Mechanisms of quinolone action and resistance: where do we stand?

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Abstract
Quinolone antibiotics represent one of the most important classes of anti-infective agents and, although still clinically valuable, their use has been compromised by the increasing emergence of resistant strains, which has become a prevalent clinical problem. Quinolones act by inhibiting the activity of DNA gyrase and topoisomerase IV – two essential bacterial enzymes that modulate the chromosomal supercoiling required for critical nucleic acid processes. The acquisition of quinolone resistance is recognized to be multifactorial and complex. The main resistance mechanism consists of one or a combination of target-site gene mutations that alter the drug-binding affinity of target enzymes. However, other mechanisms such as mutations that lead to reduced intracellular drug concentrations, by either decreased uptake or increased efflux, and plasmid-encoded resistance genes producing either target protection proteins, drug-modifying enzymes or multidrug efflux pumps are known to contribute additively to quinolone resistance. The understanding of these different resistance mechanisms has improved significantly in recent years; however, many details remain to be clarified and the contribution of less-studied mechanisms still needs to be better elucidated in order to fully understand this phenotype.

INTRODUCTION
The quinolones are a group of synthetic antibacterials with major clinical relevance, being one of the most frequently prescribed classes of antimicrobial agents in the world. Initially, quinolones were mostly used in the treatment of Gram-negative infections, but they were later modified in order to improve their pharmacokinetic properties and extend their antibacterial spectrum, becoming effective against a wide variety of Gram-negative and Gram-positive pathogens [1–5].

The founding and prototypical compound of the quinolones, nalidixic acid, was introduced into clinical use in 1962 to treat uncomplicated urinary tract infections (UTIs) and can be considered as the first generation of the quinolones [4, 6, 7]. However, the quinolones only became a widely used drug class in the 1980s with the development of a second generation of compounds, the fluoroquinolones, which displayed considerably improved activity, greater Gram-positive penetration and enhanced pharmacokinetic and pharmacodynamic properties [1, 4, 7]. The most important modifications to the quinolone structure were the introduction of a fluorine at the sixth position and a major ring substituent at position seven. The first representative of this generation was norfloxacin; however, ciprofloxacin was the first fluoroquinolone that showed significant activity outside the urinary tract [1, 7]. After almost three decades in clinical use, ciprofloxacin remains one of the most commonly prescribed antimicrobial drugs, being listed by the World Health Organization (WHO) as an essential medicine and a critically important antibiotic [8, 9]. The clinical success of ciprofloxacin led to the development of a collection of newer-generation quinolones (levofloxcin, moxifloxacin, gatifloxcin, etc.) with an even broader and different spectrum of activity and pharmacokinetic characteristics [1, 5].

Due to their potency, broad activity spectrum, oral bioavailability and generally good safety profile, fluoroquinolones have been used extensively for multiple clinical indications worldwide [3, 10]. Quinolones have been prescribed to treat...
MECHANISMS OF QUINOLONE ACTION

Quinolones act by inhibiting the activity of two essential bacterial type II topoisomerases, DNA gyrase and topoisomerase IV, which are involved in the modulation of the chromosomal supercoiling required for DNA synthesis, transcription and cell division. These enzymes modulate DNA topology by passing an intact double helix through a transient 4 bp staggered double-stranded break that they introduce in a separate segment. In order to preserve genomic integrity during this process, DNA gyrase and topoisomerase IV form covalent bonds between active site tyrosine residues and the 5’-overhangs at the DNA break, forming enzyme-cleaved DNA complexes known as cleavage complexes. Quinolones interfere with this critical process by reversibly binding to these cleavage complexes at the enzyme–DNA interface in the cleavage–ligation active site, therefore increasing the steady-state concentration of cleavage complexes by physically blocking DNA strand re-ligation. Quinolone–topoisomerase binding was recently demonstrated to occur through a water–metal ion bridge, where a noncatalytic Mg$^{2+}$ ion coordinated with four water molecules forms a bridge for hydrogen bonding between the quinolone and the serine and acidic residues that act as anchor points to the enzyme [1, 4, 10, 14].

DNA gyrase and topoisomerase IV are A$_2$B$_2$ heterotetramer enzymes composed of two pairs of identical subunits, GyrA$_2$ GyrB$_2$ and ParC$_2$ParE$_2$ (or GrlA$_2$GrlB$_2$), respectively. Despite their general functional and structural similarities, DNA gyrase and topoisomerase IV have distinct physiological functions. DNA gyrase uses the energy of ATP hydrolysis to actively introduce negative supercoils into DNA which are essential for (i) setting the super-helical density that allows chromosome condensation, (ii) relieving the torsional stress that accumulates in front of replication forks and transcription complexes, and (iii) promoting local melting for transcript initiation by RNA polymerase. Topoisomerase IV also plays a role in maintaining chromosomal super-helical density and alleviating torsional stress, although to a lesser extent than DNA gyrase since it is only able to relax positive supercoils and unable to introduce further negative supercoiling. However, the major function of topoisomerase IV is decatenation of the interlocked daughter chromosomes at the end of replication [1, 4, 5, 10, 14].

Quinolones are known to target DNA gyrase and topoisomerase IV with varying efficiencies in different bacterial species. Generally, DNA gyrase is considered the primary target of quinolones in Gram-negative species and topoisomerase IV the primary target in Gram-positives. However, this has been proven to be untrue in many cases, with examples of Gram-positive species where DNA gyrase is the primary target for quinolones and also cases of different quinolones having distinct primary targets in the same species or quinolones with similar potencies against both enzymes. Hence, the relative contribution of each topoisomerase to quinolone action still needs further investigation, on a species-by-species and drug-by-drug basis, in order to be fully elucidated [1, 5, 10, 14].

The formation of quinolone–topoisomerase–DNA ternary complexes causes the DNA replication machinery to become arrested at blocked replication forks, resulting in an inhibition of DNA synthesis, which immediately leads to bacteriostasis (at low quinolone concentrations) and eventually to cell death (at lethal concentrations) [5, 14]. Due to the positioning of DNA gyrase ahead of the DNA replication complex and of topoisomerase IV behind it, it appears that the interaction of quinolones with DNA gyrase results in a more rapid inhibition of DNA replication than with topoisomerase IV [10, 15]. Moreover, when DNA tracking systems (replication forks, transcription complexes, etc.) collide with these stabilized ternary complexes, permanent chromosomal breaks are generated [1, 10]. These double-strand DNA breaks trigger the bacterial DNA stress response, in which the RecA protein is activated by DNA damage and promotes the self-cleavage of the LexA repressor, thus de-repressing the expression of SOS response genes such as DNA repair enzymes [14, 16]. The quinolone bactericidal activity therefore results from the overwhelming of these processes and the extent to which DNA repair is incomplete [1, 10]. Indeed, fluoroquinolone bactericidal activity has been shown to be enhanced when the induction of the SOS response is prevented [17]. Although inhibition of protein synthesis does not seem to affect quinolone-mediated inhibition of DNA replication, it has been proven to reduce the quinolone bactericidal activity, with varying magnitudes between different quinolones [10]. Hence, the primary effects of the formation of quinolone–topoisomerase–DNA complexes and the following bacterial response through stress-induced protein expression seem to have a clear association in determining quinolone bactericidal activity [14]. For instance, the contribution of reactive oxygen species to quinolone-mediated cell death has been recently shown to occur in a protein synthesis-dependent manner [18]. This suggests that, in addition to the inhibition of DNA replication, other events that may affect DNA or other cellular damage may also contribute to the bactericidal activity of quinolone drugs; however, the underlying molecular mechanisms are not yet fully understood [10]. Moreover, inhibition of the catalytic functions of DNA gyrase and topoisomerase IV due to quinolone stabilization of cleavage complexes results in a loss of enzyme activity that

UTIs, respiratory tract infections (e.g. community-acquired and nosocomial pneumonia, chronic bronchitis and tuberculosis), skin and soft tissue infections, bone and joint infections, intra-abdominal infections, sexually transmitted diseases, among others [1, 3]. However, due to the extensive use of these drugs in human and veterinary medicine, and despite prescribing guidelines now recommending reserving quinolone use, the number of quinolone-resistant strains has been growing steadily, being observed in all species treated by this antimicrobial class. Although still clinically valuable, quinolone use has been compromised by the emergence of resistance, having serious implications in some clinical settings [1, 3, 5, 10–13].
affects a number of nucleic acid processes and is therefore likely to contribute to the overall toxicity of quinolones [1].

**MECHANISMS OF QUINOLONE RESISTANCE**

The acquisition of quinolone resistance may be associated with three types of mechanisms: (i) chromosomal mutations that alter the target enzymes and their drug-binding affinity; (ii) chromosomal mutations leading to reduced drug accumulation by either decreased uptake or increased efflux; and (iii) plasmid-acquired genes producing either target protection proteins, drug modifying enzymes or drug efflux pumps [1, 3–5, 10]. The cellular alterations associated with each mechanism are not mutually exclusive and can accumulate to create high levels of quinolone resistance [1, 10, 19].

**Target-site gene mutations in type II topoisomerases**

The most common mechanism of high-level resistance is due to mutations within the quinolone resistance-determining regions (QRDRs) of at least one of the genes that encode the primary and secondary targets of these drugs, the type II topoisomerases (gyrA, gyrB, parC and parE). Mutations in the QRDRs of these genes result in amino acid substitutions that structurally change the target protein and, subsequently, the drug-binding affinity of the enzyme (Fig. 1a) [1, 5]. Resistance mutations in the GyrB and ParE subunits are considerably less frequent than those in GyrA and ParC [10, 19]. Since the serine and acidic residues that anchor the water–metal ion bridge are, by far, the most common single amino acid substitutions that confer quinolone resistance, it is presumed that the disruption of this topoisomerase–quinolone interaction is the cause of resistance; however, mutations have been found throughout the A and B subunits of DNA gyrase and topoisomerase IV in quinolone-resistant strains [1, 10]. In both laboratory and clinical isolates, serine substitutions represent more than 90% of the mutant pool, with modifications at the acidic residue composing the majority of the others [1]. In *E. coli*, the most common mutation site in GyrA is at Ser83 followed by Asp87, both key residues for quinolone binding, and similar mutation frequencies are seen at equivalent positions for GyrA and ParC in other species [10, 19]. The much higher frequency of serine mutations could be fully or in part explained by the fact that mutations in this residue generally have little effect on the catalytic activity of DNA gyrase and topoisomerase IV, whereas mutations in the acidic residue can result in a five- to 10-fold decrease in the overall catalytic activity [1, 10]. The magnitude of resistance caused by single amino acid changes in a DNA gyrase or topoisomerase IV subunit varies depending on the quinolone and the bacterial species, being the resistance phenotype of a particular mutation determined by the relative sensitivities of each enzyme to a particular quinolone. Thus, the susceptibility of a wild-type isolate is determined by the more sensitive of the two target enzymes and, therefore, under quinolone selection pressure, resistance mutations will first occur in this more sensitive primary target, as mutation of the secondary less sensitive target alone is not enough for resistance due to dominance of the quinolone–primary target interaction [10]. Since fluoroquinolones currently in clinical use generally have different potencies between the two target enzymes, single target-site gene mutations typically result in an eight- to 16-fold increase in resistance, with mutation in both DNA gyrase and topoisomerase IV generally associated with higher (up to 100-fold) resistance levels [1, 4, 5, 10]. Indeed, an accumulation of mutations in one or both target enzymes has been shown to cause increasing levels of quinolone resistance and it is generally accepted that high levels of quinolone resistance require double gyrA mutations; however, high-level resistance phenotypes have been reported with single gyrA mutations [10, 19, 20]. Mutations detected in the DNA gyrase and topoisomerase IV genes of clinically important pathogens described in the literature are summarized in Table 1.

**Chromosomal mutations leading to reduced drug accumulation**

In order to interact with their cytoplasmic targets, quinolones must cross the bacterial envelope and mutations that result in a reduction of the intracellular drug concentration, either by decreased uptake (Fig. 1b) or increased efflux (Fig. 1c) or a combination of both, can confer quinolone resistance [1, 5, 10].

As opposed to Gram-positive species, the Gram-negative outer membrane represents an additional permeability barrier for hydrophilic drugs which cannot diffuse into the cell due to the core region of lipopolysaccharide (LPS) and therefore rely on outer membrane porin channels to enter the cell. Hence, mutations that result in either porin loss, porin downregulation or a modification of the size or conductance of the porin channel will result in a limited, substantially slower diffusion of quinolones and other drugs into the cell and consequently lead to bacterial antibiotic resistance (Fig. 1b1) [1, 5, 10, 21, 22]. The reduced or loss of expression of porins such as OmpF, OmpC, OmpD and OmpA has been implied in increased antibiotic resistance to quinolones and other drugs. Moreover, the overexpression of OmpX, which has been described as a downregulator of porin expression, leads to a decreased expression of the OmpC, OmpD, OmpF, LamB and Tsx porins, resulting in an increased resistance to a variety of antibiotics, including quinolones [10, 20, 21, 23–25]. Moreover, another aspect that can be or not be associated with changes in the expression and/or function of porins in reducing membrane permeability is the alteration of the outer membrane organization (Fig. 1b2). Since, depending on protonated status, quinolones are known to use both porin- and lipid-mediated pathways to enter bacterial cells, mutations that lead to LPS structure modifications are also reported to impact bacterial resistance to these and other antibiotics. In addition, OmpA in known to have a structural role in maintaining the integrity of the cell envelope; however, its exact
contribution to antibiotic resistance remains to be demonstrated [20, 22, 23, 26, 27].

Reduced influx due to porin or LPS modifications often acts together with basal or increased expression of active efflux transporters in reducing cytoplasmic quinolone concentrations, both contributing additively to resistance [1, 5, 10]. Bacterial exposure to quinolones can select mutants that overexpress efflux pumps, usually as a result of mutations in regulatory proteins and less often as a result of mutations in the structural genes associated with quinolone inclusion in pump substrate profiles [1, 5, 10]. In general, mutations affecting quinolone uptake and efflux cause only low-level resistance (about four- to eightfold increases in inhibitory concentrations) and do not usually represent a major clinical problem in the absence of additional resistance mechanisms. However, efflux systems have been shown to be of critical importance for the development of high levels of quinolone resistance and reduced intracellular concentrations of quinolones can favour the emergence and dissemination of other types of resistance [1, 4, 10].

Fig. 1. Mechanisms of quinolone resistance. (a) Chromosomal mutations within the QRDRs of the genes encoding the A and B subunits of DNA gyrase and topoisomerase IV structurally change the target protein, reducing its drug-binding affinity. (b) Chromosomal mutations leading to reduced outer membrane permeability, by either reduced porin expression (b1) or modifications in the outer membrane organization (b2), and also mutations leading to an increased expression of efflux pumps (c), contribute additively to resistance by decreasing cytoplasmic quinolone accumulation. (d) Plasmid-encoded quinolone resistance genes can produce Qnr target-protection proteins (d1), AAC(6¢)-Ib-cr acetyltransferase variants capable of modifying certain quinolones (d2) or QepA and OqxAB efflux pumps that actively extrude quinolones. LPS, lipopolysaccharide; MFS, major facilitator superfamily; RND, resistance–nodulation–division; MATE, multiple antibiotic and toxin extrusion; ABC, ATP-binding cassette; MDR, multidrug resistance. (Adapted from Correia et al. [20].)

Efflux pumps involved in quinolone extrusion have been described in both Gram-positive and Gram-negative bacteria (Fig. 1c) [2, 28]. In Gram-positive species, the major facilitator superfamily (MFS) contains the largest number of efflux systems that include quinolones in their substrate profiles [e.g. NorA, NorB, NorC, MdeA, LmrS, SdrM and QacB(III) in Staphylococcus aureus; PmrA in Streptococcus pneumoniae; Bmr, Bmr3 and Blt in Bacillus subtilis; LmrP in Lactococcus lactis; Lde in Listeria monocytogenes; EfM in Enterococcus faecium]. In addition, efflux pumps belonging to the multiple antibiotic and toxin extrusion (MATE; e.g. MepA in in Staphylococcus aureus; FepA in Listeria monocytogenes); and ATP-binding cassette (ABC; e.g. PatAB in Streptococcus pneumoniae; LmrA in Lactococcus lactis; SatAB in Streptococcus suis; EfrAB in Enterococcus faecalis) families have also been shown to confer quinolone resistance in Gram-positive species [4, 10, 21, 28]. Concerning Gram-negative bacteria, the majority of efflux pumps involved in quinolone resistance are members of the resistance–nodulation–division (RND) family, which are
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tripartite complexes formed by an inner membrane pump protein, an outer membrane channel protein and a periplasmic membrane fusion protein (e.g. AcrAB-TolC in E. coli, Salmonella spp., Enterobacter aerogenes and Enterobacter cloacaec; MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM in Pseudomonas aeruginosa; OqxAB-TolC in Klebsiella pneumoniae; CmeABC, CmeDEF in Campylobacter jejuni; AdeABC, AdeFGH, AdeJK in Acinetobacter baumannii; SmeDEF, SmeABC in Stenotrophomonas maltophilia; SdeAB, SdeXY in Serratia marcescens). A few members of the MFS (e.g. EmrAB-TolC and MdfA in E. coli), MATE (e.g. NorE, YdhE in E. coli; Emmdr in Enterobacter cloacaec; PmpM in P. aeruginosa; AbeM in A. baumannii; VcmB, VcmD, VcmH, VcmN in Vibrio cholerae; NorM in Vibrio parahaemolyticus and V. cholerae; NorA in Bacteroides fragilis; BexA in Bacteroides thetaiotaomicron) and ABC (VcaM in V. cholerae) families have also been shown to be involved in quinolone resistance in Gram-negative species [4, 10, 21, 28].

The reduced quinolone influx acts in concert with increased efflux to generate a resistance phenotype through mechanisms involving the transcriptional and post-transcriptional regulation of both efflux systems and porins [4, 10, 21, 23, 26]. Mutations in the Mar (multiple antibiotic resistance), SoxRS and Rob regulons can result in quinolone resistance, sometimes with overlapping effects since some genes are present in different regulons. For instance, mutations in the MarR and MarA regulators can result in both an increased expression of AcrAB and reduced expression of OmpF. In addition to direct regulators, mutations in the mppA gene, encoding a murein peptide-binding protein, have also been shown to increase MarA expression. Moreover, MarA can prevent ompF mRNA translation, and also activate the expression of the porin downregulator OmpX, through the activation of the small RNA (sRNA) MicF, which can likewise be activated by SoxS or the MarA homologue RamA. The OmpC porin can equally be post-transcriptionally regulated by the sRNA MicC and both ompF and ompC genes can also be transcriptionally regulated by the two-component regulatory system OmpR-EnvZ. Similarly, upon envelope stress, porin synthesis can be repressed by the RpoE-regulated sRNAs MicA, RybB and CyaR which inhibit mRNA translation of a number of OMPs including the OmpA, OmpC, OmpF, OmpX, OmpW and Tsx porins. Furthermore, LPS biosynthesis and assembly is an other important feature under the post-transcriptional control of the RpoE-regulated RybB and MicA sRNAs and LPS lipid A modifications are also regulated by the PhoP/Q two-component system through the sRNA MgrR. Changes in LPS structure and composition have been shown to be critical not only for outer membrane integrity, virulence, host survival and adaptation to specific environments, but also for resistance to quinolones and other antibiotics [4, 10, 21, 23, 26, 29–31].

**Plasmid-mediated quinolone resistance (PMQR)**

Mechanisms involving PMQR were first described in the late 1990s and have been extensively reviewed in the last decade [10, 32–40]. The first PMQR gene, *qnrA*, was described in 1998 in a broad-host-range conjugative plasmid of a ciprofloxacin-resistant *K. pneumoniae* clinical isolate [41] and, to date, about 100 Qnr variants have been identified, being classified into six distinct families: QnrA, QnrB, QnrC, QnrD and QnrVC. The Qnr proteins belong to the pentapeptide-repeat protein family and confer quinolone resistance by physically protecting DNA gyrase and topoisomerase IV from quinolone inhibition (Fig. 1d1). A second mechanism for PMQR was later discovered in 2006 and consisted of a bifunctional variant of an aminoglycoside-modifying acetyltransferase, AAC(6’)-Ib-cr, which harboured two specific amino acid substitutions, Trp102Arg and Asp179Tyr. This variant is able to acetylate the unsubstituted nitrogen of the C7 piperazinone ring that is found in quinolones such as ciprofloxacin, thereby conferring quinolone resistance by decreasing the drug’s activity (Fig. 1d2). Shortly after, a third mechanism for PMQR was added with the discovery of the plasmid-encoded efflux pumps QepA and OqxAB (Fig. 1d3). QepA belongs to the MFS family and confers decreased susceptibility to hydrophilic fluoroquinolones, especially ciprofloxacin, norfloxacin and enrofloxacin. OqxAB belongs to the RND family and has a wide substrate specificity that beyond the quinolones ciprofloxacin, norfloxacin, nalidixic acid, olaquindox and flumequine, also include the antibiotics tetracycline, chloramphenicol and trimethoprim, bile acids like benzoalkonium chloride or triclorosan, among many other compounds. PMQR determinants generally confer only low-level quinolone resistance that alone does not exceed the clinical breakpoint. However, in the presence of therapeutic levels of quinolones, PMQR provides a favourable background for selection of additional resistance
mechanisms and hence for the emergence of high levels of quinolone resistance. PMQR genes have been detected in transposons and/or integrons that are often co-located with other resistance determinants (frequently extended spectrum β-lactamase, AmpC-type β-lactamase and carbapenemase genes) in MDR plasmids of significantly varying sizes and incompatibility groups. The dissemination of multiple plasmids with such heterogeneity has been responsible for the wide geographic distribution of PMQR in a great variety of hosts. PMQR genes have also been reported with a certain frequency in chromosomal locations, reflecting the plasticity of PMQR-related mobile genetic elements. For instance, qnr genes are detected on the chromosome of Gram-negative and Gram-positive bacteria from both clinical and environmental origins. The aac(6’)-Ib-cr gene can also be chromosomal. Additionally, the qepAB gene is reported with high frequencies on the chromosome of K. pneumoniae, which represents a potential reservoir organism for the dissemination of such genes. Due to the current difficulties in the phenotypic detection of PMQR mechanisms (reliable detection depends on genetic assays), the actual prevalence of these determinants is not precisely known. Incidences varying from less than 1% to more than 50% have been reported, depending on the bacterial species and resistance mechanisms. PMQR prevalence, distribution and epidemiology data have been exhaustively compiled and reviewed recently by Martinez-Martinez et al. [37], Strahilevitz et al. [36], Rodriguez-Martinez et al. [35], Jacoby et al. [32], Yan et al. [42] and Rodriguez-Martinez et al. [40]. PMQR determinants have been found mostly in enterobacteria (particularly E. coli, Enterobacter spp., Klebsiella spp. and Salmonella spp.) with occasional reports in non-fermenting bacteria (e.g. Pseudomonas spp., A. baumannii and Stenotrophomonas maltophilia) isolated from a wide variety of human, animal and environmental samples, being qnr variants and aac(6’)-Ib-cr the most commonly reported. PMQR represents an emerging clinical problem that was not expected to occur. Although thoroughly studied in the last 10 years, much remains to be understood about these resistance mechanisms. Nevertheless, the insidiousness with which PMQR determinants can promote substantial levels of resistance together with their easy horizontal dissemination and co-selection with multiple resistance elements substantiate the need for a more cautious use of quinolone drugs in both clinical and non-clinical environments and for serious reconsiderations of actual clinical breakpoints [1–3, 5, 10, 28, 32–40, 42].

CONCLUDING REMARKS

Quinolone resistance is multifactorial and complex and even though our understanding of the interplay and biological impact of the multiple resistance mechanisms has improved significantly over the last years, we are still far from the complete elucidation of this phenotype. Due to the synthetic nature of quinolones, it was predicted that mutations in the genes encoding the target type II topoisomerases would be the only mechanism through which resistance could be acquired, and the existence of plasmid-mediated quinolone resistance was completely unexpected. However, in addition to one or a combination of target-site gene mutations, quinolone resistance has been shown to occur via plasmid-encoded modifying enzymes and/or target-protection proteins, and also due to the expression of either chromosomally or plasmid-encoded efflux pumps. Additionally, reduced quinolone influx through changes in outer membrane porin expression and/or LPS modifications have been shown to act in concert with increased efflux in conferring quinolone resistance in Gram-negative species. Although these mechanisms have been thoroughly studied, many details remain to be clarified, and the exact contribution of less studied mechanisms such as the metabolic regulation of drug resistance (reviewed in detail by Martinez and Rojo [43]), the bacterial stress responses as resistance determinants (reviewed by Poole [44]), or even quorum sensing and biofilm formation, to the global resistance phenotype to quinolones still needs further elucidation.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References


