1. INTRODUCTION
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Salmonella enterica serovar Typhi (S. Typhi) and Salmonella enterica serovar Paratyphi (S. Paratyphi A, B and C) causes typhoid and paratyphoid fever, respectively. Enteric fever (typhoid and paratyphoid fever) is an acute systemic infection caused by the bacterium. Infection is usually acquired through ingestion of food or water contaminated with Salmonella (Crump and Mintz, 2010).

Enteric fever is a major public health problem and is endemic in many developing countries, including India and has a mortality rate of 30%. This mortality rate can be reduced to 1 - 4% by appropriate treatment (Cooke and Wain, 2004; Buckle et al., 2012).

Enteric fever due to S. Typhi is traditionally believed to be more common than S. Paratyphi A, but studies from India (Sood et al., 1999; Tankhiwale et al., 2003), China (Ochiai et al., 2005) and Nepal (Karkey et al., 2013) have documented an increasing proportion of enteric fever cases due to S. Paratyphi A.

Pathogenic processes due to Salmonella are dictated by an array of virulence factors that act in tandem and ultimately manifest in the typical symptoms of the disease. Virulence genes encode products that assist the organisms in expressing its virulence in the host cells (Murugkar et al., 2003; Hensel, 2004).
Plasmid encoded fimbriae (PEF) involves in adhesion, invasion of intestine epithelial cells and colonization of Peyer’s patches (Bäumler et al., 1996; Ogunniyi et al., 1997; van der Velden et al., 1998). Only four serotypes, S. Typhimurium, S. Enteritidis, S. Choleraesuis, and S. Paratyphi C, have been reported to contain pef gene sequences (Darwin and Miller, 1999; Townsend et al., 2001).

Salmonella enterotoxin (stn) gene is associated with the manifestation of pathogenic processes in the host system and it may be responsible for loss of fluid and electrolytes from small intestine (Rumeu et al., 1997; Murugkar et al., 2003; Skyberg et al., 2006). Studies from India (Riyaz-Ul-Hassan et al., 2004) and Japan (Makino et al., 1999) indicated that stn gene is highly conserved and can be used as a molecular tool for the detection of Salmonella spp. from clinical samples. However, detailed documentation of the distributions of these genes (i.e. pef and stn) among clinical isolates of typhoidal Salmonella is not available.

Earlier treatment of choice for infections due to Salmonella included chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole (co-trimoxazole) or tetracycline (ACCoT) (Sirinavin and Garner, 1999; WHO, 2003). However, Salmonella has rapidly gained resistance to these antibiotics (Jesudason and John, 1992; Rowe et al., 1997). Later, fluoroquinolones, such as ciprofloxacin became the drug of choice for the treatment of enteric fever (Eykyn and Williams, 1987; Mandal, 1991; Parry et al., 2002; Thaver et al., 2009).
Chloramphenicol resistance is mediated by the plasmid encoded chloramphenicol acetyltransferases (CAT) (Cannon et al., 1990) or by non-enzymatic chloramphenicol resistance gene cmlA which encodes an efflux pump (Dorman and Foster, 1982). Resistance due to chloramphenicol/florfenicol exporter encoded by plasmid-borne gene floR has also been reported in Salmonella enterica serovars (Boyd et al., 2002; Chen et al., 2004; Doublet et al., 2004).

Resistance to the β-lactam antibiotic, ampicillin is mainly due to hydrolysis of the antibiotic by β-lactamase enzyme. In clinical enterobacterial isolates, ampicillin resistance occurred most commonly due to plasmid encoded TEM- and SHV-β-lactamases (Du Bois et al., 1995; Medeiros, 1997).

Trimethoprim-sulfamethoxazole resistance is mediated by alteration in the enzyme targets dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) respectively (Huovinen, 2001).

Trimethoprim-insensitive DHFRs are encoded by different dfr genes. Based on the amino acid sequences encoded by dfr genes, they are classified into two major types, dfrA and dfrB (White and Rawlinson, 2001). Most of the dfr genes have been found on transposons, plasmids and reside as gene cassettes within variable parts of integrons which assist in the spread of trimethoprim resistance (Ojo et al., 2002; Blahna et al., 2006).
Sulfonamide resistance in Gram-negative enteric bacteria is due to drug resistant variants of DHPS enzymes encoded by plasmid-borne *sul* (*sul1*, *sul2* and *sul3*) genes (Sköld, 2000; Perreten and Boerlin, 2003).

In Gram-negative microorganisms, tetracycline resistance is mainly due to efflux proteins expressed by *tet* genes (Chopra and Roberts, 2001). Different classes of tetracycline resistance determinants have been reported (Levy *et al*., 1999), in which *tet*(A) to *tet*(E) determinants are generally responsible for tetracycline resistance in *Enterobacteriaceae* (Frech and Schwarz, 1998; Chopra and Roberts, 2001). The *tet*(G) gene has been identified in the chromosome (salmonella genomic island 1) of *Salmonella enterica* serovar Typhimurium DT104 (Cloeckaert and Schwarz, 2001; Boyd *et al*., 2001). Localization of *tet* genes on mobile genetic structures such as plasmids, transposons, and integrons might accelerate the spread of tetracycline resistance among bacteria (Sandalli *et al*., 2010).

Molecular analysis of ACCoT resistant or multidrug resistant (MDR) *S. Typhi* revealed that the resistance to all the first line drugs was encoded by a conjugative plasmid of incompatibility group (Inc) HI1 (Threlfall *et al*., 1992; Parkhill *et al*., 2001).

During 1990s, the incidence of MDR *S. Typhi* in India was as high as 60%. After a decade, study reports from Pune, Nagpur, Kolkata and New Delhi showed reduced MDR
percentage (Sanghavi et al., 1999; Chande et al., 2002; Saha et al., 2002; Rai et al., 2012).

There are reports on emergence of fluoroquinolone-resistant isolates in various parts of Asia and there have been a few reports of resistance to third-generation cephalosporins in the same region (Das and Bhattacharya, 2000; Dutta et al., 2001; Gupta et al., 2001; Nagshetty et al., 2010; Thamizhmani et al., 2012). S. Paratyphi A resistant to ciprofloxacin also has been reported in Pudhucherry, India (Harish et al., 2006). *Salmonella* spp. resistant to nalidixic acid by *in-vitro* susceptibility methods has been reported to show reduced fluoroquinolone susceptibility *in-vivo*, resulting in treatment failure (Asna et al., 2003; Mandal et al., 2012b).

The exact mechanism of quinolone resistance is not fully understood but various studies have found that mutation in the quinolone resistance-determining regions (QRDRs) of *gyrA* and/or *gyrB* and/or *parC* and/or *parE* gene were found to confer resistance to nalidixic acid and reduced susceptibility to fluoroquinolones (Eaves et al., 2004; Dahiya et al., 2014).

Plasmid mediated resistance to quinolones due to proteins encoded by *qnr* (*qnrA, qnrB* or *qnrS*) genes, have been documented in several enterobacterial species including *Salmonella* (Cheung et al., 2005; Nordman and Poirel, 2005; Gay et al., 2006; Kehrenberg et al., 2006). Qnr protein was shown to protect the target enzymes, DNA
gyrase and topoisomerase IV from inhibition by the fluoroquinolone (ciprofloxacin) (Jacoby, 2005).

AAC(6′)-Ib-cr, a variant of aminoglycoside acetyltransferase (AAC(6′)-Ib) capable of modifying ciprofloxacin by N-acetylation of its piperazinyl amine and reducing the activity of ciprofloxacin, was found to emerge and reported to be more prevalent than Qnr proteins (Robicsek et al., 2006a; Park et al., 2006; Yu et al., 2011b). The plasmid mediated quinolone resistance gene, *aac(6′)-lb-cr* has been described in several enterobacterial isolates and few reports documented the presence of *aac(6′)-lb-cr* gene in non-typhoidal *Salmonella* (Xia et al., 2009; Sjölund-Karlsson et al., 2010).

Plasmid-mediated quinolone efflux pump, QepA which has the ability to confer fluoroquinolone resistance has been reported in clinical enterobacterial isolates (Yamane et al., 2008; Park et al., 2009). Study report revealed that nalidixic acid and ciprofloxacin MICs of bacteria harbouring the *qepA* gene increased 2-, and 32-fold, respectively (Yamane et al., 2007).

Broad-spectrum cephalosporins can be used as a therapeutic alternative for the treatment of MDR and fluoroquinolone resistant *Salmonella* infection (Naqvi et al., 1992; Capoor and Nair, 2010). Cephalosporin resistance due to the production of extended-spectrum β-lactamase (ESBL) is a cause of serious concern worldwide (Su et al., 2004; Schmiedel et al., 2014).
Many species of the family *Enterobacteriaceae* found to contain ESBL enzymes including different serovars of *Salmonella enterica*. Most ESBLs in *Salmonella* are derivatives of TEM and SHV β-lactamase families. Other group like, CTX-M has also been described (Pokharel *et al.*, 2006).

Infection with ESBL producing *Salmonella* can be treated effectively with fluoroquinolones (Al Naiemi *et al.*, 2008). Studies were also reported on *qnr* positivity along with ESBL production in enterobacterial isolates (Mammeri *et al.*, 2005; Nordman and Poirel, 2005). Plasmids with *qnr* genes have been found to co-transfer TEM-, SHV-, and CTX-M genes (Jacob *et al.*, 2003; Jacob *et al.*, 2006; Hopkins *et al.*, 2007).

Carbapenems are β-lactam antibiotics with broad-spectrum activity and are resistant to ESBL-mediated hydrolysis (Papp-Wallace *et al.*, 2011). Metallo-β-lactamase (MBL) enzyme confers resistant to carbapenems and almost all β-lactams except monobactams. Transferable types of MBL genes (*bla*IMP and *bla*VIM) have been reported to spread among bacterial community, which further complicates the situation (Walsh *et al.*, 2005; Savard *et al.*, 2011; Jamal *et al.*, 2013).

The purpose of the present study was to know the prevalence of plasmid encoded fimbrial gene and *Salmonella* enterotoxin gene and to assess the antibiotic resistance pattern of clinical isolates of *Salmonella* as well as to analyse the molecular mechanisms responsible for antibiotic resistance.