Introduction

Antimicrobial resistance is a complex global public health challenge, and many factors are responsible for the emergence and spread of infectious organisms that become resistant to the available antimicrobial drugs. The development of antimicrobial resistance is a natural phenomenon in microorganisms, and is accelerated by the selective pressure exerted by the use and abuse of antimicrobial agents in humans and animals. The current lack of new antimicrobial agents on the horizon to replace those that become ineffective brings added urgency to the need to protect the efficacy of existing drugs. The development and implementation of effective strategies to curtail the emergence and spread of antimicrobial resistance, and to evaluate the effect of interventions to do so, depend on the collection of accurate representative information on the extent of the problem and its impact. WHO has for many years promoted the global monitoring of antimicrobial resistance and taken steps to raise awareness among the impending public health crisis it will cause. Among a range of WHO initiatives, in 2001 the Global strategy for containment of antimicrobial resistance (WHO, 2001) was published.

Antibiotics are therapeutic agents needed for microbial eradication (Mainous et al., 2008). Unfortunately, despite public awareness and concern of health care providers, global irrational use of antibiotics is on a rise (50% to almost 100%) (Gaash, 2008; Zafar et al., 2008). Extensive irrational use of antimicrobials without medical guidance may result in greater probability of inappropriate, incorrect, or undue therapy, missed diagnosis delays in appropriate treatment, pathogen resistance and increased morbidity (Parimi et al., 2004, Matuz et al., 2007). Emergence of human pathogen resistance to antibiotics, both due to over and underuse is potentially dangerous for both individuals and societies (Matuz et al., 2007, Sahoo, 2008).

‘Self medication has been defined as obtaining or consuming drugs without the advice of a physician either for diagnosis, prescription or surveillance of treatment’ (Montastruc et al., 1997, Zafar et al., 2008). In majority of economically underdeveloped countries, nearly 60-80% of health related problems are treated through self-medication as lower cost alternative (Awad et al., 2005, Abay et al.,

2010). Self medication is a universal phenomenon and practised globally with varied frequency (Patel et al., 2013). In India, like other medicines, fluoroquinolone antibiotics are easily accessible to everyone without a prescription, and hence chances of developing resistance towards this group of antibiotics is very high (Chaudhary et al., 2015).

The fluoroquinolones have become the drugs of choice for the empirical treatment of urinary tract infection and acute diarrhoea in adults, because they are active against most of the common treatable enteropathogens, have excellent tissue and intracellular penetration, achieve high fecal concentrations, are suitable for oral administration, and have a favourable safety profile in adults (Hooper and Wolfson., 1991). Nalidixic acid was the first clinically useful quinolone, discovered by Lesher and co-workers in 1962, which was generated as by-product from chloroquine, an antimalarial agent (Appelbaum and Hunter. 2000). It was active against some Gram negative bacteria and the usefulness was limited because of its high protein binding (approximately 90%) and little half life (about 1.5 h) (Dollery,1999), as a result of which bacteria developed a rapid resistance to this agent (Pandey, 2003). In 1968, Kaminsky and Melfezer discovered anoxolinic acid, which was later on approved by the United States Food and Drug Administration (USFDA) (Pandey, 2003). Since then, efforts has been undertaken for the development and derivation of an array of significantly active drugs of this class. Certain modification at the molecular level such as lead optimization by bioisosteric replacements, homologation of side chain or branching of side chain, stereochemistry and other useful techniques of analogs design were done to develop fluoroquinolones with broad spectrum activity and minimum toxic or side effects (Appelbaum and Hunter. 2000).

Enhanced antimicrobial activity of fluoroquinolone has extended its use beyond the traditional indications for quinolone antibiotics, in the treatment of urinary tract infections. The fluoroquinolones are effective in a wider variety of infectious diseases, including skin and respiratory infections. Because of their excellent safety and tolerability, they have become alternatives to penicillin and cephalosporin derivatives in the treatment of various infections (Dana et al., 2000).

Formation of drug- enzyme- DNA complexes blocks DNA replication (Drlica et al., 2008). Quinolones have been prescribed widely to treat respiratory tract infections, including tuberculosis, urinary tract infections (UTIs), intraabdominal infections, skin and skin structure infections, sexually transmitted diseases, and bone and joints infections. They have also been used for prophylaxis in neutropenic patients with cancer, in cirrhotic patients at risk for spontaneous bacterial peritonitis, and in urologic surgery (Freidfeld et al., 2011). The increased use of quinolones steadily increased from 1994 to 2000 in many countries and this use was significantly associated with decreased overall susceptibility to ciprofloxacin in the same period. While newer class quinolones that expand the spectrum of activity to include Gram-positive bacteria and even anaerobes have been developed, quinolone resistance has increased in many bacterial species (Bratzler et al., 2013).

Resistance to quinolone are generally classified into three types: 1) chromosomal mutation altering the drug target enzyme to reduce drug binding, 2) chromosomal mutation that increases expression of native efflux pumps that transport quinolones to the outside of the bacterial cell, and 3) plasmid-acquired resistance genes producing either protection of target enzymes, drug modification, or drug efflux (Hooper, 2003)

Quinolone resistance mutations in the target enzymes generally occur first in the GyrA subunit of DNA gyrase in Gram-negative bacteria or in the ParC subunit of topoisomerase IV in Gram-positive bacteria (Jacoby, 2005). These resistance mutations occur most often in a region referred to as "quinolone resistance-determining region (QRDR)", which encompasses amino acids 51 to 106 in GyrA and 23 to 176 in ParC, with positions 83 and 87 most common in ParC (Yoshida et al., 1990; Friedman et al., 2001). These substitutions are thought to result in a reduced affinity of gyrase or topoisomerase IV for quinolones (Willmott and Maxwell, 1993; Barnard et al., 2001). In *Staphylococcus aureus* or *Streptococcus pneumoniae,* the primary mutation occurs mostly at ParC (Ng et al., 1996; Eliopoulos, 2004). In both Gram- negative and Gram-positive bacteria, combination of mutation in both GyrA and ParC generally results in progressively higher levels of resistance. Less often mutations in GyrB and ParE have also contributed resistance in clinical isolates.

Bacteria have a number of energy-dependent efflux systems in the cell membrane and envelope that can facilitate extrusion of potentially toxic agents, and many of these efflux pumps have broad substrate profiles that can include quinolones (Poole. 2005). AcrAB-TolC is the major pump contributing to quinolone resistance in *E. coli* (Jacoby, 2005). Mutation in *acrR,* which represses *acrAB,* can increase pump expression (Wang et al., 2001). In addition, mutation in *marR,* a repressor of *marA,* which activates *acrAB* and *tolC,* also cause increase of efflux (Alekshun and Levy, 1997). The product of *marA* gene also decreases the expression of OmpF, outer membrane porin protein (Cohen et al., 1989). Consequently, *marR* mutations have the dual effect of decreasing influx and increasing efflux of quinolones. Expression of *acrAB* is also induced by exposure to salicylates and bile salts, and AcrAB confers relative resistance to bile salts, thereby facilitating the ability of *E. coli* to live the intestinal tract (Thanassi et al., 1997). Efflux pumps that include quinolones among their substrates have also been associated with resistance in a number of other Gram-negative bacteria, being most extensively studied in *Pseudomonas aeruginosa.* There are atleast five known efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM and MexVW-OprM) that have shown to efflux quinolones in *P. aeruginosa* (Zhanel et al., 2004). In *S. aureus,* quinolone resistance has been associated with increased expression of NorA, NorB and NorC pumps with both *norA* and *norB* over-expression regularly found in clinical isolates (Kwak et al., 2013). Efflux also contributes to quinolone resistance in *S. pneumoniae* and mycobacteria.

Plasmid mediated quinolone resistance (PMQR) was discovered in 1998 in a clinical isolate of *Klebsiella pnemoniae* that could transfer low level quinolone resistance to Gram-negative bacteria. The responsible gene for PMQR was named *qnr*A, Qnr protein was shown to bind and protect DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin. Qnr itself provide low-level resistance to quinolones but its presence can facilitate the selection of additional resistance mutations (Martinez-Martinez et al., 1998). It was soon discovered in a growing number of organisms and is broadly distributed geographically, including other *Klebsiella pneumoniae* strains in United States (Wang et al., 2004), *E. coli* isolates in Shanghai (Wang et al., 2003), and *Salmonella enteric* in Hong Kong (Cheung et al., 2005). The gene *qnr*A was subsequently followed by discovery of *qnr*S (Hata et al., 2005), *qnr*B (Jacoby et al., 2006) *qnr*C

(Wang et al., 2009), and *qnr*D (Cavaco et al., 2009). The *qnrVC* gene from *Vibrio cholera* can also be located on a plasmid (Xia et al., 2010) or in transmissible form as part of an integrating conjugative element (Kim et al., 2010). Other PMQR mechanisms were also identified. One is *aac(6')-Ib-cr,* which is a variant of *aac(6')-Ib,* which encodes an aminoglycoside acetyltransferase (Robicsek et al., 2006). *AAC (6')-Ib-cr* confers low-level ciprofloxacin at the amino nitrogen on its piperazinyl substituent. *Aac(6')-Ib-cr* has also been associated with other PMQR genes including diverse *qnr* genes and beta-lactamase genes (Strahilevitz et al., 2009). The other PMQR mechanism is plasmid- mediated quinolone efflux. Two plasmid mediated quinolone transpoters have also been identified: OqxAB (Hansen et al., 2004) and QepA (Yamane et al., 2007).

Fluoroquinolone antimicrobial drugs are highly bio- available, broad-spectrum agents with activity against Gram-negative pathogens, especially those resistant to other class of antimicrobial drugs, first-line empiric therapy for urinary tract infections, upper and lower tract infections, enteric infections and gonococcal infection. They are particularly useful against deep infections caused by Gram- negative bacteria, including those, such as *Pseudomonas* spp., that are resistant to other orally administered antimicrobial drugs. Specific quinolone antimicrobial drugs administered to pets and food-producing animals are known to transmit cross-resistance to humans (Acar and Goldstein, 1997).

Fluoroquinolone resistant enterobacterial isolates responsible for outbreaks are being reported from different regions of the world. However, their prevalence differs in different geographical regions. Also the prevalence of plasmid mediated quinolone resistance determinants varies. Therefore, local estimation would help the clinicians to make suitable decisions for that particular region. While observing the global scenario of PMQR determinants, it has been observed that very less data is available from north- eastern part of India, especially from Assam. Studies by Ajij et al (2015) reported the prevalence of *qnr* determinants in commensals of healthy volunteers.

Another problem associated with fluoroquinolone resistance is that these antibiotics are often misused as they are available over the counter and the carriage of quinolone resistance determinants in normal gut flora demands the question to be addressed

as to whether the usage rate of these antibiotics selects the specific *qnr* genes in the environment or the organisms harbouring these genes select the specific antibiotics for their survival, vertical transfer of resistance genes and persistence within community and hospital environment.

The self medication, suboptimal dosage and irrational use of quinolone drug have led to the development of resistance against this group of antibiotics. This study will help in developing or adopting policies for use of first line drugs in hospital and in community. The prevalence of these genes are of grave concern as it may be horizontally transferred to other human pathogenic bacteria and can lead to therapeutic failure as a consequence of antimicrobial resistance. Carriage of quinolone resistance in normal gut flora by horizontal acquisition and their dissemination in community causing antibiotic resistance can also be assessed. The lack of data in this region will encourage further studies on the implications of presence, distribution, association and variation of these determinants in our quest for understanding PMQR.

Fluoroquinolone drugs are empirically used in many infections but the incidence of resistance is constantly increasing. Also there is a lack of review of resistance in records in last decades. However, in this geographical location of this country, there are no reports on prevalence rate, phenotypic and genotypic characterization of *qnr* genes. The knowledge of molecular characterization of *qnr* genes, epidemiology, genetic context, transmission dyanamics, origin and acquisition of *qnr* genes will help in fulfilling the gap and facilitating the search for a phenotypic marker in clinical microbiology laboratory which will help the clinicians to implement appropriate antimicrobial chemotherapy. Surveillance of local resistance trends will be important, and careful and selective use of quinolone will be warranted and it will provide the treatment option to the infection caused with enterobacterial isolates that are resistant to fluoroquinolones.

The increase in quinolone resistance is now threatening the clinical utility for treatment of diverse infections. There must be some intrinsic and extrinsic factors responsible for the development of quinolone resistance among the isolates that are resistant to fluoroquinolones. These mechanisms (intrinsic and acquired) of quinolone

resistance are developed during the course of antmicrobial chemotherapy or under selection pressure. Much yet to be known about these genes, whether the usage rate of these antibiotics selects the specific *qnr* genes in the environment or the organism selects these genes in a specific antibiotic pressure for their survival, vertical transfer of resistance genes and persistence within community and hospital environment. The selectivity of these *qnr* genes can be used to develop a screening tool which can highlight epidemiological, diagnostic and therapeutic insight into quinolone resistant Enterobacteriaceae.

Thus the study was carried out with following objectives:

i. To determine phenotypic markers for screening of quinolone resistant isolates in hospital and community, and to genetically characterize of quinolone resistance determinants.

ii. To study genetic environment, gene location and transferability of quinolone resistance determinants.

iii. To determine clonal dissemination/diversity of quinolone resistant isolates by DNA fingerprinting.