Chapter

Mechanisms of Resistance to Quinolones

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Abstract

Antimicrobial resistance is a worldwide problem. Various pathogenic bacteria can be resistant to one or several antibiotics, resulting in a serious public health problem. Isolation of pathogenic bacteria resistant to multiple last-generation antibiotics from hospital samples have been reported. In that sense, the isolation of pathogenic strains resistant to members of the quinolone family, from clinical samples, is an increasing phenomenon. Quinolones are a group of synthetic broad-spectrum antimicrobials, whose mechanism of action is the inhibition of DNA gyrase and topoisomerase IV, with the consequent DNA breakdown and cell death due to genotoxic damage. Three mechanisms have been determined by which bacteria can be resistant to quinolones: (1) Chromosomal mutations in coding genes (mutations that alter the objectives of the drug). (2) Mutations associated with the reduction of the intracytoplasmic concentration of quinolones. (3) Plasmid-mediated quinolone resistance genes (plasmids that protect cells from the lethal effects of quinolones). In this chapter, we analyze each of them and provide the most current connections and investigations of these processes.

Keywords: antibiotic resistance, quinolones, fluoroquinolones, DNA topoisomerase IV, genotoxic damage

1. Background

Antimicrobial resistance has become a serious public health problem in recent years. This problem has been increasing and is currently a truly global crisis that offers one of the worst forecasts of catastrophic scenarios in public health worldwide.

A sign of the seriousness of the problem is the fact that World Health Organization (WHO)'s new Global Antimicrobial Surveillance System (GLASS) reported the widespread occurrence of antibiotic resistance among 500,000 people with suspected bacterial infections across 22 countries [1].

Likewise, Centers for Disease Control (CDC)'s Antibiotic Resistance Threats in the United States (US), in 2019 (2019 AR Threats Report), reported that more than

2.8 million antibiotic-resistant infections occur in the US each year, and more than 35,000 people die as a result. Besides, 223,900 cases of *Clostridium difficile* occurred in 2017 and at least 12,800 people died [2].

Many bacteria produce important infections in human health, either due to community-acquired infections, nosocomial infections, or at intensive care units. Among these, many have an important phenotypic profile of antibiotic resistance. For example, *Staphylococcus aureus, Enterococcus* spp., *Enterobacteriaceae* (other than *Salmonella* and *Shigella*), *Pseudomonas aeruginosa,* and *Acinetobacter* spp. [3, 4].

To classify these microorganisms according to the degree of resistance and acquired resistance profiles, a group of experts in the field of antimicrobial resistance in joint work with the European Center for the Prevention of Diseases and Control (ECDC) and the CDC established the definitions and characteristics among resistant bacteria: multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) bacteria [3, 4].

To establish objective parameters of the phenotypic resistance profile in each of these bacteria, epidemiologically significant antimicrobial categories were established. These categories were established based on the documents and cut-off points of the Clinical Laboratory Standards Institute (CLSI), the European Antimicrobial Sensitivity Testing Committee (EUCAST), and the US Food and Drug Administration (FDA) [3, 4].

Based on the new limits and definitions: MDR bacteria possess acquired resistance to at least one antibiotic of three or more categories; XDR bacteria possess resistance to at least one antibiotic of almost all categories, except one or two of them; and PDR bacteria are resistant to all agents of all categories of antimicrobials [3, 4].

Antimicrobial resistance has been observed in all families of antibiotics, including the latest generation and intrahospital antibiotics such as quinolones.

The wide use of quinolones in clinical practice includes the administration of the antibiotic in prophylaxis, in neutropenic patients with cancers, in cirrhotic patients at risk for spontaneous bacterial peritonitis, and in urologic surgery, among others. In many of these cases, strains with varying degrees of resistance to quinolones have been isolated [5, 6].

2. History

In 1962, quinolones were discovered as an important treatment for various pathological manifestations. The first one was nalidixic acid, which was synthetically produced by George Lesher at the Sterling-Winthrop Research Institute. It was synthesized from the isolation of chloro-1-ethyl-1,4-dihydro-4-oxo-3-quinoline carboxylic acid years before, as a product derived from the synthesis of chloroquine [7]. Its origin dates back to the use of chloroquine as an antimalarial agent. It was until years after its development that nalidixic acid was approved for the treatment of urinary tract infections by Gram-negative bacteria. This compound does not have an important effect on Gram-positive bacteria, in addition to having a certain cytotoxic effect on the gastrointestinal tract and the central nervous system. Its effect on Gram-negative bacteria is characteristic of the first generation of quinolones [8].

3. Epidemiology

The indiscriminate prescription of quinolones worldwide has led to a rapid increase in bacterial resistance. *Acinetobacter* spp., *Campylobacter* spp., *Capnocytophaga* spp., *Clostridium* spp., *Escherichia coli, Klebsiella pneumoniae, Mycobacterium tuberculosis,*

Bacteria	Mean resistance percentage		Country with the lowest resistance percentage		Country with the highest resistance percentage	
	2015	2018	2015	2018	2015	2018
Acinetobacter spp.	43.7%	36.2%	Belgium (0%)	Norway (0%)	Greece (94.9%)	Croatia (96.1%)
Escherichia coli	22.8%	25.3%	Iceland (6.8%)	Finland (11.4%)	Cyprus (45.5%)	Cyprus (42.4%)
Klebsiella pneumoniae	29.7%	31.6%	Iceland (2.9%)	Iceland (0%)	Slovakia (70%)	Poland (68.2%)
Pseudomonas aeruginosa	19.3%	19.7%	Estonia (0%)	Malta (0%)	Romania (59%)	Slovakia (52.4%)

**The EARS-Net report does not contain information about quinolone resistance to other bacteria. Adapted from: EARS-Net 2015 and Ecdc. SURVEILLANCE REPORT. 2018 [11, 12].*

Table 1.
Profile of resistance to quinolones of European countries (2015 vs. 2018).

Neisseria gonorrhea, Proteus mirabilis, P. aeruginosa, Salmonella spp., *S. aureus,* and *Streptococcus pneumoniae,* among others, have been reported as resistant [7, 9, 10].

The ECDC collects and reports through the European Antimicrobial Resistance Surveillance Network (EARS-Net) information of seven bacterial pathogens that commonly cause infections in humans: *Acinetobacter* spp., *Enterococcus faecalis, Enterococcus faecium*, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa.* Information comparing their profile of resistance to quinolones in Europe between 2015 and 2018 can be found in **Table 1** [11, 12].

4. The structure of quinolones

The structure of quinolones derives from two types of rings, a naphthyridine core with a nitrogen molecule in positions 1 and 8. Through this structure, the compound is limited to being used as a therapy against Gram-negative bacteria. However, it has been shown that by inserting a cyclopropyl group in the first position of the nitrogen ring, an effect is achieved not only on Gram-negative bacteria but also on Gram-positive ones (**Figure 1**) [13, 14].

The second generation was developed in 1980, from the addition of a fluorine atom at position six, resulting in fluoroquinolones. These have higher activity in Gram-negative bacteria, as well as Gram-positive bacteria. Some fluoroquinolones can inhibit all Gram-negative organisms. Quinolones with piperazine on carbon 7 are effective in Gram-negative bacteria and the signaling of topoisomerase 4 (**Figure 1**) [13, 15–17].

Later, the third generation arises by adding certain molecules in the rings, such as the cyclopropyl ring in the first position of nitrogen, improving the activity in Gram-positive bacteria. Some of these modifications achieved sensitivity in organism resistant to different antibiotics, including *Streptococcus pneumoniae*. Other benefits of this generation are a longer life in serum and activity against anaerobic organisms (**Figure 1**) [7, 18, 19].

The fourth generation was later developed by incorporating nitrogen in the eighth position, resulting in a broad-spectrum antibiotic. Its action in some Gram-positive organisms is more effective compared to the other generations; however, its activity in anaerobic organisms is limited. It has a superior bacterial selectivity to avoid a high level of resistance and its toxic effects are less unfavorable than in the other generations [7, 8]. Thanks to the modifications made to the quinolones,

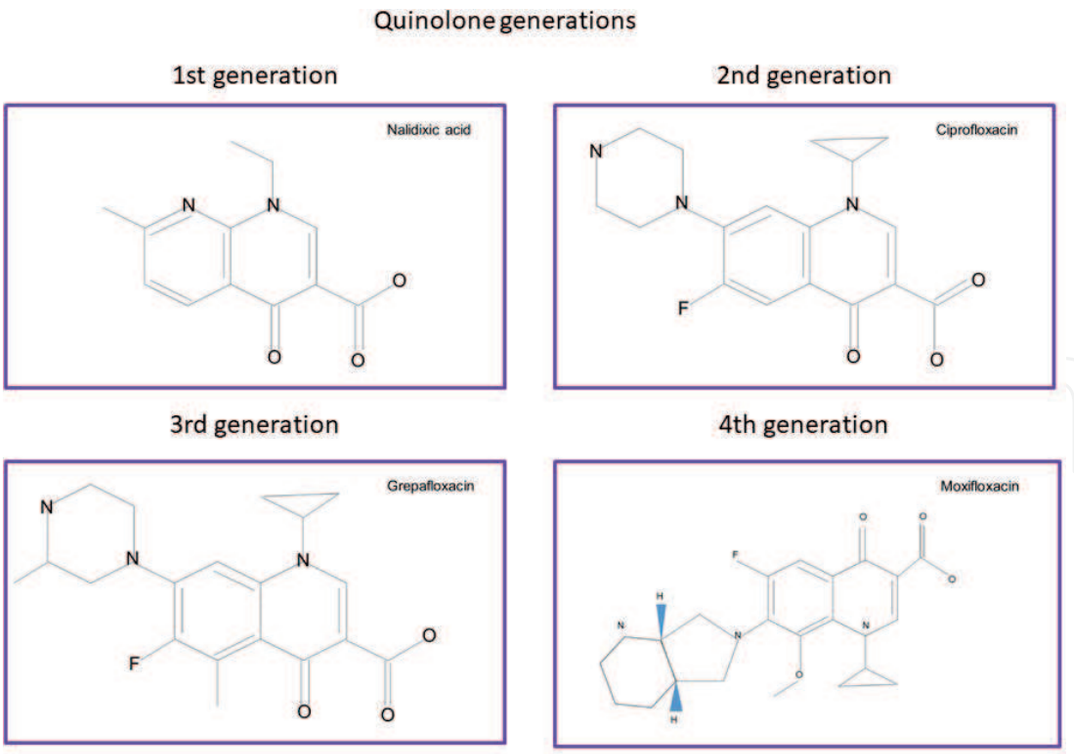

Figure 1.
Molecular structure of representative members of each quinolone generation. Based on PubChem public archive https://www.ncbi.nlm.nih.gov/pcsubstance [14, 17, 19, 20].

Generation		Compounds	Activity spectrum
1		Nalidixic acid	Gram-negative bacteria (not *Pseudomonas* spp.)
2	2a	Ciprofloxacin, enoxacin, norfloxacin	Gram-negative bacteria and atypical pathogens (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*)
	2b	Levofloxacin, lomefloxacin, ofloxacin	Gram-negative bacteria, Gram-positive bacteria (not *Streptococcus pneumoniae*), and atypical pathogens
3		Clinafloxacin, gatifloxacin, grepafloxacin, sparfloxacin.	Gram-negative bacteria, Gram-positive bacteria (*Streptococcus pneumoniae*) and improved activity against atypical pathogens
4		Gatifloxacin, gemifloxacin, moxifloxacin, trovafloxacinin	Gram-negative bacteria, Gram-positive bacteria (*Streptococcus pneumoniae*) and improved activity against atypical and anaerobic pathogens

Adapted from: Pham TDM, Ziora ZM, Blaskovich MAT [7].

Table 2.
Classification of quinolones.

an improvement in its pharmacokinetics and pharmacodynamics has been obtained, thus optimizing absorption, metabolism, and elimination, achieving lower toxicity and superiority in the mechanisms of action. It has also been possible to modify the half-life of the drug making only one dose per day necessary (**Figure 1**) [20].

Currently, nine fluoroquinolones have been approved in the US while others continue to be used in clinical trials. Information regarding the generations, compounds, and spectrum of activity can be found in **Table 2**.

It has been reported that several agents such as *Escherichia coli, Klebsiella pneumoniae, Neisseria gonorrhoeae,* or *Staphylococcus aureus* have presented significant resistance to quinolones [21]. Plasmid-mediated quinolone resistance (PMQR) was a completely unexpected event since it was thought that the only mutation would occur in genes encoding topoisomerase II identification. Currently, the resistance mechanism is multifactorial. However, the most common quinolone resistance mechanism is topoisomerase mutations [15, 22].

The excessive use of this type of drug has caused the incidence rates of hypersensitivity to increase more and more, taking the second place of antibiotics with a greater number of hypersensitivity reactions in in-hospital patients. The main agents that cause hypersensitivity are ciprofloxacin, levofloxacin, and moxifloxacin. This has positioned quinolones as the non-beta-lactam antibiotics with the highest incidence of hypersensitivity reactions [23, 24].

5. Mechanism of action

The mechanism of action of quinolones is based on the inhibition of bacterial topoisomerases II and IV. Topoisomerases are enzymes responsible for maintaining the tertiary structure of DNA during various cellular processes, such as synthesis, replication, condensation, and decondensation of DNA, among others [25–29].

Topoisomerase II, also known as DNA gyrase, is considered a negative supercoiling enzyme, which means that it cuts the two strands of DNA and propitiates that the DNA is twisted to the left producing a twist in a way contrary to the direction of the double helix. This enzyme participates in the DNA winding and relaxation during various processes, mainly in the synthesis and replication of DNA [30, 31].

The DNA gyrase consists of a heterotetramer, which is formed by two GyrA subunits and two GyrB subunits. The GyrA subunits participate in the union with the DNA and are responsible for making the double helix cuts. The GyrB subunits possess ATPase activity [30].

Topoisomerase IV is responsible for preventing the chromatids from being chained, meaning it participates in the separation of daughter chromatids after DNA replication [32].

Like DNA gyrase, topoisomerase IV is made up of a tetramer. It has two ParC subunits and two ParE subunits. These subunits possess homologous activity of GyrA and GyrB, respectively [32].

When quinolones interact and inhibit topoisomerase II and IV, it induces DNA breakdown and cell death due to genotoxic damage [27–29].

6. Resistance mechanisms

To counteract the effect of quinolones, bacteria have developed various resistance mechanisms to these antibiotics. Bacterial resistance to quinolones is mainly based on three points (**Table 3**, **Figure 2**):

1. Chromosomal mutations in coding genes (mutations that alter the objectives of the drug).

2. Mutations associated with the reduction of the intracytoplasmic concentration of quinolones.

3. PMQR genes (plasmids that protect cells from the lethal effects of quinolones) [33].

Mechanism	Description		
Chromosomal mutations in coding genes	Occurs due to errors in the replication of the genes encoding the GyrA subunits of DNA gyrase and ParC of topoisomerase IV		
Mutations associated with the reduction of the intracytoplasmic concentration of quinolones	Occurs due to mutations that lead to a decrease in the intracytoplasmic concentration of the antibiotic. It may happen through:		
	Overexpression of efflux pumps from the resistance-nodulation-cell division	Both	Reduction of the membrane permeability by downregulation of extra-membrane proteins
Plasmid-mediated quinolone resistance genes	Occurs due to the activation of plasmid-mediated quinolone resistance genes. Among them are:		
	Qnr's encode proteins that protect DNA gyrase and topoisomerase IV	AAC(6′)-lb-cr acetylates quinolones with an appropriate amino nitrogen target	QepA and OqxAB, which increase the outflow of quinolones through efflux pumps

Adapted from: Álvarez-Hernández DA, Garza-Mayén GS, Vázquez-López R. Quinolones. Nowadays perspectives and mechanisms of resistance [34].

Table 3.
Mechanisms of resistance to quinolones.

6.1 Chromosomal mutations in coding genes (mutations that alter the objectives of the drug)

The quinolone resistance associated with chromosomal mutations occurs due to errors in the replication of the genes encoding the GyrA subunits of DNA gyrase and ParC of topoisomerase IV [33, 35].

In the amino acid sequences of the GyrA and ParC subunits, there are specific regions that interact with the DNA. In these regions, there are conserved domains called quinolone resistance determining region (QRDR) [31, 35–39].

It is precisely in the sequences that code for each of the QRDR domains of the GyrA and ParC subunit genes, where such mutations occur [31, 35–39].

It has been reported that quinolone resistance may also occur due to mutations in the genes encoding the GyrB and ParE subunits; however, they do not occur so frequently and their clinical value appears to be very limited [35, 40, 41].

There is evidence that in Gram-negative bacteria, DNA gyrase turns out to be more susceptible to inhibition than topoisomerase IV. On the other hand, in Gram-positive bacteria, the opposite phenomenon occurs; that is, that topoisomerase IV is more susceptible to inhibition than gyrase. However, certain bacteria show the opposite effect, being the exception to the rule [31, 42, 43].

Therefore, we can affirm that the phenomenon of resistance in the majority of Gram-negative bacteria occurs mainly in GyrA, while in most Gram-positive bacteria the inhibition of ParC is the most important [31, 42, 43].

Summarizing, mutations that occur in the sequences encoding the QRDR domains in both GyrA-ParC and GyrB-ParE favor a decrease in the binding affinity of quinolones with the DNA–DNA gyrase and DNA-topoisomerase IV complex [33, 35].

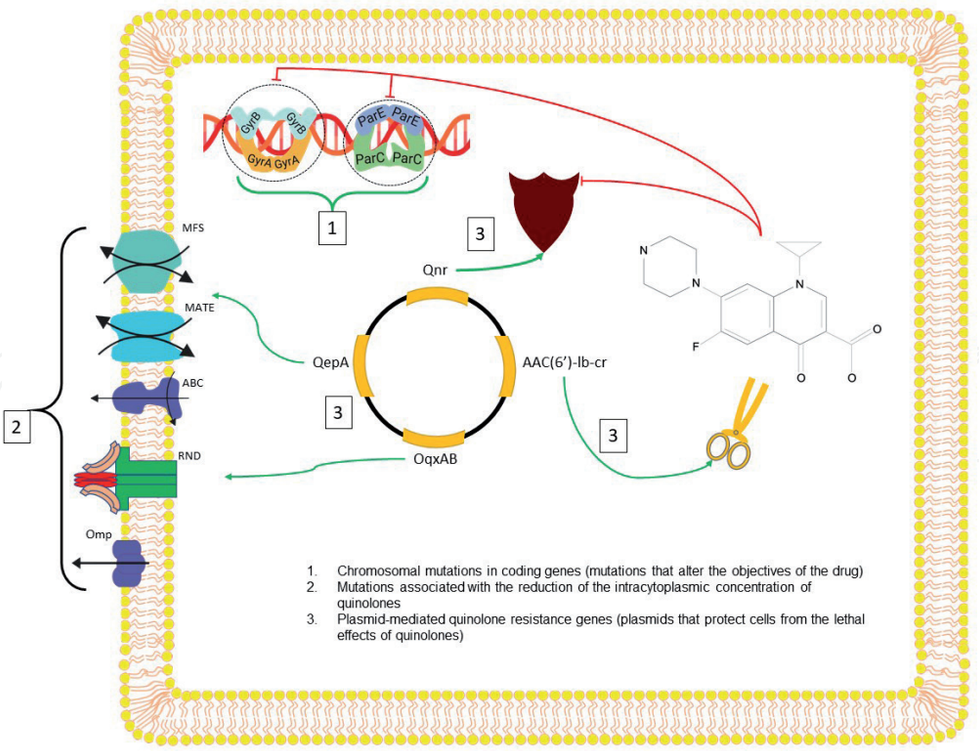

Figure 2.
Schematic representation of the mechanisms of bacterial resistance to quinolones. Based on Susana Correia et al. [22].

6.2 Mutations associated with the reduction of the intracytoplasmic concentration of quinolones

Another important quinolone resistance mechanism consists in the ability of the bacteria to decrease the intracytoplasmic concentration of the antibiotic; this decrease in concentration is determined by certain mutations.

This phenomenon is achieved through three mechanisms:

1. Efflux pumps that promote the active transport of quinolones to the outside of the bacterial cell.

2. Decreased membrane permeability toward the antibiotic.

3. A combination of both mechanisms.

It has been described that only efflux pumps participate in Gram-positive bacteria as mechanisms to reduce the intracytoplasmic concentration of quinolones since there is no evidence that the decrease in cytoplasmic membrane permeability participates in this type of bacteria [44].

On the other hand, Gram-negative bacteria do have both mechanisms and participate in a complementary way with one another, the decrease in permeability in the cytoplasmic membrane being the most important for these bacteria [45].

These two mechanisms involved in the decrease of the intracytoplasmic concentration of quinolones are not induced by the drugs themselves. There is evidence that these two mechanisms occur because of mutations in genes that encode regulatory proteins that control transcription of the outflow pump or genes that code for porin synthesis [35, 46].

6.2.1 Mutations associated with the reduction of the intracytoplasmic concentration of quinolones in Gram-positive bacteria

This resistance mechanism in Gram-positive bacteria is associated with the presence of chromosomally encoded efflux pumps that decrease the intracytoplasmic concentration of the antibiotic, giving the bacteria the characteristic of being MDR.

Efflux pumps are classified into two groups: primary active transporters and secondary active transporters [47].

The primary active transporter proteins are pumps that use ATP as a source of energy. This type of primary active transporter integrates the members of the ATP-binding cassette (ABC) superfamily [48–50].

On the other hand, the secondary active transporter proteins use the energy obtained by the difference of chemical gradients formed by either protons or ions, for example, sodium ions [48, 49].

Four types of secondary active transporter proteins have been identified: [47–49].

1. The small multidrug-resistance (SMR) family

2. The major facilitator superfamily (MFS)

3. Multidrug and toxic compound extrusion (MATE) family

4. The resistance-nodulation-cell division (RND) superfamily.

6.2.1.1 SMR (the small multidrug-resistance family)

Members of this family are proteins made up of an antiparallel dimer. Each monomer of this dimer has four transmembrane helices (TM1, TM2, TM3, and TM4). The TM 1 to M3 helices comprise the substrate binding pocket, while each TM4 helix is responsible for SMR TM4-TM4 dimerization [51–53].

The members of the SMR family are associated with resistance to various toxic compounds and some antibiotics; however, they do not appear to play a relevant role in resistance to quinolones.

6.2.1.2 MFS (the major facilitator superfamily)

Concerning efflux pumps related to the intracytoplasmic decrease in quinolone and consequently linked to resistance to this drug, they are efflux pumps that are part of the MFS. Three members of this family associated with quinolone resistance have been identified: NorA, NorB [50], and NorC [54]. Overexpression of each of three efflux pumps increases resistance to quinolones four to eight times [33].

6.2.1.2.1 NorA

The chromosomal gene that codes for NorA could be identified in 1986 from the isolation of *Staphylococcus aureus* obtained from a urine sample from a patient who had received treatment with norfloxacin at Teikyo University Hospital Japan [55]. It has been observed that NorA participates in the pumping of various quinolones, mainly ciprofloxacin and norfloxacin [56, 57].

Subsequent studies of genetic diversity described three alleles for the *NorA* gene [58]: *NorAI* (Yoshida), *NorAII* (Noguchi), and *NorAIII* (Kaatz). A correlation has been observed between the different types of NorA alleles

and specific lineages of *S. aureus*. This fact suggests that there is a correlation between the NorA variants and the population structure (lineages) of this bacterium [58].

6.2.1.2.2 NorB

It has been described that the expression of the efflux pump NorB gives certain bacteria (e.g., *Staphylococcus aureus*) the adaptability in tissue infection conditions, even in the absence of antibiotics. This fact occurs because NorB gives *Staphylococcus aureus* the ability to eliminate antibacterial substances present in the abscess and produced as a defense mechanism by the host. In this way, NorB not only participates in the quinolone resistance mechanism but also contributes to the pathophysiology of certain infections [59].

6.2.1.2.3 NorC

The efflux pump *Norc* enhances the exit of quinolones such as ciprofloxacin, garenoxacin moxifloxacin, and sparfloxacin out of the bacterial cell. Its expression is regulated negatively by MgrA [54].

Many regulatory proteins participate in a complex regulatory process in the gene expression of NorA, NorB, and NorC. One of these regulatory proteins is MgrA, which shows the ability to bind to the NorA promoter region. The overexpression of MgrA causes the inhibition of the expression of NorA, NorB, and NorC, in the opposite, resistance to quinolones is associated with a low activity of *MgrA* and the consequent overproduction of NorA, NorB, and NorC that will promote a decrease in the intracytoplasmic concentration of the drug [54, 60–62].

There is evidence that MgrA activity could be determined by environmental conditions in which the bacterium is found. Acid conditions, oxidative, as well as the presence of iron, could alter the activity of MgrA and consequently the expression of NorA, NorB, and NorC and its effect on the pumping of quinolone and its concentration in the bacterial cytoplasm [35, 59, 63–65].

On the other hand, another transcriptional regulator, called NorG, which activates the expression of NorA and NorB but suppresses the expression of NorC, has been described. It is important to understand that the regulation of the gene expression of *NorA*, *NorB*, and *NorC* results from a complex molecular framework where both activators and inhibitors participate and the balance between them, as well as the environmental and nutritional conditions in which the bacteria develops, will give as a result the resistance or the lack of it to quinolones [35, 61, 62, 66].

6.2.1.3 Other members of the MFS (major facilitator superfamily)

6.2.1.3.1 MdeA

MdeA gen was identified in an open reading frame (ORF) expression library of the *S. aureus* genome. The efflux pump protein MdeA belongs to the MFS using the proton motive force to energize the transport of its substrates [67, 68].

MdeA confers resistance to the biocides benzalkonium chloride, dequalinium, tetraphenylphosphonium, and to the dye ethidium bromide [67]. MdeA also confers resistance to multiple antibiotics among which are fusidic acid, mupirocin, novobiocin, and virginiamycin, and to some extent toward ciprofloxacin and norfloxacin [67, 68].

6.2.1.3.2 SdrM

In 2006 Yamada et al. cloned a new gene called *SA1972* isolated from *Staphylococcus aureus*. The product obtained was called SdrM and it was proven that it conferred resistance to the bacteria against, acriflavine, ethidium bromide, and norfloxacin. SdrM was classified as an efflux pump belonging to the MFS [69].

6.2.1.3.3 QacB (III)

The *qacA* and *qacB* genes that code for efflux pump proteins (QacA and QacB, respectively) are present in methicillin-resistant *Staphylococcus aureus* (MRSA). The efflux pump QacA has two isoforms, while the pump QacB has four known as QacBI, QacBII, QacBIII, and QacBIV. It has been observed that the QacBIII variant confers resistance to *S. aureus* to fluoroquinolones [70].

6.2.1.4 MATE (multidrug and toxic compound extrusion family)

6.2.1.4.1 MepA

The efflux pump MepA belongs to the multidrug and toxic compound extrusion (MATE) family. MepA gives the bacterium a phenotypic MDR profile associated with low-level resistance to some quaternary ammonium compounds. It also confers resistance to certain antibiotics, mainly toward glycylcyclines and to a lesser extent resistance to ciprofloxacin and norfloxacin [71–73].

In addition to the efflux pump described above, there are other transporters in Gram-positive bacteria that participate in the decrease in the intracytoplasmic concentration of quinolones in the bacterial cell, participating in resistance to this drug. Some of these transporters are LmrS, Bmr, Bmr3 and Blt, PmrA66, LmrP67, PatAB69, SatAB70, LmrA71, FepA, FepR, and TetR [35].

6.2.2 Mutations associated with the reduction of concentration in Gram-negative bacteria

6.2.2.1 RND (resistance-nodulation-cell division superfamily)

Gram-negative bacteria use efflux pumps belonging to the RND superfamily as the main mechanism of resistance to quinolones. The efflux pump RND pumps are a molecular complex consisting of three elements (**Figure 3**) [49, 74–77]:

1. In the inner membrane is RND pump protein.

2. An adapter protein from the MFP (membrane fusion protein) family located in the periplasmic space.

3. In the outer membrane is an outer membrane channel protein (OMP) belonging to the outer membrane factor (OMF) family.

The adapter protein MFP links the pump RND and the OMF protein [49, 74–77]. In *E. coli*, the presence of five RND efflux transporters has been reported:

1. AcrAB [78, 79]

2. AcrAD [80, 81]

3. AcrEF [82]

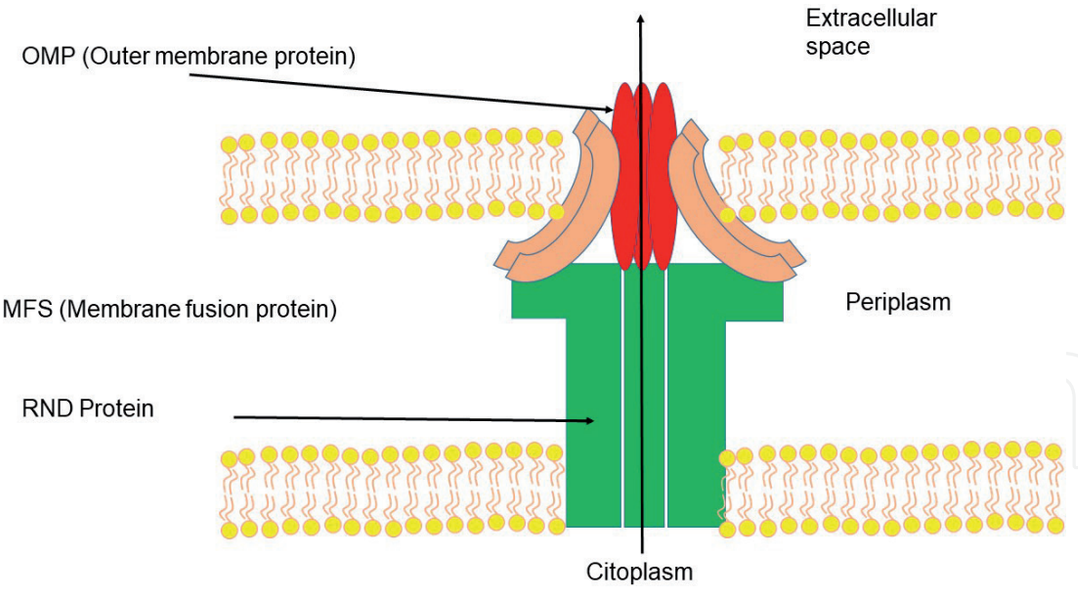

Figure 3.
Schematic representation of molecular structure of RND (resistance-nodulation-cell division superfamily). Based on Eun-Hae Kim et al. [77].

4. MdtABC [83, 84]

5. MdtEF [85, 86]

6.2.2.2 AcrAB-TolC [acriflavine (Acr) efflux system]

The AcrAB-TolC or acriflavine (Acr) efflux system consists of three elements [75, 87]:

1. The outer-membrane channel TolC

2. In the periplasmic space is the AcrA protein, which bridges these two integral membrane proteins

3. In the inner membrane is the secondary transporter AcrB.

There is evidence that the ratio between the proteins that make up this complex is 3: 6: 3, comprising an AcrB trimer, an AcrA hexamer, and a TolC trimer [75, 87].

It has been shown that various dyes can be accommodated in the transmembrane domain of the Acr efflux system, as well as doxorubicin, minocycline, and quinolone molecules [88, 89].

6.2.2.2.1 AcrAD

AcrAD is an antibiotic efflux pump complex of the RND type. It provides resistance to aminoglycosides such as amikacin, gentamicin, and tobramycin. There is no known effect on quinolone resistance [80, 90].

6.2.2.2.2 AcrEF

AcrEF is an antibiotic efflux pump complex of the resistance-nodulation-cell division (RND) type. It provides resistance to cephalosporins, cephamycins, fluoroquinolones, and penams [91, 92].

6.2.2.2.3 MdtABC

MdtABC is an antibiotic efflux pump complex of the resistance-nodulation-cell division (RND) type. It provides resistance to aminocoumarins, which have a mechanism of action similar to quinolones [93, 94].

6.2.2.2.4 MdtEF

MdtEF is an antibiotic efflux pump complex of the RND type. It provides resistance to fluoroquinolones, macrolides, and penams [82].

6.2.2.3 Other members of the RND (resistance-nodulation-cell division superfamily)

6.2.2.3.1 MexAB-OprM efflux system

MexAB-OprM efflux system is an antibiotic efflux pump complex of the RND type. It provides resistance to multiple antibiotics, including aminocoumarins, carbapenems, cephalosporins, cephamycins, diaminopyrimidines, fluoroquinolones, macrolides, monobactams, penams, phenicols, peptides, sulfonamides, and tetracyclines [95, 96].

6.2.2.3.2 MexCD-OprJ with type A NfxB mutation

MexCD-OprJ with type A NfxB mutation is an antibiotic efflux pump complex of the RND type. It provides resistance to the aminocoumarins, cephalosporins, diaminopyrimidines, fluoroquinolones, macrolides, penams, phenicols, and tetracyclines [97].

6.2.2.3.3 MexCD-OprJ with type B NfxB mutation

MexCD-OprJ with type B NfxB mutation is an antibiotic efflux pump complex of the RND type. It provides resistance to the aminocoumarins, aminoglycosides, cephalosporins, diaminopyrimidines, fluoroquinolones, macrolides, penams, phenicols, and tetracyclines [97].

6.2.2.3.4 MexEF-OprN

MexEF-OprN is an antibiotic efflux pump complex RND. It provides resistance to diaminopyrimidines, fluoroquinolones, and phenicols [98].

6.2.2.3.5 MexXY-OprM

MexXY-OprM is an antibiotic efflux pump complex RND. It provides resistance to the acridine dye, aminoglycosides, carbapenems, cephalosporins, cephamycins, fluoroquinolones, macrolides, penams, phenicols, and tetracyclines [96, 99, 100].

6.2.2.3.6 CmeABC

CmeABC is an antibiotic efflux pump complex RND. It provides resistance to cephalosporins, fluoroquinolones, fusidic acid, and macrolides [101, 102].

6.2.2.3.7 AdeIJK

AdeIJK is an antibiotic efflux pump complex RND. It provides resistance to carbapenems, cephalosporins, diaminopyrimidines, fluoroquinolones, lincosamides, macrolides, penems, phenicols, rifamycins, and tetracyclines [103].

6.2.2.3.8 AdeABC

AdeABC is an antibiotic efflux pump complex RND. It provides resistance to glycylcyclines and tetracyclines [104, 105].

6.2.2.3.9 AdeL

AdeL is an antibiotic efflux pump complex RND. It provides resistance to fluoroquinolones and tetracyclines [106].

6.2.2.3.10 SmeDEF

SmeDEF is an antibiotic efflux pump complex RND. It provides resistance to fluoroquinolones, macrolides, phenicols, and tetracyclines [107].

Other molecular complexes associated with decreasing the intracytoplasmic concentration of antibiotics in Gram-negative bacteria include:

6.2.2.4 Members of the MFS (major facilitator superfamily) in Gram-negative bacteria

6.2.2.4.1 EmrAB-TolC

EmrAB-TolC is an antibiotic efflux pump belonging to MFS. It provides resistance to fluoroquinolones [108].

6.2.2.4.2 MdfA

MdfA is an antibiotic efflux pump belonging to MFS. It provides resistance to benzalkonium chloride, fluoroquinolones, rhodamine, and tetracyclines [109, 110].

6.2.2.5 Other Gram-negative mechanisms

Other molecular complexes associated with decreasing the intracytoplasmic concentration of antibiotics in Gram-negative bacteria include:

6.2.2.5.1 Porin OprF

The OprF porin channel is permeable to quinolones and other antibiotics, promoting its outflow and decreasing intracytoplasmic concentration and consequently is a mechanism of antibiotic resistance for the bacteria [111, 112].

6.3 Plasmid-mediated quinolone resistance genes (plasmids that protect cells from the lethal effects of quinolones)

In 1998 at the University of Alabama, from the isolation of *Klebsiella pneumoniae* from a urine sample, Martinez et al. managed to identify a plasmid they named *pMG252*. They demonstrated that this plasmid induced bacterial resistance to

fluoroquinines and nalidixic acid. This resistance phenomenon could be induced in a variety of bacteria deficient in outer-membrane porins. They also described that this plasmid promoted the acceleration of resistance development and its propagation. The gene responsible for this resistance was called *qnr*, later it became *qnrA* [113, 114].

In 2002, Tran and Jacoby, working with the qnr plasmid, managed to identify an integron-like environment upstream from qacEΔ1 and sulI. The product obtained from this gene was a 218-aa protein called QnrA. This protein belonging to the pentapeptide repeat family shared sequence homology with the immunity protein McbG. Previous studies suggested that McbG protects DNA gyrase from the action of various genotoxic chemicals [115].

Based on the mechanism of action of quinolones (the inhibition of topoisomerases I and IV) and the similarity of QnrA to McbG, Tran and Jacoby determined the ability of QnrA to induce resistance against quinolones by topoisomerase protection [115].

In 2005, two independent teams managed to determine the same activity as QnrA for two other proteins identified as QnrB [116] and QnrS [117].

Subsequent studies of the qnrA plasmid found that this plasmid was able to promote greater resistance than expected and that is how, in 2006, Ari Robicsek et al. discovered another mechanism of action of resistance to quinolones mediated by the enzymatic action of aminoglycoside acetyltransferase, AAC(6′)-Ib-cr. They also reported that the quinolone resistance mechanism was determined by reduction of the activity of ciprofloxacin by N-acetylation at the amino nitrogen on its piperazinyl substituent [118].

In 2007, three groups of researchers separately demonstrated another resistance mechanism encoded by plasmids. These works, in correlation with Martinez's works, involve quinolone efflux pumps mediated by plasmids QepA [119, 120] and OqxAB [121].

In summary, there are three mechanisms for PMQR:

1. The plasmid genes *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS,* and *qnrVC* encode proteins from the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition. The *qnr* genes are generally associated with mobilizing or transposable elements in plasmids and are often incorporated into sul1-type integrons.

2. The second mechanism mediated by plasmids involves acetylation of quinolones with an appropriate amino nitrogen target by a variant of the common aminoglycoside acetyltransferase AAC(6′)-Ib-cr.

3. Improved outflow produced by plasmid genes for QepAB and OqxAB pumps.

7. Concluding remarks

Bacterial resistance to antibiotics is a serious problem worldwide and offers the bleakest outlook and prognosis. The number of reports of isolation of multiresistant strains is increasing, including antibiotics of the latest generation or exclusive intrahospital use. In this sense, isolates of strains resistant to practically all members of the quinolone family have been reported.

The implementation of appropriate practices in the use of antibiotics plays an important role in the fight against this serious global problem. The proper management of antibiotics must include limiting their use in the livestock, agricultural, and food industries; as well as the correct medical prescription, avoiding self-medication, and always seeking adherence to the full antibiotical treatment scheme.

The knowledge of the molecular mechanisms associated with resistance to quinolones and other antibiotics offers us great possibilities for molecular epidemiological monitoring of the emergence of new resistant strains, as well as their distribution. This knowledge offers the pharmaceutical industry the tools for the development of new drugs. It is important to consider that the development time of new drugs is exceeded by the speed of appearance of new resistant strains.

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