Review of literature

Anti-infective chemotherapy is the branch of science that deals with administering chemical agents to treat infectious diseases. Ehrlich was the first to discover successfully the purely synthetic revolutionary antimicrobial drug salvarsan in 1910 (Patrick, 2003). Afterwards, beta-lactam antibiotic penicillins and sulfonamide have been the successful and promising drugs in clinical usage (Applebaum et al., 2000).

Quinolones and fluoroquinolones are a class of synthetic antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections (Abraham, 2003), respiratory tract infections (RTI), sexually transmitted diseases (STD) and skin infections (Guneysel et al., 2009). They are primarily used against many infections, viz. urinary tract infections and are clinically useful against prostatitis, skin infection and bones and penicillin resistant sexually transmitted diseases. These agents are also used for the treatment of bacterial enteric infections, prophylaxisin the immunocompromised neutropenic host. New quinolones are also used as an alternative antibacterial therapy, especially in areas where the prevalence of penicillin resistant and macrolide-resistant organisms exists (Abraham, 2003).

2.1 Historical Background:

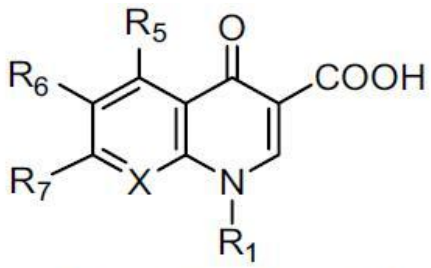
Nalidixic acid was the first clinically useful quinolone, discovered by Lesher and co-workers in 1962, which was generated as by-product from choloquine, antimalarial agent (Applebaum et al., 2000). It was active against some gram negative bacteria and the usefulness was limited because of its high protein binding (approximately 90%) and little half-life (about 1.5h) (Dollery, 1999). As a result, bacteria developed a rapid resistance to this agent (Pandey, 2003 and Sarkozy, 2001). In 1968, Kaminsky and Melfezer discovered anoxolinic acid, which was later approved by the United States Food and Drug administration (USFDA) (Pandey, 2003). Since then, efforts have been undertaken for the development and derivation of an array of significantly active drugs of this class.

Certain modifications at the molecular level such as lead optimization bybioisteric replacements, homologation of side chain or branching of side chain, stereochemistry or other useful techniques of analog design were done to develop of fluoroquinolones with broad spectrum activity and minimum toxic or side effects. New antibiotics were developed and achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets. The development was based on the following aspects: Increasing activity against resistant strains of microbes, anaerobes and typical organisms, reducing rate of emergence of resistance, improving pharmacokinetics and pharmacodynamics profile, targeted towards selectivity of drugs. Flumequine was patented in 1973, the first fluroquinolone, after which many fluroquinolones have been patented and are still used today, including norfloxacin (1978), pefloxacin (1979), enoxacin (1980), fleroxacin (1981), ciproflaxicn (1981) and ofloxacin (1982) (Applebaum et al., 2000). Advantages of these compounds over previous ones are their spectrum of activity. In 1980 an analog of naldixic acid, enoxacin was derived with significantly increased spectrum of gram negative or gram positive bacteria (Patrick, 2003).

Ciprofloxacin was successful and widely marketed in 1986, and since then the fluroquinolones were used for the treatmernt of wide variety of infections (Applebaum et al., 2000). This class of compounds has upgraded pharmacokinetic properties and additionally broad and strong activity against different parasites, bacteria and mycobacteria, including safe strains compared to already existing bactericidal medications (De Almeida et al., 2007 and Anquetin et al., 2006).

2.1.1 Generation and development of Quinolones

Based on 4-quinolone nucleus (Fig 1), the quinolones comprise a relatively large and expanding group of synthetic compounds. Price was the first to prepare an alkaloid having quinolone structure but it possessed no biological activity. In 1960, Barton et al**,** isolated 6- chloro-1H- ethyl-4-oxo-quinoline- 3-carboxilic acid during antimalarial research, which showed antibacterial activity.



quinolones: X= CH or C-R_8
naphthyridones: X= N

Figure 1. Common structure of 4-quinolones.

Image adopted from Emami et al. (2005). Iranian Journal of Pharmaceutical Research (2005) 3: 123-136

In 1962, during the process of synthesis and purification of choloroquine (antimalarial agent), a napthyridine derivative, nalidixic acid 1 was discovered which possessed bactericidal activity (Lescher et al., 1962). However, its clinical use was limited to the treatments of UTIs caused by the majority of Gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*. The clinical usefulness of nalidixic acid, in the treatment of other infections than UTIs, was limited by its low serum concentrations and high minimum inhibitory concentrations (MIC 4-16 mg/L) (Wise, 1984). Thereafter, novel compounds of this family, such as oxoclinic acid 2 , pipemedic acid 3 and cinoxacin 4 were synthesized and introduced into clinical practice (Figures 2 and 3).

In spite of the fact that the clinical ramifications of the quinolones were kept just for UTIs (Norris et al.,1988), these early operators, in any case, demonstrated priceless in the treatment of uncomplicated UTIs, for example, cystitis. Nalidixic acid has a few basic components held by the more current compounds, and its activity depends on a 4-oxo-1, 8-naphthyridin-3-carboxylic acid core. Two noteworthy gatherings have been created from the essential structure: quinolones and napthyridones (Domagala, 1994). The presence of a nitrogen at position 8 recognizes the quinolones (Fig 1). The quinolones and napthyridones were further enhanced by the expansion of gatherings to the N-1, C-5, C-6 and C-7 positions of their separate essential particles. Until the advancement of flumequine, the primary monofluoroquinolone in 1976, none of the prior mixes had offered any critical upgrades over nalidixic acid. Flumequine was the principal compound to be created with a fluoro bunch at position, and gave the primary signs that changes of the essential chemical structure could enhance gram-

positive activity (Appelbaum et al., 2000). Its range of activity embraced the Enterobacteriaceae, including some strains that were resistant to nalidixic acid with useful acitivity against uncomplicated gonorrhea, albeit with a two- or three dose regimen. In 1978, norfloxacin 6, a –fluorinated quinolone with a piperazinyl side-chain at position 7, was developed. Norfloxacin 6 had a longer half-life than the earlier compounds (3-4 h), less protein binding (50%) and improved Gram-negative activity (Koga et al., 1980). The addition of fluorine atom at position 6 was one of the earliest changes of the basic structure. The single alteration provides a more than 10-fold increase in gyrase inhibition and upto 100-fold improvement in MIC.

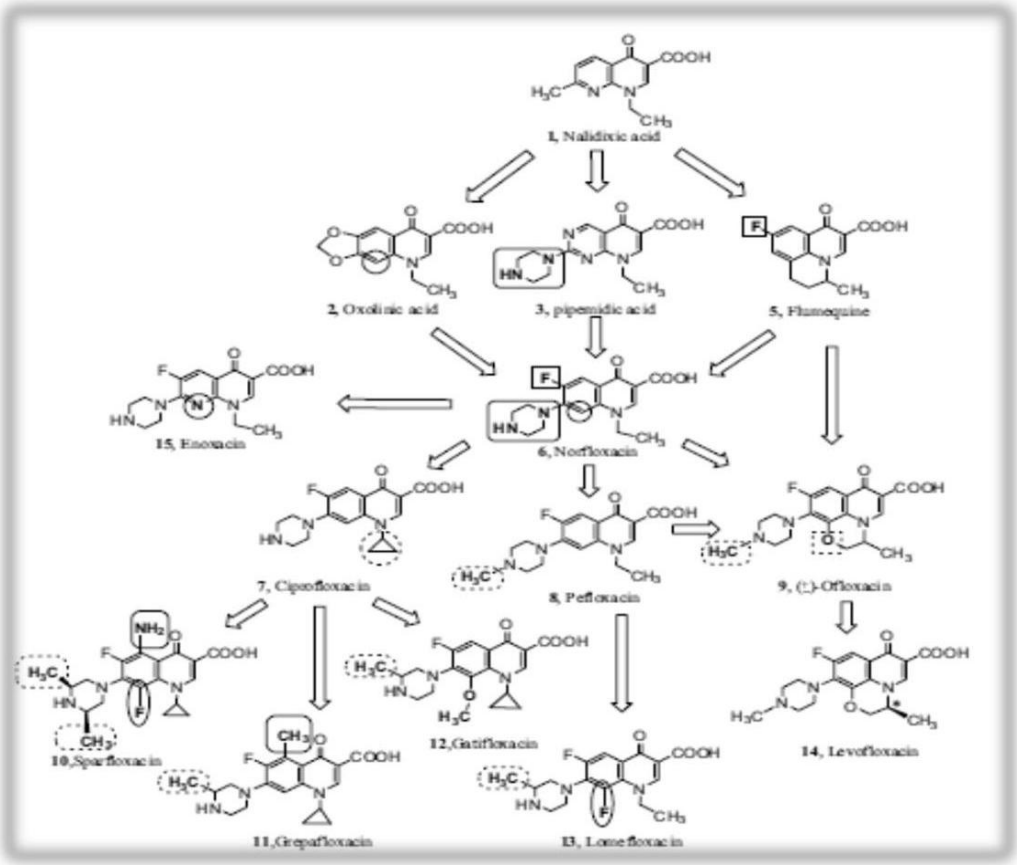


Figure 2: Structural development of 7- piperazinylquinolone from primary quinolones.
(Image adopted from Emami et al. (2005). Iranian Journal of Pharmaceutical Research (2005) 3: 123-136).

During the 1980s, an incredible number of fluoroquinolones were created. Notwithstanding the fluorine atom at the C-7 position, a cyclopropyl gathering was acquainted with the N-1 position and is best exemplified by ciprofloxacin, which was initially combined in 1983 (Figures 2 and 3). This builds the intensity of the medication and numerous resulting quinolones have a cyclopropyl bunch. The benzopyridone core (quinolone) turned out to be more responsive for synthetic control which can be useful to upgrade antibacterial strength. Subsequent discovery of fluorine molecule and piperazinyl ring on the quinolone ring specifically norfloxacin, ciprofloxacin, pefloxacin, ofloxacin altered the chemistry of fluroquinolones. These agents show powerful action against the Gram-negative bacteria, however not against the Gram-positives bacteria or anaerobes. In the 1990s,

further alterations of the quinolones resulted in the discovery of novel compounds that not only showed the potency against the Gram-negative bacteria but also against the Gram-positives. A number of other structural manipulations have been tried to improve the anti-gram-positive activity of fluroquinolones. One of the first additions was an NH2 group at position C-5, which resulted in a general increase in anti-gram-positive activity (Applebaum et al., 2000).

Table 1: Clinical development of Quinolone Drug

Date	Quinolone
1960-1969	Nalidixic acid
1970-1975	Cinoxacin
1975-1985	Norfloxacin
1985-1990	Ciprofloxacin, Oflxacin
1990-1995	Temafloxacin, sparfloxacin
1995-2000	Grepafloxacin, levofloxacin, trovafloxacin
2000-2005	Moxifloxacin, possibly gemifloxacin and garenoxacin in 2003 or later

This is seen with sparfloxacin which otherwise has a very similar structure to ciprofloxacin. Sparfloxacin also has fluorine at position C-8, a piperazine at position C-7 and is alkylated (Jaillon et al., 1996). Grepafloxacin is also substituted at position C-5 but by a CH3 group which improved anti-Gram-positive potency compared with ciprofloxacin (Wiedemann and Heisig, 1997). Other new quinolones containing 7-piperazinyl group are gatifloxacin, lemofloxacin and levofloxacin (the (S)-enantiomer of ofloxacin). Gatifloxacin is a racemic compound that bears a C-7 3-mitheylpiperazinyl and a C-8 methoxy substituents. Gatifloxacin is active against penicillin-resistant *S. penumoniae* and has proven highly effective in the treatment of lower respiratory tract infections. Pyrrolidine rings (five-membered) are also common substituents at position 7 (Fig 4), and are associated with low water solubility and low oral bioavailability, so in-vivo activity may be compromised. Introduction of methyl groups on the pyrrolidine ring helps to overcome some of these physical properties. Naphthyridine derivatives, tosufloxacin and gemifloxacin (Fig 4) are example of the advantages and disadvantages associated with a

pyrrolidine ring at position 7 (Lowe and Lamb, 2000). The addition of azabicyclo groups onto position 7 (Fig 4) has resulted in agents (moxifloxacin and trovafloxacin) with significant anti-Gram-positive activity, marked lipophilicity and half-lives of >10 h (Brighty and Gootz, 1997).

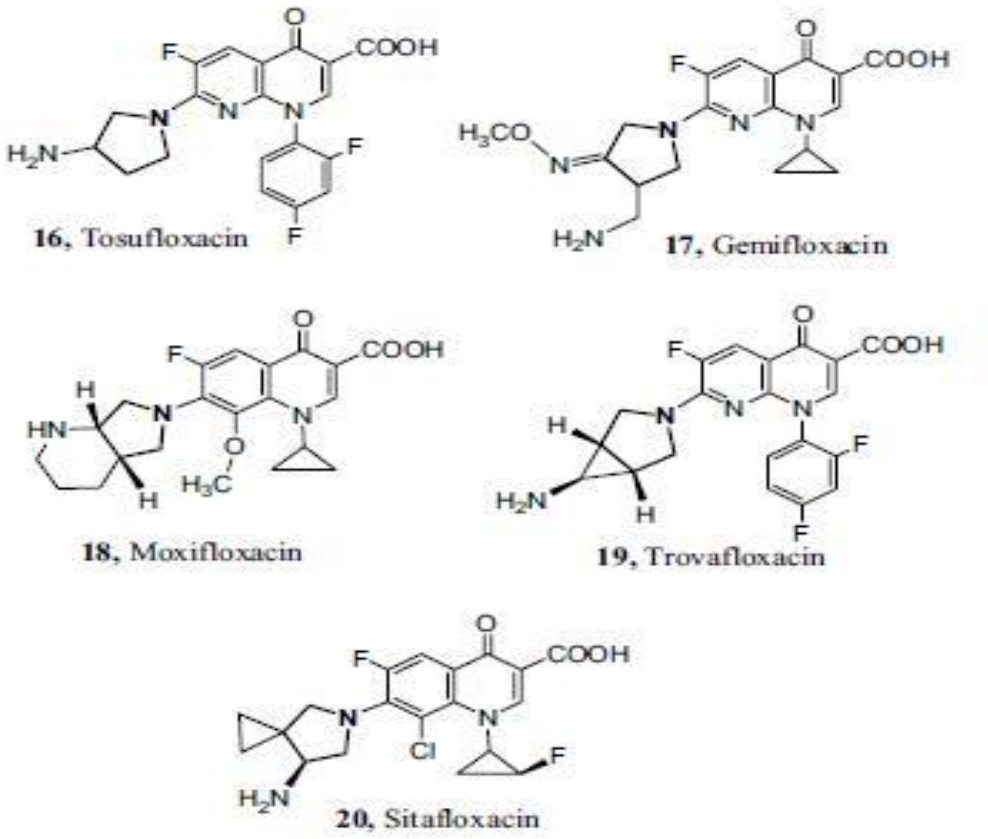


Figure 3:Quinolone containing pyrrolidine skeleton at C-7 position.

(Image adopted from Emami et al. (2005). Iranian Journal of Pharmaceutical Research (2005) 3: 123-136).

Sitafloxacin is another new fluoroquinolone that is in limited development due to photosensitivity. Unique structural features of sitafloxacin, which is being developed as a single enantiomer, include a novel aminopyyrolidine substituent at C-7 and a fluorocyclopropyl group at N-1 (Applebaum et al., 2000). Manipulation of the group at position 8 has also been shown to play a role in altering oral pharmacokinetics, broadening the spectrum of activity and reducing the selection of mutants (Ma et al., 1999). Whilst alkylation has been shown to increase further anti-gram-positive activity, it also improves tissue penetration and increases the half-life by increasing

lipophilicity, as with grepafloxacin, levofloxacin and sparfloxacin. In addition, some of the new compounds, such as trovafloxacin (having 2, 4 difluorophenyl group at position 1), also showed promising activity against the anaerobes (Spangler, 1996). Thus, continuous efforts were directed to further modify the quinolone pharmacophore with more complex newer fluroquinolones, namely danofloxacin, pazufloxacin, clinafloxacin and prulifloxacin (Fig 5). Recently, non-fluorinated quinolones, such as garenoxacin (Fig 6) have been developed, which further opens novel avenues in the development of quinolone antibiotics (Dalhoff and Schmitz, 2003). The tragets in fluroquinolone research during the last few years include improving the pharmacokinetic properties, increasing the activity against Gram-positive cocci and anaerobes, and against fluoroquinolone-resistant strains and improving activity against non-fermentative Gram-negative species (Fung- Tomc et al., 2000).

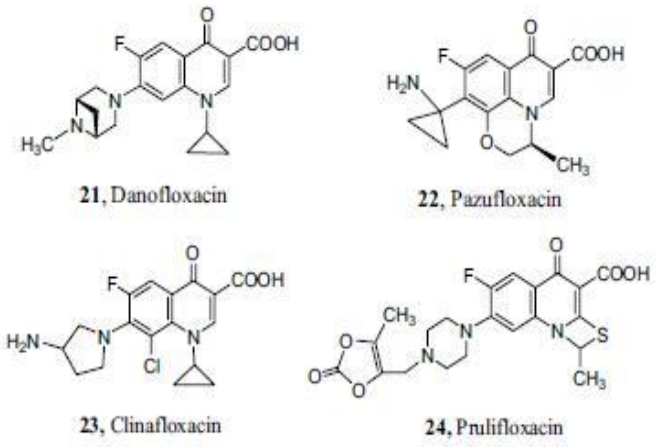


Figure 4: Quinolone with modifications at C-7 and N-1 position. (Image adopted from Emami et al. (2005). Iranian Journal of Pharmaceutical Research (2005) 3: 123-136).

Table 2: Comparison of quinolone Generations

Generation	Drug	Characteristic features
First	Nalidixic acid, Oxolinic acid, Pepemidic acid	Active against some Gram negative bacteria. Highly protein bound drugs. Short half-life.
Second	Norfloxacin, Enoxacin, Ciprofloxacin, Ofloxacin, Lomeofloxacin	Protein binding (50%). Longer life than previous agents. Improved activity against Gram negative bacteria.
Third	Temafloxacin, Sparfloxacin, Grepafloxacin	Active against Gram negative bacteria. Also, active against Gram positive bacteria.
Fourth	Clinafloxacin, Trovafloxacin, Moxifloxacin, Gatifloxacin	Show extended activity against both Gram positive and Gram negative bacteria. Active against anaerobes and atypical bacteria.

2.2 Mechanisms of Quinolone action:

Quinolones rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death. As a general rule, gram-negative bacterial activity corresponds with inhibition of DNA type IV topoisomerase. These enzymes play essential roles in most nucleic acid processes, help to control levels of DNA under- and overwinding, and remove knots and tangles from the bacterial chromosomes. Gyrase and topoisomerase IV adjust the topological condition of DNA by passing an in place twofold helix through a transient two-fold stranded break that they create in a different portion of DNA. Movement through the synergist cycle is driven by ATP authoritative and hydrolysis (Hawkey, 2003).

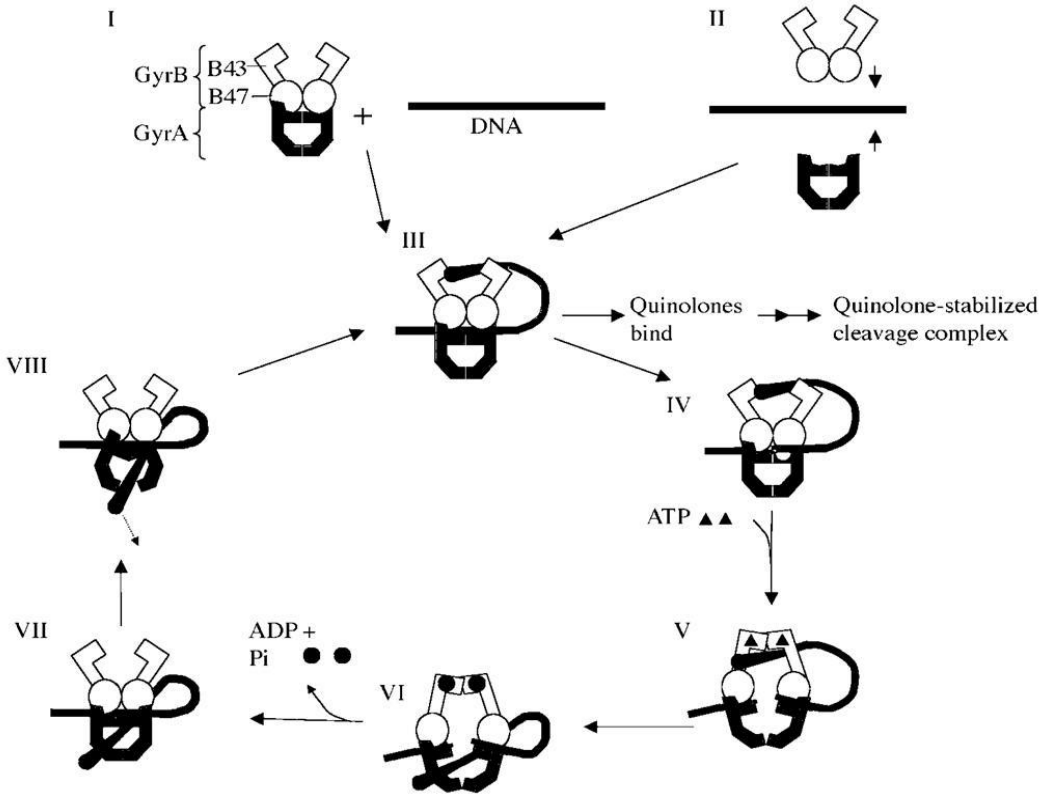


Figure no 5: Mechanism of action of quinolone antibiotic

(Image adopted from Hawkey, 2003. *Journal of Antimicrobial Chemotherapy* (2003) 51, *Suppl. S1*, 29–35).

To kill bacterial cells, fluroquinolones must get accumulated intracellularly. In Gram negative bacteria, this is accomplished by passive diffusion, with the porin outer membrane proteins playing a role. Fluroquinolone uptake into Gram-positive bacteria, such as *Bacillus subtilis*, appears to involve passive diffusion (Zhanel et al., 2002). Once inside bacterial cells, the fluoroquinolones have two targets: DNA gyrase and topoisomerase IV. DNA gyrase is made up of two each of the subunits encoded by gyrA and gyrB. Similarly, topoisomerase is made up of two each unit of the subunits encoded by *par*C (also termed grlA) in *Staphylococcus aureus*. As a result, these enzymes bind at similar sites on the DNA. The active tyrosine residue in the enzymes: DNA gyrase and topoisomerase IV bind near alpha helics. These enzymes being emphatically charged can without much of a stretch tie to contrarily charged DNA (Somasundaram and Manivannan., 2013). The amino acids of these proteins, in the

region in which quinolone resistance transformations in gyrA and gyrB were regularly confined, are exceedingly moderated. Furthermore, parC and parE transformations appeared to bring about extra increment in quinolone resistance within the sight of gyrA changes (Hooper, 2000).

2.2.1 DNA gyrase:

In 1976, a bacterial enzyme DNA gyrase (E.C. 5.99.1.3) was discovered and was found to possess the unique ability to catalyze the introduction of negative superhelical turns into closed-circuit double stranded DNA. Since then, this enzyme has been the focus of a great deal of attention concerning its structure, mechanism of action, interaction with anti-bacterial agents and physiological role. Besides the natural movement of gyrase, it serves as the valuable model framework for the investigation of DNA-protein cooperations and organic vitality coupling. DNA gyrase has a place with a class of proteins called DNA topoisomerases that have the property of catalyzing interconversions between various topological types of DNA. All topoisomerases are able to relax negatively supercoiled DNA, but only gyrase can introduce negative supercoils into DNA (Watson and Crick, 1953).

DNA gyrase was first introduced in 1976 by Gellert and co-workers, who were attempting to establish the *Escherichia coli* host factors required for bacteriophage X site-specific integration. One of the important facts about these host factors were shown to be an ATP-dependent enzyme capable of introducing negative supercoils into closed-circular duplex DNA, and was named DNA gyrase. The discovery of gyrase has been preceded by work on two classes of DNA synthesis inhibitors, the quinolones (e.g. nalidixic acid, oxolinic acid and ciprofloxacin) and the coumarins (e.g. novobiocin, coumermycin, chlorobiocin). DNA gyrase has since been found to be the cellular target of each of these antibacterial classes. Subsequently, gyrase has been found to be composed of two separate proteins, coded for by genes previously identified as genetic loci determining resistance to either nalidixic acid or coumermycin (m1A and mu). With the discovery of gyrase, these genes are referred to as *gyr*A and *gyr*B, and are located at 48 and 83 minutes respectively, on the standard *E. coli* K-12 chromosome map, but in some bacteria they are adjacent to each other and oriA; this configuration is known as Quinolone resistance determining region (QRDR). Temperature sensitive and drug resistance mutations of both the

*gyr*ase proteins have been identified and found to map to the loci indicated here. The gyrase enzymes have also been identified in other organisms. In number of cases, where both *gyr*A and *gyr*B genes have been identified, they have been found to be contiguous within the genome. For example, the *Bacillus subtilis* gyrase genes are both located near to the origin of replication and are separated by only 214 base pairs. One exception to this is *E. coli* where the *gyr*ase genes are widely separated. The two gyrase subunits can be purified to near homogeneity from *E. coli* and both the genes have been cloned into plasmids that allow their overproduction (Hawkey, 2003). The synthesis of gyrase is itself controlled by the level of DNA supercoiling within the cell. Agents that block DNA gyrase activity, and thus decrease the level of intracellular supercoiling, can increase the *in vivo* rate of synthesis of A and B subunits up to tenfold. A systematic deletion analysis of the gyrase promoters indicates that a DNA sequence some 20 bp long, that includes the 10 consensus region, the transcription start point , and the first few bases of the genes, are responsible for the property of induction by DNA relaxation. Both the *gyr*A and *gyr*B genes of *E. coli* have been sequenced and have been found to encode proteins of 874 (M, 97,000) and 804 (M, 90,000) amino acids, respectively. The molecular mass values are in close agreement with those predicted from SDS-polyacrylamide electrophoresis (Mizuuchi et al.,1996). The A protein seems to be have its major role in the breakage and reunion of DNA, while the B protein has an ATPase activity. The *Micrococcus luteus* DNA gyrase appears to resemble the *E. coli* enzyme very closely. From the results of protein cross-linking experiments, the active enzymes from *M. luteus* is thought to be a tetramer of A2B2. The tetramer structure has subsequently been confirmed for *E. coli* by small-angle neutron scattering, which yielded an estimated molecular mass of 353 kDa (the A2B2 structure has calculated molecular mass of 374 kDa). In general, both the subunits are required for all the reactions of gyrase, although the A subunit does appear to bind DNA in the absence of the B and the B subunit of gyrase is found to have a weak ATPase activity; the ATPase activity of the gyrase B protein is only fully stimulated in the presence of the A protein and DNA (Mizuuchi et al., 1980).

DNA gyrase is responsible for introducing negative supercoils into DNA and for relieving topological stress arising from the translocation of transcription and replication complexes along DNA. It acts by wrapping DNA into positive supercoil

and then passing one region of duplex DNA through another via DNA breakage and re-joining. This is an ATP-dependent process. In the presence of ATP, the process is driven forward, increasing supercoiling. In the absence of ATP, the process is driven back gain, relaxing the DNA. Keeping the DNA chromosome wound into loops facilitates the movement of replication forks (Hawkey, 2003).

2.2.2 Topoisomerase IV:

Topoisomerase IV is a homologue of DNA gyrase, comprising four subunits, two of C and two of E, encoded by the *par*C and *par*E genes, respectively. The topoisomerase IV locus was portrayed in 1990. Notwithstanding, various quinolone resistance markers had as of now been depicted and mapped to this locus. In *Staphylococcus aureus*, the flq locus, now alluded to as grlA, is proportionate to parC in other microscopic organisms, for example, *Streptococcus pneumoniae*. In *E. coli*, the nfxD locus is presently parE. The reaction mechanism of topoisomerase IV is similar to that of gyrase but topoisomerase IV binds to DNA crossovers rather than wrapping DNA. Topoisomerase IV is primarily involved in decatenation, the unlinking of replicated daughter chromosome (Hawkey, 2003).

2.2.2.1 Mode of action:

When fluoroquinolones bind to these enzymes, they block the activity of these enzymes and hence stop replication, transcription, repair as well as recombination. The fluoroquinolones bind to the enzyme-DNA complex and form a stable ternary complex. The complex of drug, topoisomerase IV and DNA collides with DNA replication complex and forms a physical barrier that blocks the further progression of the replication fork. On the other hand, the complex of drug, DNA gyrase and DNA blocks the passage of RNA polymerase and leads to the premature termination of transcription (Somasundaram and Manivannan, 2013).

It has been observed that these drugs preferably bind to either DNA gyrase or topoisomerase IV. In gram negative bacteria like *Escherichia coli*, fluoroquinolones bind to DNA gyrase as an essential target and topoisomerase IV as the auxiliary target. Conversely, in gram positive bacteria like *Staphylococcus aureus*, fluoroquinolones bind to topoisomerase IV as the essential target and DNA gyrase as the optional target. Nonetheless, some studies demonstrated that the DNA gyrase may

go about as the essential focus in Gram-positive microorganisms (eg. *S. pneumonia*) for some quinolones, such as sparfloxacin and nadifloxacin (Ruiz, 2003). In *Mycbacterium tuberculosis,* DNA gyrase was observed to be the unique target for fluoroquinolones. The cell is at last killed by the development of irreversible complex of medication, chemical and DNA and afterward the arrangement of double stand break up the DNA by denaturation of topoisomerase and after that the DNA is scratched, once at every strand to cut the DNA (Somasundaram and Manivannan., 2013).

2.3 Quinolone resistance- mechanism of action:

The fluoroquinolones have been used to treat a great variety of infections, including gonococcal infections, osteomyelitis, enteric infections or respiratory tract infections, and as prophylaxis in neutropenic patients, surgery or to prevent spontaneous bacteria peritonitis in cirrhotic patients, among others (Davis et al., 1996). In addition, quinolones alongside other hostile to bacterial operator have been widely utilized as a part of veterinary practice, either for medicinal reasons or for development promoters. As a consequence of their wide range of movement, quinolones have been broadly utilized. As of late, ciprofloxacin was brought up as the most devoured antibacterial operator around the world. This abnormal state of utilization and to some level of abuse in the feeling of superfluous utilizes or utilization of quinolones with proactivity in some creating nations have been rebuked for the fast advancement of microorganisms imperviousness to these operators (Hart and Kariuki., 1998).

Till date, two principal mechanisms of quinolone resistance have been built up: alterations in the targets of quinolones and decreased accumulation inside the bacteria due to impermeability of the membrane and/or an overexpression of effluxpump systems. Both of these mechanisms are chromosomally mediated. Furthermore, mobile elements have been described carrying the *qnr* gene which confers resistance to quinolones. These mobile elements have the potential for horizontal transfer of quinolone resistance genes.

2.3.1 Target alterations

Quinolones act by repressing the activities of type II topoisomerases, DNA gyrase and topoisomerase IV. DNA gyrase is a tetrameric chemical made out of two A

subunits and two B subunits, encoded by gyrA and gyrB, individually. The principle capacity of this compound is to catalyze the negative supercoiling of DNA. Topoisomerase IV is an A2B2 chemical too, encoded by parC and parE (alluded to as grlA and grlB in *Staphylococcus aureus*). These subunits are exceptionally homologous to GyrA and GyrB, separately. The fundamental part of topoisomerase IV is by all accounts connected with decatinating the daughter replicons (Drlica and Zhao, 1997). The quinolone targets are fundamentally diverse in gram-negative and gram-positive microorganisms. For Gram-negative bacteria it is the DNA gyrase, whereas in the Gram-positive it is the topoisomerase IV. However, some studies indicate that the DNA gyrase may act as the primary targets in Gram-positive microorganisms for some quinolones, such as sparfloxacin and nadifloxacin, have similar affinity for both targets. Most of the writing with respect to the components of activity and imperviousness to the quinolones alludes to studies done on the Enterobacteriaceae, particularly *Escherichia coli* (Takei et al., 2001). Amino acid substitutions required in the improvement of quinolone resistance in these microorganisms have been portrayed for GyrA/GyrB and ParC/ParE. The prevalence of mutations in their particular encoding qualities is connected with in vitro and in vivo beginnings of the strains. Thus when comparing the presence of mutations in the DNA gyrase of quinolone-resistant *E. coli* strains obtained in vitro, results showed a similar proportion of mutations in *gyr*A and *gyr*B (Nakamura et al., 1989), whereas, in studies using clinical isolates, the results showed an exclusive prevalence of mutations in *gyr*A (Vila et al., 1995; Everett et al., 1996)

Table 3: Mutation described in subunit *Par*C and *Par*E

Codon	**Wild Amino acid**	**Mutation derived**
	*Par*C	
78	Gly	Asp
80	Ser	Ile
84	Glu	Lys, Val, Gly
	*Par*E	
445	Leu	His

Table 4: Mutation described in subunits *Gyr*A and *Gyr*B of resistant strain of *E. coli*

Codon	Wild amino acid	Mutation derived
	*Gyr*A	
51*	Ala	Val
67*	Ala	Ser
81	Gly	Cys, Asp
82*	Asp	Gly
83	Ser	Leu, Trp, Ala, Val
84	Ala	Pro, Val
87	Asp	Asn, Gly, Val, Tyr, His
106*	Gln	Arg, His
*Gyr*B		
426	Asp	Asp
447	Lys	Glu

"*" Only described in mutants obtained in vitro

2.3.1.1 Alterations in the DNA gyrase

Alterations described in the GyrA of *E. coli* are predominantly in the so-called quinolone-resistance determining region (QRDR), 21 between position 67 and 106 (Table 3). Mutations in codons 67, 81, 82, 83, 84, 87 and 106 of gyrA have been seen to be in charge of the improvement of quinolone resistance in *E. coli* (Yoshida et al., 1988). However some of the mutations within QRDR (e.g. in *E. coli* mutations at positions 67, 82 and 106), have only been described in laboratory obtained quinolone-resistant mutants (Tavio et al., 1999). Additionally in position 51, a region outside the QRDR, has been proposed as a novel point transformation bringing about diminished vulnerability to the quinolones. The presence of a single mutation in the above-mentioned positions of the QRDR of *gyr*A usually results in high-level resistance to nalidixic acid. To obtain high levels of resistance to fluoroquinolones, the presence of additional mutation(s) in *gyr*A and/or in other targets such *par*C is required. Thus, it has been proposed that the MIC of nalidixic acid could be used as a generic marker of resistance for the quinolone family in Gram-negative bacteria (Hakanen et al., 1999). However, nalidixic acid vulnerable, ciprofloaxin-resistant (NalS CipR) phenotypes have been depicted in two lab mutants of *E. coli*. In *E. coli* the phenotype is connected with the nearness of the substitutions Gly-81 to Asp or Asp-82 to Gly 22-26. Be that as it may, in unconstrained mutants of *Salmonella* Typhimurium, a change from Gly-81 to Ser does not influence the MIC of any of the six tested quinolones (counting nalidixic corrosive and ciprofloxacin) (Reyna et al., 1995).

The NalS CipR phenotype has also been described as *Campylobacter jejuni*, although the molecular basis underlying it remains unknown (Bachoual et al., 2001). In fact, the only NalS CipR *C. jejuni* isolate in which the presence of mutations in *gyr*A and *gyr*B were analyzed showed as single mutation in codon 86 (equivalent to Ser-83 in *E. coli*) resulting in the substitution Thr to Ile, the most frequently found alteration among quinolone-resistant isolates of *C. jejuni* (Ruiz et al., 1998). The conceivable contribution of compensatory mutations in other *gyr*A codons were recommended to clarify this current isolates phenotype. In any case, the conceivable hyper susceptibility to nalidixic acid of the parental strain because of expanded uptake ought to likewise be considered. Susceptibility to nalidixic acid, yet imperviousness to various fluoroquinolones, (for example, ciprofloxacin or norfloxacin) is by all accounts regular for *Strenotophomonas maltophilia* (Valdezate et al., 1999; Ribera et al., 2002). In a study involving 109 isolates of *S. maltophilia*, 88% were susceptible to nalidixic acid, whereas only 20.2% were susceptible to norfloxacin (Valdezate et al., 1999). Interestingly, it has been shown that the development of quinolone resistance in the microorganism is not related to the presence of mutations in the *gyr*A or *par*C genes (Ribera et al., 2002). This fact suggests the possibility of potent efflux pumps playing a role in the resistance to quinolones for these microorganisms. In this line of thought, a study by Alonso and Martinez. (1997) showed the in vitro display of a quinolone-resistant mutant selected with tetracycline. Recently, the SmeDEF efflux system (which is capable of pumping quinolones out of the bacteria) has been characterized in *S. maltophilia* (Alonso and Martinez 1997). The most frequent mutation observed in quinolone resistant *E. coli* is at codon 83 of *gyr*A (Yoshida et al., 1988). Moreover, it seems to be the most frequently found in most clinical and laboratory quinolone-resistant isolates of other *Enterobacteria*, such as *Citrobacter freundii* or *Shigella* spp. or in pathogens such as *Neisseria gonorrhoeae* or *Acinenobacter baumannii* (Vila et al., 1995; Vila et al., 1999). In *E. coli* or different microorganisms, for example, *S.* Typhimurium or *A. baumannii*, codon 83 is situated in Hinf I restriction site, empowering changes at this position to be effectively identified with a mix of PCR and RFLP examination (Ruiz et al., 1997). In clinical isolates, the second most regularly observed mutation is at codon 87 at *gyr*A. Strains with a two-fold transformation are at codon 83 and 87 have higher MIC's of quinolones. This fact is true for other Gram-negative microorganisms, such as *C.*

freundii, Pseudomonas aeruginosa or *N. gonorrhoeae*. Substitutions in the positions equivalent to aforementioned amino acids 83 and 87 of *E. coli* have also been the most frequently described in quinolone-resistant Gram-positive microorganisms (Schmitz et al., 1998).

In quinolone-resistant *S.* Typhimurium strains, a transformation has been depicted in codon 119, bringing about the mutation of Ala to Glu or Val. This codon, outside the QRDR, has been embroiled in the improvement of nalidixic acid resistance (Griggs et al., 1996). A mutation in this codon creating the substitution Ala-119 to Ser has additionally been portrayed for *A. baumannii*. However, in *A. baumannii*, this mutation was found in both quinolone-resistant and quinolone-susceptible isolates, suggesting that other mechanisms may be responsible for the changes in quinolone susceptibility observed. Different amino acid substitutions at the same position result in different quinolone susceptibility levels, indicating that the final MIC is a function of the specific substitution (Yonezawa et al., 1995). This is likely because of the mechanism of interaction between the quinolones and their targets. It has been proposed that amino acid 83 (numeration for *E. coli*) of GyrA connects with the radical in position 7.20. This model additionally applies for 80 and 84 (numeration for *E. coli*) of ParC. Accordingly, distinctive amino acids substitutions at these focuses would influence in various ways the liking for the quinolone atom. Additionally mutations in different positions may influence the entire protein structure, influencing the communication with quinolones. In *Gyr*B of *E. coli* , substitutions resulting in resistance to quinolones have been described at positions 426 (Asp 426 to Asn) and 447 (Lys-447 to Glu) (Yoshida et al., 1991). Substitutions at position 426 appear to present resistance to all quinolones, while those at position 447 result in an expanded level of resistance to nalidixic corrosive, yet a more prominent susceptibility to fluorinated quinolones. Mutations in comparable positions have been portrayed for Gram-positive microorganisms. In *S.* Typhimurium, the amino acid substitution Ser to Tyr at position 463 has been related to the development of quinolone resistance (Gensberg et al., 1995).

2.3.1.2 Alteration in topoisomerase IV

In the *par*C gene of *E. coli*, among different microorganisms, the most well-known substitutions happen at codons 80 and 84 (Heisig., 1996; Kumagai et al., 1996). In *E.*

coli, another substitution (Gly-78 to Asp) has been depicted both in clinical isolates and laboratory got quinolone-resistant mutants (Table 3) (Kumagai et al., 1996). A substitution depicted in the *par*C quality of in vitro mutants of *Shigella flexneri* influences position 79 (Asp to Ala) (Chu et al., 1998). Other substitution similarly situated has been found in different microorganisms both Gram-negatives [such as *Haemophilus influenzae* (Asp to Asn)] and Gram-positives [such as *Streptococcus pneumoniae* (Asp to Asn)] (Jones et al., 2000). Although, in every case they were found concomitantly with other mutations either in *Gyr*A or *Par*C, to date only described in grlA of *S.aureus*, influences codon 116, delivering a change from Ala to Glu or Pro 52, 55. This codon is a simple of codon 119 of GyrA in *S.* Typhimurium (Griggs et al., 1996). Likewise, mutations in different codons, for example, 23 (Lys to Asn), 69 (Asp to Tyr), 176 (Ala to Gly) or 451(Pro to Gln) have been portrayed in *S. aureus* (Ince and Hooper, 2001). The part of amino acids substitutions in ParE, bringing about the improvement of quinolone resistance in clinical isolates of Gram-negative microorganisms seems, by all accounts, to be immaterial (Ruiz et al., 1997). In fact, only one substitution (Leu-445 to His) has been depicted in *par*E of single quinolone-safe in vitro mutant of *E. coli*. Additionally, this mutation just appears to influence the MIC of quinolones within the sight of a concomitantmutation in *gyr*A (Breines et al., 1997). Modifications in this subunit have likewise been depicted in both clinical and research facility acquired quinolone-resistance Gram-positive microorganisms. In *S. pneumoniae* for instance, the mutations discovered delivered changes from Asp-435 to Asn or from His-102 to Tyr, whereas in *S. aureus* the amino acid changes Pro-25 to His, Glu-422 to Asp, Asp-432 to Asn or Gly, Pro-451 to Ser or Gln and Asn-470 to Asp have been described (Fournier and Hooper., 1998). However, it is possible that some or all of these substitutions may not play any role in the development of quinolone resistance, as has been suggested in *S. pneumoniae* (Jones et al., 2000).

2.3.2 Decreased uptake

Decreased quinolone uptake may be associated with two factors: an increase in the bacterial impermeability to these anti-bacterial agents or the over expression of efflux pumps. Quinolones may cross the external membrane in two diverse routes: through particular porins or by dispersion through the phospholipid bilayer. The level of dispersion of a quinolone is significantly connected with, and reliant on, its level of

hydrophobicity. All quinolones may cross the external membrane through the porins, yet just those with more prominent level of hydrophobicity may diffuse through the phospholipid bilayer. Hence adjustments in the creations of porins and/or in the lipopolysaccharides may change susceptibility profiles. In lipopolysaccharide-imperfect mutants, expanded helplessness to hydrophobic quinolones has been portrayed, without modifications in the level of imperviousness to the hydrophilic quinolones. Modifications in the film porousness are typically connected with diminished relationship of porins. This has been depicted in both *E. coli* and Gram-negative microscopic organisms (Hirai et al., 1986).

The outer membrane of *E. coli* possesses three main porins (OmpA, OmpC and OmpF). A diminishing in the level of expression of OmpF identified with the expansion in the resistance to some quinolones (Cohen et al., 1989) yet does not influence the MIC of others, for example, tosufloxacin or sparfloxain (Mitsuyama et al., 1992). In addition, a diminished expression of OmpF results in an decrease in susceptibility to an assortment of hostile to antibacterial agents, for example, B-lactams, tetracyclines and chloramphenicol (Cohen et al., 1989). Some chromosomal loci, for example, MarRAB (constituted by three genes: marR that encodes a represser protein, marA encoding a transcriptional activator and marB which encodes a protein with an obscure capacity) or SoxRS (this operon encodes for two proteins, SoxR, a general protein and SoxS a transcriptional activator) direct both the levels of expression of OmpF and some efflux pumps in *E. coli*. It has been shown that chloramphenicol, tetracycline and other substrates such as salicylate, may induce the expression of MarA, producing an increase in the expression of micF, an anti-sense regulator that includes a post-transcriptional repression of the synthesis of OmpF. The expression of micF may also be regulated by the SoxRS operon. In *E. coli,* the MarRB and SoxRS operons additionally direct the level of expression of efflux pumps frameworks, for example, AcrAB (Oethingher et al., 1998). Mutations influencing MarR prompt the constitutive expression of this operon, prompting the improvement of a multiresistant phenotype. As of late, Baucheron et al. (2002) working with strains of *S.* Typhimurium conveying amino acids susbtitutions either in GyrA (Ser-83 to Ala and Asp-87 to Asn), ParC (Ser-80 to Ile) and GyrB (Ser-464 to Phe), have demonstrated the high significance of the AcrAB efflux pump in the improvement of quinolone resistance in *S.* Typhimurium. This study demonstrated

that interruption or hindrance (with Phe-Arg-B-naphthylamide) of the AcrAB operon results in a lessening in the MIC of a tried quinolones (e.g. MIC of ciprofloxacin diminished from 32 mg/L to 2-4 mg/L; MIC of enrofloxacin diminished from 64 mg/L to 2 mg/L; MIC of marbofloxacin diminished from 32 mg/L to 2-4 mg/L). The outer membrane composition of such microorganisms, for example, *A. baumannii* or *P. aeruginosa*, has been connected with their natural resistance. Wild-sort strains of *A. baumannii* show MICs of ciprofloxacin extending somewhere around 0.125 and 1 mg/L. Conversely, wild-sort *E. coli* strain show MICs of ciprofloxacin extending somewhere around 0.007 and 0.25 mg/L. This outcome has been interpreted as characteristic resistance or because of the overexpression of the efflux pump(s). Curiously, this extent is not rationed while analyzing the MIC of nalidixic acid. The outer membrane of *P. aeuroginosa* has low non-particular penetrability to little hydrophobic atoms (Yoshimura and Nikaido., 1982) which may represent the inborn resistance for these microorganisms against quinolones. In fact, the outer membrane of *P. aeruginosa* is 10-to 100 foldless penetrable to antibiotics than that of *E. coli*. Distinctive efflux frameworks appeared to pump out quinolones, for example, MexAB-OprM, MexCD-OprJ or MexEF-OprN have been depicted in *P. aeruginosa* (Cohen et al., 1989). A fourth efflux framework named MexXY fit for pumping out quinolones have likewise been depicted, however no open perusing outline relating to an outer membrane protein has been discovered downstream of mexXY. Actually, it might be that OprM (which is encoded downstream of MexAB) may go about as the outer membrane protein of this efflux framework (Mine et al., 1999). It has been accounted for that the disturbance of OprM produces a more noteworthy impact in the weakness levels to some antimicrobial operators, than the disruption of MexA or MexB. This may be due to the presence of a weak promoter in the mexB gene upstream of the oprM gene, which facilitates the expression of oprM in the absence of expression of the other components of the MexAB-OprM operon. This would infer that OprM may add to the intrinsic resistance levels to hostile to antimicrobial agents by collaboration with other internal and periplasmic membrane components (Zhao et al., 1998). Other efflux pump connected with expanding levels of quinolone resistance has additionally been described in *E. coli* and other Gram-negative microorganisms (Putman et al., 2000). In addition, late studies examining entire genomes have reported the high number of putative efflux pumps which may have the capacity to pump out against bacterial agents that are available in microorganisms.

For instance, in *E. coli*, distinctive putative medication transporters have been discovered (Nishino and Yamaguchi., 2001). Efflux pumps have additionally been portrayed in Gram-positive microorganisms, the best described being NorA, from *S. aureus*. NorA is an ATP-dependent efflux pump equipped for pumping out hydrophilic quinolones like enoxacin or norfloxacin, however not influencing the hydrophobic quinolone, for example, sparfloxacin (Yoshida et al., 1990). This efflux pumps can likewise expel different molecules like essential colors, puromycin or chloramphenicol (Chou et al., 1993). Two distinctive DNA arrangements encoding firmly related NorA efflux pumps have been depicted in *Bacillus subtilis*. Their overexpression gives a comparative resistance range to that of NorA (Ahmed et al., 1995). The presence of NorA-like efflux frameworks has additionally been depicted or proposed in other Gram-postive microorganisms, for example, *S. pneumoniae* or *Streptococcus viridans* (Table 4). To date, distinctive substances equipped for hindering the activity of some efflux pumps, for example, reserpine or CCCP have been depicted. Shockingly, such compounds can't be utilized as a part of clinical practice because of their high toxic quality. A novel compounds, for example, Phe-Arg-B-naphthylamide (Ribera et al., 2002) are presently utilized as efflux pump inhibitor.

2.3.3 Transferability of quinolone resistance

Quinolone resistance acquired

Another mechanism of quinolone resistance is naturally occurring plasmid-mediated quinolone resistance (PMQR) determinants. The discovery in 1998 of *qnr*, a plasmid-mediated horizontally transferable gene encoding quinolone resistance, focused light on these phenomena. *qnr* is a naturally occurring allele encoding a pentapeptide repeat protein that confers reduced susceptibility to nalidixic acid or fluoroquinolone. The *qnr* families (such as *qnr*A, *qnr*B and *qnr*C) are defined by a 30% or more difference in nucleotides or derived amino-acids. Within each family, *qnr* alleles differ in one or more amino-acids. *Qnr* genes found on a bacterial chromosome are named after the host organism or assigned to a family if the gene is at least 70% identical to an established *qnr* family (e.g. Sa*qnr*A3 from the Chromosme of *Shewanella algae*) (Jacoby et al., 2008).

Qnr proteins are capable of protecting DNA gyrase from quinolones and have been in circulation for at least 20 years (Jacoby et al., 2009). During this time, they have achieved global distribution in a variety of plasmids environments and bacterial genera.

Two additional mechanisms of resistance that were predicted to occur were subsequently found. *AAC(6')-Ib-cr*, a variant aminoglycoside acetyltransferase capable of reducing ciprofloxacin activity, is also carried on plasmids and may be even more prevalent than *Qnr* proteins (Robicsek et al., 2005). Quinolone extrusion, a prevalent chromosomally encoded mechanism of resistance, has also been found to be plasmid borne (Wiegand et al., 2005). These mechanisms provide the low-level quinolone resistance shown in vitro to facilitate the emergence of higher-level resistance in the presence of quinolones at therapeutic levels. The understanding of the structure of *acc(6')-Ib* importantly advanced the understanding of the *acc(6')-Ib-cr*, mechanisms of action and various new plasmids carrying variants of *acc(6')-Ib-cr* genes have been reported (Vetting et al., 2008).

2.3.3.1 Pentapeptide repeat proteins

Discovery of *qnr* Genes

***qnr*A:** The discovery of PMQR in the late 1990's was a serendipitous work by Martinez-Martinez and colleagues. They were studying pMG252, a plasmid from a multiresistant strain of *Klebsiella pneumoniae* that was isolated from aurine specimen from a patient from a University of Albama at Birmingham in 1994. A quinolone was included as a control in a study of ability of pMG252 to increase resistance to β-lactam antibiotics in porin-deficient strains of *Klebseiella pneumoniae*. Unexpectedly, an increase in the quinolone MIC was found. The effect of the plasmid was increased 4-to-16 fold in this porin-deficient isolate, but even in the *Escherichia coli* strain with intact porins, pMG252 increased the quinolone MIC's between 8 and 64 fold. Although this increase from the baseline was not to the level designated to represent clinical resistance (the resistance breakpoint), the plasmid also facilitated the selection of higher-level quinolone resistance. A wildtype *E. coli* strain carrying pMG252 plated onto agar containing nalidixic acid or ciprofloxacin was 100 times expected to give rise to spontaneous resistant mutants than was a plasmid free strain. The plasmid

did not have a general mutator effect since the frequency of other genetically defined mutations was equivalent to that of plasmid frees *E. coli*. Subsequent cloning of the gene responsible for this phenotype revealed it to be a 657-bp open reading frame, and the protein which it encoded was named Qnr, for quinolone resistance. Later, this protein has been renamed QnrA1, since related proteins have been identified (Martinez-Martinez et al., 1998).

A *Klebsiella oxytoca* isolate from Anhui Province, China was reported to carry a variant of *qnr*A differing from the originally detected gene by four amino acids. This variant was designated *qnr*A2 (Nordmann and Poirel., 2005). While searching for a chromosomal orthologue of *qnr*A in the genome sequences of environmental organisms, Poirel et al. (2005) identified three additional variants (*qnr*A3, *qnr*A4 and *qnr*A5) of this gene in *Shewanella algae*, varying from *qnr*A1 in two to four codons. At about the same time, *qnr*A3 was also detected in clinical *salmonella* isolates (Cheung et al., 2005).
Camban and co-workers searching for *qnr*A determinants among isolates of Enterobacteriaceae with reduced susceptibility to quinolones found *qnr*A6 in *Proteus mirabilis* isolates (Cambau et al., 2006).

***qnr*S:** In succeeding years, search for plasmids exhibiting transferable resistance to quinolones led to the discovery of four additional similar proteins, QnrS (Hata et al., 2005), QnrB (Jacoby et al., 2006), QnrC (Wang et al., 2009) and QnrD (Cavaco et al., 2009). In October 2003, a single clone of *Shigella flexneri* 2b caused a food-borne outbreak of enterocolitis in Aichi prefecture, Japan. One of eight strains of this clone was resistant to ciprofloxacin. This strain was found to harbor a unique conjugative plasmid that transferred a quinolone resistance. Cloning of that plasmid revealed an open-reading frame encoding a 218-amino acid protein of the pentapeptide repeat family. This protein shares only 59% amino acid identify with QnrA1 and was named QnrS (Hata et al., 2005).

While searching for *qnr* genes among clinical non-Typhi *Salmonella* isolates from the United States, a *qnr*S variant (*qnr*S2) was detected on a plasmid from *Salmonella enterica* seovar Anatum that codes for a protein that is 92.2% identical in amino acid sequence to *Qnr*S1 (Gay et al., 2006). At about the same time, *qnr*S2 was found on a plasmid isolated from the activated sludge basin of a wastewater treatment plant in

Germany (Jung et al., 2009). *qnr* from veterinary clinical *E. coli* isolates in Guangdong, China, that was earlier reported as *qnr*S1, differed from *qnr*S1 in one codon and has thus been renamed as *qnr*S3. In order to avoid such confusion and to bring order into *qnr* numbering, a database of *qnr* allele designations have been established at hhtp://www.lahey.org/*qnr*studies. A fourth *qnr*S variant in a *Salmonella* isolate from Denmark was also reported (Torpdahl et al., 2009).

***qnr*B:** While investigating strains of *K. pneumoniae* from India, some of which contained *qnr*A, Jacoby and colleagues found that several could transfer low-level quinolone resistance but were negative by PCR for *qnr*A. The PMQR gene responsible for this phenotype coded for a 214-or-226-amino acid protein and was termed *qnr*B1. The QnrB1 protein shares 43% and 44% amino acid identifies with QnrA and QnrS, respectively (Jacoby et al., 2006). The repertoire of *qnr*B variants is broader than that of *qnr*A and *qnr*S. The first variant, *qnr*B2, was found among several isolates of Enterbacteriaceae from the United States. The *qnr*B2 gene codes for a 214-amino acid protein that differs from *qnr*B1 in five codons. Later on B3, B4 and B5 were also reported whch differ from *qnr*B1 in 2, 14 and 6 codons respectively. The search for variants extended upto *qnr*B13, *qnr*B14 and *qnr*B15, which were reported among *Citrobacter freundii* strains from South Korea (Tamang et al., 2008). The detection of *qnr*B in a collection of isolates of Enterbacteriaceae from Kuwait city, Kuwait. Cattoir et al. (2008) identified *qnr*B7 in *Enterobacter cloacae* and *qnr*B8 in *C.freundii*. These genes differ from *qnr*B1 by 4 and 11 amino acids, respectively. *qnr*B19, which differs from *qnr*B1 by six amino acids was found in an *E. coli* isolate from Colombia by Cattoir et al. (2008).

*qnr*B10 (Quiroga et al., 2007), discovered in *C. freundii*, differs from *qnr*B1 by five amino acids and *qnr*B12 (Kehrenberg et al., 2008), identified in *Citrobacter werkmanii*, differs from *qnr*B1 by two amino acids, was found among several cephalosporin-resistant isolates of Enterobacteriaceae in western China. *qnr*B9, *qnr*11, *qnr*16, *qnr*17 and *qnr*18 were all found among different isolates of *C. freundii*.

***qnr*C:** A clinical strain of *Proteus mirabilis* from Shanghai, China was isolated from an outpatient with an urinary tract infection. Plasmid harbored by this strain, on conjugation showed a low-level quinolone resistance and was negative by PCR for the known *qnr* genes. Subsequent cloning and sequencing of the new gene showed

that a 666-bp gene codes for a 221-amino acids and designated as *qnr*C1. It shared 64%, 42%, 59% and 43%identity with *Qnr*A1, *Qnr*B1, *Qnr*S1 and *Qnr*D respectively (Wang et al., 2009).

***qnr*D:** Four *Salmonella enterica* isolates obtained from humans in the Henan Province of China showed reduced susceptibility to ciprofloxacin that was transferrable on a small plasmid of about 4.3 Kb, which is *E. coli* conferred a 32-fold increase in the MIC of ciprofloxacin. This plasmid was negative for *qnr*A, *qnr*B, *qnr*S, *acc(6')-Ib-cr* and *qepA* The plasmid encodes a 214-amino acid pentapeptide repeat protein designated *Qnr*D. *qnr*D showed 48% similarity to *qnr*A1, 61% similarity to *qnr*B1 and 32% similarity to *qnr*S1 (Cavaco et al., 2009).

2.3.3.1.1 About *Qnr* proteins:

The qnr proteins have a place with the pentapeptide repeat family, which is characterized by a progression of pair of 5-amino acid repeats. In the pentapeptide repeats, no position is totally moderated, however each of the buildups of an individual pentapeptide displays a propensity for a limited number of amino-acids with the repetitive general motif approximately represented by the sequence. A(D/N)LXX and more precisely represented by [Ser, Thr, Ala or Val][asp or Asn][Leu or Phe][Ser, Thr or Arg][Gly] (Vetting et al., 2008). Qnr proteins, however often have a cysteine at position i^{-2} (with position i representing the central amino acid of each repeat). A characteristic feature of the *qnr* proteins is that they are formed by the two domains of pentapeptide repeats separated by single amino acids, usually glycine. The primary structure of QnrA, QnrB and QnrS are comparable, with nine pentapeptide repeat units associated by a single glycine, trailed by a cysteine, with variable number of units (22 in QnrS, 28 in QnrA and 29 in QnrB, QnrC and QnrD). These contrast from MfpA, a pentapeptide repeat protein found in *Mycobacterium smegmatis* that is also fit for gyrase protection from quinolone. In that protein, there is a kink in the helical axis halfway through the β-helix however no putative glycine "pivot" (Hegde et al., 2005). As the three-dimensional structure of Qnr has not yet been unraveled, the contribution of the glycine moiety to capacity is obscure. In like manner, it is difficult to predict increments in movement from changes in essential structure among the many Qnr variants. For instance, when the

Vibrio parahaemolyticus qnr homologue, VPA0095, was cloned onto a plasmid, it didn't give fluoroquinolone resistance unless cysteine 115 was transformed to a tyrosine (Saga et al., 2005). At the point when the same amino acid substitutions were made in QnrA1 or QnrS1, however the capacity to secure against quinolone declined rather than increase (Cattoir et al., 2007). Mutations in the amino acid that are monitored among Qnr proteins had a comparative impact of diminishing in quinolone resistance yet the adjustment in the MIC differed among QnrA1, QnrB1 and QnrS1 with the same substitution (Rodriguez-Martinez et al., 2009). A phylogenetic analysis of Qnr alleles recommends that recombination has had essential influence in their evolution yet that a particular part in protection against quinolones is not clear (Baquirin and Barlow., 2008).

2.3.3.2 Chromosomal *qnr* Genes:

Genes for pentapeptide repeat proteins with sequence similarity to plasmid-borne *Qnr* proteins have been found on the chromosomes of both gram-positive and gram-negative bacteria. Some of these proteins additionally have the essential structure of two pentapeptide strings of variable length associated by a single amino-acid. For example, Efs*qnr*, found on the chromosome of *E. faecalis* strains V583 and Jh2-2, encodes a 211-amino acid pentapeptide repeat protein that shares 25% identity and 40% similarity with QnrA. The protein is divided into two pentapeptide repeat domains of 9 and 33 unists, each connected by a single asparagine (Arsene and Leclercq., 2007).

***qnr*A-like genes:** Gram-negative species were at first screened for *qnr*-like genes in a search for the repository of Qnr determinants (Poirel et al., 2005). The determination that *qnr*A3 has a chromosomal location in *Shewanella algae* was based upon entire genome restriction with the ribosomal endonuclease I-Ceul took after by two-fold hybridization with rRNA and qnrADNA tests to the same high-molecular weight band. This got further backing from the identical G+C content of *qnr*A3 and the genome of *S. algae* and the absence of basic region 1 that is a piece of the sull-type integrin, which was beforehand connected with the assembly of *qnr*A1 onto plasmids (Poirel et al., 2005). Ensuing in silico examinations recognized *qnr*-like qualities in the genomes of different individuals from the Shewanellaceae (Sanchez et al., 2008), including *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Photobacterium profundu* and

other *Vibrio* spp. also, *Shewanella* spp. Correspondingly to *S. algae* they are not flanked by insertion groupings or genetic structures known to mobilize resistance genes (Poirel et al., 2005). The encoded proteins have at most 67% identity with QnrA1 and are made of two domains of 11 and 32 units connected by a single glycine. Upon expression in a heterologous *E. coli* host system they conferred an increased MIC of quinolones similar to that obtained with the recombinant plasmid that expressed the *qnr*A3 determinant from *S. algae* (Poirel et al., 2005). Comparative perceptions were made for a *qnr*-like arrangement in *Vibrio parahaemolyticus* (Saga et al., 2005). Despite the fact that in the silico examination, the *qnr*-like quality is not distinguished in *Vibrio cholerae* and other *Vibrio* spp., another study looking at the ciprofloxacin-resistant *V. cholerae* O1 clone from a cholera epidemic in Brazil recognized a *qnr*-like quality encoding a protein with 69% sequence identity to that of the *P. profundum* Qnr-like peptide. The gene was designated *qnr*VC1, but according to recommended nomenclature, it might better be termed Vc*qnr*1. VC1 had a G+C content of 36.8%, which is considerably different from that of *V. cholerae* genome (47.5%) suggesting dissemination through horizontal gene transfer. It also has an affiliated *aat*C recombination site, unlike other *qnr* genes, and is incorporated as a cassette into a chromosomal class 1 integron (Fonseca et al., 2008).

Arsene and Laclercq. (2007) have identified in the genome of *Enterococcus faecalis* V583 a *qnr*-like gene, named *E. faecalis qnr* (*qnrE faecalis*), encoding a putative pentapeptide repeat protein that shares 25% identity with Qnr. To assess its potential role in the intrinsic resistance of *E. faecalis* to fluoroquinolones, *qnrE. faecalis* was inactivated in *E. faecalis* JH2-2 by insertion of the thermosensitive vector pG1KT. This strain was complimented by *qnrE. Faecalis* cloned in the multi-copy plasmid pORI23. The effects of its overexpression were also studied. Inactivation of the *qnr E. faecalis* gene resulted in two fold decreases in the MIC's of ofloxacin and ciprofloxacin. When the gene was complimented or overexpressed, MIC's of fluoroquinolones increased four-to-nine fold, leading to MIC's of ofloxacin and ciprofloxacin equal to 32 g/ml and 8 g/ml, respectively. The *E. faecalis* Qnr (*QnrE.faecalis*) protein was produced and purified. Qnr *E.faecalis* protein protected *Escherichia coli* DNA *gyr*ase from inhibition by ofloxacin. The *qnr E.faecalis* gene was then introduced into *E. coli* DH10B, *Staphylococcus aureus* RN4220 and *Lactococcus lactis* IL-1419 to study it's heterologous expression. MIC's of the

various fluoroquinolones tested increased 4-to-16 fold, showing that *qnr E.faecalis* conferred resistance to fluoroquinolones in various bacterial backgrounds. Overexpression of *qnrE.faecalis* in enterococci or mobilization of the gene to other bacterial species may be anticipated as a possible new mechanism for the fluoroquinolone resistance.

*qnr*B-like genes-*qnr*B12 was found in three epidemiologically and clonally unrelated *Citrobacter werkmanii* isolates of poultry origin from Germany by Kehrenberg et al. A chromosomal location of this gene was clear due to some reasons: repeated plasmid transformation and conjugation experiments failed, Southern blot hybridization studies of *I-Ceul*-digested genomic DNA gave a signal only with the largest I-Ceul fragment (approximately 800 Kb) in each strain, and further S1 nuclease digestion followed by pulsed-field gel electrophoresis did not identify any large *qnr*B-carrying plasmid which might comigrate with this 800-kb I-CeuI fragment (Kehrenberg et al., 2007). Sm*qnr*, identified in the genome of *Stenotrophomonas maltophilia* strain R551-3, codes for a 219-amino acid protein that shares about 60% amino acid identity with QnrB. SmQnr has two domains of 5 and 28 pentapeptide repeats separated by a glycine. An even closer homologue of *qnr*B has been found in DNA sequences in a marine metagenome, but its location on a bacterial chromosome or on a mobile genetic element is unknown (Sanchez et al., 2008).

***qnr*S-like:** In silico analysis of the genome sequence of *Vibrio splendidus* identified an open reading fram encoding a 218-amino acid protein sharing 84% and 87% amino acid identities with QnrS1 and QnrS2, respectively. When overexpressed in *E. coli* cells, recombinant plasmids with the *V. splendidusqnr* genes conferred an eight fold increase in the MIC of nalidixic acid and 4- to-16 fold increases in the MIC's of fluoroquinolones (Cattoir et al., 2007).

2.3.3.3 Mechanism of action of pentapeptide repeats protein families conferring quinolone resistance

The vast majority of the pentapeptide repeat-containing proteins currently listed in the Pfam database of the Wellcome Trust Sanger Institute (www.sanger.ac.uk/cgi-bin/Pfam) are found in prokaryotes. The pentapeptide repeat proteins are of particular interest because they confer some level of quinolone resistance. Some analogous

pentapeptide repeat proteins that also confer fluoroquinolone resistance are McbG and MfpA.

McbG:

McbG is a pentapeptide repeat protein offering 19.6% amino acid similarity to Qnr. It protects DNA gyrase against the impact of a microcin (Garrido et al., 1998). Microcins are a class of small inhibitory proteins (under 10 kDa) that vary in their mechanism of activity. One of these, microcin B17 (MccB17), is a bacterial toxic substance that, similar to the quinolones, hinders DNA gyrase (however at an alternate site other than that of the quinolones). Organisms producing MccB17 additionally deliver McbG, which shields them from the impact of this poison, and mcbG has been found on resistance plasmids in clinical isolates. In host *E. coli* J53 cells, plasmid mediated mcbG created a slight increment in the MIC of sparfloxacin. Furthermore, within the sight of mcbG, mutants with resistance to sparfloxacin emerged at a concentration of sparfloxacin that was two-fold higher than that of J53 R-. Thus, the pentapeptide repeat protein McbG like Qnr proteins appears to provide some antiquinolone protection. The effect, however, was much smaller than that of the Qnr proteins (Heddle et al., 2001). Also, McbG did not appear to affect the susceptibility of *E. coli* J53 to ciprofloxacin or nalidixic acid.

MfpA and $MfpA_{Mt}$:

MfpA, a pentapeptide protein shares 18.9% amino acid similarity to QnrA. The *mfp*A gene was first identified in the chromosome of *Mycobacterium smegmatis* (Montero et al., 2001). When expressed on a multicopy plasmid, this quality brought about an expansion in the MIC of ciprofloxacin for this organism of somewhere around four and eight fold, and the inactivation of the gene on the *M. smegmatis* chromosome brought about expanded ciprofloxacin susceptiblty. The three-dimensional structure of this gene variant in *Mycobacterium tuberculosis*, MfpAMt, showed that the pentapeptide repeat arrangement encodes quadrilateral β-helix (Hegde et al., 2005). MfpAMt is prominent among pentapeptide repeat proteins in that (like QnrA) the primary buildup in the pentapeptide (deposit i-2) is regularly a cysteine (Vetting et al., 2006).

Hegde and coworkers have shown that MfpAMt inhibits ATP-dependent DNA supercoiling and ATP-independent relaxation reactions catalyzed by *E. coli* DNA gyrase. The apparent median inhibitory concentration (IC50) values were calculated to be ~1.2 M (based on active dimer) for both reactions in assay containing 3 units of DNA gyrase. In brief, molecules mobilized on a sensor surface alter the refraction of polarized light. When a sample is passed over a sample surface, and molecules interact with immobilized molecules, the level of light outflow changes in extent to the mass of bound material. Tests utilizing this procedure showed that MfpA associates straight forwardly with DNA gyrase. Both the three-dimensional structure of this MfpA homologue and its charge dissemination intently look like those of B-form DNA. This displacement may generate some resistance to fluoroquinolones, as DNA gyrase bound to MfpA will not participate in the deleterious quinolone-gyrase-cleaved DNA complex that is the basis for quinolone cell killing (Hegde et al., 2005).

2.3.3.4 Origins of *qnr* genes

Before the report of discovery of *qnr* genes in 1998, *qnr* number, variety and geographical and bacteriological penetration of these or similar genes existed for a considerable time (Kehrenberg et al., 2006). This raises questions about the origin and purpose of these genes before being recruited to protect bacteria from antimicrobial agents. Postulating that *qnr* genes originated on the chromosome of organisms occupying a human, veterinary or environmental reservoir, Poirel and colleagues screened the genome sequence of 48 gram-negative species from a wide range of genera for *qnr*A. Variants of *qnr*A (*qnr*A3 to *qnr*A5) were located on the chromosome of *Shewanella algae*. The quinolone MIC's of this organism were four-to-eight fold higher than those of *Shewanella putrefaciens*, a closely related organism lacking a chromosomal *qnr*A gene. It suggested that *S. algae* is a possible reservoir of *qnr*A. *Shewanella* spp. are water dwellers present in both marine and freshwater environments (Poirel et al., 2005).

Later, *qnr*A3-positive *Klebsiella pneumoniae* and *Kluyvera ascorbate* strains isolated from the feces of an immune compromised outpatient in Paris were analyzed. The sequence is immediately downstream from *qnr*A3 in the *S. algae* chromosome, supporting the notion that the *qnr*A3 has been excised from chromosomal DNA of *S. algae* or similar organism (Lascols et al., 2008). These pentapeptide repeat proteins

showing 40 to 67% amino acid identity to *qnr*A were present in other water borne *Shewanella* spp. Further studies found *qnr*B-like and *qnr*S2 genes in water and other environmental isolates (Saga et al., 2005). Sanchez et al. and Venter et al. found a gene encoding a pentapeptide repeat in a sequence database of microbial populations collected from seawater samples from the Sargasso sea (Venter et al., 2004). The protein designated marine metagene QnrA, was 88% similar to QnrB5 and QnrB19, but its functionality was not examined. It was noted that metagene *qnr* is 98% similar to *qnr*B8 and the first 214 amino acids are 99% identical to *Qnr*B8. In addition, BLAST analysis revealed the sequence that is closely linked to *qnr*B genes on plasmids downstream of metagene *qnr*, further supporting the aquatic origin of this PMQR determinant.

*qnr*S2 genes have also been found on plasmids carried by environmental organisms in 2006, Cattoir et al. sampled water from urban sites in the Seine River and found *Aeromonas punctuate subsp*. *punctata* and *Aeromonas media* strains carrying plasmids that transfered quinolone resistance. These plasmids were shown to carry *qnr*S2 (Cattoir et al., 2008). The same gene has also been found in plasmids from *Aeromonas allosaccharophilia* found in lake Lugano in 2005 (Picao et al., 2008) and together with a Tn1721-like transposon, on plasmid pGNB2 in sludge basin bacteria (from a German waste water treatment plant) in 2004. This plasmid also conferred decreased susceptibility to only quinolones (Bonemann et al., 2006).

All the above findings suggest that some *qnr* genes in circulation likely originated in the chromosomes of water dwelling environmental organisms. The recent detecting in *qnr*-bearing plasmids in water organisms suggests that fresh water inhabited areas may be a reservoir in which pathogens acquire these elements. Quinolones is excreted un-metabolized by mammals into waste water. The carboxylic acid of quinolones is degradable by sunlight in aqueous solution (Torniainen et al., 1997), but when these compounds enter the aquatic environment via sewage water, photo-degradation may be of only minor importance. Elimination can also occur via adsorption to sediments, degradation by terrestrial fungus or environmental *Mycobacterium* spp., but a substantial quantity of active drug may remain in the environment. For example, ciprofloxacin and other fluoroquinolones have been found at concentrations of up to 0.005 µg/ml in water sources including water downstream from a waste water

treatment plant in the United States and in the Seine River in France. Thus, it is possible that environmental quinolone accumulation has contributed to the success of these genes, perhaps by helping to maintain a reservoir of aquatic organisms for which a low-level quinolone resistance gene provided a survival advantage (Batt et al., 2006).

2.3.3.5 Biological functions of*qnr*:

The native function of *qnr* genes is obscure. Ellington and Woodford proposed that Qnr could be an antibody, shielding DNA gyrase and topoisomerase IV from some normally naturally occurring toxins (Ellington and Woodford., 2006). There are known characteristic DNA gyrase toxins. These incorporate CcdB, a toxin encoded on the F plasmid (Critchlow et al., 1997). ParE (particular from ParE subunit of topoisomerase IV), situated on the wide host-range RK2 plasmid (Jiang et al., 2002) and MccB17 a post transitionally changed peptide created from the plasmid-borne MccB17 operon. Each of these toxins is paired with an inhibitor (antitoxin) that protects cells from death and is encoded in the same operon: CcdA, *Par*D and McbG (which is in fact a pentapeptide repeat protein) (Heddle et al., 2001). Qnr may thus be analogous to one of the anti-toxins. Qnr may alternatively serve a function similar to that of the chromosomally encoded non-pentapeptide-repeat protein GyrI, a DNA gyrase regulator that also is capble of some anti-toxin and anti-quinolone effect. Plasmid-carried *qnr*B alleles having upstream LexA binding site, leading to increased *qnr*B expression levels upon exposure to quinolones, mitomycin and possibly other DNA-damaging agents as part of the SOS response. Thus, if such induction is also seen in an organism with a chromosomal *qnr*B progenitor, it suggests that *Qnr*B may have a native function in protection from naturally occurring DNA-damaging agents (Malik et al., 2006).

2.3.3.6 *Acc(6')-Ib-cr*

Discovery of *acc(6')-Ib-cr*

After the discovery of QnrA, Robicsek and associates were examining the wonder of imbalance in the level of quinolone resistance exchanged with various qnr plasmids. Wild-type *E. coli* strains had a MIC of ciprofloxacin of around 0.008µg/ml, and most qnr plasmids decided a MIC of ciprofloxacin of 0.25µg/ml for *E. coli*. Likewise it

was observed that specific plasmids from clinical *E. coli* strains gathered in Shanghai gave around four fold larger amounts of ciprofloxacin resistance (1.0µg/ml). It was observed that this abnormal state resistance was not created by an expanded level of expression of *qnr*A. By random transposon mutagenesis of plasmid DNA, they found that the gene in charge of the incremental resistance was an aminoglycoside acetyltransferase, *acc(6')- Ib*, which confers resistance to tobramycin, amikacin and kanamycin. Sequencing showed this allele to be unique among the approximately 30 known variants of *acc(6')-Ib* in two codon changes, Trp102Arf amd Asp179Tyr, which was found to be necessary and sufficient for the ciprofloxacin resistance phenotype. An acetylation assay showed the capacity of this *aac(6')-Ib* variant [designated as *AAC(6')-Ib-cr*, for ciprofloxacin resistance] to acetylate ciprofloxacin at the amino nitrogen on its piperazinyl substituent (Robicsek et al., 2006).

2.3.3.6.1 Resistance Activity of *AAC(6')-Ib-cr*

The increase in MIC conferred by *AAC(6')-Ib-cr* is smaller than that conferred by Qnr proteins, and its specific quinolone target is selective, only ciprofloxacin and norfloxacin, both of which have piperzinyl secondary amines. Other quinolones lacking an unsubstituted piperazinyl nitrogen were unaffected. Although the increase in the MICs of ciprofloxacin and norfloxacin was modest (three fold to four fold), the effect on the MPC was marked. In the presence of *acc(6')-Ib-cr*, resistant clones of wild-type *E. coli* strain J53 could still be recovered at concentrations of 1.6 µg ciprofloxacin per ml, a level approximating the peak serum concentration of free ciprofloxacin during therapy (Ellington et al., 2006).

2.3.3.6.2 AAC(6')-Ib-cr Protein

Kinetic studies of purified ACC(6')- IB and its cr variations showed that the mutant compound has just somewhat lessened effectiveness (with respect to that of wild sort catalyst) for the acetylation and kanamycin (Vetting et al., 2008). The acetylation of ciprofloxacin, although less effective than that of kanamycin, was adequate in bacterial cells to deliver a diminish susceptibility phenotype equal to that of cell presented to artificially incorporated N-acetyl ciprofloxacin (Robecseik et al., 2006), recommending that complete ciprofloxacin acetylation has happened under states of bacterial development. Deadend quinolone (pefloxacin) and aminoglycoside (lividomycin) substrates, for which the target sites of medication acetylation, are

either blocked (pefloxacin) or absent (lividomycin), both created a focused hindrance of acetylation of the genuine substrates kanamycin and ciprofloxacin, demonstrating an functional overlap in the coupling site of both classes of substrate of ACC(6')- Ib-cr (Vetting et al., 2008). The X-beam crystallographic structure of the wild-type protein with kanamycin and acetylco-chemical A has been explained and permitted the development of an molecular model of ciprofloxacin official to the cr variation (Vetting et al., 2008). In the model, the Asp179Tyr, mutation had the greatest effect resulting in a stacking interaction with the quinolone rings to enhance drug-binding. The Trp102Arg mutation was more distantly positioned, serving to stabilize the positioning of Tyr179. This model is consistent with the magnitude of the effects of the individual mutations, with Asp179Tyr having a partial resistance phenotype, Trp102Arg having little detectable resistance phenotype. An alternative model of positioning of ciprofloxacin that emphasizes plasticity in the enzyme active site proposes that Arg92 (equivalent to Arg102) has a direct interaction with the carboxyl group of ciprofloxacin but does not account for the relative effects of the two individual mutations. A direct structural analysis ACC(6')-Ib-cr with both acetyl coenzyme A and ciprofloxacin substrates is needed (Maurice et al., 2008)

2. 3. 3.3. 6. 3 Genetic Environment of *acc(6')-Ib-cr* Plasmids

Acc(6')-Ib-cr like its parent *acc(6')-Ib,* is in an integrin cassette with an associated attC site. It is hence found in various integrons, but especially on IncF11 plasmids expressing CTX-M-15 that have spread rapidly so that CTX-M-15 has become the predominant ESBL is many countries around the world (Boyd et al., 2004; Machado et al., 2006; Cordeiro et al., 2008; Pitout et al., 2008; Sabtcheva et al., 2009). *Acc(6')-Ib-cr* has been associated with other PMQR genes including *qnr*A1 (Jiang et al., 2008; Frank et al., 2011), *qnr*B2 (Pomba et al., 2009), *qnr*B4 (Ma et al., 2009), *qnr*B6 (Jiang et al., 2008), *qnr*B10 (Quiroga et al., 2007), *qnr*S1 (Jiang et al., 2008), *qnr*S2 (Picao et al., 2008) and *qep*A (Ma et al., 2009) and with other lactameses including CTX-M-1 (Soge et al., 2006), CTX-M-14 (Jiang et al., 2008), CTX-M-24 (Jiang et al., 2008), DHA-1 (Ma et al., 2009), SHV-12 (Ma et al., 2009) and KPC-2 (Chmelnitsky et al.,2008).

2.3.4 Plasmid-Mediated Quinolone Efflux

Antimicrobial efflux mechanisms can act on single or multiple agents and can be carried on both plasmids and chromosomes. Different mechanisms have been depicted, and they are progressively being perceived as important determinants of antimicrobial resistance in a variey of organism. Efflux determinants of quinolone resistance in gram-negative microorganisms are to a great extent multidrug transporters of the resistance-nodulation-cell division (RND) family encoded by endogenous chromosomal genes. This family of traditionally chromosomal efflux pumps is ubiquitous in gram-negative bacteria, serving to remove unwanted compounds from the cytoplasm and membrane. Two plasmid mediated quinolone transporters have now been found: OqxAB and more recently QepA (Putman et al., 2000).

OqxAB:

A conjugative plasmid, pOLA52, conferring resistance to the antibiotic olaquindox (a quinoxaline derivative that is used in agriculture as a growth promoter) was found in *E. coli* strains isolated from swine manure. The resistance component was recognized to be a multidrug efflux pump. Hansen and colleagues reported OqxAB has a place with the RND family (Hansen et al., 2004). It presents resistance to different specialists too, including chloramphenicol. Upon expression in an *E.coli* strain without a local acrA quality, pOLA52 presented 8 and 16 fold increments in the MIC's of nalidixic corrosive and ciprofloxacin, separately. In the single prevalence study performed by Kim et al. (2009), oqxAB was uncommon. Ten of 556 (1.8%) *E. coli* strains isolated between 1995 and 1998 in Denmark and Sweden were shown to have an MIC of olaquindox of ≥64μg/ml; in 9 of the 10 strains, the oqxA gene was detected. Plasmid-mediated OqxAB was recently detected in a human clinical *E. coli* isolate from South Korea. OqxAB genes are also present on the chromosome of *K. pneumoniae*, with different levels of expression being correlated with differences in susceptibility to olaquindox (Kim et al., 2009).

QepA:

The novel efflux pump of QepA was found to be encoded on plasmid pHPA, discovered in an *E. coli* strain from a urine specimen from an inpatient in Hyogo Prefecture, Japan, by Yamane et al. (2007). This plasmid showed a different resistance profile for aminoglycosides, fluoroquinolones and wide range B-lactams. qepA encoded a 511-amino acid protein putatively having a place with the 14-transmembrane section significant facilitator superfamily of transporters. The G+C contect of QepA is 72%, higher than that of chromosomes of Enterobacteriaceae (half). As per phylogenetic investigation, QepA has a place with the 14-transmembrane section family transporters of gram-positive Actinomycetales yet not those of gram-negative bacteria. Yamane and coworkers found that *qep*A cloned in pSTV28 increased the MIC's of several compounds in an *E. coli* trans-conjugant. The MIC's of nalidixic acid, ciprofloxacin and norfloxacin expanded 2-, 32-and 64-fold separately; a two-fold increment was observed for erythromycin, acriflavine and ethidium bromide, while the MIC's of other antibiotic classes and regular transporter substrates did not change. Since its revelation, a variation of qepA having two amino acids substitution has been found. This variant (named QepA2) conferred a phenotype similar to that of the QepA determinant, now called QepA1. Sequence analysis revealed that the qepA gene is located in a 10-kb region, with a flanking sequence that is in large part identical in different plasmids. However, *qep*A1-positive isolates from Japan (Yamane et al., 2007), Belgium and South Korea were flanked by two copies of IS26 and associated with the *rmt*B gene, encoding an aminoglycoside ribosomal methylase, whereas qepA2 was flanked by a novel insertion sequence element (ISCR3C) and rmtB was not found (Cattoir et al., 2008).

2.3.4.1 Genetic Environment of Efflux-Encoding Plasmids

The oqxAB plasmid analyzed inan *E. coli* isolate from Denmark is a 52-kb IncX1 plasmid (Sorensen et al., 2003). *qep*A1 has been found on 113 and 168-kb IncF1 plasmids from Belgium, while in *qep*A2 was discovered on a 90-kb IncF1 plasmid from France. Ruiz and coworkers have reported the association of *qep*A with IncFII and IncN plasmids (Ruiz et al., 2012).

2.4 Status of research related to quinolone resistance

Fluroquinolones are the only class of antimicrobial agents in clinical use that directly inhibit bacterial DNA synthesis by inhibiting two bacterial enzymes, DNA gyrase and topoisomerase IV. Resistance to quinolones occurs by mutation in chromosomal genes that encode the subunits of DNA-gyrase and topoisomerase IV and expression of efflux pumps (Drlica and Zhao., 1997 and Zhao et al., 1997). In the superseding years, mutations in the chromosomal genes were reported by many authors all over the world.

Fluoroquinolone was considered as first line drug for the treatment of typhoid fever. However, some *Salmonella enterica* serovar exhibit decreased susceptibilities to fluoroquinolones due to mutation in *gyr*A and *par*C. The fact that topoisomerase IV was the primary quinolone target in gram positive species was supported by the studies carried out by Ng et al. (1996). Decreased susceptibility to fluoroquinolones is associated with specific point mutations in *gyr*A amlong several species of Enterobacteriaceae was described by Weigel et al. (1998). The relationships between DNA gyrase and topoisomerase IV as primary or secondary targets in *E. coli* was reported by Hooper (2000). Mutation in *par*C that resulted in higher MICs of ciprofloxacin, norfloxacin, levofloxacin and trovafloxacin was reported in *Streptococcus pneumoniae* (Fukuda et al., 1999). Sorlozano et al. (2007) described the acquisition of mutation within the QRDR of the *par*E at position 458 in Enterobacteriaceae. FQs have proven to be among the most effective second line anti-mycobacterial drugs but the increasing incidence of FQ resistance in *Mycobacterium tuberculosis* were reported by many authors (Wang et al., 2007; Malik et al., 2006).

Another critical factor contributing to fluoroquinolone resistance is the over-expression of efflux pumps. The impacts of efflux pumps on fluoroquinolone activity in *S. pneumoniae* were reported by Piddock et al, (1998). Poole (2005) demonstrated that transporters belonging to four of the five families of multidrug resistance system the ATP binding cassette (ABC) family, the major facilitator superfamily (MFS), the resistance-nodulation division (RND) family, and the multidrug and toxic compound extrusion (MATE) family, are capable of extruding quinolones in both gram positive and gram negative bacteria.

Several mechanisms associated with transferable plasmid mediated fluoroquinolone resistance mechanisms are known: 1. Qnr, 2. Aminoglycoside acetyltransferase *aac(6')-Ib-cr*, 3.OqxAB, QepA. The emergence of plasmid-mediated quinolone resistance was first found in strains of *Klebsiella pneumoniae* in one region of the United (Martinez- Martinez et al., 1998). An overview for *qnr*A by PCR of more than 350 gram-negative isolates gathered basically in the 1990s and incorporated a wide geographic range and an assortment of genera of gram-negative microorganisms discovered *qnr*A in just six isolates (four *E. coli* and two *Klebsiella* spp. isolates), all from the same center in Albama where the original strain had been detected and all collected between July and December 2004 (Jacoby et al., 2008). Since that early study, more epidemiological surveys have been reported. The earliest known qnr alleles are a *qnr*-B8 like quality in a *C. freundii* isolate from Brooklyn, NewYork and a *qnr*-B9 like gene in a *K. pneumoniae* isolate from Cordoba, Argentina both collected in 1988 (Jacoby et al., 2006). By and large, studies have been confined to a restricted geological area and a constrained scope of genera. Most studies utilized PCR techniques to analyze clinical isolates of Enterboacteriaceae gathered in the 1990's or mid 2000's, crossing periods running from a couple of months to over 10 years. The avarage prevalances of *qnr*A, *qnr*B, *qnr*S and *acc(6')- Ib-cr* in the database were 1.5%, 4.6%, 2.4% and 10.8% , separately. Early overviews searched for *qnr*A and in late studies *qnr*S, *qnr*B, *acc(6')- Ib-cr* and *qep*A were likewise included. *E. coli* has been the most well-known species screened for PMQR. Nonetheless, in most of the overviews, qnr was more predominant among *Enterbacter* spp. and *Klebsiella* spp. than in *E. coli* strains (Jiang et al., 2008; Jung et al., 2009). Then again, studies that incorporated the balanced dissemination of isolates of Enterbacteriaceae, it was apparent that *acc(6')- Ib-cr* is most normal among *E. coli* strains (Park et al., 2007; Jiang et al., 2008). The determination criteria for isolates included in surveys could potentially bias prevalence data. Surveys for the most part have been performed with isolates gathered over a brief period or isolates that are resistant to different drugs, most usually quinolones or ESBLs. In some studies, strains were collected just with regards to an outbreak. The occurrence of PMQR genes has expanded for last decades. A few studies were conducted over adequately long periods to survey patterns. For example, in a survey in Paris, France, no *qnr* genes were found in 2002, and 10 were found in 2005 (Poirel et al., 2005; Mammeri et al., 2005). In addition, 41

of 1,147 *K. pneumoniae* bloodstream isolates collected in Taiwan from 1999 through 2005 were *qnr*B4 positive; no *qnr*B4 genes were found in 1999 to 2000, but in 2005, 14 isolates (7.6%) were positive. In a cohort of clinical *Enterobacter* spp. isolates from Jerusalem, Israel, that were collected from 1990 through 1993, none of 94 isolates had *qnr*; in isolates from 1994 through 2005, 33 out of 485 (6.8%) isolates had *qnr*. Findings were similar for *K. pneumoniae* (Strahielevitz et al., 2009). A similar scenario was seen with *acc(6')-Ib-cr*. The cr variant was not found among 150 *acc(6')-Ib*-positive strains collected between 1981 and 1991. In a study from Ljubljana, Slovenia from 2000 through 2002*, acc(6')-Ib-cr* was detected in 1 of 17 *Klebsiella* isolates, whereas in 2003 to 2005, the prevalence increased significantly to 24 of 57 isolates (Ambrozic Avgustin et al., 2007). In Calgary, Canada, the prevalence of *acc(6')-Ib-cr* significantly increased from 5 of 121 isolates (4.1%) in 2004 to 52 of 346 *E. coli* strains (Pitout et al., 2008). In Jerusalem, *E. coli* isolates bearing *acc(6')-Ib-cr* emerged in 1998, and since then*, acc(6')-Ib-cr* progressively penetrated into multiple clinical *E. coli* clones (Warburg et al., 2009). A survey of the *qnr, acc(6')- Ib-cr*, *qep*A qualities among 461 unselected, consecutive bloodstream isolates collected in Seoul, South Korea, in two periods, 1998 to 2001 and 2005 to 2006, substantiate the findings from earlier studies. There was a noteworthy increase in the rate of ciprofloxacin resistance over time. In spite of the fact that *qnr*B was the most prevalent PMQR gene, there was a overall increase in the prevalence of PMQR genes, speaking to an expanding assorted genes in PMQR genes instead of a predominance of single gene (Kim et al., 2009). It was also found that in strains with PMQR gene, there was a huge increase in the *gyr*A and/or *par*C resistance mutations after some time, yet in strains without PMQR qualities, these mutations remained stable over the long run. This epidemiological affiliation underpins the part of PMQR qualities in advancing larger amounts of resistance by transformation in clinical settings, as has been exhibited in the research facility. Qnr qualities have as of now been found in every single populated mainland (and also the waters in the middle of them) and in most clinically basic Enterobacteriaceae. These species incorporate *E. coli*, *Klebsiella* spp. (*K. pneumoniae* and *Klebsiella oxytoca*), *Enterobacter* spp. (*Enterobacter cloacae*, *Enterobacteraerogenes*, *Enterobacter amnigenus* and *Enterobacter sakazakii*), *Citrobacter freundii* and *Providencia stuartii*. Among these, *qnr* has been all the more ordinarily identified in *Enterobacter* spp. taken after by *K.*

pneumoniae and less so in *E. coli*, where *acc(6')- Ib-cr* is by all accounts more common. Up to this point, absent from this point were *Proteus* spp and clinically important non-enetric gram-negative microscopic organisms (e.g., *Pseudomonas aeruginosa* and *Acinetobacter* spp.). All three have been included in small surveys of isolates of human origin, without PMQR genes being detected. *qnr* was also not found among multidrug resistant *Acinetobacter baumannii* isolates. Interestingly, in a recent survey of isolates from zoo animals, *qnr*B was detected in *Pseudomonas fluorescence* from a turtle and in *Proteus mirabilis* from feces of Bengalese finches (Ahmed et al., 2007). *Qnr*A has also recently been found in an isolate of *A. baumannii*. Epidemiological studies have been useful in supporting genetic data indicating a linkage of PMQR with other resistance genes, particularly ESBLs. Various investigators have demonstrated higher *qnr* prevalence's among ESBL-positive strain. For example, Strahilevitz and coworkers found that among clinical isolates of *Enterobacter* spp. and *Klebsiella pneumoniae*, the relative risk for ceftazidime resistance (a surrogate for ESBL presence) in *qnr*-positive *K. pneumoniae* isolates was 1.8 (95% confidence interval, 1.3 to 2.5), and in *Enterobacter* isolates, it was 3.5 (95% confidence interval, 2.7 to 4.5) (Strahilevitz et al., 2009). Further work demonstrated that the ceftazidime resistance in *qnr*-positive *Enterobacter* strains was associated with a true ESBL mediated mechanism. Because fluoroquinolones remain one of the few options for treating infections caused by such organisms, it is of concern that a substantial fraction of the ceftazidime-resistant *qnr*-carrying isolates in this study were susceptible to ciprofloxacin according to CLSI criteria. Similarly, in a French survey of ESBL-positive isolates of Enterobacteriaceae, 43% of *qnr*A-positive isolates tested as ciprofloxacin susceptible. Whether such *qnr*A-positive, fluoroquinolone-"susceptible" isolates can be effectively treated with fluoroquinolones requires further investigation. A close association of *acc(6')-Ib-cr* with CTX-M-15, arising from epidemiological studies in hospital as well as community settings has emerged worldwide in recent years (Pitout et al., 2008). Despite continued efforts to control its spread, non-typhi *Salmonella enterica* persists as the most common food-borne pathogen in the United States. Unlike most Enterobacteriaceae species in which *qnr* genes have been detected, non-typhi serotypes of *Salmonella enetrica* are carried largely in the intestinal tract of food animals and are transmitted to humans through the food chain. Therefore, quinolone

use in agriculture may drive the dissemination of *qnr*-mediated resistance in these pathogens, and mapping of *qnr* in non-typhi serotypes of *S. enetrica* could serve as a marker of the route of infiltration of antibiotic resistance from the food animal industry to humans. *qnr*A determinants were also detected from multidrug resistant Enterobacteriaceae from blood cultures in Liverpool, United Kingdom. In the subsequent years, several distantly related plasmid mediated Qnr determinants were described in Enterobacteriaceae. *qnr*S was detected by Hata and coworkers., 2005 in a single clone of *Shigella flexneri* 2b which caused a food borne outbreak of enterocolitis in Aichi Prefecture, Japan (Hata et al., 2005). A variant of *qnr*S (*qnr*S2) was detected on a plasmid from *Salmonella enteritica* serovar Anatum by Gay et al., 2006 in United States (Gay et al., 2006). At about same time in Germany *qnr*S2 was found on a plasmid isolated from the activated sludge basin of a wastewater treatment plant (Bonemann et al., 2006). Jacoby and colleagues were the first who found that several *K.pneumoniae* could transfer low-level quinolone resistance and the PMQR gene responsible for this phenotype was termed *qnr*B1 (Jacoby et al., 2006). The first variant, *qnr*B2 and latter on other variants *qnr*B3, *qnr*B4 were also detected among several isolates of Enterobacteriaceae from United States (Jacoby et al., 2006; Robicsek et al., 2006). *qnr*B as predominant determinant in commensal enterobaceria isolates from healthy children was reported in Peru and Bolivia (Pallecchi et al., 2009) and in Korea (Kim et al., 2010). Wang et al. (2009) first isolated *qnr*C from plasmid which transferred low-level quinolone resistance and negative by PCR for the known *qnr* genes, from a clinical strain of *Proteus mirabilis* in Havard Medical College, Boston, Massachusetts. From the same Medical College, Robicsek et al. reported variants of *aac(6')Ib-cr* which acetylates ciprofloxacin (Robicsek et al., 2006). In the year 2009 from China, Cavaco and co-workers reported a small plasmid of 4.3 kb which encodes 214-amino-acid pentapeptide repeat protein and designated as QnrD (Cavaco et al., 2009). *qnr*D has also been reported in *Morgenella morganii* and *Proteus mirabilis* from Italy (Koncan et al., 2010) and *M. morganii* from Algeria (Iabadene et al., 2008). Paiva et al. (2012) reported for the first time *qnr*B19, *qnr*S1 and *aac(6')-Ib-cr* among uropathogenic *E. coli* in Brazil. Later on *qnr*B19 was also reported from *E. coli* of animal origin in Germany (Schink et al., 2012). In Iran, Firoozeh et al. (2014) detected the presence of *qnr*A and *qnr*B among *E. coli* isolates.

The efflux pump Qep*A* was first discovered in an *E. coli* strain from a urine specimen in Hyogo Prefecture, Japan (Yamane et al., 2007). Less data was accessible about the epidemiology of the PMQR pump QepA when it was new found. A review performed in Japan discovered qepA in 2 (0.3%) of 751 *E. coli* isolates (cut off of MIC of norfloxacin, ≥0.025 µg/ml) gathered from 140 healing centers somewhere around 2002 and 2006 (Yamane et al., 2008). A second expansive overview was done by PCR in France. A solitary *E. coli* isolates among 121 (0.8%) ESBL-positive Enterobacteriaceae strains isolated in 2007 was certain for a variation named *qep*A2 (Cattoir et al., 2008). In investigation of pig homesteads in China, *qep*A was found in 16 of 101 (15.8%) segregates, including, surprisingly, *K. pneumonia* and *E. cloacae* (Ma et al., 2009). Few as of late distributed studies demonstrated a wide distribution of the gene. Two extra studies screened isolates from Seoul, South Korea, for *qep*A. Four clonally unrelated strains of 621 (0.6%) *E. coli* blood stream isolates were observed to be sure in one study (Cheong et al., 2001), and two E. aerogenes separates of 223 (0.9%) *E. cloacae, E. aerogenes, C. freundii* and *Serratia marcescens* disconnects with lessened weakness to quinolones were *qep*A positive in the second overview (Park et al., 2007).qepA was not found in a large survey of non-typhi *Salmonella enetrica* isolates collected in the United States from 1996 to 2006 (Sjolund -Karlsson et al., 2009). Cheong and coworkers reported *qepA* from *E. coli* blood stream isolates in Korea. *qepA* was also found in a survey of non-Typhi *Salmonella enteric* in United States (Sjolund -Karlsson et al., 2009).

2.4.1 Status in India

Pazhani and co-workers found fluoroquinolone resistant *Shigella dysenteriae* type1 and *Shigella flexneri* type 2a which had mutations in *gyr*A and *par*C genes during shigellosis outbreak in Ahmedabad (Pazhani et al., 2008). In Andaman and Nicobar Island, *Salmonella enterica* serovar Typhi was isolated which showed high MICs (>256µg/ml) against nalidixic acid, norfloxacin and ciprofloxacin had mutations in *gyr*A and *par*C (Thamizhmani et al., 2012). Divya et al. (2015) reported *Shigella* spp. with mutation in *gyr*A and *par*C which showed high level of resistance to

ciprofloxacin and norfloaxcin. Taneja et al. (2015) reported *Shigella* spp. with mutation in *gyr*A and *par*C with high MIC against ciprofloxacin.

Magesh and co-workers reported the presence of *qnr*A, *qnr*B and *aac(6')-Ib-cr* from South India among *K.pneumoniae* isolates (Magesh et al., 2011). *qnr*A, *qnr*B, *aac(6')-Ib-cr* and *qepA* were also found among *E. coli* isolates from hospital waste water treatment plant (Diwan et al., 2012). Pathak et al. (2013) reported *aac(6')-Ib-cr* and mutations in *gyr*A and *par*C among multi drug resistant *E. coli* from Central India. The association of *qnr* genes along with ESBL genes in pathogenic *Klebsiella pneumonia*e were shown by Tripathi et al. (2012).

2.5 Methods of Detection of PMQR

As it is known, the resistance phenotype does not recognize PMQR and other resistance mechanisms. PMQR gene present low-level quinolone resistance that is beneath the CLSI breakpoint for non-susceptibility, like that gave by initial step DNA gyrase mutations, transporters that prohibit quinolones, and diminished levels of expression of porins. The phenotype of low-level nalidixic acid resistance and diminish ciprofloxacin susceptibility sometimes observed among qnr-positive strains is neither sensitive nor specific. In this manner, screening for qnr qualities is for the most part done by PCR amplification of the target genes. To encourage a higher throughput, various gatherings have utilized multiplex PCR for the identification of qnrA, qnrB and qnrS (Robicsek et al., 2006). Later, additional sets of primers were used, including multiplex PCR with degenerate primers for *qnr*B, to overcome the wider variability within this group. For example, primers which were used did not fully match all *qnr*B genes, and the reverse primers mismatched at the 3'end with *qnr*B5, *qnr*B10 and *qnr*B19. *qnr*B5, was however, detected using these primers. Similarly, the reverse degenerate primer for *qnr*B did not completely align with *qnr*B17 (Gay et al., 2006). Also, false-positive amplicons have been reported for multiplex PCR procedures that are not seen with simplex PCR using the same individual primer pairs within the multiplex primer mixture, emphasizing the importance of extensive DNA sequence confirmation (Kim et al., 2009). Because a comparison of the detection methods has not been performed, the prevalence studies were potentially subject to detection bias. Because the difference between *acc(6')-Ib-cr* and *acc(6')-Ib* is in only two necleotides, screening for *acc(6')-Ib-cr* has

traditionally involved *acc(6')-Ib* amplification followed by sequencing or restriction analysis (Pitout et al., 2008). To overcome this cumbersome and costly method, Warburg et al. (2009) employed the gap-ligase chain reaction for the G to T change in the cr variant at nucleotide 535, one of the two defining mutations of *acc(6')-Ib-cr*, to screen a large database for this gene. In this technique, two same-directional primers, separated by a gap of several nucleotides, were chosen. These primers hybridize to complementary strands of target DNA and are extended by a DNA polymerase and subsequently ligate into a single long oligonucleotide when the mutation of interest, which corresponds to the 3' end of the first primer, is present. This oligonucleotide can then be amplified. All *acc(6')-Ib-cr* positive control strains were identified, and by optimizing the assay conditions, it was able to use unquantified extracts of whole-cell DNA and maintain specificity.

2.5.1 Molecular detection of quinolone resistance

A large portion of the studies have utilized DNA sequencing of PCR-amplified DNA for the identification of changes in the target enzymes. The primers, however, are for the most part species- specific. In this way, PCR sequencing tests for quinolone resistance are accessible just in research laboratories that have DNA sequencing facility. Since numerous research facilities don't have the equiptment, time or ability to sequence gene for the examination of mutations significant to fluoroquinolone resistance, different strategies have been utilized for the particular discovery of mutations in the genes or modifications in the enzymes.

The correlation between fluoroquinolone resistance and mutations in the *gyr*A and *par*C genes of *P. aeruginosa* clinical isolates from the urine of urinary tract infection patients and their rapid detection were done by denaturing high-performance liquid chromatography (DHPLC). PCR products were mixed with an equal amount of DNA amplifies from a corresponding wild-type reference sample. Heteroduplexed PCR products were analyzed using the WAVE system with a DNA sep HT catridge. (Matsumoto et al., 2012).

Detection of genes coding for resistance can be determined by PCR strategies. The approach of real- time PCR has allowed the better detection of molecular mechanism of AMR. Such techniques can be specifically connected for identification of MRSA

and VRE in clinical materia. Two continuous polymerase chain reaction assays were created to identify changes in codons 83 and 87 in *gyr*A and in codons 80 and 91 in *par*C, the principal site that causes quinolone resistance in pathogenic *Escherichia coli* and *Shigella* spp. Two multiplex PCR tests were produced to recognize changes in the QRDRs of *gyr*A and *par*C. In the principal measure (named Gyr), Taqman MGB tests were intended to recognize wild-type nucleotide arrangements comparing to the codons of Ser80 and Agr87 in parC. Selective probes for both assays were composed utilizing Primer Express (form 3.0; Applied Biosystems, Foster City, CA, USA) to recognize changes in the a respectable halfway point of each of the focused on codon. Template DNA was set up from each isolate by boiling in 100 mL of 10 mM Tris-HC1 (pH 8.0) for 10 minutes. The primers were incorporated by Bioneer (Seoul, Korea) and the tests were blended by Applied Biosystems. For every assay set, the probe for one allele was named with FAM (6-carboxy-fluorescein) and the other was marked with VIC (4, 7, 2 0-trichloro – 70 – phenyl 6-carboxyfluorescein). The RT-PCR test produced for this study can all the while distinguish changes in particular regions of the *gyr*A and *par*C genes (Kim et al., 2012).

Hees and coworkers performed the analysis of the gyrase A (*gyr*A) gene in *E. coli* isolates by denaturing gradient gel electrophoresis (DGGE) and clonal relatedness was determined by the single-enzyme amplified fragment length polymorphism (seAFLP) technique. DGGE revealed 11 different *gyr*A sequence patterns and based on AFLP analysis, there was proof of choice of ciprofloxacin-resistant strains under antibiotic pressure, as well as the occurrence of genetically indistinguishable ciprofloxacin-resistant and susceptible *E. coli* isolates. Clonal dissemination of ciprofloxacin-resistant *E. coli* was observed, however did not prevail (Hees et al., 2011).

Detection of mutation in the QRDRs region of *Gyr*A and *Par*C can also be done by direct sequencing of amplified product of *gyr*A and *par*C genes. The obtained sequences were analyzed with the Lasergene software (DNASTAR) and the predicted amino acid sequences of *Gyr*A and *Par*C were analyzed for amino acid changes by comparison with wild-type *Gyr*A of *E. coli* K-12. Eight types of mutation and six types of mutation were observed in *gyr*A and *par*C (Liu et al., 2012).

Two mismatch amplification mutation (MAMA) assays were produced and used to encourage fast identification of *gyr*A and *par*C mutations. Two duplex PCR assays (gyrA83+parC80 examine and gyrA87+parC84 measure) were produced for the concurrent identification of mutations in Ser83 codon of *gyr*A subunit and ser80 codon of *Par*C subunit, repectively. Universal *gyr*A and *par*C forward primers (Li et al., 2012) were utilized together with the reverse primers (MAMA primers) outlined in this study for the enhancement of *gyr*A (Ser83 and Asp87) and *par*C (Ser80 and Glu84) genetic regions. The reverse primers were complimentary to the wild type alleled of *gyr*A and *par*C arrangements of *K. pneumonia* strain ATCC 13883, except for a mismatch at the antepenultimate (- 3) nucleotide of the 3'end of every MAMA preliminary, which was incorporated to enhance allele discrimination. The MAMA primers template mismatches incorporated into this study were C:C (in gyrA83 and 87), A:G (in ParC80), G:A (in parC84). The determination of the mismatches was based on past perception of their consequences for the general PCR yield (Kwow et al., 1990). The presence of single primer template mismatch has negligible impact on the PCR yield; consequently the wild-type gene can be amplified productively. On instance of mutation(s), PCR efficiency will be extremely lessened because of the presence of extra mismatch(es) at the 3' end of the MAMA primers which won't bind to the template ; in this way, amplification of the target gene failed. The performance of the primers was assessed utilizing monoplex PCR before use in the duplex PCR assays which were finally optimized for the simultaneous detection of mutation in Ser83 codon of GyrA subunit with Ser80 codon of ParC subunit and in Asp87 codon of GyrA subunit with Glu84 codon of ParC subunit.

PCR products were analysed on a 2% agarose gel prestained with 0.5 ug/mL ethidium bromide in 0.5x TBE buffer.

Detection of genes coding for resistance can be determined by PCR methods. The advent of real-time PCR has revolutionized detection as well as understanding of molecular mechanisms of AMR. Such methods can be directly applied for detection of MRSA and VRE in clinical material.

Various methods have been reported to detect point mutations in target genes, including sequence specific oligonucleotide probe hybridization, sequencing of the target genes, RFLP, radioisotopic or nonradioisotopic SSCP analysis, mismatch

amplification mutation assay PCR and allele-specific PCR in combination with RFLP. Fasching et al. (1991) developed probes to detect mutation in staphylococcal *gyr*A genes associated with resistance, but the probes have proved difficult to use and not all resistant-strains demonstrate differential binding of the probes. Most of the studies have employed DNA sequencing of PCR-amplified DNA for the detection of alterations in the target enzymes. The primers, however, are mostly spieces specific. Thus, PCR sequencing tests for quinolone resistance are available only in laboratories that have DNA sequencing capability. Since numerous research centers don't have the equiptment, time, or mastery to sequence gene for the examination of mutations pertinent to fluoroquinolone resistance, different strategies have been utilized for the particular identification of mutations in the genes or modifications in the enzymes. (Fasching et al., 1991).

Tokue et al. (1994) studied 36 *S. aureus* isolates by non-radioisotopic SSCP (nRI-SSCP) for the presence of point mutations in the *gyr*A gene. Direct DNA-sequencing analysis of the PCR-amplified DNA fragments confirmed the results obtained by nRI-SSCP. The authors identified seven mutational types which were separated from the wild-type in a single electrophoretic step within 2h after PCR amplification. The authors concluded that the use of the nRI-SSCP methods allows relatively rapid analysis of DNA from a large number from strains. Since the electrophoretic pattern is theoretically specific for each mutation, identification of a specific mutation is possible by comparison of the mobilities of the sample DNAs with that of control DNA carrying known mutations. Thus, nRI-SSCP analysis is a simple and useful method not only for the detection of point mutations associated with quinolone resistance but also for the investigation of epidemiologic markers (Tokue et al., 1994).

In an extended study, Wang et al. (1998) analysed mutations in the *grl*A and gyrA genes of 344 clinical strains of *S. aureus* by combination of nRI-SSCP, restriction fragment lengthanalysis, and direct sequencing. Five sorts of single-point changes and four sorts of twofold mutations were seen in *grl*A genes, while four sorts of single-point changes and four sorts of twofold mutations were found in the *gyr*A qualities. Six of nine sorts of *grl*A changes and a wide range of *gyr*A mutations were discernable from the wild-sort by nRI-SSCP examination. Despite the fact that the

force of direct sequencing was more noteworthy for the recognition of *gyr*A mutations, the authors reasoned that SSCP investigation is a fast, basic and viable strategy for location of point mutations in both the *grl*A gene and the *gyr*A gene of *S. aureus* strains.

Meanwhile, several investigators have applied SSCP analysis to the detection of mutations, mainly in *gyr*A, in several bacterial species. Takenouchi et al. (1999) studied *gyr*A point mutations in 335 clinical *P. aeruginosa* isolates by nRI-SSCP analysis and direct sequencing. By SSCP analysis, 18 band patterns could be differentiated, with each pattern corresponding to a distinct mutation. The band patterns were reproducible and particular from each other. Since SSCP investigation is basic and fast, it may likewise be appropriate for epidemiological observation of of organism involved in outbreaks for epidemiological resistance studies (Takenouchi et al., 1999).

A mismatch amplification mutation assay (MAMA) PCR protocol was produced by Zirnstein et al. (1999) that distinguishes the most usually experienced *gyr*A mutation in quinolone-resistant *Campylobacter jejuni* isolates. Since the Thr86-Ile modification was the most generally experienced change prompting fluoroquinolone resistance, the authors have built up a MAMA protocol for the identification of this exceptional adjustment. A conserved forwardprimer, MAMA *gyr*A1and a reverse mutation detecton primer, MAMA *gyr*A5, were utilized together as a part of a PCR to create a 256-bp PCR product that was a positive sign of the presence of the uncommon adjustment Thr86-Ile in *C. jejuni gyr*A. Primer GZgyrA4, a conserve reverse primer, was utilized as a part of conjunction with primer MAMA *gyr*A1, to create a positive PCR control result of 368 bp with any *C. jejuni gyr*A gene. Isolates with the wild-sort amino acid 86 codon were not intensified with the converse mutation primer MAMAgyrA5, while detaches with the changed amino acid 86 codon created a 256-bp PCR product with MAMA gyrA5 reverse mutation primer and the MAMA gyrA1 forward mutation primer. Conserved primers GZgyrA4 and MAMA gyrA1 generated a 368-bpgyrA PCR product with DNA isolated from all isolates. At the point when the MAMA protocol is utilized, isolates must be affirmed as *C. jejuni*, since false-positive PCR items can come about when *E. coli* detaches are utilized. While the MAMA PCR assay described by Zirnstein et al. (1999) is undoubtedly simpler than

DNA sequencing for use in determining the presence of mutations relevant for fluoroquinolone resistance, it does have a disadvantage. Different adjustments, other than the most generally experienced Thr-86-Ile change, can't be recognized in gyrA. Be that as it may, it ought to be easy to build up extra MAMA PCR mutation detection primers. The MAMA PCR technique is a basic and fast other option to SSCP and DNA sequencing for the location of essential changes inside the QRDRs (Zirnstein et al., 1999).

Giraud et al. developed a rapid assay combining allele-specific PCR and RFLP (AS-PCR-RFLP) for the screening of point mutations responsible for all amino acid changes encoded by the *Salmonella enetrica* sarovor Typhimurium *gyr*A gene at codons 81, 83 and 87. These modifications were most oftentimes experienced in fluoroquinolone-resistant clinical and laboratory strains. The PCR amplification was performed by three primers. The forward primer, STGYRA1, and the reverse primer, STGYRA-HinfI/87, were relied upon to create a 195-bp fragment with a HinfI restriction site at the codon comparing to Ser83. As already depicted for *E. coli*, the reverse primer STGYRA-HinfI/87, whose sequence is distinctive by 1 base from the gene sequence, presented a artificial HinfI cleavage site including the Asp87 codon as indicated by the primer determined restriction site change technique. A second allele-particular forward primer, AS-81, whose 3'- terminal nucleotide compares to the first nucleotide of codon 81, allowed the amplification of a 80-bp fragment only in the presence of this nucleotide, a fragment that likewise contained both the natural and the artificial HinfI cleavage destinations. The AS-PCR-RFLP is by all accounts a basic and quick contrasting option to SSCP and DNA sequencing for the location of vital alterations inside the QRDRs. In contrast to the MAMA PCR method, several mutations can be detected. This method has the potential to be used as a quick screening method in laboratories where systematic sequencing of the target gene is not suitable (Giraud et al., 1999).

2.6 Genetic environment of *qnr* determinants:

The appearance and spread of antibiotic resistance in bacterial pathogens is significant example of evolution of bacterial resistance. But these are like assembly

platforms which integrate exogenous open reading frames (ORFs) by site-specific recombination and change them to functional bacterial genes by ensuring the correct gene expression (Figure 6). However these integrons are composed of three key elements which are compulsory for the capture of exogenous genes: intI; this is responsible for encoding an integrase which belongs to the tyrosine recombinase family; attI site (a primary recombination site); and Pc promoter, an outward-oriented promoter that directs transcription of the captured bacterial resistance genes (figure 6) (Hall et al., 1995). However in these integrin encoded integrases enzyme can recombine distinct units of circularized DNA (Collis et al., 1993) which is well known as gene cassettes. Integration occur downstream of the resident Pc promoter at the attI site, allowing expression of the genes in the cassette in the integrons (Mazel. 2006). There is an attC site in each integrons which is containing single genes and an imperfect inverted repeat at the 3' end. These are also known as 59 be and are a diverse family of nucleotide sequences that function as recognition sites for the specific integrase. Their length varies from 57 bp to 141 bp (figure 6) (Mazel. 2006).

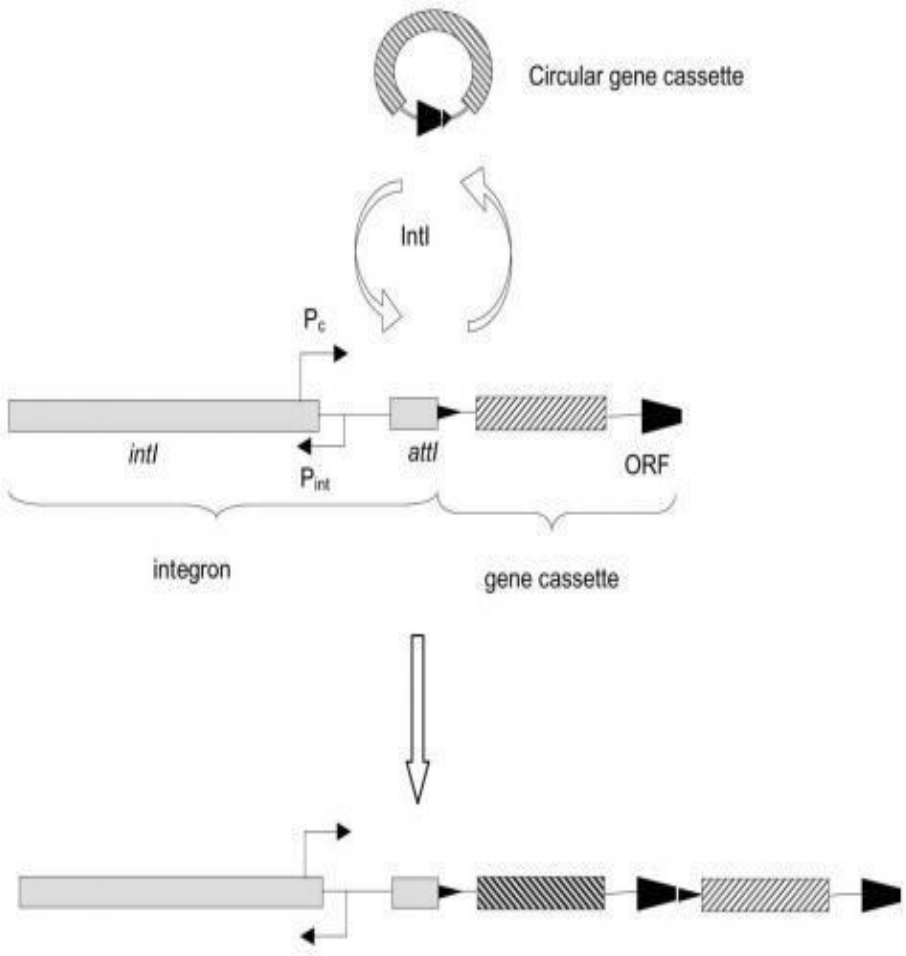


Figure 6: Generalized structure of the integron/gene cassette system.

(Image adopted from Michael et al., 2004, Am. Nat. 2004. Vol. 164, pp. 1–12).

The intI gene encodes the IntI protein. The promoter for cassette associated genes is Pc. The filled arrow represents a 59-be site. The integrin-associated recombination site is attI.

Genes for quinolone resistance have been found on plasmids fluctuating in size and incompatibility specificity, showing that the spread of different plasmids has been in charge of dispersal of this resistance around the globe. The prompt genetic

environment of each gene type, in any case, is sufficiently comparative to recommend a set number of events took after by transposition, recombination, replicon combination and determination, and deletion and insertion of DNA to produce the differing qualities of plasmid structures seen today. Now and then the plasmids are similar to the point that an expansive dissmenation of the same plasmid appears likely. For instance, *qnr*A1 plasmids can differ in size from 20 to 320 kb (Cambau et al., 2006) and have a place with atleast three plasmid incongruently (Inc) bunches (Poirel et al., 2005a). *qnr*A1 is normally connected with ISCR1 (Toleman et al., 2006), albeit 6.2% of *qnr*-A1 positive strains in a study from South Korea were negative for ISCR1 by PCR (Park et al., 2007). As a rule, a solitary duplicate of ISCR1is discovered downstream from qnrA1, yet in pMG252 and related plasmids, the qnrA1 quality is sectioned by two duplicates of ISCR1 (Robicsek et al., 2006). The *qnr*A1 ISCR1 complex is inserted in turn into a sull-type integrin containing several other resistance gene cassettes (figure 7) and AmpC-lactamases are often found on the same plasmid. *qnr*A1 was discovered in an isolate from Alabama on plasmid pMG252 also experiencing the uncommon FOX-5 B-lactamase. Plasmids carrying *qnr*A1 and FOX-5 have subsequently been found in specimens from Delaware, Kentucky, New York, North Carolina and Tennessee (Wang et al., 2004; Rodriguez- Martinez et al., 2009;) and from as far away as Brazil (Castanheira et al., 2008). In isolates from Canada, France, Thailand and Turkey, *qnr*A1 is associated with VEB-1βlactamase (Mammeri et al., 2005; Nazic et al., 2011; Poirel et al., 2005b; Poirel et al., 2005c), while in samples from other countries, *qnr*A1 and extended spectrum Blactamase (ESBL) SHV-12 and various CTX-M enzymes are linked (Wu et al., 2007; Yang et al., 2008). *qnr*B1 was discovered on a 340-kb multiresistance plasmid from India (Jacoby et al., 2006) and was subsequently reported for isolates from Algeria (Iabadene et al., 2008), Denmark (Torpdahl et al., 2009), South Korea (Park et al., 2007), Nigeria (Soge et al., 2006) and Scotland (Murray et al., 2008). It is associated not with ISCR1 but with another putative transposase, Orf1005.

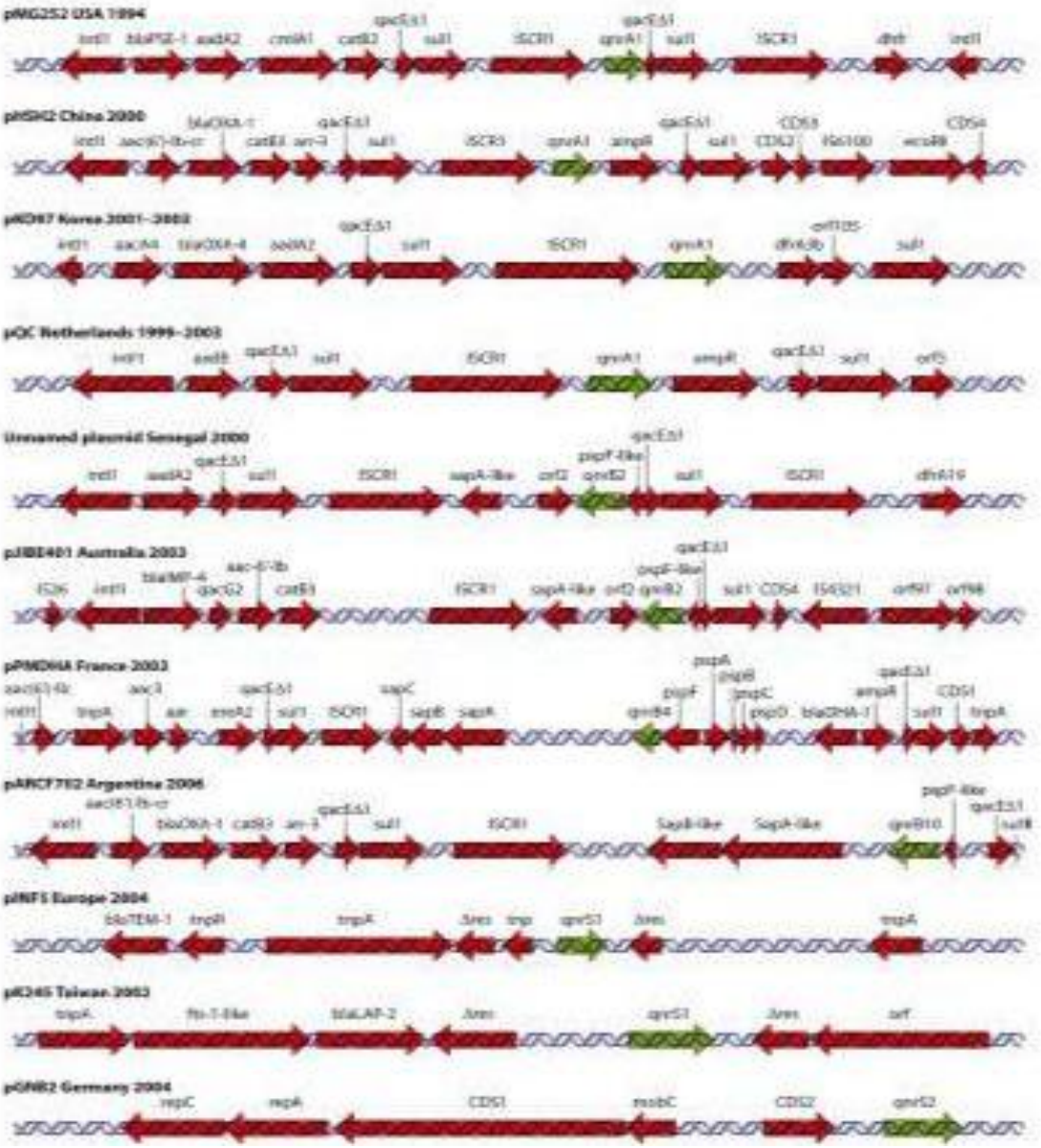


Figure 8: Schemetic presentation of the genetic environment of *qnr*B19 genes.

(Image adopted from Schink et al., 2012. *J Antimicrob Chemother* February 14, 2012)

The *qnr*B2 allele has an even wider distribution, having been found originally in isolates from the United States (Jacoby et al., 2006) and subsequently from China (Martinez et al., 2008), Brazil (Mirarini et al., 2008), Hungary (Szabo et al., 2008), Israel (Chmelnitsky et al., 2008), South Korea (Park et al., 2007), The Netherlands (Veldman et al., 2008), Portugal, Scotland, Senegal, Switzerland, Taiwan and the United Kingdom. *qnr*B2 is linked to a single copy of ISCR1 that surround the gene (Fig 7.). The *qnr*B4 allele has also been associated with or linked to the ESBLs SHV-12 (Pai et al., 2007; Cattoir et al., 2008a;), CTXM-3 (Jiang et al., 2002; Lassine et al., 2008), CTX-M-9, CTX-M-14 and CTX-M-15. In addition, *qnr*B4 alleles are firmly connected to eight qualities or pseudogenes irrelevant to anti-microbial resistance: *sap*A and *sap*B (both peptide transport framework permeases); *sap*C, *psp*A, *psp*B, *psp*C and *psp*D (all phage stun proteins); and *psp*F (a transcriptional activator for the psp operon) (Fig 7).

The ISCR1 element is also capable of mobilizing *qnr*A1 in multiple drug resistance regions, generally located on plasmids that mediate resistance to multiple antibiotics (Frank et al., 2011). Another study carried out to determine the *qnr* genes among *Escherichia coli* of animal origin showed that they harboured conjugative *qnr*B19 in two plasmids p*QNR*2078 and p*QNR*2086. The *qnr*B19 gene was flanked by copies of the insertion sequence IS26 (Shink et al., 2012). The resistance gene *qnr*B19 was flanked by copies of IS26 located in the same orientation. The IS26 element upstream of *qnr*B19 and the immediate flanking regions showed homology to a previously described plasmid *Salmonella* Typhimurium, which harboured the *qnr*B19-carrying transposon Tn2012. Sequence alignment of p*QNR*2078 with plasmids from the database identified a fragment of orf1 of the transposon Tn1721 downstream of the right-hand IS26 and part of a tnpA gene of Tn1721 upstream of the left-hand IS26 (Figure 8).

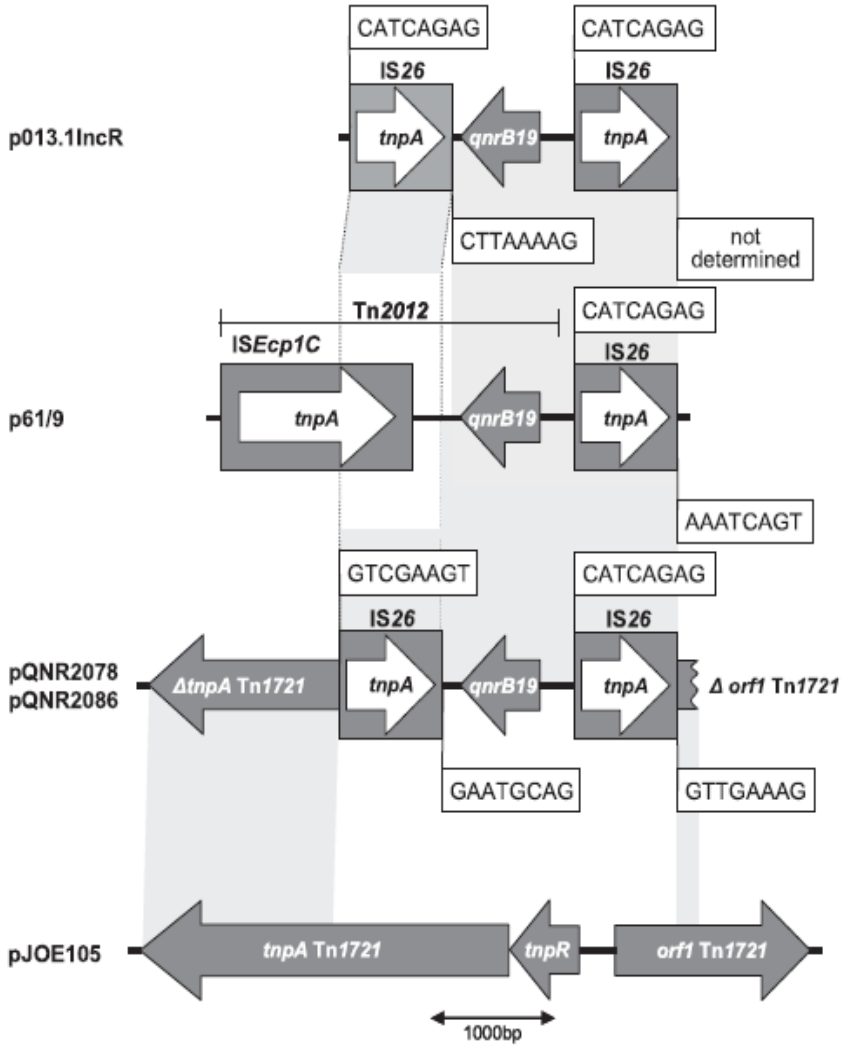


Figure 8: Schemetic presentation of the genetic environment of *qnr*B19 genes.

(Image adopted from Schink et al., 2012. *J Antimicrob Chemother* February 14, 2012)

2.7 Therapeutic option:

A study was carried out among patients suffering from bacteraemia due to quinolone resistant *Escherichia coli* in a teaching hospital in South Korea which analyzed the correlation between the consumption of fluoroquinolone and the prevalence of QREC bacteremia, to define risk and prognostic factors for the acquisition of QREC bacteremia, to estimate the antibiotic susceptibility of QREC isolates from the community and hospital. QREC bacteremia was treated with third generation

cephalosporins alone (30 cases) or third-generation cephalosporins with aminoglycosides (10cases) (Cheong et al., 2001).

Another study carried out in Vietnam in 1993 among Nalidixic-acid resistant *Salmonella* Typhi which reported that the typhoid fever caused by MDR *Salmonella* Typhi could be treated by ofloxacin and ceftriaxone (Wain et al., 1997).

A study was conducted to detect and analyze the presence of plasmid-mediated quinolone resistance (PMQR) determinants [*qnr*, *acc(6')-Ib-cr* and *qep*A] among *Citrobacter freundii* isolates from patients in Anhui province, PR China during 2009-2010. *Qnr* genes were detected in eigjht isolates out of 31 *Citrobacter freundii*. Isolates carrying *qnr* genes were resistant to fluoroquinolone but were susceptible to imipenem (Shao et al., 2011).

Bouchillon and coworkers carried out a study for monitoring antimicrobial resisance trends among hospitalized patients in 33 countries. FQ resistant gram negative UTI pathogens showed good activity against amikacin, ertapenem, imipenem and pipericillin-tazobactum (Bouchillon et al., 2012).

Nandihal (2015) observed good efficacy of nitrofurantoin against fluoroquinolone resistant *E. coli* and *Klebsiella* spp. So nitrofurantoin can be a good choice for the treatment of UTI caused byquinolone resistant enterobacterial isolates. Imipenem was also found to act against *E. coli* and *Klebsiella* spp.