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Mechanisms of Fluoroquinolone Resistance: An Update 1994-1998

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Abstract

Fluoroquinolone resistance is mediated by target changes (DNA gyrase and/or topoisomerase IV) and/or decreased intracellular accumulation. The genes (*gyrA/gyrB/parC/parE*) and proteins of DNA topoisomerase IV show great similarity, both at the nucleotide and amino acid sequence level to those of DNA gyrase. It has been shown that there are hotspots, called the quinolone resistance determining region (QRDR), for mutations within *gyrA* and *parC*. Based on the *Escherichia coli* co-ordinates, the hotspots most favoured for giving rise to decreased susceptibility and/or full resistance to quinolones are at serine 83 and aspartate 87 of *gyrA*, and at serine 79 and aspartate 83 for *parC*. Few mutations in *gyrB* or *parE/grlB* of any bacteria have been described. Efflux of fluoroquinolones is the major cause of decreased accumulation of these agents; for *Staphylococcus aureus*, the efflux pump involved in norfloxacin resistance is NorA, and for *Streptococcus pneumoniae*, PmrA. By analysis of minimum inhibitory concentration (MIC) data derived in the presence and absence of the efflux inhibitor reserpine, it has been shown that up to 50% of ciprofloxacin-resistant clinical isolates of *S. pneumoniae* may possess enhanced efflux. This suggests that efflux may be an important mechanism of clinical resistance in this species. In *Pseudomonas aeruginosa*, several efflux operons have been demonstrated genetically and biochemically. These operons are encoded by *mex* (Multiple EffluX) genes: *mexAmexB-oprM*, *mexCD-OprJ* system and *mexEF-oprN* system. The *E. coli* efflux pump is the *acr*AB-*tol*C system. Both the *mar* operon and the *sox* operon can give rise to multiple antibiotic resistance. It has been shown that mutations giving rise to increased expression of the transcriptional activators *marA* and *soxS* affect the expression of a variety of different genes, including *ompF* and *acrAB*. The net result is that expression of OmpF is reduced and much less drug is able to enter the cell; expression of *acrAB* is increased, enhancing efflux from the cell.

From studies with many different types of bacteria and different classes of antibacterial agent, it is clear that there are essentially 3 types of resistance mechanism deployed by bacteria to evade the action of an antibiotic. Firstly, there is prevention of access of the drug to the target; this can either be by reducing entry of the drug into the cell or by enhancing the pumping of the drug out of the cell, i.e. efflux. Secondly, bacteria can produce novel enzymes that inactivate or modify the drug, and classic examples of these are β-lactamases or aminoglycoside-modifying enzymes. Thirdly, the target site can be altered so that the interaction of the drug is reduced such that increased concentrations of the drug are required to achieve the same level of inhibition of enzyme activity.

For fluoroquinolones, resistance is mediated by target changes or reduced intracellular accumulation. As yet there has been no bacterial enzyme described in the literature capable of modifying, hydrolysing or altering, in any way, the fluoroquinolone molecule.

Over the last decade there have been sporadic reports of plasmid-mediated resistance, with early reports describing a mutator plasmid; when this plasmid was present, the frequency of resistance for selecting anti-

Fig. 1. The quinolone resistance determining region (QRDR) of 9 bacterial species (reproduced courtesy of Springer-Verlag).

Table I. Mutations in *gyrA* giving rise to fluoroquinolone

Organism	Amino acid substitution	Reference
Acinetobacter baumanni	Gly 81 → Val	4
	Ser 83 → Leu	4
Aeromonas salmonicida	Ser 83 → Ile	5
	Ser 83 → Ile; Ala 67 → Gly	5
A. baumanni	Glu 87→ Gly	4
Campylobacter fetus	Asp91→Tyr	6
C. jejuni	Ala 70 → Thr	7
	Thr 86→Phe, Lys	8
	Asp 90→Asn	9
C. lari	Thr 86 → Ile	7
	Asp 90 → Ala, Asn	7,9
	Pro 104 → Ser	Everett et al. unpublished
Enterobacter cloacae	Ser 83 → Leu	Everett et al. unpublished
	Ser 83 → Tyr, Phe	10,11
	Asp 87→His, Gly, Val, Ala, Asn	10, 11
Escherichia coli	Ala 67 → Ser	12
	Gly 81 → Cys, Asp	13, 14
	Ser 83 → Leu, Trp, Ala	13, 15-17
	Ser 83→Phe, Tyr, Val, Ile	18
	Ala 84 → Pro	13
	Asp 87 → Asn, Val, Thr, Gly, His	13, 15, 19, 20
	Asp 87→Tyr	21
	Asp 87→Ala, Phe, Ser, Lys	18
	Gln 106 → His, Arg	12, 17
Enterococcus faecalis	Ser 83 → Ile, Ang, Asn	22, 23
	Glu 87→Gly	24
Haemophilus influenzae	Ser 84→Leu, Tyr	25
	Asp 88→Asn, Tyr	25
Helicobacter pylori	Asn 87 → Lys	26
	Ala 88 → Val	26
	Asp 91 → Gly, Asn, Tyr	26
	Asp 91→ Asn	26
	Ala 97 → Val	26
Klebsiella pneumoniae	Ser 83→Tyr, Phe	10
	Asp 87→Gly, Asn, Ala	27
Mycobacterium avium	Ala 90 → Val	28
M. smegmatis	Ala 90 → Val	29
	Asp 94 → Gly	29

biotic-resistant strains, not just quinolone-resistant strains, increased. In 1997, plasmid-mediated resistance capable of being transferred to other Enterobacteriaceae was described in *Klebsiella pneumoniae*.[1] The mechanism of resistance has yet to be described and so the clinical relevance and epidemiology of this resistance is not known.

All of the mechanisms described herein are chromosomally mediated. There are many different genes and loci in *Escherichia coli* that can contain mutations that give decreased susceptibility to quinolones, not just those encoding target proteins or decreased accumulation; for instance, those involved in the SOS response or different metabolic functions, e.g. *crp*.[2] It is clear that the role of many gene products, their potential interaction with quinolones, and how they may or may not be involved in resistance, is still poorly understood.

Quinolone resistance can be detected in many ways; for many laboratories it is sufficient to phenotypically determine the disk sensitivity or the minimum inhibitory concentration (MIC) of the agent for the bacterium. In laboratories investigating the mechanisms of fluoroquinolone resistance, there is usually a combination of phenotypic and genotypic characterisation. Phenotypically, the interactions of DNA gyrase and/or topoisomerase IV with the quinolone can be investigated. For Gram-negative bacteria, the outer membrane protein profiles of resistant and susceptible bacterial strains can be compared. The concentration of drug accumulated in the presence and absence of various metabolic inhibitors is used to detect the presence of active efflux mechanisms. For bacteria where a chromosomal map is available, the gene involved in resistance can be mapped. Considerable work has utilised cloned wildtype *gyrA* or *gyrB*, *parC* or *parE* and their introduction into the bacterium of interest. Dominance of the wildtype gene over the gene containing a mutation allows screening for reversion to susceptibility as an indication as to whether there is a mutation in *gyrA* or one of the other genes. More recently, it has become possible to clone the

Table I. Contd

Organism	Amino acid substitution	Reference
M. tuberculosis	Gly 88 → Cys	30
	Ala 90 → Val	30
	Ala 90→Pro	31
	Ser 91 → Pro	30
	Asp 94 → Asn, His, Gly, Tyr, Ala	30
Neisseria gonorrhoeae	Ser 83 → Phe	32
	Asp 87 → Asn	33
	Ser91→Phe, Tyr	34
	Asp95→Asn	34
	Ser 91→Phe	35
	Ala 75→Ser	36
	Asp 95→Asn, Gly	36
Pseudomonas aeruginosa	Thr 83 → Ile	37, 38
	Asp 87 → Tyr, Asn, Gly, His	37
Shigella dysenteriae	Ser 83 → Leu	39
Serratia marcescens	Ser 83→Arg	43
	Asp 87→Tyr	43
Streptococcus pneumoniae	Ser 84→Tyr, Phe	44, 45
	Glu 88→Lys	44
Salmonella enteritidis	Ser 83→Phe	40, 41
S. hadar	Ser 83→Phe	40, 41
S. typhi	Ser 83 → Phe	46
		47
S. typhimurium	Ala 67 → Pro; Gly 81 →Ser	48
	Gly 81 →Ser	48
	Ser 83 → Phe, Tyr	41, 48
	Ser 83 → Ala	49
	Asp 87 →Gly, Tyr, Asn	41, 49
	Ala 119 → Glu	41
Staphylococcus aureus	Asp 73→Gly	50
	Ser 84 → Leu, Ala, Phe	51-53
	Ser 84→Val	54
	Ser 85 → Pro	55
	Glu 88 → Lys, Gly	50, 52, 53, 55
S. epidermidis	Ser 84 → Phe	56
S. haemolyticus	Ser 84→Leu	42

* Codon position based on *E. coli* sequence.

Table II. Mutations in *gyrB* affecting fluoroquinolone activity

Organism	Amino acid substitution	Reference
Escherichia coli	Asp 426→Asn	58
	Lys 447→Glu	58
Staphylococcus aureus	Asp 437→Asn	55
	Arg 458→Gln	55
	AA 432→	59
	Pro 456→Ser	60
Streptococcus pneumoniae	Glu 474→Lys	61
Salmonella typhimurium	Ser 464→Tyr	62

gene of interest, or use the polymerase chain reaction (PCR) to amplify the quinolone resistance determining region (QRDR) of that gene, and then apply a variety of rapid screening techniques (particularly when examining a large number of clinical isolates) e.g. restriction fragment length polymorphism (RFLP) or single-stranded conformational polymorphism (SSCP), coupled with DNA sequencing.

1. DNA Gyrase

Most work has focused on DNA gyrase, specifically the A subunit of the enzyme.[2] The 3-dimensional crystal structure of the N terminal portion of *E. coli* GyrA has been published.[3] Quinolones interact with the enzyme to prevent DNA resealing after cleavage by GyrA. From the 3-dimensional crystal structure, an electrostatic GRASP representation can be made. From analysis of this representation, Tony Maxwell has proposed that DNA curves around a 'saddle' on DNA gyrase and over a 'hump' (A. Maxwell, personal communication). This model requires some distortion of the DNA to allow an interaction with the active site at tyrosine 122, the region that is responsible for the DNA breakage-reunion interaction. It has been shown that there are hotspots (QRDR) for mutations within *gyrA*, all focused at a locus encoding amino acids very close to tyrosine 122 in the N terminal region of GyrA. Comparing the amino acid sequences of the QRDR from different bacteria (ranging from *E. coli* to *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and *Enterococcus faecalis*), and basing the analysis on the *E. coli* co-ordinates, the hotspots most favoured for giving rise to decreased susceptibility and/or full resistance to quinolones occur at serine 83 and aspartate 87 (fig. 1). Other loci have also been described. However, mutations at these sites are far less frequently observed (table I). Using the 3-dimensional crystal structure of the N terminal region of GyrA and mapping the various mutations described for *E. coli*, it has been shown that the majority of mutations giving rise to resistance occur at the dimer interface and binding site of DNA gyrase (A. Maxwell, personal communication); the current working model is that mutations in this area affect the binding between DNA GyrA and DNA over the 'saddle' and 'hump'.

The crystal structure of the *E. coli* GyrB N terminal region has also been determined.[57] From an analysis of mutants there are also hotspots in the *gyrB* gene for mutations that give rise to decreased susceptibility to quinolones. These mutations all give rise to amino acid substitutions in a very small region of GyrB near to the locus that has been shown to be cleaved *in vitro*, and involved in interacting with GyrA. So far, few mutations in *gyrB* of any bacteria have been described compared with those in *gyrA* and *parC* (see below). It is considered that mutation in *gyrB* is a rare contributor to fluoroquinolone resistance (table II).

2. DNA Topoisomerase IV

Recently, DNA topoisomerase IV has been demonstrated as an alternative target for quinolones. Most fluoroquinolones preferentially interact with DNA gyrase as the primary target in Gram-negative or Gram-positive bacteria. However, ongoing research suggests that some newer fluoroquinolones with extended spectra of activity interact equally with both DNA gyrase and topoisomerase IV.[61] As for DNA gyrase, DNA topoisomerase IV is a tetrameric enzyme comprised of 2 A subunits and 2 B subunits. The genes and proteins of topoisomerase IV show great similarity, both at the nucleotide and amino acid sequence level, to those of DNA gyrase. Most work on topoisomerase IV has been with the enzymes from *Streptococcus pneumoniae* and *Staphylococcus aureus*.[44,45,63] Aligning the DNA sequences of *gyrA* and *parC* of *S. pneumoniae* and using the *E. coli* co-ordinates, it is clear that the mutation hotspots in *gyrA* (serine 83 and glutamate 87) are the same in *parC* (serine 79 and aspartate 83) (table III). It has been proposed that the interactions between fluoroquinolones and ParC will be similar to those between GyrA and fluoroquinolones (A. Maxwell personal communication). Recently, Pan and Fisher[61] have shown that high level resistance of *S. pneumoniae* to clinafloxacin requires stepwise and multiple mutations in *gyrA* and *parC*, giving rise to mutants that require high fluoroquinolone MIC values for inhibition. The role of efflux in contributing to resistance in these mutants was not examined.

Similar homology is observed between the *gyrB* and *parE* genes. As for mutations in *gyrB*, similar hotspots have been identified in *parE* of *S. pneumoniae* (and *grlB* of *S. aureus*) (table IV). However, it is unclear as to exactly what role mutations in *parE* contribute to clinical resistance, and mutations in this gene are probably as rare as those in *gyrB*.

Table III. Mutations in *parC/grlA* giving rise to fluoroquinolone resistance

Organism	Amino acid substitution	Reference
Acinetobacter baumannii	Ser 80→Leu	64
	Glu 84→Lys	64
Escherichia coli	Gly 78→Cys	65
	Ser 80→Ang, Ile	65-68
	Glu 84→Gly, Lys	66, 67
Enterobacter cloacae	Ser 80→Ile	11
	Glu 84→Glu,Lys	11
Enterococcus faecalis	Ser 80→Ang, Ile	23
	Glu 84→Ala	23
Haemophilus influenzae	Ser 84→Ile	25
	Glu 88→Lys	25
Klebsiella pneumoniae	Ser 80→Ile, Ang	27
	Glu 84→Gly, Lys	27
Neisseria gonorrhoeae	Asp 86→Asn	34
	Ser 87→Ile	69
	Ser 88→Pro	34
	Gly 85→Cys	70, 71
	Glu 91→Gly	34, 70
	Ang 116→Leu	70, 71
Pseudomonas aeruginosa	Ser 80→Leu	72
	Glu 84→Lys	72
Staphylococcus aureus	Ser 80→Phe	73
	Ser 80→Tyr	59, 63, 74
	Ser 81→Pro	50
	Glu 84→Lys	59, 63, 74
	Ala 116→Pro, Glu	73
Streptococcus pneumoniae	Ser 79→Tyr, Phe	44
	Asp 83→Gly	44
	Asp 84→His	45
	Ser 80→Tyr	45

3. Altered Accumulation: Efflux by Gram-Positive Bacteria

The cell envelope of Gram-positive bacteria is a relatively simple structure compared with that of Gram-negative bacteria. It consists of a cytoplasmic membrane, where many of the membrane metabolic activities and enzymes reside, followed by a small area through which lipoteichoic acids link the cytoplasmic membrane with a large layer of peptidoglycan. From experimental work on the accumulation of fluoroquinolones by bacteria, it has been shown that most of the drug is accumulated by cells within 1 to 2 minutes of antibiotic exposure until an equilibrium, or steady-state concentration is achieved.[77] If an efflux inhibitor, such as carbonyl cyanide m-chlorophenylhydrazone (CCCP) or reserpine, is added at this equilibrium stage there is an apparent increase in the concentration of fluoroquinolone accumulated. This is due to inhibition of the efflux pump (which is present in all wildtype cells examined to date), allowing higher concentrations of quinolone to be retained within the cell. In some resistant bacteria it has been shown that the concentration of fluoroquinolone accumulated is far lower than in wildtype bacteria; when an efflux inhibitor is added, the concentration of fluoroquinolone accumulated increases far more than for wildtype cells, showing that the low concentrations of drug accumulated are due to enhanced efflux. For *S. aureus* it has been shown that the efflux pump involved in norfloxacin resistance is NorA.[78] It has also been shown that fluoroquinolone-resistant mutant *S. aureus* with enhanced efflux occur prior to mutations in *grlA* (topoisomerase IV sub unit A) and *gyrA*.[79] While work in other bacterial species has demonstrated the presence of multiple efflux pumps, to date only NorA has been described for staphylococcal fluoroquinolone efflux. However, it cannot be ruled out that other pumps exist that have yet to be described.

Recently, an efflux protein, PmrA, of *S. pneumoniae* has been reported.[80] By analysis of MIC data derived in the presence and absence of the efflux inhibitor reserpine, it has been shown that up to 50% of ciprofloxacin-resistant clinical isolates of *S. pneumoniae* may possess enhanced efflux.[81,82] These data suggest that efflux may be an important mechanism of clinical resistance in this species.

4. Altered Accumulation: Efflux by Gram-Negative Bacteria

The Gram-negative cell envelope is more sophisticated than that of Gram-positive bacteria. In Gram-negative cells, there is a cytoplasmic membrane after which there is a periplasmic space and a thinner layer of peptidoglycan, linked via Brauns lipoprotein to an outer membrane. As for Gram-positive bacteria, the cytoplasmic membrane houses most of the metabolic activities of the cell envelope. However, in Gram-negative bacteria the outer membrane contains pore-forming proteins that allow solutes into the cell. In *P. aeruginosa*, several efflux operons have been demonstrated genetically and biochemically. These operons are encoded by *mex* (Multiple EffluX) genes. The most

Table IV. Mutations in *parE/grlB* giving rise to fluoroquinolone resistance

Organism	Amino acid substitution	Reference
Escherichia coli	Leu 445→His	75
Streptococcus pneumoniae	Asp 435→Asn	76
	Pro 454→Ser	61
Staphylococcus aureus	Asp 432→Val	60
	Asn 470→Asp	59

widely studied operon is the *mexAmexB-oprM* operon, which gives rise to resistance to ciprofloxacin, nalidixic acid, tetracycline and chloramphenicol.[83] The basic model comprises MexB, embedded in the cytoplasmic membrane, as the efflux pump; the accessory protein, MexA, links MexB to the outer membrane. The outer membrane protein is OprM, and it is through this linked 3-protein system that drugs are expelled. In wildtype *P. aeruginosa*, the efflux pump gives rise to the inherent resistance to many antibiotics that is associated with this species. Enhanced efflux has been associated with fluoroquinolone resistance and resistance to other newer agents. Other efflux pumps that can give rise to fluoroquinolone resistance in *P. aeruginosa* are the *mexCD-OprJ* system[84] and *mexEF-oprN* system.[85]

In *E. coli*, it has been shown that there is linkage between the regulation of the porin proteins in the outer membrane, which allow drugs and other solutes to enter the bacterial cell, and the efflux pump. The efflux pump described for *E. coli* that is most likely to be involved in decreased susceptibility to fluoroquinolones is the *acrAB-tolC* system, where AcrB is the efflux protein in the cytoplasmic membrane, AcrA is the accessory protein, and TolC is the outer membrane protein.[86] It has long been known that both the *mar* operon and the *sox* operon can give rise to multiple antibiotic resistance. It has been shown that mutations giving rise to increased expression of the transcriptional activators, *marA* and *soxS*, affect the expression of a variety of different genes, including *ompF* and *acrAB*. The net result is that expression of OmpF is reduced and much less drug is able to enter the cell; expression of *acrAB* is increased, thus enhancing efflux from the cell.[87]

The concept of efflux and any associated role in antibiotic resistance is a relatively new and flourishing area of research. Preliminary evidence from *S. aureus* suggests that increased expression of NorA is the first step in resistance. It is proposed that enhancement of a chromosomal regulatory network allows bacteria to survive while mutations in target genes can accumulate. It is already known that by enhancing the expression of efflux pumps in a variety of different bacterial species gives rise to a multidrug resistant (multiple antibiotic resistant) phenotype. In addition, while increases in the MIC values of the affected agents for some bacteria, such as *E. coli*, have been modest, for *P. aeruginosa* and *S. aureus* they have been sufficient to allow the bacterium to withstand concentrations greater than the recommended breakpoint concentration of many agents.

Inhibitors of specific efflux proteins are being developed, but no information is available as to whether these will be for use in combination with fluoroquinolones or any other agent, or as stand-alone agents.

5. Conclusions

It has been shown by several researchers (for example, Everett et al.[19]) that to give rise to a highly fluoroquinolone-resistant bacterium, multiple mutations in several genes, including *gyrA*, *parC* and those encoding for efflux, are required. Although it was widely predicted that high level resistant bacteria that are usually exquisitely susceptible to fluoroquinolones would occur rarely, highly resistant *E. coli* have been isolated in locations where there has been heavy and frequent use of fluoroquinolones. With the continued increase in the clinical use of these agents, not always appropriately, bacteria will continue to respond with multiple and novel mechanisms of resistance with which to evade antimicrobial action.

References

1. Martínez Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet 1998; 351: 797-9
2. Piddock LJV. Mechanisms of resistance to fluoroquinolones: state-of-the-art 1992-1994. Drugs 1995; 49 Suppl. 2: 29-35
3. Cabral JHM, Jackson AP, Smith CV, et al. Crystal structure of the breakage-reunion domain of DNA gyrase. Nature 1997; 388: 903-6
4. Vila J, Ruiz J, Goni P, et al. Mutation in the *gyrA* gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 1995; 39: 1201-3
5. Oppegaard H, Sorum H. GyrA mutations in quinolone-resistant isolates of the fish pathogen *Aeromonas salmonicida*. Antimicrob Agents Chemother 1994; 38: 2460-4
6. Taylor DE, Chau ASS. Cloning and nucleotide sequence of the *gyrA* gene from *Campylobacter fetus* subsp fetus ATCC27374 and characterization of ciprofloxacin-resistant laboratory and clinical isolates. Antimicrob Agents Chemother 1997; 41: 665-71
7. Wang Y, Huang WM, Taylor DE. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob Agents Chemother 1993; 37: 457-63
8. Ruiz J, Goni P, Marco F, et al. Increased resistance to quinolones in *Campylobacter jejuni*: genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol Immunol 1998; 42: 223-6
9. Charvalos E, Peteinaki E, Spyridaki I, et al. Detection of ciprofloxacin resistance mutations in *Campylobacter jejuni gyrA* by non-radioisotopic single-stranded conformational polymorphism and direct sequencing. J Clin Lab Anal 1996; 10: 129-33
10. Deguchi T, Yasuda M, Kawamura T, et al. In-vitro antimicrobial activity of HSR-903, a new fluoroquinolone against clinical isolates of *Klebsiella pneumoniae*, and *Enterobacter cloacae* with fluoroquinolone resistance associated amino acid alterations in *gyrA* and *parC*. J Antimicrob Chemother 1997; 40: 907-9
11. Deguchi T, Yasuda M, Nakano M, et al. Detection of mutations in the *gyrA* and *parC* genes in quinolone-resistant clinical isolates of *Enterobacter cloacae*. J Antimicrob Chemother 1997; 40: 543-9
12. Yoshida H, Komjima T, Yamagishi, J, et al. Quinolone-resistant mutations of the *gyrA* gene of *Escherichia coli*. Mol Gen Genet 1988; 211: 1-7
13. Yoshida H, Bogaki M, Nakamura M, et al. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. Antimicrob Agents Chemother 1990; 34: 1271-2
14. Cambau E, Bordon F, Collatz E, et al. Novel *gyrA* point mutation in a strain of *Escherichia coli* resistant to fluoroquinolones but not to nalidixic acid. Antimicrob Agents Chemother 1993; 37: 1247-52
15. Oram M, Fisher LM. 4-Quinolone resistance mutations in the DNA gyrase of *Escherichia coli* clinical isolates identified by using the polymerase chain reaction. Antimicrob Agents Chemother 1991; 35: 387-9

16. Cullen Me, Wyke AW, Kuroda R, et al. Cloning and characterization of a DNA gyrase A gene from *Escherichia coli* that confers clinical resistance to 4-quinolones. Antimicrob Agents Chemother 1989; 33: 886-94
17. Hallett P, Maxwell A. Novel quinolone resistance mutations of the *Escherichia coli* DNA gyrase A protein: enzymatic analysis of the mutant proteins. Antimicrob Agents Chemother 1991; 35: 335-40
18. Yonezawa M, Takahata M, Banzawa N, et al. Analysis of the NH2-terminal 83rd amino-acid of *Escherichia coli gyrA* in quinolone-resistance. Microbiol Immunol 1995; 39: 243-7
19. Everett MJ, Jin Y-F, Ricci V, et al. Contribution of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* isolated from humans and animals. Antimicrob Agents Chemother 1996; 40: 2380-6
20. Ouabdesselam S, Hooper DC, Tankovic J, et al. Detection of *gyrA* and *gyrB* mutations in quinolone resistant clinical isolates of *Escherichia coli* by sscp analysis and determination of levels of resistance conferred by two different single *gyrA* mutations. Antimicrob Agents Chemother 1995; 39: 1667-70
21. Conrad S, Oethinger M, Kaifel K, et al. *gyrA* muations in high-level fluoroquinolone resistant clinical isolates of *Escherichia coli*. J Antimicrob Chemother 1996; 38: 443-55
22. Korten V, Huang WM, Murray BE. Analysis by PCR and direct sequencing of *gyrA* mutations associated with fluoroquinolone resistance in *Enterococcus faecalis*. Antimicrob Agents Chemother 1994; 38: 2091-4
23. Kanematsu E, Deguchi T, Yasuda M, et al. Alterations in the *gyrA* subunit of DNA gyrase and the *parC* subunit of DNA topoisomerase IV associated with quinolone resistance in *Enterococcus faecalis*. Antimicrob Agents Chemother 1998; 42: 433-5
24. Tankovic J, Mahjoubi F, Courvalin P, et al. Development of fluoroquinolone resistance in *Enterococcus faecalis* and role of mutations in DNA gyrase gyrA gene. Antimicrob Agents Chemother 1996; 40: 2258-61
25. Georgiou M, Munoz R, Roman F, et al. Ciprofloxacin-resistant *Haemophilus influenzae* strains possess mutations in analogous positions of gyrA and parC. Antimicrob Agents Chemother 1996; 40: 1741-4
26. Moore RA, Beckthold B, Wong S, et al. Nucleotide sequence of the *gyrA* gene and characterization of ciprofloxacin-resistant mutants of *Helicobacter pylori*. Antimicrob Agents Chemother 1995; 39: 107-11
27. Deguchi T, Fukouka A, Yasuda M, et al. Alterations in the gyrA subunit of DNA gyrase and the parC subunit of topoisomerase IV in quinolone-resistant clinical isolates of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 1997; 41: 699-701
28. Cambau E, Sougakoff W, Jarlier V. Amplification and nucleotide sequence of the quinolone resistance determining region in the *gyrA* gene of mycobacteria. FEMS Microbiol Lett 1994; 116: 49-54
29. Revel V, Cambau E, Jarlier V, et al. Characterization of mutations in *Mycobacterium smegmatis* involved in resistance to fluoroquinolones. Antimicrob Agents Chemother 1994; 38: 1991-6
30. Takiff HE, Salazar L, Ruerrero C, et al. Cloning and nucleotide sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother 1994; 38: 773-80
31. Williams KJ, Chan R, Piddock LJV. gyrA of ofloxacin-resistant clinical isolates of *Mycobacterium tuberculosis* from Hong Kong. J Antimicrob Chemother 1996; 37:1032-4
32. Deguchi T, Yasuda M, Nakano, M, et al. Rapid screening of point mutations of the Neisseria gonorrhoeae *parC* gene associated with resistance to quinolones. J Clin Microbiol 1997; 35: 948-50
33. Deguchi T, Yasuda M, Asano M, et al. DNA gyrase mutations in quinolone-resistant clinical isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 1995; 39: 561-3
34. Ison CA, Woodford PJ, Madders H, et al. Drift in susceptibility of *Neisseria gonorrhoeae* to ciprofloxacin and emergence of therapeutic failure. Antimicrob Agents Chemother 1998; 42: 2919-22
35. Tanaka M, Takahashi K, Saika T, et al. Development of fluoroquinolone resistance and mutations involving gyrA and parC proteins among *Neisseria gonorrhoeae* isolates in Japan. J Urol 1998; 159: 2215-9
36. Tanaka M, Matsumoto T, Sakumoto M, et al. Reduced clinical efficacy of pazufloxacin against gonorrhea due to high prevalence of quinolone-resistant isolates with the *gyrA* mutation. Antimicrob Agents Chemother 1998; 42: 579-82
37. Kureishi A, Diver JM, Beckthold B, et al. Cloning and nucleotide sequence of *Pseudomonas aeruginosa* DNA gyrase *gyrA* gene from strain PAO1 and quinolone-resistant clinical isolates. Antimicrob Agents Chemother 1994; 38: 1944-52
38. Pumbwe L, Everett MJ, Hancock REW, et al. Role of *gyrA* mutation and loss of OprF in the multiple antibiotic resistance (MAR) phenotype of *Pseudomonas aeruginosa* G49. FEMS Microbiol Lett 1996; 143: 25-8
39. Rahman M, Mauf G, Levy J, et al. Detection of 4-quinolone resistance mutations in the *gyrA* gene of *Shigella dysenteriae* Type 1 by PCR. Antimicrob Agents Chemother 1994; 38: 2488-91
40. Ouabdesselam S, Tankovic J, Soussy CJ. Quinolone resistance mutations in the gyrA gene of clinical isolates of salmonella. Microb Drug Resist 1996; 2: 299-302
41. Griggs DJ, Gensberg K, Piddock LJV. Mutations in *gyrA* gene of quinolone-resistant salmonella serotypes isolated from humans and animals. Antimicrob Agents Chemother 1996; 40: 1009-13
42. Yonezawa M, Takahata M, Banzawafutakuchi N, et al. DNA gyrase gyrA mutations in quinolone-resistant clinical isolates of *Staphylococcus haemolyticus*. Antimicrob Agents Chemother 1996; 40: 1065-6
43. Kim JH, Cho EH, Kim KS, et al. Cloning and nucleotide sequence of the DNA gyrase *gyrA* gene from *Serratia marcescens* and characterization of mutations in *gyrA* of quinolone-resistant clinical isolates. Antimicrob Agents Chemother 1998; 42: 190-3
44. Janoir C, Zeller V, Kitzis MD, et al. High-level fluoroquinolone resistance in *Streptococcus pneumoniae* requires mutations in *parC* and *gyrA*. Antimicrob Agents Chemother 1996; 40: 2760-4
45. Tankovic J, Perichon B, Duval J, et al. Contribution of mutations in *gyrA* and *parC* genes to fluoroquinolone resistance of mutants of *Streptococcus pneumoniae* obtained in-vivo and in-vitro. Antimicrob Agents Chemother 1997; 40: 2505-10
46. Brown JC, Shanahan PMA, Jesudason MV, et al. Mutation responsible for reduced susceptibility to 4-quinolones in clinical isolates of multiresistant *Salmonella typhimurium* in India. J Antimicrob Chemother 1996; 37: 891-900
47. Wain J, Hoa NTT, Chinh NT, et al. Quinolone-resistant *salmonella typhi* in Vietnam: molecular basis of resistance and clinical response to treatment. Clin Infect Dis 1997; 25: 1404-10
48. Reyna F, Huesca M, Gonzalez V, et al. *Salmonella typhimurium gyrA* mutations associated with fluoroquinolone resistance. Antimicrob Agents Chemother 1995; 39: 1621-3
49. Heisig P, Kratz B, Halle E, et al. Identification of DNA gyrase A mutations in ciprofloxacin-resistant isolates of *Salmonella typhimurium* from men and cattle in Germany. Microb Drug Resist 1995; 1: 211-8
50. Wang T, Tanaka M, Sato K. Detection of *grlA* and *gyrA* mutations in 344 *Staphylococcus aureus* strains. Antimicrob Agents Chemother 1998; 42: 236-40
51. Sreedharan S, Oram M, Jenson B, et al. DNA gyrase *gyrA* mutations in ciprofloxacin-resistant strains of *Staphylococcus aureus*: close similarity with quinolone resistance mutations in *Escherichia coli*. J Bacteriol 1990; 172: 7260-2
52. Goswitz JJ, Willard KE, Fasching CE, et al. Detection of *gyrA* gene mutations associated with ciprofloxacin resistance in methicillin-resistant *Staphylococcus aureus*: analysis by polymerase chain reaction and automated direct DNA sequencing. Antimicrob Agents Chemother 1992; 36: 1166-9
53. Tokue Y, Sugano K, Saito D, et al. Detection of novel mutations in the *gyrA* gene of *Staphylococcus aureus* by nonradioisotopic single-stranded conformational polymorphism analysis and direct DNA sequencing. Antimicrob Agents Chemother 1994; 38: 428-31
54. Takenouchi T, Ishii C, Sugawara M, et al. Incidence of various *gyrA* mutants in 451 *Staphylococcus aureus* strains isolated in Japan and their susceptibilities to 10 fluoroquinolones. Antimicrob Agents Chemother 1995; 39: 1414-8
55. Ito H, Yoshida H, Bogaki-Shonai M, et al. Quinolone resistance mutations in the DNA gyrase *gyrA* and *gyrB* genes of *Staphylococcus aureus*. Antimicrob Agents Chemother 1994; 38: 2014-23
56. Sreedharan S, Peterson LR, Fisher LM. Ciprofloxacin resistance in coagulase-positive and -negative staphylococci: role of mutations at serine 84 in the DNA gyrase A protein of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob Agents Chemother 1991; 35: 2151-4
57. Tsai FTF, Singh OMP, Skarzynski T, et al. The high-resolution crystal structure of a 24-kDa gyrase B fragment from *E. coli* complexed with one of the most potent coumarin inhibitors, clorobiocin. Proteins 1997; 28: 41-52
58. Nakamura S, Nakamura M, Kojima T, et al. *gyrA* and *gyrB* mutations in quinolone-resistant strains of *Escherichia coli*. Antimicrob Agents Chemother 1989; 33: 254-5
59. Tanaka M, Zhang YX, Ishida H, et al. Mechanisms of 4-quinolone resistance in quinolone-resistant and methicillin resistant *Staphylococcus aureus* isolates from Japan and China. J Med Microbiol 1995; 42: 214-9

60. Takahashi H, Kikuchi T, Shoji S, et al. Characterization of *gyrA*, *gyrB*, grlA and grlB mutations in fluoroquinolone-resistant clinical isolates of *Staphylococcus aureus*. J Antimicrob Chemother 1998; 41: 49-57
61. Pan XS, Fisher LM. DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1998; 42: 2810-6
62. Gensberg K, Jin YF, Piddock LJV. A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolates of *Salmonella typhimurium*. FEMS Microbiol Lett 1996; 137: 293
63. Ferrero L, Cameron B, Crouzet J. Analysis of *gyrA* and GrlA mutations in stepwise selected ciprofloxacin-resistant mutants of *Staphylococcus aureus*. Antimicrob Agents Chemother 1995; 39: 1554-8
64. Vila J, Ruiz J, Goni P, et al. Quinolone-resistance mutations in the topoisomerase IV *parC* gene of *Acinetobacter baumannii*. J Antimicrob Chemother 1997; 39: 757-62
65. Lee S, Lee Y. Laboratory developed fluoroquinolone resistant *Escherichia coli* has a new missense mutation in QRDR of *parC*. J Microbiol 1998; 36: 106-10
66. Vila J, Ruiz J, Goni P, et al. Detection of mutations in *parC* in quinolone-resistant clinical isolates of *Escherichia coli*. Antimicrob Agents Chemother 1996; 40: 491-3
67. Heisig P. Genetic evidence for a role of parC mutations in development of high-level fluoroquinolone resistance in *Escherichia coli*. Antimicrob Agents Chemother 1996; 40: 879-85
68. Kumagai Y, Kato J, Hoshino K, et al. Quinolone-resistant mutants of *Escherichia coli* DNA topoisomerase IV *parC* gene. Antimicrob Agents Chemother 1996; 40: 710-4
69. Trees DL, Sandul AL, Whittington WL, et al. Identification of novel mutation patterns in the *parC* gene of ciprofloxacin-resistant isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 1998; 42: 2103-5
70. Deguchi T, Yasuda M, Nakano M, et al. Quinolone-resistant *Neisseria gonorrhoeae* – correlation of alterations in the *gyrA* subunit of DNA gyrase and the *parC* subunit of topoisomerase IV with antimicrobial susceptibility profiles. Antimicrob Agents Chemother 1996; 40: 1020-3
71. Deguchi T, Yasuda M, Nakano M, et al. Uncommon occurrence of mutations in the *gyrB* gene associated with quinolone resistance in clinical isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 1996; 40: 2437-8
72. Nakano M, Deguchi T, Kawamura T, et al. Mutations in the *gyrA* and *parC* genes in fluoroquinolone-resistant clinical isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1997; 41: 2289-91
73. Fournier B, Hooper DC. Effects of mutations in GrlA of topoisomerase IV from *Staphylococcus aureus* on quinolone and coumarin activity. Antimicrob Agents Chemother 1998; 42: 2109-12
74. Yamagishi JI, Kojima T, Oyamada Y, et al. Alterations in the DNA topoisomerase IV *grlA* gene responsible for quinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1996; 40: 1157-63
75. Breines DM, Ouabdesselam S, Ng EY, et al. Quinolone resistance locus *nfxD* of *Escherichia coli* is a mutant allele of the parE gene encoding a subunit of topoisomerase IV. Antimicrob Agents Chemother 1997; 41: 175-9
76. Perichon B, Tankovic J, Courvalin P. Characterization of a mutation in the *parE* gene that confers fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997; 41: 1166-7
77. Piddock LJV. Mechanism of quinolone uptake into bacterial cells. J Antimicrob Chemother 1991; 27: 399-403
78. Kaatz GW, Seo SM, Ruble CA. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1993; 37: 1086-94
79. Roland GE, Gracheck SJ. Differential selection of *grlA* and *gyrA* mutants in *Staphylococcus aureus* to clinafloxacin and ciprofloxacin as determined by *Haemophilus influenzae* I restriction fragment length polymorphism [abstract C65]. Proceedings of the 35th Interscience Conference of Antimicrobial Agents and Chemotherapy; 1995; New Orleans, Louisiana. Washington DC: American Society for Microbiology, 1995
80. Gill MJ, Brenwald N, Wise R. Identification of an efflux pump gene, pmrA, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1999; 43: In Press
81. Brenwald NP, Gill MJ, Wise R. Prevalence of a putative efflux mechanism among fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1998; 42: 2032-5
82. Piddock LJV, Johnson M, Ricci V, et al. Activity of new fluoroquinolones for fluoroquinolone-resistant pathogens of the lower respiratory tract. Antimicrob Agents Chemother 1998; 42: 2956-60
83. Poole K, Krebes K, McNally C, et al. Multiple antibiotic-resistance in *Pseudomonas aeruginosa* evidence for involvement of an efflux operon. J Bacteriol 1993; 175: 7363-72
84. Poole K, Gotoh N, Tsujimoto H, et al. Overexpression of the mexC-mexD-oprJ efflux operon in nfxB type multidrug resistant strains of *Pseudomonas aeruginosa*. Mol Microbiol 1996; 21: 713-24
85. Kohler T, MicheaHamzehpour M, Henze U, et al. Characterization of MexE-MexF-OprN, a positively regulated multi-drug efflux system of *Pseudomonas aeruginosa*. Mol Microbiol 1997; 23: 345-54
86. Fralick JA. Evidence that TolC is required for functioning of the Mar/AcrAB efflux pump of *Escherichia coli*. J Bacteriol 1996; 178: 5803-5
87. Okusu H, Ma D, Nikaido H. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. J Bacteriol 1996; 178: 306-8

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