International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 4; October 2017; Page No. 73-78

Study of antibiotic susceptibility of *Salmonella* serovars in patients suffering from enteric fever

Javed Iqbal, Altaf Hussain Sheikh, Shoket Ali
Environmental Biotechnology Laboratory, Department of Post-Graduate Studies and Research in Biological Sciences, Rani Durgavati University Jabalpur, Madhya Pradesh, India

Abstract
Enteric fever is still an important public health problem in developing countries including India. A changing antibiotic susceptibility pattern of *Salmonella* serovars and emergence of multi drug resistance has increased to a great concern. Aim of the study was to investigate the antibiotic susceptibility pattern of Salmonella species. Study was carried out at the Department of Post-Graduate Studies & Research in Biological Science, R.D University, Jabalpur. A total of 40 clinical samples were collected from typhoid patient of different hospitals. Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method and results were interpreted by National Committee for Clinical Laboratory (NCCLS) guideline. All the 16 isolates of *Salmonella* were tested against 14 antimicrobial compound namely cefotaxime, norfloxacin, aztreonam, ceftriaxone, nalidixic acid, nitrofurantoin, cefuroxime, gentamycin, amikacin, ciprofloxacin, ofloxacin, ceftazidime, cefixime and cefdinir. All the mentioned drugs are the common drug of choice for the treatment of typhoid fever. Seven isolates of *Salmonella* were found resistant to 14 of these antimicrobial compounds. However, 7 isolates were found resistant to cefotaxime, norfloxacin, aztreonam, ceftriaxone, nalidixic acid, nitrofurantoin, cefuroxime, gentamycin, amikacin, ciprofloxacin, ofloxacin, ceftazidime, cefixime and cefdinir. It was observed that *Salmonella* isolated from stools sample had developed simultaneous resistance to commonly used antibiotics. It was observed that *Salmonella* isolated from stool had developed simultaneous resistance to commonly used antibiotics. This has led to enormous morbidity in and around Jabalpur region. The area around Jabalpur is representative of tropics, where tropical diseases are abundantly present. The illiteracy, bad food habits temperature and water logging are main cause of food borne and water borne diseases, typhoid is much common.

Keywords: *salmonella typhi*, multidrug resistance, Jabalpur, microbial sensitivity test

Introduction
Salmonella was named for a notable American veterinary pathologist, Daniel E. Salmon. (Dolmen and Wolfe, 2003) [3]. Salmonellosis caused by species in the genus *Salmonella* was described in 1984 as a "new and significant threat to the public health" by the World Health Organization and *Salmonella* has remained a major food borne pathogen associated with different types of food (FAO, 1984) [4]. *S. typhi* and *S. paratyphi* are causes of typhoidal and para-typhoidal infections, where as non-typhoidal *Salmonella* with 2300 serotypes (*S. muenchen*, *S. lexington* and *S. hirschfeldii*) are causes of non-typhoidal infections such as gastroenteritis and septicemia. *S. typhi* and *S. paratyphi* A, B and C are the predominant types of *Salmonella* responsible for enteric fever particularly in the summer (Lesser and Miller, 2005) [10]. Non-typhoidal *Salmonella* strains are usually isolated from animal sources (Miller and Pegues, 2000) [12].

Typhoid fever caused by *Salmonella enterica* Serovor *typhi* (*S.typhi*), remains a significant problem in developing countries. It is estimated that there are more than 16 million cases of typhoid each year, resulting in at least 600,000 deaths, with annual attack rates ranging from 358 to 1100 per 100,000 populations (Ivanoff *et al*., 1994). In the pre-antibiotic era mortality rates of over 20% were not uncommon. The introduction of first chloramphenicol and subsequently ampicillin and co-trimoxazole greatly improved the outlook, decreasing mortality rates to as low as 1%. This progress has been seriously compromised by emergence of multi-drug resistant (MDR) typhoid, carrying resistance genes on the plasmid, which has returned the progress of the disease to that found in the pre-antibiotic era (Hart *et al*., 2003) [6].

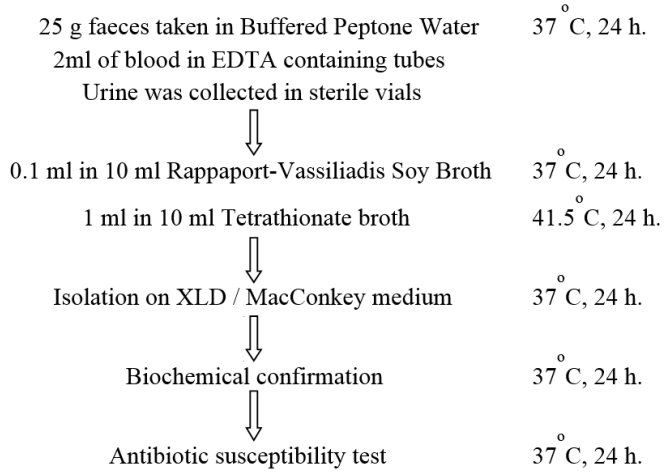
In recent years, several treatment failures with fluoroquinolones have also been reported, due to decreased susceptibility to ciprofloxacin (Asna *et al.*, 2003). In most strains of serovar *typhi*, resistance to the quinolones has been attributed to point mutations in the genes encoding DNA gyrase (*gyrA* and *gyrB*) or DNA topoisomerase IV (*parC* and *parE*) enzymes, which are located within the quinolone resistance determining region (QRDR) of the chromosomes of bacteria (Giraud *et al*., 1999) [5]. Recently, MDR serovar *typhi* showing resistance to nearly all of the commonly available 'first line' antimicrobial agents used for the treatment of these and other infections have been isolated in Kenya (Kariuki *et al*., 2000) [9]. The state of Madhya Pradesh is poor in health care infrastructure and number of cases of morbidity has been seen in past few years. The suggested cause of lethality was pinpointing the fact of MDR. The more common MDR diseases are tuberculosis, leprosy and malaria and of course number of attempts was made together the samples from different sources around Jabalpur region.With best of our knowledge, as such no database is present for this area about the antibiotic resistance. It is the initial work; we have taken

14 commonly used antimicrobial drugs. As these were more common of bacterial resistance, seen by clinicians in the area. This study provides baseline information to evaluate the bacterial resistance against different antibiotics.

Material and Methods

The present study was carried out at the Department of Post-Graduate Studies & Research in Biological Science, R.D University, Jabalpur during the period from March to June, 2014. The methodology followed is as under-

Experimental design



Sample collection

A total of 40 clinical samples were collected from typhoid patient of different hospitals like N. S. C. Bose Govt. Medical Hospital, Govt. Hospital Narsinghpur, Govt. Hospital Katni, Govt. Hospital Sagar, and Govt. H. G. Hospital located in and around Jabalpur region. 25 grams of stool samples were taken in 225 ml of buffered peptone water. The urine was collected in sterile vials whereas blood samples in EDTA containing tubes from typhoid positive patients by visiting different hospitals in Jabalpur region.

Isolation of *Salmonella* species

The standard procedures (ISO-6579; 2002) followed for the isolation of *Salmonella* from clinical samples is described as under-

Non selective pre-enrichment

Buffered peptone water is used as pre-enrichment medium. Weigh out 25 gm stool sample, and put it into a sterile flask and add 225 ml of buffered peptone water to obtain 1 part sample + 9 part buffer. Mix it well and incubate at 37ºC for 18-24 hr.

Selective enrichment: For selective enrichment two media was used.

i) Tetrathionate broth and ii) Rappaport Vassiliadis soy (RVS) peptone broth

Transfer 1.0 ml of Pre-enrichment in 10 ml of Tetrathionate broth and incubate it at 37ºC±0.5ºC for 18-24 hr. Tetrathionate broth is a selective enrichment of *Salmonella*. It is inhibitory to *S. typhi, S. pullorum* and *S. gallinarum.* Transfer 0.1 ml of Pre-enrichment culture medium in 10 ml of Rappaport Vassiliadis broth and incubate at 41.5ºC±0.5ºC for 18-24 hr.

ii) Sub-cultivation on Xylose Lysine Desoxycholate (XLD) agar plates

Sodium desoxycholate is the selective agent and phenol red is the pH indicator. The indicative principle is based on lactose, sucrose and xylose fermentation, H_2S production and lysine decarboxylation. If H_2S is produced from sodium thiosulphate, black FeS (ferrosulfide) will develop. *Salmonella* ferments xylose, but not lactose and sucrose, decarboxylate lysine and produces H_2S. *Salmonella* suspect colonies grow as red colonies with a black center. Prepared XLD (HiMedia) was also used (55.8 g in 1000 ml of distilled water).

Take 10µl of inoculated and incubated selective enrichment (Rappaport Vassiliadis soy peptone broth and Tetrathionate broth) and spread it onto the XLD agar plate, incubate it at 37ºC for 18-24 hr.

Antibiotic susceptibility testing

Antibiotic susceptibity is tested by clario combi disc. The type and concentrations of antibiotics in the discs were as followed:-

Cefotaxime (30 µg), Norfloxacin (10 µg), Aztreonam (30 µg), Ceftriaxone (30 µg), Nalidixic acid (30 µg), Nitrofurantoin (300 µg), Cefuroxime (30 µg), Gentamycin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Ceftazidime (30 µg), Cefixime (5 µg), Cefdinir (5 µg).

Preparation of plates

Sterile XLD agar and Nutrient Agar Medium are poured into plates kept on a leveled surface. The depth of the medium should be approximately 4mm. After the medium has solidified, dry the plates for 30 minutes in an incubator to remove excess moisture from the surface.

Preparation of inoculum

Select 4 to 5 similar colonies and transfer them into a tube containing 5 ml nutrient broth Incubate the broth culture at 35-37ºC for 2 to 5 hours to obtain moderate turbidity. Dilute the broth culture of actively growing organism with sterile broth or saline to obtain a tubidity equivalent to that of barium sulphate standard (prepared by adding 0.5 ml of 1.175% $BaCl_2$ 2 H_2O solution to 99.5ML OF 0.36 N H_2SO_4).

Inoculation

Take 30 µl of culture inoculum in micropipette and add it on the agar surface. With the help of swab streak the agar surface of the plate in three directions, turning the plate by 60ºbetween each streaking. Replace the lid of the petridish and keep it at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculums. Confluent growth is desirable for accurate results.

Application of combi disc

Remove one combi disc from its container with the help of a flaimed forceps and carefully place it on the surface of one medium. Finally press it lightly with the forceps to make

complete contact with the surface of the medium. Allow the plates to stand at room temperature for 30 minutes before proceeding for the next step.

Incubation and measurement of Inhibition zone

Incubate the plates at 35-37ºC for 16 to 18 hours. Measure the diameter of the zone of inhibition at the end of the incubation period. Measure only those zones that are showing complete inhibition and record the zone diameter to the nearest millimeter.

The criteria for designating sensitivity, resistant, intermediate and total resistant Salmonellae were taken from the standard protocol of Clario Combi disk.

Results

Isolation of Salmonellae

A total 40 clinical samples of blood, urine and stool were collected from 40 patients admitted during March to April in different hospitals. Out of these, only 16 samples showed the presence of Salmonellae after screening with selective enrichment technique. All the 16 isolates of *Salmonella* were tested against 14 antimicrobial compound namely cefotaxime, norfloxacin, aztreonam, ceftriaxone, nalidixic acid, nitrofurantoin, cefuroxime, gentamycin, amikacin, ciprofloxacin, ofloxacin, ceftazidime, cefixime and cefdinir. The test results are presented in Table 1, 2, 3 and 4. All the mentioned drugs are the common drug of choice for the treatment of typhoid fever. Seven isolates of *Salmonella* were found resistant to 14 of these antimicrobial compounds. These resistant strains of *Salmonella* are marked as EBL S1 *S. typhi*, EBL S3 *S. houtnae*, EBL S4 *S. typhi*, EBL S6 *S. bongori*, EBL S8 *S. typhi*, EBL S11 *S. typhimurium*, EBL S13 *S. typhi* as presented in Fig 1, 2 & 3.

However, 7 isolates were found resistant to cefotaxime, norfloxacin, aztreonam, ceftriaxone, nalidixic acid, nitrofurantoin, cefuroxime, gentamycin, amikacin, ciprofloxacin, ofloxacin, ceftazidime, cefixime and cefdinir. It was observed that *Salmonella* isolated from stools sample had developed simultaneous resistance to commonly used antibiotics. This has led to enormous morbidity in Jabalpur region. Numbers of reports have come out in recent years indicating the growing concern and seriousness of this problem (Mary *et al.,* 1992). In one study from Andhra Pradesh reported strains of *S. typhi* isolated from blood cultures of patients with typhoid fever showing multiple drug resistance. Sabherwal *et al.* (1992) [14] reported 730 strains of *S. typhi* isolated in, 1989-1990. Out of those 218 isolates were resistant to chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole. Agerwal *et al.* (1992) [1] observed that between July, 1990 and March, 1991 91.3% of the 241 isolates were resistant to chloremphenicol, ampicillin and trimethoprim-sulfamethoxazole.

The emergence of drug resistance among *Salmonella* and the alarming ability of *Salmonella* to acquire high level resistance to the commonly used antimicrobial created the difficulty of treating infections caused by these organisms (Rowe *et al*., 1990 and Mantel, 1990) [13, 11]. In Jabalpur region, antimicrobials can be procured are available from the chemist shop or even in general stores without legal prescription. This encourages patients to buy antimicrobial from the counter and use them without consultation with the doctor. By doing so, wrong antimicrobial drug which helps in selection of resistance in bacteria rather than curing the patient. This self medication is often taken for wrong duration which further helps in selection of resistant bacteria against these antimicrobial. Very often antimicrobial are prescribed without determining the causative organism or its susceptibility to antimicrobial. When antimicrobial are prescribed for infection due to viruses or resistant bacteria, thus also result in increased resistance among bacteria including *Salmonella* serovars.

Tables (1- 4) Antibiotic susceptibility test

Table 1

Serovars	***S. typhi***		***S. cholerasius***		***S. houtnae***		***S. typhi***	
Antibiotics (µg/disc)	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**state**	**Zone diam.(mm)**	**state**
CX/30	27	S	-	S	26	S	25	S
NF/10	-	R+	17	I	05	R	13	R
AT/30	13	R	22	S	-	S	-	R+
FR/30	-	R+	-	S	17	S	15	R
NA/30	-	R+	14	R	-	R+	-	R+
FU/300	9	R	10	R	-	R+	-	R+
CR/30	17	R	13	R	11	R	11	R
GM/10	11	I	-	S	12	R	-	R+
AK/30	09	R	-	S	16	R	18	I
Cl/5	-	R+	22	S	15	R	16	R
OF/5	-	R+	-	S	-	R+	-	R+
CZ/30	-	R+	-	S	21	S	-	R+
FX/5	12	R	27	S	-	R+	19	I
CN/5	-	R+	-	S	14	R	18	R

Table 2

Serovars	*S. typhi*		*S. bongori*		*S. salmae*		*S. typhi*	
Antibiotics (µg/disc)	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**State**	**Zone diam. (mm)**	**State**
CX/30	21	I	23	I	-	S	23	S
NF/10	-	S	14	R	17	I	10	R
AT/30	26	S	15	R	16	S	-	R+
FR/30	-	S	18	R	12	I	-	R+
NA/30	13	R	-	R+	14	R	-	R+
FU/300	15	R	-	R+	16	R	08	R
CR/30	09	R	-	R+	14	I	12	R
GM/10	10	R	19	I	16	S	10	I
AK/30	13	R	-	R+	13	S	-	R+
Cl/5	-	S	16	R	14	R	-	R+
OF/5	-	S	10	R	18	I	09	R
CZ/30	19	S	-	R+	-	S	07	R
FX/5	-	S	-	R+	-	S	13	R
CN/5	-	S	13	R	19	S	11	R

Table 3

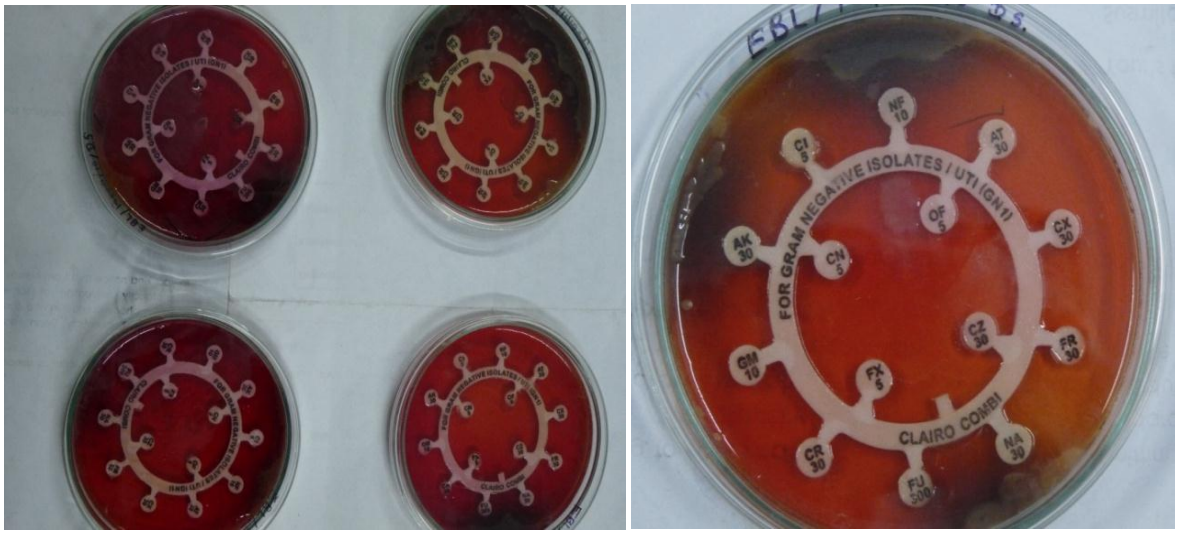
Serovars	*S. typhi*		*S. indica*		*S. typhimurium*		*S. arizonae*	
Antibiotics (µg/disc)	**Zone diam. (mm)**	**State**	**Zone diam. (mm)**	**State**	**Zone diam. (mm)**	**state**	**Zone diam.(mm)**	**State**
CX/30	-	S	-	S	10	R	-	S
NF/10	14	R	-	S	-	R+	-	S
AT/30	-	S	12	R	-	R+	-	S
FR/30	25	S	-	S	12	R	-	S
NA/30	18	I	08	R	-	R+	-	S
FU/300	14	S	12	R	-	R+	16	S
CR/30	-	S	-	S	-	R+	-	S
GM/10	15	R	-	S	11	R	-	S
AK/30	15	R	16	S	10	R	-	S
Cl/5	-	R+	-	S	-	R+	12	S
OF/5	-	S	14	S	-	R+	-	S
CZ/30	21	S	-	S	-	R+	-	S
FX/5	12	R	-	S	05	R	-	S
CN/	-	S	-	S	-	R+	18	S

Table 4

Serovars	*S. typhi*		*S. bongori*		*S. arizonae*		*S. diarizonae*	
Antibiotics (µg/disc)	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**state**	**Zone diam. (mm)**	**State**	**Zone diameter (mm)**	**state**
CX/30	-	R+	-	S	-	S	-	S
NF/10	-	R+	08	R	21	S	-	S
AT/30	11	R	-	S	-	S	20	S
FR/30	-	R+	-	S	23	S	-	S
NA/30	-	R+	-	R+	-	S	-	S
FU/300	10	I	12	R	18	R	-	S
CR/30	-	R+	18	R	19	R	08	R
GM/10	08	R	16	R	-	S	16	I
AK/30	-	R+	-	R+	11	R	14	R
Cl/5	-	R+	-	S	14	R	-	S
OF/5	-	R+	17	I	-	S	12	S
CZ/30	-	R+	12	R	-	S	-	S
FX/5	-	R+	16	R	-	S	-	S
CN/5	-	R+	-	R+	-	S	18	R

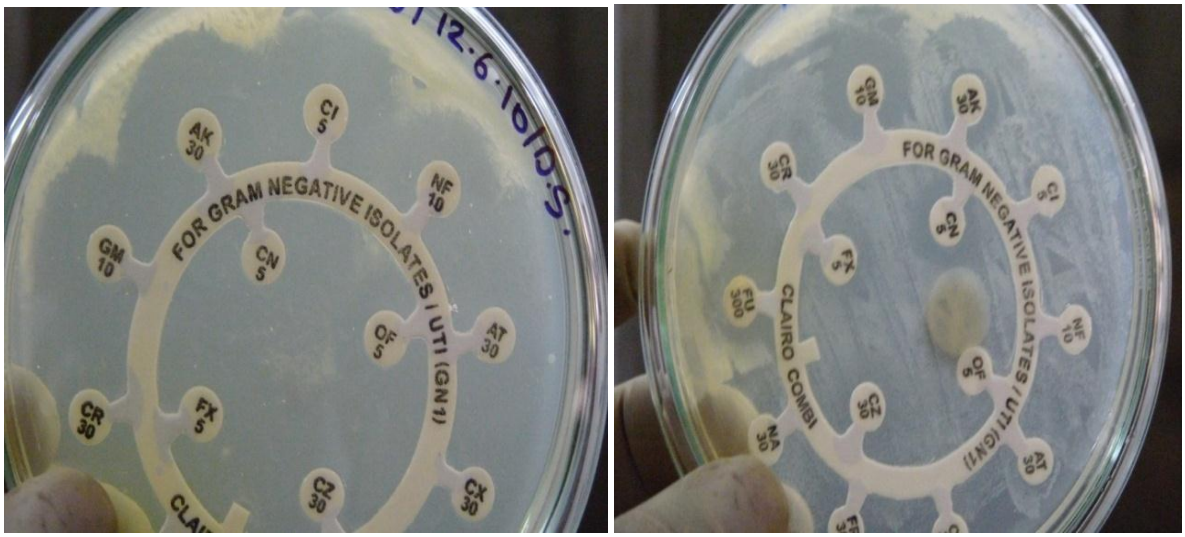
CX - Cefotaxime, NF- Norfloxacin, AT- Aztreonam, FR- Ceftriaxone, NA- Nalidixic acid, FU- Nitrofurantoin, CR- Cefuroxime, GM- Gentamycin, AK- Amikacin, Cl- Ciprofloxacin, OF- Ofloxacin, CZ- Ceftazidime, FX- Cefixime, CN- Cefdinir.

S = Sensitive, R = Resistant, I = Intermediate, R+ = Total Resistant, - = no inhibition zone Samples = EBL S1 - EBL S16



(a) **(b)**

Fig 1: (a & b) Antibiotic susceptibility test by using Clario combi disc on XLD medium



(a) **(b)**

Fig 2: (a & b) Antibiotic susceptibility test by using Clario combi

(a) **(b)**

Fig 3: (a & b) Antibiotic susceptibility test by using Clario combi disc on NAM.

Acknowledgements
Author would like to acknowledge to all the patients involved in this study.

References

1. Agarwal U, Brahme RB, Dhanuijay AG, Jalgaronkar PD, Pathak AA, Saoji AM. Antibiogram phage types and biotypes of *Salmonella typhi* isolated in Nagpur. Indian J. Med. Res. 1992; 95(A):14.
2. Asna SM, Haq JA, Rahman M. Nalidixic acid-resistant *Salmonella enteric*a serovar *typhi* with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Jpn J Infect Dis, 56: 32-33.
3. Dolman CE, Wolfe RJ. Suppressing the diseases of animals and man. Countway Library of Medicine. 2003; 9:691.
4. Food and Agriculture Organization [FAO]. Food inspection. Food and nutrition paper. Rome, Italy. 1984, 107-12.
5. Giraud E, Brisabois A, Martel JL, Chaslus DE. Comparative studies of mutations in animal isolates and experimental in vitroand in vivo-selected mutants of *Salmonella* species suggest a counter selection of highly fluoroquinolone resistant strains in the field. Antimicrob. Agents Chemother. 1999; 43:2131-2137.
6. Hart CA, Kariuki S, Mirza SH. Multi-drug resistant Typhoid. Post-Graduate Doctor Caribbeon. J of Special supplemen. 2003; 19(1):1-6.
7. ISO 6579 (4th ed) Microbiology General guidance on methods for the detection of *Salmonella*, International Organization for Standardization, Geneve, Switzerland4th ed.
8. Ivanoff B, Levine MM, Lambert PH. Vaccination against typhoid fever present status. J of Bull World Health Organization. 1994; 72:957-971.
9. Kariuki S, Gilks C, Revathi G, Hart CA. Genotypic analysis of multidrug resistant *Salmonella enterica* Serovar *typhi*, Kenya. Emerg. Infect. Dis J. 2000; 6:649-651.
10. Lesser C, Miller SI. Salmonellosis In Kasper, Fauci, Braunwald and Isselbacher, Eds. Harrisons principles of internal medicine. 2005; 16:897-902.
11. Mantel BK. Modern treatment of multi-drug resistant typhoid fever. Lancet. 1990; 336:1383.
12. Miller SI, Pegues DA. *Salmonella* species, including *Salmonella typhi*. Text book of principles and practice of infectious disease. 2000; 5:2344-2362.
13. Rowe B, Ward LR, Threlfall EJ. Spread of multi-drug resistant *Salmonella typhi.* The Lancet. 1990; 336:1065.
14. Sabherwal U, Chaudhary U, Saint S. Multi-resistant *Salmonella* typhi in Haryana in 1989-1990. J. Med Res. 1992; 95(A):12.