287.
22. Choudhary A, Gopalakrishnan R, Nambi PS, Ramasubramanian V and Ghafur KA et al. Antimicrobial susceptibility of *Salmonella enterica* serovars in a tertiary care hospital in Southern India. Indian J. Med. Res. 2013; 137: 800-802.

23. Capoor MR and Nair D. Quinolone and cephalosporin resistance in enteric fever. J. Global Infect Dis. 2010; 2: 258-262.
24. Saha SK, Talukder SY, Islam M and Saha S. A highly ceftriaxone resistant *Salmonella typhi* in Bangladesh. Pediatr. Infect. Dis. J. 1999; 18:387.
25. Murdoch DA, Banatvaia N, Bone A, Shoismatulloev BI, Ward LR, and Threlfall EJ. Epidemic ciprofloxacin-resistant *Salmonella typhi* in Tajakistan. Lancet 1998; 351:339.
26. Raza S, Tamrakar R, Bhatt CP, and Joshi SK. Antimicrobial susceptibility patterns of *Salmonella typhi* and *Salmonella paratyphi* A in a tertiary care hospital. J Nep Health Res Council. 2013.
27. Hasan B, Nahar SG, Akter L and Saleh AA. Antimicrobial sensitivity pattern of *Salmonella typhi* isolated from blood culture in a referral hospital. Bang J Med Microbiol 2011; 5:16-20.