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Chapter 13

The Mechanisms of Action and Resistance to Fluoroquinolone in Helicobacter pylori Infection

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Additional information is available at the end of the chapter

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1. Introduction

The discovery of the *Helicobacter pylori* (*H. pylori*) infection and its role in various diseases (from chronic gastritis to gastric cancer) has been a radical change in the therapeutic conduct of patients suffering from this condition [1, 2].

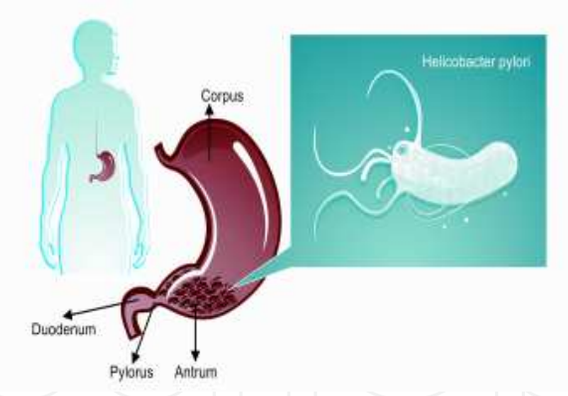

Figure 1. *Helicobacter pylori* infection

Unfortunately though, the purpose of inducing a cure of all first intention treated patients, as is the case in most ordinary infectious diseases, has not occurred in the *H. pylori* infection (Figure 1).

Current guidelines recommend triple therapy of a double dose proton pump inhibitor and two antibiotics chosen from among amoxicillin, clarithromycin and metronidazole for four to seven days, which is conducive to an eradication rate between 70-80%. Resistance to antibiotics, to

clarithromycin and metronidazole in particular, is a major factor contributing to failure of the therapy [3, 4]; resistance to clarithromycin may lead to a decrease in efficiency to 70%, whereas the infection may persist up to about 20% of patients under treatment [5]. Such limitations have resulted in development of alternative therapies - "rescue" therapies – using medicines which have not been used as a first or second intention, such as fluoroquinolones (moxifloxacin, levofloxacin) and rifabutin (a derivative of rifamycin), which are commonly used in treatment of mycobacterial infections and are also highly efficient against *H. pylori* [5, 6].

Quinolones have been the focus of considerable scientific and clinical interest, since their very development in the early 1960s. This happened because such drugs possess many of the attributes of an ideal antibiotic, combining high potency, a broad spectrum of activity, good bioavailability, oral and intravenous formulations, high serum levels, a large volume of distribution, indicating concentration on a tissue level and potential low incidence of adverse reactions.

Nalidixic acid was the first quinolone to be developed. Subsequent medicines have been derived by manipulation at the level of their side chain and nucleus [7]. Development of the fluoroquinolone class may be described in terms of generations, each generation sharing similar characteristics or antimicrobial spectrum. The activity of first-generation medicines is more effective against gram-negative aerobic bacteria and less effective against gram-positive aerobic bacteria or anaerobic bacteria. Original fluoroquinolones are second-generation agents and they owe their name to the addition of a fluorine atom in the C6 position; they provide improved coverage against gram-negative bacteria and moderately improved coverage against gram-positive ones. Third-generation agents have greater efficacy against gram-positive bacteria, particularly against pneumococci, which is combined with good activity against anaerobic bacteria. Fourth generation fluoroquinolones provide superior coverage against pneumococci and anaerobic bacteria [8].

2. Clinical indications of fluoroquinolones

Fluoroquinolones such as levofloxacin are indicated in: acute bacterial sinusitis (diagnosed according to national guidelines on treatment of infections of the respiratory tract and where use of antibacterial agents commonly recommended for initial treatment of this infection is considered inappropriate or in case of their failure to cure the infection), acute bacterial exacerbations of chronic bronchitis (diagnosed according to national guidelines on treatment of respiratory tract infections and where use of antibacterial agents commonly recommended for initial treatment of this infection is considered inappropriate or in case of their failure to cure the infection), community acquired pneumonia (where use of antibacterial agents commonly recommended for initial treatment of this infection is considered inappropriate), uncomplicated urinary tract infections (including pyelonephritis), chronic bacterial prostatitis and skin and soft tissue infections.

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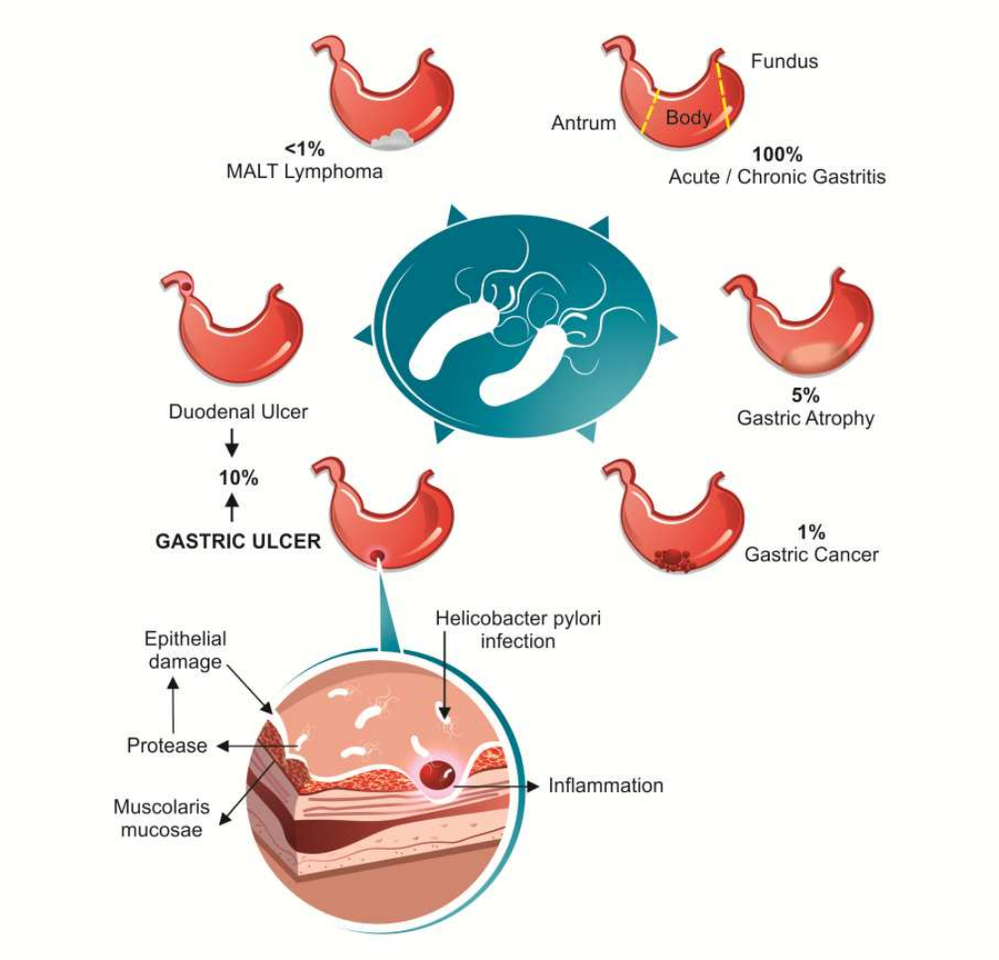

Figure 2. Diseases involving *Helicobacter pylori*

At the same time, as mentioned before, fluoroquinolones are implicated in *H. pylori* eradication. Indications for a *H. pylori* eradication therapy are defined in consensus guidelines. These indications include peptic ulcer disease, low-grade gastric mucosa-associated lymphoid tissue lymphoma, atrophic gastritis and after resection of early gastric cancer (Figure 2).

The following are included in the *H. pylori* elimination:

First-line treatment: should be considered as the first-line therapy in areas with high prevalence of *H. pylori* resistance to clarithromycin, in the frame of the new sequential ten-day scheme. Sequential therapy includes a dual therapy (proton pump inhibitor and amoxicillin for five days), followed by triple therapy with a proton pump inhibitor, clarithromycin and metronidazole for five days. The eradication rate achieved by sequential therapy has shown higher values, than those obtained with standard therapy [9, 10]. However, sequential therapy has been shown ineffective in eradicating *H. pylori* in patients with dual resistance to clarithromycin and metronidazole [11, 12]. A new aspect of the scheme as concomitant therapy is a fourth medicine – a non-bismuth-containing regimen (proton pump inhibitor, clarithromycin, amoxicillin and metronidazole), which seems more appropriate for patients in highly endemic

areas and with dual resistance. Clinically speaking, this is much easier than sequential therapy and can improve compliance, because medicines are administered concurrently, instead of changed in mid therapy An intention-to-treat analysis demonstrated that sequential or concomitant therapy with a PPI, amoxicillin, clarithromycin and an imidazole agent has similar rates for eradication of *H. pylori* infection [10]. As far as dual resistance is concerned, several attempts have been made such as extension of sequential therapy and continuation with amoxicillin therapy for all 14 days, in order to improve effectiveness of standard triple therapy and proton pump inhibitors. A hybrid sequential-concomitant therapy has been recently designed by Hsu et al. [13]. Data have shown promising success rate: 99% by per-protocol analysis and 97% by intention-to-treat analysis. However, it should be noted this may not be effective in all geographic areas and results will have to be confirmed in areas where different patterns of resistance are present.

Second-line therapy: bismuth is a component of quadruple therapy and/or rescue therapy recommended by the Maastricht IV/Florence Consensus Report [7]. Several multicentre studies regarding quadruple therapy using a single triple medicine (bismuth, metronidazole and tetracycline), in a capsule pharmaceutical formulation together with a proton pump inhibitor have shown good efficacy in *H. pylori* eradication [14-16]. With regard to adverse reactions, bismuth induced toxic effects are still one of the unnecessary safety concerns in relation to quadruple therapy [17]; therefore, a reasonable bismuth dose regimen needs to be established to ensure maximum eradication. In patients where first-line treatment has failed, e.g., clarithromycin-based triple therapy, levofloxacine-based triple therapy (levofloxacin, amoxicillin and a proton pump inhibitor), a meta-analysis has shown this therapy to be superior to quadruple therapy and be accompanied by fewer adverse reactions than rescue therapy [18]. In addition, the study has shown that antibiotics (e.g., levofloxacin) in this triple therapy should not be changed randomly and then switched to first-line treatment. As far as antibiotic resistance is concerned, growth rates of levofloxacin resistance, particularly in developing countries, are to be taken into account, since resistance to quinolones is related to the status of patients having used fluoroquinolones for other indications [19].

Third-line treatment: the third-line treatment standard for refractory *H. pylori* infection has not been established. The Maastricht IV/Florence Consensus Report recommends conducting anti-*H. pylori* therapy according to results of susceptibility testing after failure of second-line therapy, whenever possible [7]. Unfortunately, antimicrobial sensitivity data for patients in whom eradication therapy has failed are not yet widely available in clinical practice.

Practitioners need a few simple strategies for empirical management. A prospective study has assessed the efficacy and safety of levofloxacin, amoxicillin, bismuth and rabeprazole, as a quadruple therapy, with regard to third-line treatment in patients where eradication of *H. pylori* infection has failed. In this study, the ten-day quadruple rescue therapy, based on levofloxacin and amoxicillin, ensured better eradication with a significant additional clinical benefit involving improvement of tolerability due to fewer adverse reactions [18]. Other alternative agents that are candidates for third-line treatment are rifabutin and quinolones, which also have promising results [20-22], although the optimal dose and the combination require further study (Table 1).

Treatment			Regimens									Duration of treatment
			A	C	M	T	L	R	F	B	PPI	
First line therapy	Standard triple therapy		1g b.i.d.	0. 5g b.i.d.							SD b.i.d.	7-14 days
	Concomitant therapy		1g b.i.d.	0. 5g b.i.d.	0. 5g b.i.d.						SD b.i.d.	7-10 days
	Bismuth-containing quadruple therapy				0. 25g q.i.d.	0. 5g q.i.d.				0. 48g q.i.d.	SD b.i.d.	10-14 days
	Sequential therapy	First phase	1g b.i.d.								SD b.i.d.	5 days
		Second phase		0. 5g b.i.d.	0. 5g b.i.d.						SD b.i.d.	5 days
	Hybrid therapy	First phase	1g b.i.d								SD b.i.d.	7 days
		Second phase	1g b.i.d.	0. 5g b.i.d.	0. 5g b.i.d.						SD b.i.d.	7 days
Second line therapy	Bismuth-containing quadruple therapy				0. 5g t.i.d.	0. 5g q.i.d.				0. 48g q.i.d.	SD b.i.d.	10-14 days
	Levofloxacin-based triple therapy		0. 5g b.i.d.				0. 5g q.i.d.				SD b.i.d.	10 days
Third line therapy	Culture guided therapy		Sensitivity tests – two antibiotics selected							0. 48 q.i.d.	SD b.i.d.	NA
	Levofloxacin-based quadruple therapy		0. 5g q.i.d.				0. 5g q.i.d.			0. 48 q.i.d.	SD b.i.d.	10 days
	Rifabutin-based triple therapy		1g b.i.d.					0. 15g b.i.d.			SD b.i.d.	14 days
	Furazolidone-based quadruple therapy					1g b.i.d.			0. 2g b.i.d.	0. 24g b.i.d.	SD b.i.d.	NA

A = amoxicillin, C = clarithromycin, M = metronidazole, T = tetracycline, L = levofloxacin, R = rifabutin, F = furazolidone, SD = standard dose, B = bismuth, PPI = proton pump inhibitor

Table 1. Recommended regimens for the treatment of *H. pylori* infections in adults

3. Safety of fluoroquinolones

3.1. Overview

As a class of medicines, fluoroquinolones are generally well tolerated; most adverse reactions are mild, self-limiting and rarely require treatment discontinuation [23]. The most frequently occurring adverse reactions class consists of gastrointestinal trouble (nausea, vomiting,

diarrhoea, constipation and abdominal pain, about 7% of all adverse reactions). Less common are adverse reactions include those involving the central nervous system (less than 5%), kidney disorders (approximately 4. 5%), skin hypersensitivity reactions and photosensitivity reactions (approximately 2%). In rare cases, convulsions, psychosis and tendonitis have been reported [24]. Some of these events may not be directly attributed to fluoroquinolone therapy, but to other underlying conditions in the patient, including additional therapies not related to the microbial infection but still contributing to adverse reactions. Phototoxicity could be observed over a longer period in relation to administration of lomefloxacin, sparfloxacin and clinafloxacine, which has been determined to be a dose-dependent phenomenon requiring direct or indirect exposure to UVA rays and also closely correlated with the presence of a habide in the C8 position.

Serious adverse reactions have been developed following introduction to the use of the following three agents: temafloxacin, grepafloxacin and trovafloxacine.

Temafloxacin syndrome has been characterized by haemolytic anaemia, renal failure, hepatotoxicity, disseminated intravascular coagulation and hypoglycaemia [28]. Approximately two thirds of patients with "temafloxacin syndrome" develop acute renal failure. In addition, slight hepatobiliary changes have been observed in half of patients, whereas coagulopathy has been observed in a third of patients. Development of such adverse reactions resulted in withdrawal of temafloxacin from the market in 1992 [26]. Adverse reactions to temafloxacin not shown in the clinical trials conducted were observed at a rate of 1/3500 patients after placement on the market. In contrast, rare adverse reactions similar to those seen in temafloxacin have been reported with ciprofloxacin. This is significant, considering that ciprofloxacin has the largest database regarding fluoroquinolone safety information. Further analysis of such specific temafloxacin events have demonstrated that data involving norfloxacin and ofloxacin have been similar to those of ciprofloxacin. Specifically, such effects have been observed in 1/17000 patients treated with ciprofloxacin, in 1/25000 patients treated with norfloxacin and in 1/30000 patients treated with ofloxacin [25].

Grepafloxacin was placed on the market in 1997 and subsequently withdrawn in 1999 because of serious cardiovascular reports of adverse reactions in patients who had been administered this medicine [27]. The association between grepafloxacin and serious cardiovascular adverse reactions became evident after extended clinical use. Starting with grepafloxacin placement on the market to its withdrawal in 1999, torsades de pointes were observed in 7/3.7 million patients [27]. Trovafloxacin was authorized based on clinical efficacy trials conducted on batches including more than 6000 patients treated with this medicine. In these studies, 5% of patients discontinued trovafloxacin treatment because of adverse events, the most common of which included those on the central nervous system and gastrointestinal tract levels. As with temafloxacin, adverse reactions had not been clearly associated with use of the medicinal product before extended clinical use. Serious adverse reactions associated with trovafloxacin included liver eosinophilia and hypoglycaemia [29], observed post-marketing.

All the adverse reactions described above have resulted in limiting the use of these medicines to hospital use only, in cases of severe life-threatening infections [29]. Whereas withdrawal of temafloxacin and grepafloxacin from the market has raised concerns about the safety of

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fluoroquinolones, in considering the evaluation of their safety and efficacy, several aspects have required balancing. Despite rigorous preclinical studies, once a medicine is placed on the market for widespread clinical use, the likelihood of observing rare but serious adverse reactions is significantly increased [29]. It is important to recognize the low incidence of adverse reactions and serious adverse reactions in other agents of the same class, which generally demonstrates the relative safety of this class of medicines [23].

3.2. Categorization of adverse effects

Adverse reactions of levofloxacin consist of: disorders such as tachycardia, ventricular arrhythmia and torsades de pointes (reported predominantly in patients with risk factors for QT prolongation), ECG QT prolonged, leukopoenia, eosinophilia, thrombocytopenia, neutropoenia, dizziness, headaches, drowsiness, convulsion, tremors, paraesthesia, vertigo, impaired hearing, bronchospasm, dyspnoea, diarrhoea, nausea, vomiting, abdominal pain, dyspepsia, flatulence, constipation, increased blood creatinine, rashes, pruritis, urticaria, tendon disorders including tendinitis (e.g., in the Achilles tendon), arthralgia, myalgia, anorexia, fungal infections (and proliferation of other resistant microorganisms), hypotension, asthenia, increased hepatic enzymes (aspartate aminotransferase/alanine aminotransferas, alkaline phosphatise and gamma-glutamyl transpeptidase) and increased blood bilirubin [30].

Many of the fluoroquinolone adverse reactions are associated with quinolone pharmacore in positions one, seven and eight. In the following sections, the discussion focuses on class specific reactions regarding structural modifications in positions one, seven and eight [25].

Adverse reactions on the central nervous system level: although much information about the pathophysiology of fluoroquinolone induced central nervous system adverse reactions remain less well defined, one hypothesis suggests that drug interactions with the gamma-aminobutyric acid (GABA) receptor, an inhibitory neurotransmitter, may explain the stimulant effects at this level.

Affinity for the GABA receptor seems to be triggered by the R7 side chain substituent, unsubstituted piperazinyl and pyrrolidinyl moieties in particular. In this respect, agents with an unsubstituted piperazinyl ring (ciprofloxacin, enoxacine and norfloxacin) display high-affinity binding to GABA as well as interference with GABA binding to its receptor. It has been demonstrated that biphenyl acetic acid, which is an active metabolite of fenbufen, a nonsteroidal anti-inflammatory medicine, enhances binding of fluoroquinolones to GABA receptors.

Concurrent administration of fenbufen and fluoroquinolones has been shown to be capable of inducing seizures in mice; certain researchers however have observed that alterations in GABA receptor binding mediated by quinolone are weak and may not fully explain reactions at the central nervous system level [31]. It was noted that seven Japanese patients developed seizures following concurrent administration of enoxacine and fenbufen. Studies have also shown that penetration of quinolones at the CNS level does not seem to be correlated with the incidence of adverse reactions at this level [25]. A possible understanding of such discrepancies is that fluoroquinolones may also induce excitatory effects by means of direct activation of N-methyl-D-aspartate (NMDA) and adenosine-receptor mechanisms. It is therefore possible that the

events mentioned may occur at this level only under special circumstances, when sufficient penetration is possible in the CNS, coupled with threshold antagonism of inhibitory pathways (GABA) and stimulation of excitatory pathways (NMDA, adenosine).

Ofloxacin and levofloxacin, its levorotatory isomer, have been observed to induce a number of adverse reactions at the central nervous system level, including headaches (9% ofloxacin, 6% levofloxacin), dizziness (5% ofloxacin, 3% levofloxacin) and less common events such as confusion, impaired thinking, insomnia and psychosis, in rare cases. Such reactions were induced even in the absence of concomitant medications such as NSAIDs [25] and they tend to occur more frequently with ofloxacin than levofloxacin.

Genetic Toxicology: quinolones have been shown to inhibit mammalian cell topoisomerase II, a mechanism correlating with the occurrence of in vitro cytotoxicity, on the level of these cells [30]. Substitutions and positions one, seven and eight have the greatest potential cytotoxicity and the effect is additive. However, chromosomal disruption or clastogenicity usually occurs only at very high concentrations of the medicine (300 to 10000 times the therapeutic dose) and in post-marketing studies was not shown to have carcinogenic potential.

Cardiovascular adverse reactions: cardiovascular adverse reactions, particularly prolongation of the heart rate corrected QT interval (the QTc interval) have been reported with quinolone therapy [30]. Sparfloxacin increases the QTc interval in up to 3% of patients [32]. Such significant results involving serious cardiac events have led to the withdrawal of grepafloxacin. Sparfloxacin is not recommended for administration in patients with a history of QT prolongation or patients receiving concomitant therapy likely to increase the interval, induce bradycardia or cause torsades de pointes (e.g., class Ia and III antiarrhythmics, bepridil, cisapride, erythromycin, terfenadine or tricyclic antidepressants). It seems possible that this effect may be more predictable with medicines administered concurrently with quinolones inhibiting cytochrome P450-mediated metabolizing, because of drug accumulation. So far, no specific structural change has been associated with adverse cardiovascular outcomes, including those possibly affecting cytochrome P450-involving metabolism. Currently, the only possible specific structural changes that may be associated with increased incidence of serious cardiovascular events in relation to grepafloxacin or sparfloxacin therapy consist of a methyl or amino moiety in position C5 (of grepafloxacin, sparfloxacin, respectively) [32]. In light of experience acquired with sparfloxacin and grepafloxacin concerning adverse cardiovascular outcomes, more recent members of this class of medicines have been studied with particular focus on these reactions [30].

4. Basic antimicrobial activity

4.1. Pharmacokinetics

Fluoroquinolones have favourable pharmacokinetic properties, which have encouraged their extensive use. They are well absorbed and show good tissue penetration, which favours their use in many clinical syndromes. Whereas ciprofloxacin requires frequent administration, the long half-life of new generation fluoroquinolones allows use in daily single doses.

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Most fluoroquinolones are eliminated renally. Moxifloxacin elimination involves the liver and this is one of the fluoroquinolones lacking effectiveness in treatment of genitourinary infections. In general, because they are not as highly bound to plasma proteins and because of CYP1A2 enzyme-limited inhibition of CYP450, drug interactions are somewhat minimized [33-35]. Fluoroquinolones have been shown to interact with xanthines, theophylline and caffeine, which is matter of concern with older generation agents [36]. Concurrent use of fluoroquinolone and warfarin may result in excessive anticoagulation [37]. Probably the most common interactions involve cationic di- and trivalents. Administration with antacids may result in subtherapeutic fluoroquinolone levels, thus raising potential therapeutic failure. This may be particularly relevant in an "inpatient setting", because of the frequent fluoroquinolone and antacids association; co-administration of agents mentioned previously has raised the issue of emergence of resistance to fluoroquinolones [38, 39].

In terms of levofloxacin involved in *H. pylori* eradication therapy, oral administration is rapidly and almost completely followed by levofloxacin absorption, with a peak concentration achieved within one hour. Absolute bioavailability is approximately 100% and food has a low impact on levofloxacin absorption.

Approximately 30-40% of levofloxacin is bound to plasmatic proteins and a therapy involving a 500mg dose once daily for several days has shown non-significant accumulation. However, there is modest but predictable accumulation after therapy involving 500mg twice daily, with a balance reached within three days. Levofloxacin metabolism is very scarce and its metabolites are "desmethyl-levofloxacine" and "levofloxacin N-oxide". These metabolites contribute to less than 5% of the urine-excreted dose. Levofloxacin is stereochemically stable and undergoes no chiral inversion. Following oral or intravenous administration, levofloxacin is relatively slowly eliminated from plasma (t1/2 = 6-8 hours). The chief route of excretion is mainly renal (>85% of the dose administered). There are no major differences in levofloxacin pharmacokinetics in terms of oral versus intravenous administration, which leads to the conclusion that oral and intravenous administration are interchangeable.

4.2. Pharmacodynamics

Pharmacokinetics (PK) regards the time course of antimicrobial concentrations in the body; on the other hand, pharmacodyamics (PD) provides insight into the relationship between such concentrations and the effect on antimicrobial level.

Traditionally, doses of antibiotic therapeutic schemes only determined the pharmacokinetics; however, pharmacodynamics plays an equally, if not more important, role.

In this period of increased antimicrobial resistance, pharmacodynamics is becoming perhaps the most important because it can be used in the design of a dosage regimen to prevent resistance [40, 41].

The primary parameter of antibiotic activity is the minimum inhibitory concentration (MIC). MIC is the lowest concentration of an antibiotic able to completely inhibit in vitro microorganism growth. Whereas MIC is a reliable indicator of antibiotic potency, it has no relevance in terms of the time course of antimicrobial activity.

Pharmacokinetic parameters quantify the time course of an antibiotic level. The three pharmacokinetic parameters most important in evaluating effectiveness of the antibiotic are the peak serum level (Cmax), the minimum level (Cmin) and the area under the serum concentration time curve (AUC). Whereas these parameters quantify the serum level over a certain period of time, they describe the destruction activity of an antibiotic.

Integration of pharmacokinetic parameters and MIC provides three pharmacokinetic/pharmacodynamic (PK/PD) parameters quantifying the activity of an antibiotic: the peak/MIC ratio, the T>MIC and the ratio 24h-AUC/MIC

- the peak / MIC ratio is Cmax divided by MIC
- the T>MIC (time above MIC) is the percentage of a dosing interval when the serum level reaches MIC
- the 24h-AUC/MIC ratio is determined by dividing the 24-hour-AUC by MIC [40, 41].

The three pharmacodynamic properties of antibiotics best describing the destruction activity are time-dependent, concentration-dependent and the presence of persistent effects. The destruction rate is determined by either the time needed for destruction (time-dependent) or the effect of increased concentration (concentration-dependent). Persistent effects include post-antibiotic effect, which is the persistence of suppression of bacterial growth after exposure to antibiotics.

For fluoroquinolones, the best dosing regimen would maximize the concentration, as higher concentrations cause a superior and faster degree of destruction, therefore the "24h-AUC/MIC ratio" and the "peak/MIC ratio" are important predictors for antibiotic efficacy.

In this case, as far as fluoroquinolones are concerned, the "24h-AUC/MIC ratio" for Gram-negative bacteria (*H. pylori* included) is about 125 and 40, respectively, for Gram-positive bacteria. For Gram-negative bacteria, however, the "24h-AUC/MIC ratio" for the group of medicines described above, the reports vary widely in the literature [40, 41].

4.3. Mechanisms of action

The process of fluoroquinolone interference in cell replication, transcription and DNA repair consists in disabling DNA gyrase (previously topoisomerase II) and topoisomerase IV, two processes essential to bacterial enzymes (Figure 3).

Essentially a tetramer of two A and two B subunits, DNA gyrase is subject to encoding performed by gyrA and gyrB [42]. It brings in negative DNA supercoils, also removing both positive and negative supercoils and acts on chromosomal material by catenating and decatenating it [43].

The other bacterial enzyme mentioned above is topoisomerase IV, which is a homologue of DNA gyrase. This consists of two E and two E subunits, both subject to parC- and parE-encoding [42]. Similarly to its homologue, topoisomerase IV this is also able to remove both negative and positive supercoils; however, its main involvement remains contribution to separating of the daughter chromosome [33, 43] (Figure 4).

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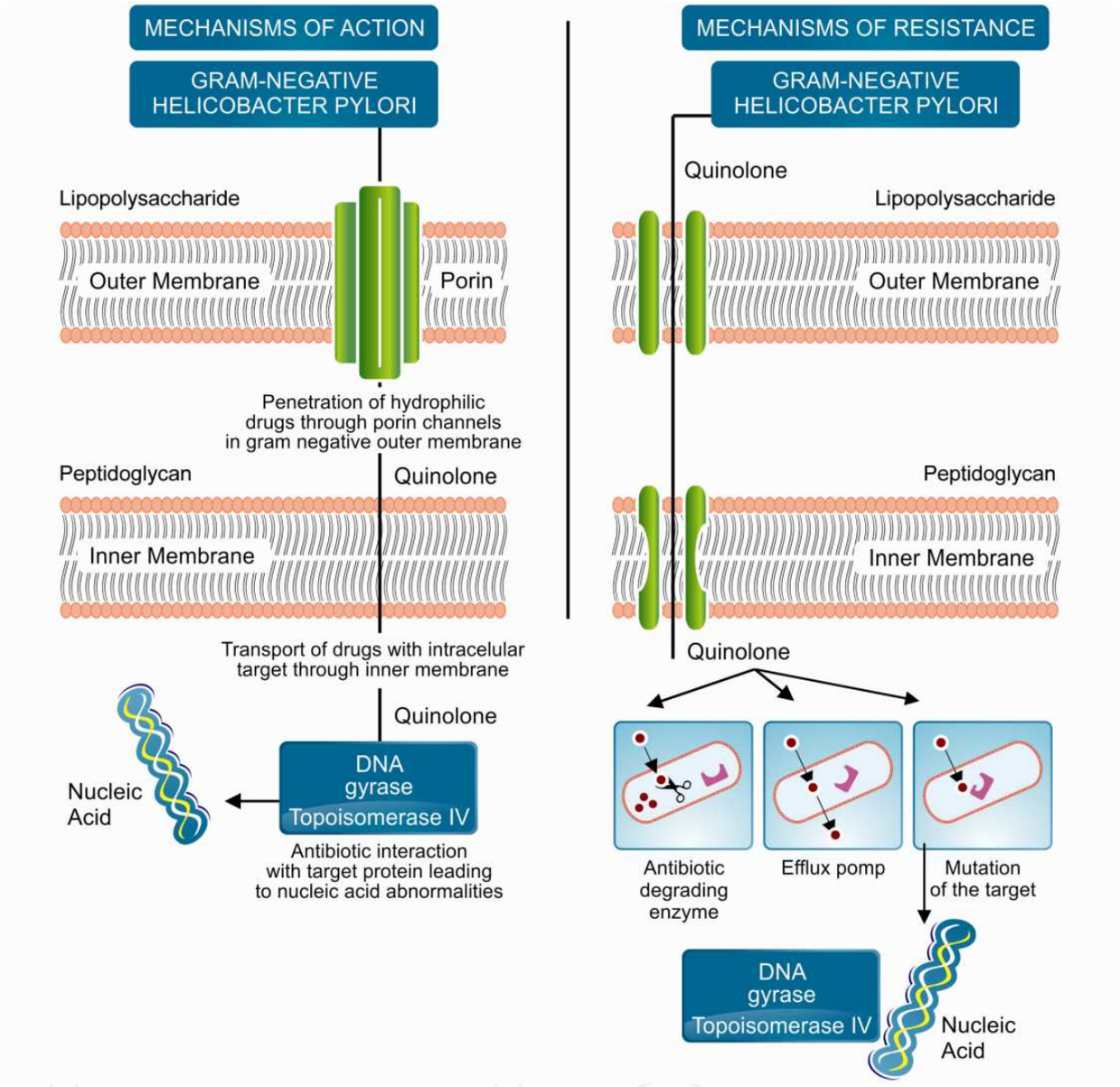

Figure 3. Mechanisms of action/Mechanisms of resistance

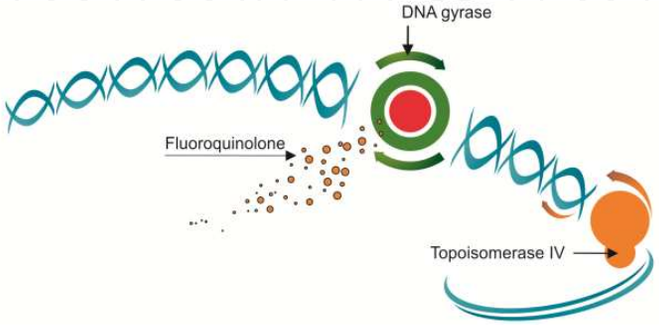

Figure 4. Fluoroquinolones bind the two nuclear enzymes, inhibiting DNA replication

The binding of fluoroquinolones to the complex made up of enzyme–DNA determines the change in enzyme conformation. This results in enzyme cleaving of the DNA, while ligation of broken DNA strands is prevented by the continued presence of the fluoroquinolone. Continuation of DNA replication is inhibited by the process in which the enzyme on the DNA is "trapped" by fluoroquinolone as a complex of fluoroquinolone–enzyme–DNA. DNA cleavage is generally held responsible for the destructive action of fluoroquinolones on bacteria [42, 43].

5. General mechanisms of fluoroquinolone resistance

Regarding quinolone resistance reported during treatment, it has been observed that, in the context of its rather extended use during the twenty years after its placement on the market, it was not commonly observed except for cases involving Staphylococcus aureus and notably P. aeruginosa and S. aureus, which were resistant to methicillin.

These two species have been observed to rapidly develop resistance to fluoroquinolones, which has been attributed to clonal spread occurring in immunocompromised patients and nursing home settings [44].

Starting around 1995 however, increased quinolone resistance has been noted in most Gram-negative (*H. pylori*) and Gram-positive species. At the same time, it has become evident that species were broadly varied (from ≤0.015 up to ≥128 mg/l [45-47]) by minimal concentrations (MICs) that inhibited 90% of the strains under study; this showed the common character of resistant subpopulations over the same period of time after placement on the market, which had however not been noticed.

In light of more recent studies involving surveillance of the issue, resistance rates have been shown to further increase and therefore have an impact on the management of patients.

5.1. Interaction with bacterial DNA gyrase and topoisomerase IV

From among antimicrobial agents in common clinical use, fluoroquinolones are the only class that directly inhibit synthesis of bacterial DNA. There are two bacterial enzymes with distinct and essential roles in DNA replication, called DNA gyrase and topoisomerase IV, which are inhibited by fluoroquinolones. The respective process involves binding quinolones to the complex with DNA of each such enzyme, which results in a ternary complex of topoisomerase-quinolone-DNA. Subsequently, this complex induces the generation of double-stranded breaks in DNA, further blocking progress of the DNA replication enzyme complex.

The final result of this action is damaged bacterial DNA and ultimately death of bacterial cells [48-51].

The responsibility for quinolones resistance lies with mutation in chromosomal genes. This occurs by means of two mechanisms: the altered target mechanism, on one hand, involving encoding DNA-gyrase and topoisomerase IV subunits and the altered permeation mechanism,

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on the other hand, regulating expression of proteins or cytoplasmic membrane efflux pumps, both of which make up the channels for outer membrane diffusion.

A further mechanism for the generation of low-level quinolone resistance has been considered, reduced target expression [52].

5.2. Response of the SOS gene network

Inhibition of bacterial type II topoisomerases activates repair mechanisms in response. This is because each piece of DNA damage activates an SOS gene network, which initiates the generation of repair proteins of various kinds [53-57]. There are over 40 genes making up this so-called SOS system, which is under the control of regulatory RecA and LexA proteins. The former is in charge of generating a signal triggering the SOS response, whereas the latter has a repressor function. The process consists of gene repressor LexA binding, which results in the unmasking of its autoproteolytic activity and subsequent ending of repression of the 40 SOS genes. The respective LexA binding takes place in the sequence up from qnrB (but not qnrS or qnrA). Therefore, in response to DNA damage, the SOS-system regulates qnrB as well [58]. Furthermore, the SOS response has recently been shown to promote qnrB expression [59].

Bacterial DNA-topoisomerases are protected from quinolone inhibition by the QnrB peptide, which also renders low-level resistance to quinolone. On the contrary, high-level resistance emerges with facilitation of the Qnr-determinants. It should also be noted that, in the case of *E. coli*, this particular effect directly depends on the increased ability for mutation due to action of nonessential polymerases Pol II, Pol IV and Pol V over the de-repression of the genes polB, dinB and umuDC, respectively, mediated by the LexA-cleavage.

In this way, the same signal of the SOS response triggers both increased mutation ability and qnrB-mediated quinolone resistance.

Because of the RecA/LexA-dependent manner in which ciprofloxacin upregulates the qnrB quinolone resistance gene, the development of quinolone resistance is integral to their action mode in bacteria harbouring qnrB.

LexA positive wild-type strains are much more liable to elicit ciprofloxacin resistant mutants than their mutant counterparts [60, 61]. The reverse is also true – prevention of LexA cleavage results in bacterial inability to develop fluoroquinolone resistance [60, 61].

The ability of the SOS response to induce persistent fluoroquinolone should also be mentioned [62].

From the above outcomes inference may occur in the role of fluoroquinolones as more than simply selectors of resistant variants, as well as on the active role of bacteria themselves in their own genomes' mutation.

Resistance to quinolone is acquired by means of both mutations in the target site and by the SOS system induced de-repression of genes, whose products increase rates of mutation.

The emergence of resistance may generally be reduced by interference with the response of bacterial stress [63].

In *E. coli*, ciprofloxacin has recently been shown to stimulate recombination of divergent DNA sequences that is independent from the SOS system.

Genetic variation may also be increased by fluoroquinolones by means of a second mechanism, which is SOS independent [64] and may also favour acquisition, evolution and the spread of resistance determinants.

Besides quinolones as DNA damaging agents, the SOS gene network response is triggered by other factors as well, such as beta-lactams interfering with penicillin binding protein 3 [65, 66], zidovudine or trimethoprim [67] and rifampicin [60].

As shown by these data, persistence and evolution of resistance in general is facilitated by induction of the SOS response by means of any of these medicines classes.

Given the above, speculation becomes possible concerning the ability of these agents to also affect quinolone activity and/or development of resistance via the expression of qnrB as promoted by the SOS system.

In turn, the SOS system further promotes horizontal dissemination of antibiotic-resistance genes [68] or mutations, thus contributing to the spread of antibiotic resistance.

5.3. Plasmid mediated resistance

Usually, genetic information for the efflux resistance mechanisms or the targeted site is chromosomally encoded.

In this context however, there have been reports of resistance to fluoroquinolones mediated by plasmids, which renders such resistance transferable (Figure 5).

This involves a number of mechanisms, such as:

1. Qnr
2. Aminoglycoside acetyltransferase AAC(6_)-Ib-cr
3. OqxAB, QepA [69-72]

Plasmid-mediated resistance to quinolone was first found in 1998, emerging in strains of Klebsiella pneumoniae in one US region [73]. The emergence was determined to be induced by one of the members of the pentapeptide repeat (PPR) family of proteins, Qnr (subsequently referred to as QnrA).

Later on, several related plasmid-mediated Qnr determinants were communicated in Enterobacteriaceae (QnrB, QnrC, Qnr D, QnrS) that were distantly related [74, 75].

These have been further found in the entire world and almost invariably in association with expanded spectrum β-lactamases production [76-78].

Gram-positive bacteria M. smegmatis Mycobacterium tuberculosis, M. avium [79], E. faecalis [80], C. perfringens, Listeria monocytogenes, C. difficile and E. faecium [81] have been found to display Qnr-like peptides (that share a 16 to 22% amino-acid identity with QnrA).

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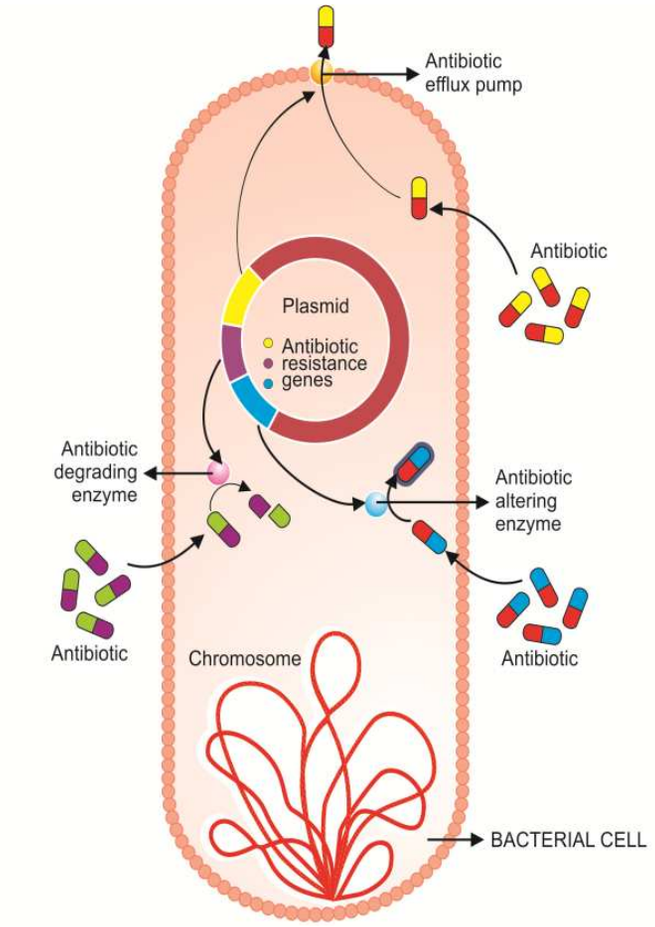

Figure 5. Plasmid mediated resistance

Stenotrophomonas maltophilia has recently shown the emergence of a new quinolone resistance gene of the PPR family that was chromosomally encoded, which has accordingly been called Smqnr [82]. Serratia marcescens has never been found to display the smaqnr gene[83].

Quinolone inhibition is prevented by the interaction of topoisomerase IV and DNA-gyrase with Qnr [69, 84].

The Qnr protein induces resistance to nalidixic acid and diminished susceptibility to fluoro-quinolone resistance or low-levels of fluoroquinolone resistance [84].

Identification of Qnr-genes in isolates susceptible to ciprofloxacin and isolates displaying resistance to quinolones has made it possible to infer from laboratory results that because of chromosomal mutation, presence of Qnr-genes promotes higher-level resistance.

That is why there exists the possibility that development of quinolone resistance in clinically relevant species of both Gram-negative and Gram-positive bacteria can be fostered by presence of qnr genes.

In addition, the qnrA and qnrB genes usually make up integrons containing genes such as aminoglycoside inactivating enzymes or β-lactamases, which are responsible for resistance to other antibiotics.

Integrons do not contain qnrS genes, but these genes however associate with TEM-1 type β-lactamases-containing transposons [85].

As a result, the association of genes that encode resistance to both quinolone and other medicine classes like aminoglycosides and β-lactams are favourable to selection and subsequent dissemination by chemically unrelated medicines classes of strains that are resistant to fluoroquinolones.

The reverse is also found concerning fluoroquinolones selecting and disseminating aminoglycoside or β-lactam resistant strains (please see the sections related to resistance to fluoroquinolones for issues regarding the tight correlation between and quinolone resistance and production of extended spectrum β-lactamases (ESBL)).

The chromosome of Shewanella algae, a bacterium found in environmental water has also been found to display Qnr genes.

The discovery of other qnr homologs in the genome sequences characterizing several Photobacterium profundum and Vibrio spp. suggests the possibility of water-borne Vibrionaceae as a source of qnr genes and also a reservoir [86-88].

Recent in vitro tests have shown the possibility for transfer of the plasmid borne Shewanella algae qnr gene to Enterobacteriaceae [86].

A further discovery envisages a plasmid-encoded determinant of resistance to quinolones, a variant of the aac(6_)Ib gene that encodes an aminoglycoside acetyltransferase.

The process of acetylation of both medicine classes is catalysed by AAC(6_)-Ib-cr, the bifunctional fluoroquinolone and aminoglycoside active variant [89].

This variant enzyme has become able to acetylate norfloxacin and ciprofloxacin as well as to determine a fourfold reduction of ciprofloxacin activity [90, 91].

Because of the absence in position C-7 of a piperazinyl substituent, levofloxacin and moxifloxacin do not undergo acetylation.

Interestingly, S. marcescens, the first clinical isolate determined as ciprofloxacin resistant, was found prior to the introduction of quinolone treatment in a patient treated with an aminoglycoside and a β-lactam. In that context, ciprofloxacin MIC during pre-therapy was 0.06, whereas the respective post-therapy MIC was 4mg/L.

The strain in question underwent changes in the composition of its outer-membrane and produced an aminoglycoside acetyltransferase [92].

It is possible that Qnr- determinants are less widespread than AAC(6_)-Ib-cr.

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ESBL production is associated with the production of both AAC(6_)-Ib-cr- and Qnr-, which may therefore be considered a second mechanism for co-selection of drug-resistance induced by exposure to agents that are chemically unrelated.

A third type of plasmid-mediated resistance to quinolones has been recently identified, consisting of the quinolone efflux pumps Qep and OqxAB, [70-72, 93, 94].

The QepA and OqxAB proteins are responsible for the induction of resistance to hydrophilic fluoroquinolones such as ciprofloxacin, norfloxacin and enrofloxacin, leading to a 32- to 64-fold MIC increase [93-96].

As far as QepA is concerned, in addition to quinolones, this extrudes a restricted range of agents such as ethidium bromide, erythromycin and acrifliavine.

In turn, OqxAB is responsible for the export of a wider range of agents among which are tetracyclines, chloramphenicol, ethidium bromide, olaquindox, trimethoprim and disinfectants such as triclosan [85, 96, 97].

However, the issue here is the presence of a transposable element also consisting of an aminoglycoside ribosome methyltransferase and the qepA gene [94]. This allows for the possibility for both aminoglycosides to select QepA determinants and for quinolones to select aminoglycoside resistance, which is also true in what concerns aac(6_)Ib gene mediated resistances.

A third mechanism for cross-resistance consists of extrusion by efflux-pumps of chemically unrelated agents.

To conclude, it appears that, even in the absence of exposure to this medicine, class resistance to fluoroquinolones can emerge. This can be explained by the action of several co-selection mechanisms, which all support emergence of quinolone resistance.

Identification in 50–70% of *E. coli* clinical isolates displaying high-level quinolone resistance (MICs up to 1500-fold higher than expected) of the multidrug efflux pump AcrAB, as well as of known plasmid- and chromosomally- mediated resistance mechanisms, makes it reasonable to infer the existence of additional mechanisms inducing quinolone resistance that have yet to be discovered

5.4. Other resistance mechanisms

In order to reach its target, all antibacterial agents that interact with an intracellular target have to cross the bacterial cell wall and then the cytoplasmic membrane. The process continues with active efflux of most antibacterial agents taken up.

This explains why permeation barriers and efflux pumps affect fluoroquinolones as well, whether accompanied by target modifications or just by themselves.

As indicated earlier, there are many, many Gram- positive and Gram-negative mutant strains resistant to fluoroquinolone, which did not display mutation in the region determining quinolone resistance (QRDR).

For instance, absence of classical QRDR mutations was observed in 70% of *E. coli* mutant variants recovered from besifloxacin selection plates [99]. At the same time, 39% of wild type *E. coli* accumulated higher levels of ciprofloxacin than high-level ciprofloxacine-resistant isolates. To this, one must add the gyrA mutations detected in all [100].

In addition, fluoroquinolone susceptibilities of *E. coli* were also affected by chemically unrelated substances such as salicylate, tetracycline and cyclohexane.

In this respect, it was determined that 21 of 57 clinical isolates of *E. coli* showing high level fluoroquinolone-resistance displayed tolerance to cyclohexane, which suggests a presence of elevated broad spectrum efflux activity [101]. Efflux of a wide range of chemically unrelated compounds, among which are different medicine classes of antibacterials, is determined by the so-called mar (multiple antibiotic resistance) genes [102], which suffer the influence of an assortment of chemically unrelated substances.

The role of the mar genes is the regulation of accumulation of quinolones and thus their intracellular concentrations, which is achieved by changing the expression of efflux pumps and porins [100, 102].

To this, one must add the extrusion of quinolones out of the bacteria by AcrAB, a different efflux pump.

The mar gene exerts partial control over the pump, which seems the most important mechanism of resistance for mar mutant variants [103].

Salicylate stimulates fluoroquinolone resistance selection because the production of MarA, a positive regulator of acrAB transcription, is induced by salicylate and tetracycline.

Resistance is visible in either mar expression alone or if combined with type II topoisomerase mutations [102]. The combination of topoisomerase mutations with AcrAB over-expression results in high-level resistance to fluoroquinolone. In this respect, increased production of AcrA has been noted in over 60% of high-level ciprofloxacine-resistant isolates [104-106].

Patterns of quinolone resistance may be altered by further nontopoisomerase resistance mechanisms, over which the mar exerts no control. The quinolone entry into the cell is decreased because of the nfxB gene action to code for a modified outer cell membrane protein F [107].

Fluoroquinolone activity is further affected by the action of soxRS gene products involved in bacterial adaptation to superoxide stress [101].

Fluoroquinolone-resistant *E. coli*, other Enterobacteriaceae and nonfermenters display a relatively wide range of diminished antibiotic accumulation, efflux and target enzyme modification [100, 108].

Because of their limited substrate specificity, increased expression of efflux pumps is associated with cross-resistance between fluoroquinolones and antibacterials of chemically unrelated medicine classes. This is the case of, for instance, MexAB, which induces resistance to nonfluorinated and fluoroquinolones, chloramphenicol and tetracycline in MexCD, rendering

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resistance to fluoroquinolones, trimethoprim, triclosan and erythromycin in MexEF and providing resistance to triclosan, imipenem, chloramphenicol and triclosan in MexXY, which gives resistance to fluoroquinolones, aminoglycosides and erythromycin.

There are a number of reviews available, which provide a comprehensive view on the impact of fluoroquinolone resistance and extrusion [108-111].

A fourth type of cross-resistance can be represented by the selection of a fluoroquinolone resistant or even multidrug-resistant phenotype by exposure to a broad range of chemically unrelated drug classes. All the above are illustrations which underline the complex character of mechanisms inducing resistance to fluoroquinolone, selection by fluoroquinolones and co-selection of resistance by chemically unrelated classes of antibacterials and antiseptics.

All general mechanisms of fluoroquinolone resistance have been presented for an overview of the issue. Regarding fluoroquinolone resistance in the case of *H. pylori* infection, this is due mainly (99%) to mutations in the QRDR of gyrA (Figure 3).

Antibiotic bacterial resistance is a result of the inhibition of binding between the enzyme and the antibiotic, determined by point mutations in QRDR of gyrA. In various studies, the following *H. pylori* loci have been found to be involved: (1) position 88 (Ala88Val), (2) position 91 (Asp91Gly, Asn, Ala, or Tyr) and (3) position 87 (Asn87Lys). In 100% of levofloxacin resistant isolates there have been observed mutations in both position 91 and 87. In addition, a new mutation has been identified, which consists of Tyr substituting Asn in position 87. Position 86 (Asp86Asn) is involved in infrequent mutations; the same position usually associates with mutations at positions 87 and 91, which diminishes its role in MIC values. In a similar manner, it is most likely that gyrB constantly associating with gyrA 87-91 mutations reduce to a minimum the role gyrB mutations hold in emergence of quinolone resistance. Actually, the involvement of gyrA and gyrB gene mutations has been observed in levofloxacin resistance as 83.8% and 4.4%, respectively.

There are also other factors that are involved in levofloxacin resistance, such as occurrence in codon 87 of gyrA of an amino acidic polymorphism, which consists of the presence of various asparagine-threonine residues. Specifically, presence of threonine in the J99 strain and asparagine residues in the 26695 strain associated with a higher antibiotic susceptibility has been identified due to the complete sequencing genome of two strains, namely the J99 and the 26695. Other Helicobacter types interestingly preserve the presence of threonine residue in codon 87, which therefore indicates the likelihood of the occurrence of a "philogenic" type evolution of the Helicobacter species.

6. Clinical and social implications of fluoroquinolone resistance

The increased incidence of fluoroquinolone resistance is a major reason for concern in medicine. Identification of and subsequent familiarisation with plasmid-mediated quinolone resistance (PMQR) has revealed a new and more dangerous mechanism of resistance allowing bacteria to adapt to and survive therapeutic concentrations of fluoroquinolones. As mentioned

above, PMQR only provides low-level resistance, not enough to enable classification as clear resistance (MIC ≥4g/ml), according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria for quinolone resistance. With these low MICs, such isolates, although transporting mutations conferring low sensitivity to quinolones, are to be classified as sensitive (MIC ≥1g/ml), meaning that physicians can further prescribe this class of medicines.

This in itself is a dilemma, because PMQR allows such "sensitive" organisms to survive even under therapeutic concentrations, easily circulating their genes afterwards. Continued exposure to these antibiotics determines high selection for plasmid-carrying pathogens, then rapidly conducing to general development of high-level clinically significant degrees of resistance. It has been shown that PMRQ-conferred low resistance levels can still remain undetected by current CLSI criteria and therefore are still conducive to failure of therapy. This is reason for concern with regard to the safety of prescribing fluoroquinolones for treatment of PMRQ gene-bearing organisms, even if they do not qualify as "resistant".

In such cases, the problem arises whether clinical breakpoints should be reviewed with regard to plasmid-carrying pathogens.

There is a strong association between fluoroquinolone resistance and resistance to other antibiotics, particularly wide spectrum β-lactamases and aminoglycosides. This indicates that gene-carrying plasmid organisms conferring quinolone resistance increase the likelihood of developing multi-drug resistant bacteria and prescription of a quinolone may be selective of not only quinolone resistance but also resistance to other classes of medicines.

The discovery of plasmid-mediated resistance genes in some non-Typhi serotypes of Salmonella enterica in animals has raised a major public health concern. The presence of such resistance genes from plasmid-mediated resistance genes in some non-Typhi serotypes of Salmonella enterica suggests an unsettling potential for horizontal transmission of resistance genes among animals and of infection-causing human pathogens, by means of food. Fluoroquinolone resistance prolongs hospitalisation and may further determine complications because of the selected therapy. The following can be mentioned among strategies implemented in some geographic areas: prohibited use as animal food and restricted use of fluoroquinolones in agriculture and their use for therapeutic purposes only, development of programmes for antibiotics management in hospitals (by drug rotation, cycling and restriction) as well as carrying out educational campaigns addressing physicians and patients, whose aim should be to increase awareness of inappropriate antibiotics.

The current breakpoints allow continuation of the quinolone treatment, whereas the organisms carrying these plasmid-mediated resistance genes remain undetected, which results in further dissemination of these plasmids, because of the selection pressure. The aim is a review of CLSI quinolone and fluoroquinolone breakpoints, against the background of the new mechanism (PMQR). Lower clinical breakpoints will help physicians to detect the low-level-resistance phenotype as rendered by such genes as well as avoidance of resumed prescription of quinolones as a treatment.

Identification of the PMQR mechanism is indicative of an increased risk of spreading resistance not only to fluoroquinolones but also, because of co-transmission, to other significant antimi-

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crobial classes. Tackling this issue by judicious use of antibacterials and re-evaluation of clinical breakpoints will constitute an important step in preserving the efficiency of this important class of medicines.

Concerning the *H. pylori* infection, an encouraging strategy to approach cases of multiple failures in prior *H. pylori* eradication is quinolone-based treatment as a rescue therapy. According to European guidelines, before selecting a third-line treatment, which is based on microbial sensitivity to antibiotics, culture is recommended. Quinolones for third-line therapy should be selected based on results of drug susceptibility tests or analysis of gyrA.

If available, further alternatives have also been suggested for rescue therapy, consisting of furazolidone-based therapy, triple rifabutin-based therapy or high-dose amoxicillin/PPI therapy.

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