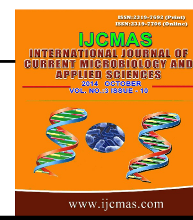

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 487-495**
http://www.ijcmas.com

Original Research Article

Mutation at quinolone resistance-determining region of gyrA gene of *Salmonella typhi* isolated from tertiary health care hospitals of Amravati, Maharashtra

S.G.Dahikar[*1], S.S.Deshmukh[2] and P.V. Thakare[3]

[1]Department of Microbiology, Shri Shivaji Science College, Amravati, India
[2]Principal, J.D.P.S. College, Daryapur, India
[3]Department of Biotechnology, S.G.B. Amravati University, Amravati, India
**Corresponding author*

Keywords

Salmonella enterica serovar Typhi, antimicrobial resistances, quinolone resistance, typhoid outbreak

A B S T R A C T

The problem of typhoid fever is exacerbated as the causative agent, Salmonella enterica subspecies enterica serovar Typhi (*S. typhi*), rapidly develops resistance to drugs used in treatment. Antimicrobials play a role in emergence and persistence of epidemic MDR strains and the MDR *S. typhi* is on the rise. A total of 309 Salmonella enterica serovar Typhi strains isolated from Amravati City, in 2010-2011, were tested for antibiotic susceptibility test. Antibiotic sensitivity was carried out by Kirby - Bauer method. Selected *S. enterica* serovar typhi isolates were tested for MICs by E-test for ciprofloxacin The study indicates that MDR *S. typhi* is on the rise. With increasing resistance to ciprofloxacin and the possibility of re - emergence of sensitivity to chloramphenicol, the policy of empirical treatment of enteric fever needs to revise. Thus MIC of *S. typhi* isolates for ciprofloxacin was found to be 0.25 µg/ml. The molecular analysis of quinolone resistance determining region (QRDR) revealed the most prevalent amino acid substitution was Asp 87→Tyr with point mutations in gyr A. Thus it is necessary to monitor mutation in DNA topoisomerase associated with increases in quinolones resistance.

Introduction

Typhoid fever is a major health problem as the causative agent *Salmonella enterica Serovartyphi* and S.*paratyphi* has developed resistance to many antimicrobial agents (Murray, 1986 and Miriagou *et al.,* 2006). They are transmitted by the ingestion of food or water contaminated with human feces from an infected person. A few people can become carriers of *S. typhi* and continue to shed the bacteria in their feces for years (Gil *et al.,* 2009).

Salmonella enterica epidermics often involved rapid dissemination of predominant epidemic strains over a large geographical distance (Imberechts *et al.,* 2000). It is estimated that more than 33 million cases of typhoid fever occur annually causing more than 500,000 deaths in 2003 (Khan *et al.,* 2008).

Antimicrobial resistant typhoid has been observed for over half a century and is now

common in many areas. Chloramphenicol resistant *S. typhi* was first reported in 1950, shortly after the drug was introduced for treatment of typhoid. By early 1970s *S. typhi* resistant to both chloramphenicol and ampicillin had been observed and multidrug resistant *S. typhi* emerged soon after. The rate of MDR of *S. typhi* can fluctuate over time and geographical space, as can the precise combination of drug resistance genes and phenotypes. Fluoroquinoloes reduce the duration of fever. Among fluoroquinolones ciprofloxacin is most active drug against typhoid (DuPont, 1993).

A single mutation in the *gyr* A gene is sufficient to confer resistance to nalidixic acid and reduce susceptibility to fluoroquinolones, and a second mutation leads to high-level fluoroquinolone resistance (MIC range 8 to > 32 $\mu g\ ml^{-1}$) (Renuka *et al.,* 2005). Resistance to ciprofloxacin is mediated either by chromosomal mutations in *gyr*A gene, or by decreased cell wall permeability. Such mutations confer resistance not only to ciprofloxacin but also to the entire class of fluoroquinolone agents (Therelfall *et al.,* 1997).

Present study deals with analysis of mutations in gyraseand a mechanism of fluoroquinolone resistance and the prevalence of multiple antimicrobial resistances among *S. enteric* serovarTyphi isolated from Amravati city were determined.

Materials and Methods

Five hundred and three typhoid positive samples were collected from general hospital and private pathology laboratory for evaluation of possible series of bacterial infection during the period of March 2010 to June 2011.The specimen were preferably collected at the onset of fever, at when the organism were likely to be present in the blood stream. When fever was intermittent, blood samples were drawn. Collected samples were brought to the laboratory for further analysis. Blood sample of positive Widal test were culture on various differentiating media i.e. Bismuth Sulphite agar (BSA), *Shigella Salmonella* (SS) agar, Xylose Lysine Dioxycholate (XLD) agar. Identification and confirmation of *Salmonella* strains were carried out on the basis of morphological, cultural, biochemical and enzymatic reactions. Out of these 309Widal positive samples were selected for further study.

Antimicrobial susceptibility testing

Antibiotic sensitivity test of *Salmonella typhi* isolate from different health care centers was determined for 13 antibiotics. The selection of antibiotics was based on the data (about commonly prescribed antibiotics) collected from the tertiary health care centres of Amravati city. Based on this data, the antibiotics were selected for present study namely ciprofloxacin, ofloxacin, norfloxacin, co-trimoxazole, levofloxacin, aztreonam, gatifloxacin, nitrofurantoin mainlybelonging to quinolone group of antibiotic along with ampicillin, chloramphenicol, gentamycin, streptomycin and tetracycline. *S. enterica*serovarTyphi isolates were tested for susceptibility to antimicrobials.Antibiotic susceptibility pattern of the isolates were determined by the Bauer – Kirby disc diffusion method according to Clinical and Laboratory Standards Institutes (CLSI) guide lines (CLSI, 2008).

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was

performed by Epsilometer Test "E" Test.The antibiotic ciprofloxacin was used for determining the MIC against ciprofloxacin resistant isolates. MIC breakpoint for susceptibility of *Salmonella typhi* providing by CLSI guidelines (CLSI, 2008) were used for interpreting "E" test MIC values.

Isolation of genomic DNA

Genomic DNA was extracted from overnight grown cultures of the selected bacterial isolates. Bacterial growth culture of 0.5 Mc-Farland standards was centrifuged at 10,000 rpm for 3 minutes. Repeat same step for one more time for sufficient pellet. Bacterial pellet was washed twice with distilled water and the pellet was resuspended in 100 µl nuclease free water. The pellet was kept at 85 ^{0}C water bath for 1 minute to bring about the cell lysis.

PCR amplification was performed using a 50 µl reaction mixture containing 100 ng of template DNA, 20 µmol of *gyr*A primers, 200 µM of dNTPs, 1.5 mM of $MgCl_2$, 1U of *Taq*DNA polymerase (MBI Fermentas) and 10 µl of 10x *Taq*polymerase buffer. The sequences of quinolone resistant genes of *gyr*A (Table 1) used were as follows:

Table.1 Primers used for PCR amplification and sequencing of genes coding for the quinolone resistance

Primer	Primer sequences
gyrA- F	5'-ATGAGCGACCTTGCGAGAGAAATTACACCG-3'
gyrA- R	5'-CTTCTGTAGTCGTAACTTCCCGACTACCTT-3'

Amplification was carried out with an initial denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec., extension at 72°C for 1 min and final extension at 72°C for 10 min in Plam cycler (Corbette).

Result and Discussion

All the 309 Widal test positive samples were separated out. Pure culture were identified by morphology, colony characters and different biochemical test such as Indole, Methyl red, Vogues Proskauer and Citrate utilization. All the isolated pure cultures gave the 100% standard results. In triple sugar iron tests acid, gas and H_2S production were detected.

MIC results showed the effect of various concentrations of ciprofloxacin on the survival of *S. typhi* isolates. Ciprofloxacin resistant isolates of *S. typhi* were tested against 15 different concentrations of ciprofloxacin ranges 0.001µg/ml to 240 µg/ml. All the isolates were found to be resistant to 0.01 µg concentration. Thus MIC of *S. typhi* isolates for ciprofloxacin was found to be 0.25 µg. Similar observations were recorded in case of *S. paratyphi* isolates where the MIC was 0.25 µg.

Molecular identification of Ciprofloxacin resistant isolates by polymerase chain reaction

The ciprofloxacin resistance in *S. typhi* is due to accumulation of mutations in specific site of *gyr* A. Clinical isolates with resistance 0.25 µg/ml to ciprofloxacin were selected for the analysis of mutation in QRDR. DNA was isolated from the selective strains. Using the isolated DNA and PCR primers *gyr* A (F and R) the QRDR sequences was amplified. The amplified DNA of size 600bp yield single

band was sequenced by Chromas Biotech Ltd (Banglore) using ABI 3710 sequencers using Sanger's method of sequencing. The sequences were provided in Fasta format and chromatogram files with extension *.abi analysed using Chromos Lite (V.2.4).

Alignment of Sequences

BLAST Search

The nucleic acid sequences were searched using NCBI (National Center for Biotechnology Information) data base BLAST (Basic Local Alignment Search Tool) to find out the similarity matrices with the sequences already deposited in the NCBI nucleotide database. The sequences already deposited in NCBI database for QRDR region GI: AL513382.1 was used as standered sequence for further analysis.

BioEdit

Standard alignment tool Bioedit (V7.2.0) was used for alignment and analysis of mutations.

Multiple sequence alignment using BLAST tool for *gyr*A DNA sequence of sample 1 with MIC 0.25 µg/ml had mutation at position Asp 87, Val 73, Ala 119 and Gly 133. BLAST nucleotide sequence alignment are shown in (Fig.1) The *gyr* A DNA sequence of sample 2 had mutations at two sites i.e. Asp 87 and Tyr 86. The mutation at Asp87 was common in both the strains.

Salmonella is one of the leading cause of food borne illness in countries around the world. Treatment of *Salmonella* infections in both animal and human, have become more difficult due to the increased prevalence of MDR *Salmonella* strains. To control the spread of multidrug resistant *Salmonella*, it is important to first understand mechanism responsible for drug resistance and how drug resistance is transmitted to and between *Salmonella* strains (Alcaine, 2007).

Table.2 Percentage zone of inhibition by antibiotics against *Salmonella* isolates

Sr. No.	Antimicrobials	Resistance	Percentages	Sensitive	Percentages	MAR Index
1	Aztreonam	231	74.75	78	25.24	0.0575
2	Gatifloxacin	33	10.67	276	89.92	0.0082
3	Nitrofurantoin	162	54.42	147	47.57	0.0403
4	Co-trimoxazole	162	54.42	147	47.57	0.0403
5	Ciprofloxacin	130	42.07	179	57.92	0.0323
6	Ofloxacin	62	20.06	247	79.93	0.0154
7	Norfloxacin	82	26.53	227	73.46	0.0204
8	Levofloxacin	70	22.65	239	77.34	0.0174
9	Ampicillin	303	98.05	06	1.94	0.0754
10	Chloramphenicol	178	57.60	131	42.39	0.0443
11	Gentamycin	85	27.05	224	72.39	0.0211
12	Streptomycin	108	34.95	201	65.04	0.0268
13	Tetracycline	286	92.55	23	7.44	0.0711

```
                                     10        20        30        40
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  GGCCTGAAGCCGGTACACCGTCGCGTACTTTACGCCATGA
                              G  L  K  P  V  H  R  R  V  L  Y  A  M   52
Sample1_gyrA gene            GGCCTGAAGCCGGTACACCGTCGCGTACTTTACGCCATGA
                              G  L  K  P  V  H  R  R  V  L  Y  A  M   52
Sample2_gyrA gene            GGCCTGAAGCCGGTACACCGTCGCGTACTTTACGCCATGA
                              G  L  K  P  V  H  R  R  V  L  Y  A  M   52

                                     50        60        70        80
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  ACGTATTGGGCAATGACTGGAACAAAGCCTATAAAAAATC
                             N  V  L  G  N  D  W  N  K  A  Y  K  K  S 66
Sample1_gyrA gene            ACGTATTGGGCAATGACTGGAACAAAGCCTATAAAAAATC
                             N  V  L  G  N  D  W  N  K  A  Y  K  K  S 66
Sample2_gyrA gene            ACGTATTGGGCAATGACTGGAACAAAGCCTATAAAAAATC
                             N  V  L  G  N  D  W  N  K  A  Y  K  K  S 66

                                     90       100       110       120
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  TGCCCGTGTCGTTGGTGACGTAATCGGTAAATACCATCCC
                               A  R  V  V  G  D  V  I  G  K  Y  H  P  79
Sample1_gyrA gene            TGCCCGTGTCGTTGGTGACGAAATCGGTAAATACCATCCC
                               A  R  V  V  G  D  E  I  G  K  Y  H  P  79
Sample2_gyrA gene            TGCCCGTGTCGTTGGTGACGTAATCGGTAAATACCATCCC
                               A  R  V  V  G  D  V  I  G  K  Y  H  P  79

                                    130       140       150       160
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  CACGGCGATTCCGCAGTGTATGACACCATCGTTCGTATGG
                              H  G  D  S  A  V  Y  D  T  I  V  R  M   92
Sample1_gyrA gene            CACGGCGATTCCGCAGTGTATTACACCATCGTTCGTATGG
                              H  G  D  S  A  V  Y  Y  T  I  V  R  M   92
Sample2_gyrA gene            CACGGCGATTCCGCAGTGTAATACACCATCGTTCGTATGG
                              H  G  D  S  A  V  *  Y  T  I  V  R  M   92

                                    170       180       190       200
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  CGCAGCCATTCTCGCTGCGTTACATGCTGGTGGATGGTCA
                             A  Q  P  F  S  L  R  Y  M  L  V  D  G  Q 106
Sample1_gyrA gene            CGCAGCCATTCTCGCTGCGTTACATGCTGGTGGATGGTCA
                             A  Q  P  F  S  L  R  Y  M  L  V  D  G  Q 106
Sample2_gyrA gene            CGCAGCCATTCTCGCTGCGTTACATGCTGGTGGATGGTCA
                             A  Q  P  F  S  L  R  Y  M  L  V  D  G  Q 106

                                    210       220       230       240
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  GGGTAACTTCGGTTCTATTGACGGCGACTCCGCGGCGGCA
                               G  N  F  G  S  I  D  G  D  S  A  A  A  119
Sample1_gyrA gene            GGGTAACTTCGGTTCTATTGACGGCGACTCCGCGGCGGCC
                               G  N  F  G  S  I  D  G  D  S  A  A  A  119
Sample2_gyrA gene            GGGTAACTTCGGTTCTATTGACGGCGACTCCGCGGCGGCA
                               G  N  F  G  S  I  D  G  D  S  A  A  A  119

                                    250       260       270       280
                             ....|....|....|....|....|....|....|....|
Standard|AL513382.1|S.typhi  ATGCGTTATACGGAGATCCGTCTGGCGAAAATCGCCCACG
                              M  R  Y  T  E  I  R  L  A  K  I  A  H   132
Sample1_gyrA gene            ATGCGTTATACGGAGATCCGTCTGGCGAAAATCGCCCACG
                              M  R  Y  T  E  I  R  L  A  K  I  A  H   132
Sample2_gyrA gene            ATGCGTTATACGGAGATCCGTCTGGCGAAAATCGCCCACG
                              M  R  Y  T  E  I  R  L  A  K  I  A  H   132
```

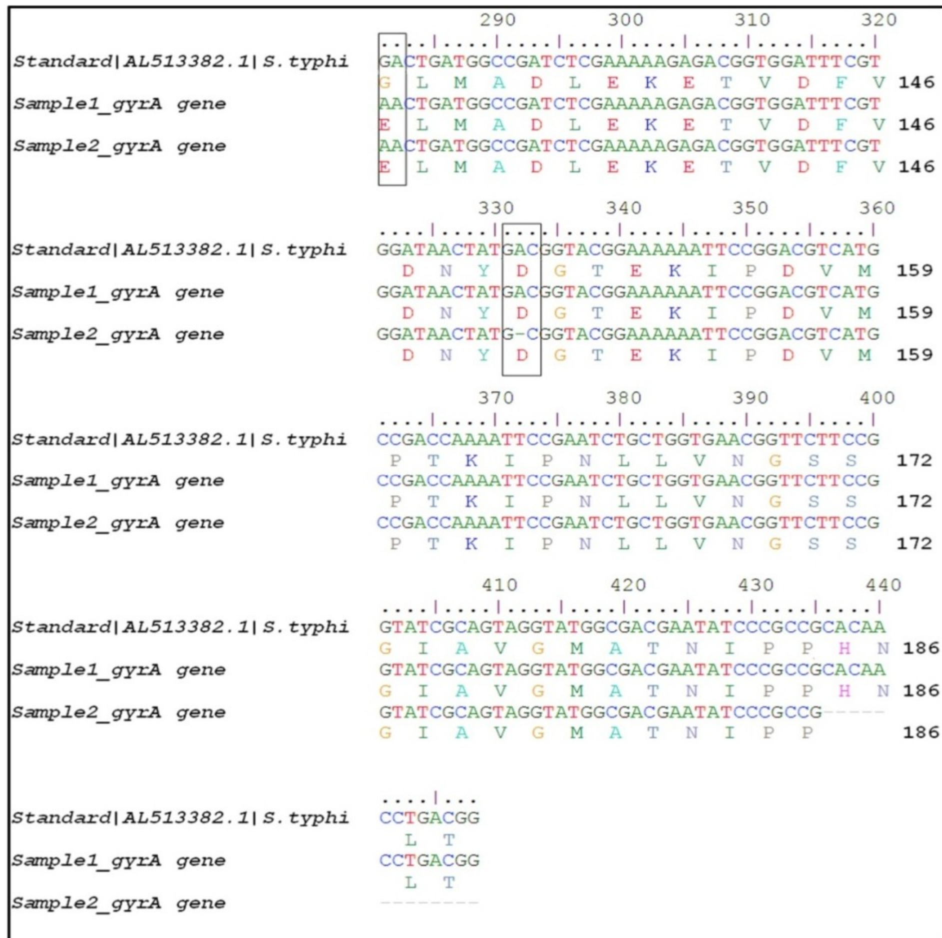

Figure.1 Mutations observed in *S. typhii* isolates

The clinical diagnosis of typhoid fever is difficult, as the presenting symptoms are often diverse and similar to those observed with other febrile illness. The defective diagnosis of typhoid fever requires the isolation of *Salmonella typhi* or paratyphi from the patients concerned. Widal test is helpful for rapid diagnosis of typhoid fever during epidemics (Wallace, 1972). Medical doctors are not familiar with microbiological tools; they rely the clinical picture or Widal test for the diagnosis of typhoid fever (Lunguya *et al.,* 2012).

Present study shows the presence of MDR *Salmonella* strains circulating in Amravati which present an increasing public health problem and the data reported herein will contribute towards epidemiological monitoring and investigations of *Salmonella* infection in Amravati. The isolates were analyzed to determine the antibiotic susceptibility pattern and the resistance was found against 13 different antibiotics (Table 2). In the present study the *S. typhi* strains showed highest resistant to ampicillin 98%, tetracycline 93% and chloramphenicol 57%. Similar results were reported by Hila *et al.* (2011) in their dynamic of antimicrobial resistance of *Salmonella typhimurium* isolated during the year 1985-2004 but having the slight difference in chloremphenicol resistance.

The resistance pattern showed 54.42% resistance to co-trimoxazole. 98% strains were found resistant to ampicillin and 57% resistance towards chloramphenicol. Similar

resistance pattern were studied by Yousefi-Mashouf and Mashtaghi (2007), Arora *et al.,* (2010).

Resistance to chloramphenicol was reported to emerge in only two years after its introduction in 1948 and was not until 1972 that typhoid fever causes by chloramphenicol resistant *S. typhi* become a major problem. Outbreak occurred in Mexico, India, Vietnam, Thailand, Korea and Peru. In present study sensitivity of chloramphenicol was also noted i.e. 42.39%. Chloramphenicol sensitivity documented in literatures ranges from 19.7-100% (Madhulika *et al.,* 2004).The less we use a particular drug, the probability of the organism becoming sensitive to the drug increases. Gentamycin is another commonly used antibiotic. In present investigation it shows the lowest resistance. The sensitivity shown by aztreonam and gentamycin was 24.25% and 72.49% respectively. Similar kind of result was reported by Mushtaq (2006), Ajayi and Egbebi (2011). The low resistance against gentamycin despite its heavy use could definitely attribute to intrinsic factor.

Five different quinolones were selected for testing in this study. It was found that the isolates had high resistance to the ciprofloxacin and low resistance or highest sensitivity to fourth generation gatifloxacin. Here we describe the emergence of novel quinolone resistance pattern among *Salmonella* isolates oriented from tertiary health care centers from Amravati. Population with such a resistance pattern has not been previously described.

Sensitivity of other quinolones i.e. norfloxacin, levofloxacin, ofloxacin and gatifloxacin were 73.46%, 77.34%, 79.93% and 89.92% respectively. Ofloxacin and gatifloxacin shows the highest sensitivity towards *Salmonella typhi*. Isolates with reduced susceptibility to fluroquinolones may further develop full resistance (Acharya *et al.,* 2011).

Genetic data demonstrating that alteration in *gyr*A or *gyr*B are alone sufficient to cause quinolone resistance and that DNA gyrase is a primary target of the quinolones *in vivo*. In other species the occurrence of single first step resistance mutations in *gyr*A also suggest that DNA gyrase is the primary target (Hooper, 1998).Quinolones are the most commonly prescribed antibacterial, their use is threatened by an increasing prevalence of resistance. The most common causes of quinolone resistance are mutations of a specific serine or acidic residue in the A subunit of gyrase or topoisomerase IV.

In the present study Asp 87 was substituted by Tyr. Griggs *et al.* (1996) studied the four veterinary isolates had substitutions at Asp 87; in three Asp 87 → Gly was found, resulting in the exchange of negatively charged residue with a small polar molecule and another had an Asp 87 → Tyr substitutions, substituting the negatively charged residue with large polar amino acid. Substitution in *E. coli* of Asp 87 → Asn, Val or Gly all lead to the loss of negative charge, which suggests that, the charge at this residue is important in the quinolone – gyrase interaction.The tyrosine is much larger than glycin. The MICs of quinolones associated with the substitutions in *Salmonella* are the same.

A single novel mutation was found in present study was outside the QRDR resulting in Ala 119 → Ala (A →C) this mutation was present in *gyr*A gene of the *Salmonella* isolate. Griggs *et al.* (1996) reported a similar mutation but Ala 119 get substituted with Glu. The substitution outside the region of the gene sequenced i.e. Ala 119 mutation is a silent mutation.

In present study the sequence of quinolone resistance determining region of these gene revealed that the presence of *gyr*Amutations at codon corresponds to Val 73 and Tyr 86. One of which was associated with a mutation at Asp 87. One mutant selected had both *gyr*A mutation at codon 86 and 87. Single *gyr*A mutation was also found highly resistant *in vitro* selected mutants (Giraud *et al.,* 1999).

Whatever the molecular mechanism of resistance of such strains, the main concern is detection of such isolates in clinical practice to prevent fluoroquinolone treatment failures. Ciprofloxacin drug MICs should be determined for all *Salmonella typhi* isolates. There is also a clear need to re-evaluate the clinical breakpoints for this pathogen. The major mutation in QRDR region was due to Asp 87 → Tyr and was predominant in the region.

References

Acharya, D., Bhatta, D.R., Malla, S., Dumre, S.P., Adhikari, N., Kandel B. P. 2011. *Salmonella enteric* serovarparatyphi A: an emerging cause of febrile illness in Nepal. *Nepal Med. Coll. J.,* 13(3): 69–73.

Ajayi, A.O., Egbebi A.O. 2011. Antibiotic susceptibility of *Salmonella typhi* and *Klebsiella pneumonia* from poultry and local birds in Ado-Ekiti, Ekiti-State, Nigeria. *Ann. Biol. Res.,* 2(3): 431–437.

Alcaine, S.D., 2007. Antibiotic resistance transmission and molecular ecology of *Salmonella enterica* subtypes from New York State. M.S. Thesis, Cornella University, New York.

Arora, D., Gupta, P., Gill, G., Chawla, R., Singla R. 2010. Changing trends in the antibiograms of *Salmonella* isolates in Nothern area of Punjab. *Int. J. Pharm. Pharm. Sci.*, 2(3): 135–137.

Clinical and Laboratory Standards Institute (CLSI). 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacterial isolates from animals; Approved standard -3rd Ed., CLSI document M13 -A3. CLSI, Wayen, Pennsylvia 19087-1898 USA.

DuPont H.L., 1993. Quinolones in *Salmonella typhi* infection. *Drug,* 45 (3): 119–124.

Gil, R., Álvarez, J.L., Gomez, C., Álvaro, A., Gil, A. 2009. Epidemiology of typhoid and paratyphoid fever hospitalizations in Spain (1997–2005). *Hum. Vaccines.,* 5(6): 420–424.

Giraud E., Brisabois, A., Martel, J.L., Chaslus-Dancia, E. 1999. Comparative studies of mutations in animal isolates and experimental *in vitro*- and *in vivo*- selected mutants of *Salmonella* spp. suggest a counterselection of highly fluoroquinolone – resistant strains in the field. *Antimicrob. Agents Chemother.,* 43(9): 2131–2137.

Griggs, D.J., Gensberg, K., Piddock, L.J.V. 1996. Mutations in *gyrA*genes of quinolone-resistant *Salmonella* serotype isolated from humans and animals. *Antimicrob. Agents Chemother.,* 40(4): 1009–1013.

Hila N., Devolli, A., Puto, K., Mali, S., Brahimaj, Z., Peqini, E., Dervishi, A. 2011. The dynamic of antimicrobial resistance of *Salmonella typhimurium*isolates. *J. IMAB,* 17: 111–115.

Hooper, D.C., 1998. Bacterial topoisomerases, anti-toposisomerase, and anti-toposiomerase resistance. *Clin. Infect. Dis.,* 27: S54– S63.

Imberechts H., Hooghe, I.D., Bouchet, H.,

Godard, C.L., Pohl, P. 2000. Apparent loss of enrofloxacin resistance in bovine *Salmonella typhimurium* strains isolated in Belgium, 1991 to 1998. Veter. Rec., 147: 76–77.

Khan, K.H., Ganjewala, D., Rao, K.V.B. 2008. Recent advancement in typhoid research - a review. *Adv. Biotech.,* 7(4): 35–41.

Lunguya, O., Phoba, M.F., Mundeke, S.A., Bonebe, E., Mukadi, P., Muyembe, J.J., Verhaegen, J., Jacobs, J. 2012. The diagnosis of typhoid fever in the democratic republic of the Congo. *Trans. R. Soc. Trop. Med. Hyg.,* 106 (6): 348–355.

Madhulika, U., Harish, B.N., Parija, S.C. 2004. Current pattern in antimicrobial susceptibility of *Salmonella typhi* isolates in Pondicherry. *India. J. Med. Res.,* 120: 111–114.

Miriagou V., Carattoli, A., Fannings S. 2006. Antimicrobial resistance islands: resistance gene clusters in *Salmonella* chromosome and Plasmids. *Microb. Infect.,* 8(7): 1923–1930.

Murray, B.E. 1986. Resistance of *Shigella*, *Salmonella,* and other selected enteric pathogens to antimicrobial agents. *Rev. Infect. Dis.,* 8(2): 172–181.

Mushtaq, M.A., 2006. What after ciprofloxacin and ceftriaxone in treatment of *Salmonella typhi*. *Pak. J. Med. Sci.,* 22(1): 51–54.

Renuka, K., Sood, S., Das, B.K., Kapil, A. 2005. High-level ciprofloxacin resistance in *Salmonella enterica* serotype Typhi in India. *J. Medi. Microbiol.,* 54(10): 999–1000.

Therelfall, E.J., Graham, A., Cheastry, T., Ward, L.R., Rowe, B. 1997. Resistance to ciprofloxacin in pathogenic Enterobacteriaceae in England and Wales in 1996. *J. Clin. Pathol.,* 50: 1027–1035.

Wallace, G.J. 1972. Typhoid and its serology. *Br. Med. J.,* 1: 921–922.

Yousefi-Mashouf, R., Moshtaghi, A.A. 2007. Frequency of typhoidal and *Salmonella* species and detection of their drug resistance patterns. *J. Res. Health Sci.,* 7(1): 49–56.