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Antimicrobials that affect the synthesis and conformation of nucleic acids

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Summary

Several antimicrobials act by inhibiting the synthesis of nucleic acids (rifamycins, sulfamides, diaminopyridines), modifying their conformation (quinolones, coumarins) or causing irreversible lesions (nitroimidazoles, nitrofurans). The resistance mechanisms are: a reduction in intracytoplasmic accumulation, modification of the target or the production of a new low-affinity target and, more rarely, enzyme inactivation. Although the mechanisms affecting the targets are specific to each family and can lead to high-level resistance, the reduced permeability of the membrane and the increased efflux are non-specific and result in low-level cross-resistance between several families. The genetic mediation is usually chromosomal for rifamycins and quinolones, although plasmid-mediated resistant genes have been observed. On the other hand, for sulfamides and trimethoprim, plasmid-borne genes are frequent. Resistance to nitroimidazoles and nitrofurans is still not widely understood.

Keywords

Diaminopyridines – Imidazoles – Nitrofurans – Quinolones – Resistance – Rifampicin – Sulfamides.

Introduction

Several families of antimicrobials act by inhibiting the synthesis of nucleic acids (rifamycins, sulfamides, diaminopyridines), modifying their conformation (quinolones, coumarins) or causing irreversible lesions (nitroimidazoles, nitrofurans). Some are antibiotics in the strictest sense of the word because they are produced naturally and others are synthetic antimicrobials.

They can be classified according to the different stages in the synthesis of nucleic acids:

– synthesis of puric and pyrimidic nucleotides for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) inhibited by sulfamides and diaminopyridines

– replication of DNA inhibited by quinolones and coumarins, and transcription of DNA into RNA inhibited by rifamycins.

Finally, certain antimicrobials directly destroy nucleic acids, such as nitrofurans and nitroimidazoles. The principal molecules are shown in Figure 1. The resistance mechanisms and their genetic mediation are summarised in Table I.

Inhibition of the synthesis of nucleic bases

Two main families inhibit the synthesis of purine and pyrimidine nucleotides by inhibiting the synthesis of folic acid: sulfamides and diaminopyridines (Fig. 2). These are synthetic molecules.

Sulfamides

Sulfamides were the first antimicrobials, discovered in 1935 by G. Domagk (Nobel Prize, 1939).

The sulfamides (sulfamethoxazole, sulfadiazine, sulfamidine, sulfadoxine, sulfadimerazine, sulfachlor-

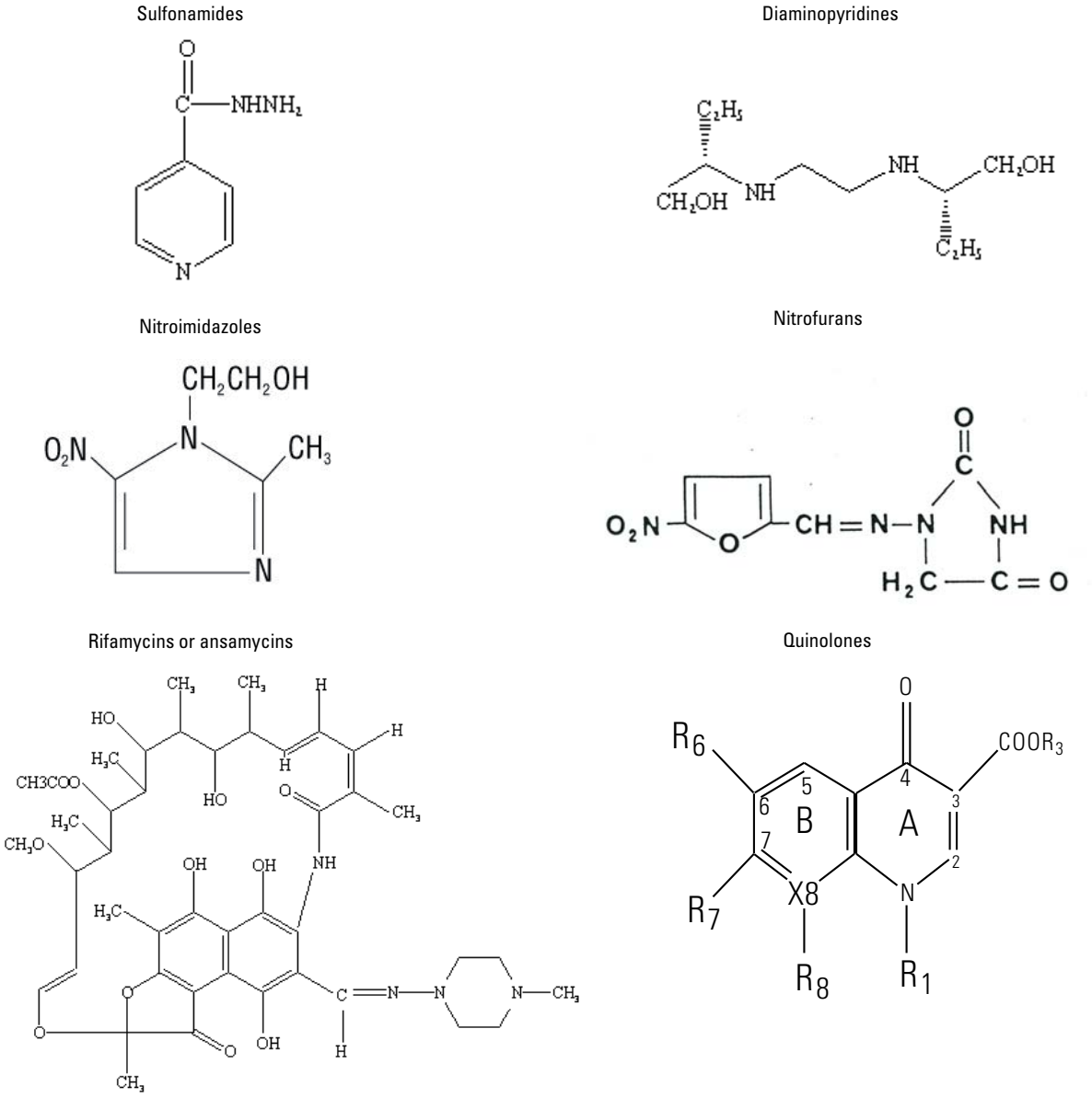


Fig. 1
Structure of the main antimicrobials acting on the synthesis or conformation of nucleic acids

pyridazine, etc.) and the sulfones (dapsone) inhibit dihydropteroate synthase (DHPS). This enzyme enables bacteria to synthesise dihydrofolic acid from para-aminobenzoic acid (PABA) and dihydropteridine. Sulfamides are structurally similar to PABA and act by competing with the PABA (14, 18) (Fig. 2). In eukaryotic cells, the folic acid is captured directly in the external environment and therefore the sulfamides are not active.

Sulfamides are broad-spectrum antimicrobials that act on Gram-positive bacteria (including streptococcus, staphylococcus, corynebacterium, *Listeria*, *Nocardia* and mycobacteria) and Gram-negative bacteria (enterobacteria, *Neisseria*). Sulfamides also act on more complex cells such as fungi (*Pneumocystis jirovecii*) and parasites (*Isospora belli*, toxoplasma and *Plasmodium*).

The mechanisms of resistance to sulfamides are (1):

– reduction in the intra-bacterial concentration of sulfamides: this stems from either a reduction in the permeability of the Gram-negative bacterial cell wall by reducing the quantity of porines (22), or hyper-expression of the resistance, nodulation and cell division (RND) efflux pumps (23). These mechanisms are not specific to sulfamides because they also concern the efflux of other molecules with a low molecular weight, including hydrophilics such as beta-lactamines, aminoglycosides and quinolones. Mutations in the genes that regulate the synthesis of OmpF (Mar operon) or expression of the efflux pumps (Acr operon) are traditionally observed in enterobacteria (11, 22, 23). Such resistance is chromosome-mediated;

Table I
Gene-mediated resistance to antimicrobials acting on the synthesis and conformation of nucleic acids

Antibiotics	Chromosome-mediated resistance (gene)	Plasmid-mediated resistance (gene)
Sulfamides	Reduction in the permeability of the cell wall of Gram-negative bacteria (e.g. OmpF-) Hyperexpression of RND efflux pumps (e.g. Acr) Structural modification of the target DHPS (*folP1*) Hyperproduction of DHPS	Production of DHPS enzymes resistant to the action of sulfamides (*sul1, sul2, sul3*)
Diaminopyridines	Reduction in the permeability of the cell wall of Gram-negative bacteria (e.g. OmpF-) Hyperexpression of RND efflux pumps (e.g. Acr) Structural modifications of the DHFR (dhfr) Hyperproduction of DHFR	Production of DHFR enzymes resistant to the action of trimethoprim (*dhfr1* and *dhfr2*)
Quinolones	Mutations in structural genes of type II topoisomerases, (*gyrA, parC, gyrB* or *parE*)	Protection of the target by the Qnr proteins (*qnrA, qnrB, qnrC, qnrD, qnrS*)
	Reduction in the permeability of the cell wall of Gram-negative bacteria (e.g. OmpF-) Hyperexpression of efflux RND pumps (e.g. Acr) in Gram-negative bacteria and MPS pumps in Gram-positive bacteria (e.g. NorA, Pmr)	Enzyme inactivation (*aac-6'-Ib-cr*) Efflux pumps (*qepA oqxAB*)
Coumarins	Mutations in sub-unit B of the DNA gyrase (*gyrB*)	
Rifamycins	Modification of the target by *rpoB* mutations Hyperefflux or impermeability	Inactivation by ADP ribosylase (*arr-3*)
5-Nitroimidazoles	Reduction in intra-bacterial accumulation by reducing the influx or efflux	Reduction in nitroreductase activity (*nimA, nimB*)
Nitrofurans	Reduction in intra-bacterial accumulation by reducing the influx or efflux Reduction in nitro-reductase activity (mutations of *nfsA* and *nfsB*)	

DHPS: dihydropteroate synthase
DHFR: dihydrofolate reductase

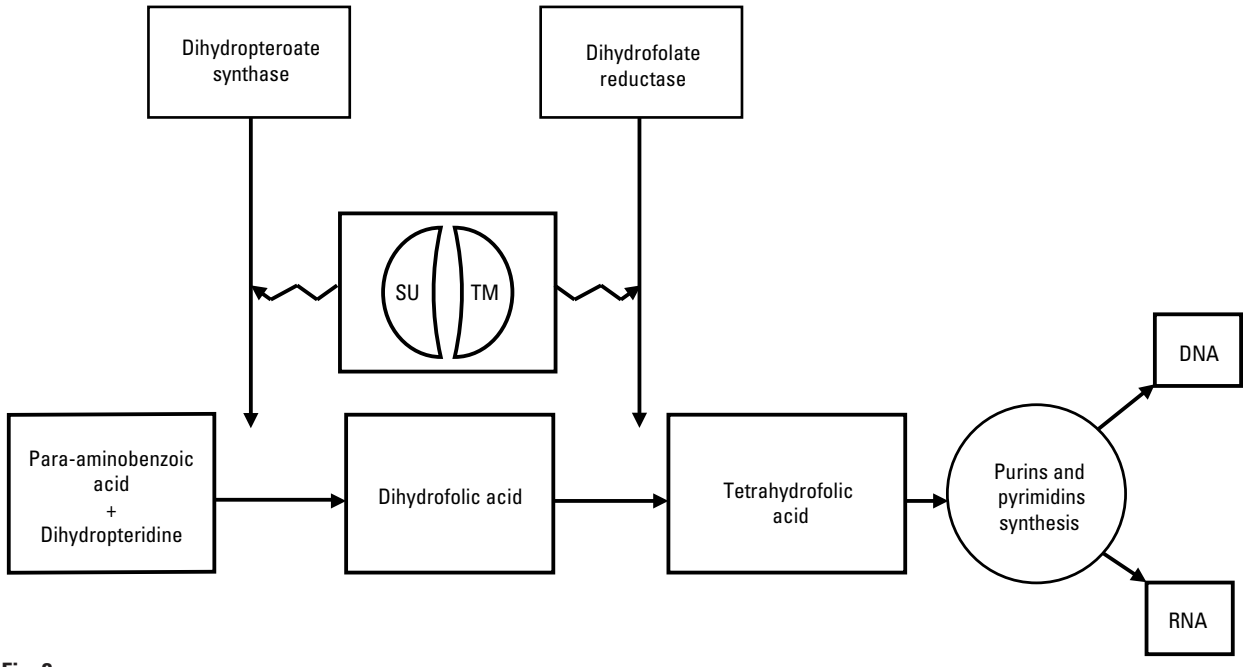


Fig. 2
Diagram of the mechanism of action of sulfamides and diaminopyridines

– structural modification of the DHPS: these modifications are secondary to structural mutations of the chromosomal gene. For example, in strains of *Mycobacterium leprae* resistant to dapsone (5) there are *folp1* mutations in positions 53 (Thr53Ala, Thr53Ile) and 55 (Pro55Leu). In *Neisseria meningitidis* (9) or *Streptococcus pneumoniae* (27), insertions of several nucleotides are found in the chromosomal gene forming a sort of mosaic gene, resulting from recombination with genes from other bacteria;

– production of DHPS enzymes resistant to sulfamides: these enzymes are coded by genes (*sul1*, *sul2*, *sul3*) acquired from other cells and transferred via transposons or integron genetic structures (14). The gene *sul1* characterises class 1 integrons within transposons often carried by plasmids with resistance to multiple antibiotics (multidrug-resistant [MDR] plasmids) (10);

– hyperproduction of DHPS.

Diaminopyridines

Diaminopyridines are derived from antiparasitic molecules. Trimethoprim is used mainly as an antibacterial, while pyrimethamine is used as an antiparasitic.

Diaminopyridines act by inhibiting dihydrofolate reductase (DHFR). This enzyme is used to synthesise tetrahydrofolic acid from dihydrofolic acid. Diaminopyridines therefore act in synergy with sulfamides, but at a later stage, by inhibiting the synthesis of folic acid (Fig. 2) (1).

Trimethoprim is a broad-spectrum antimicrobial that acts on Gram-positive bacteria (including streptococcus and enterococcus, staphylococcus, corynebacterium, *Listeria*, *Nocardia*) and Gram-negative bacteria (enterobacteria). However, some Gram-negative species are intrinsically resistant to trimethoprim, e.g. *Neisseria*, *Acinetobacter*, *Pseudomonas* and *Campylobacter/Helicobacter*. Trimethoprim and pyrimethamine act on *Pneumocystis jirovecii* and *Isospora belli* but pyrimethamine is more active on toxoplasma and *Plasmodium*.

The mechanisms of resistance to diaminopyridines are:

– reduction in the intra-bacterial concentration in Gram-negative bacteria linked to a reduction in the influx or hyper efflux, as for sulfamides (11, 22, 23);

– production of the enzyme DHFR resistant to the action of trimethoprim. These enzymes are coded by genes (genes 17 to 18 *dhfr* as described) acquired from other bacteria and transferred via transposons on MDR plasmids, sometimes in integron genetic structures (10);

– structural modifications of the DHFR, following missense mutations of the chromosomal gene, for example the mutation Ile100Leu in *S. pneumoniae* (27);

– increase in the synthesis of the target (DHFR) (14).

Note that the presence of thymine or thymidine in the culture medium can result in the observation of false resistance to trimethoprim.

Inhibition of DNA replication

Quinolones and fluoroquinolones

Fluoroquinolones are currently among the most heavily prescribed antimicrobials in the world. These antimicrobials are in such common use because of their pharmacodynamic (spectrum) and pharmacokinetic properties (13).

Nalidixic acid belongs to the pyridone-ß-carboxylic or 4-quinolone family. This synthetic molecule, derived from anti-malarials, is the first of the quinolones and was first used in 1962. The success and development of this family are associated with the addition of a fluorine in C6 of the pyridine cycle and C7 of the piperazyl cycle, opening the door in the 1980s to a new group of quinolones, including the fluoroquinolones.

Antimicrobial activity

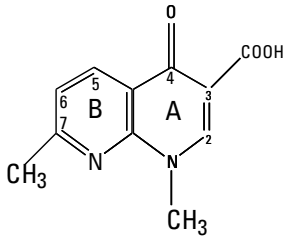
The quinolones can be divided into four groups according to their structure (Fig. 3) (all synthetic chemical molecules) and their antimicrobial activity (3, 4).

The 'classic' quinolones (nalidixic acid, oxolinic acid, pipemidic acid, a first-generation quinolone with a piperazine at C7, and flumequine, a first-generation quinolone with a fluorine at C6) act only on Gram-negative bacteria, especially enterobacteria (*Escherichia coli*, *Proteus*, *Klebsiella*, etc.), *Neisseria*, *Haemophilus* spp., *Moraxella* spp. and *Branhamella* spp.

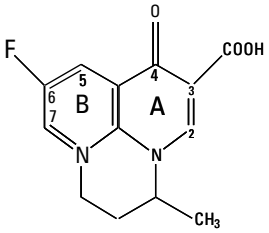
The fluoroquinolones (enrofloxacin, marbofloxacin, ciprofloxacin, norfloxacin) have in common a piperazine radical at C7 and a fluorine at C6 of the B-cycle, which makes them at least 100 times more active than the classic quinolones. They therefore act on other Gram-negative bacilli, such as *Pseudomonas aeruginosa* and legionella, as well as on Gram-positive bacteria such as staphylococcus. Levofloxacin also forms part of this group because of its structure (levogyre isomer of ofloxacin) and its antimicrobial activity.

The new fluoroquinolones (pradofloxacin, moxifloxacin, gatifloxacin) were developed to broaden the spectrum of

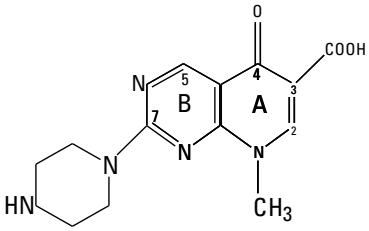
Classic quinolones



Nalidixic acid

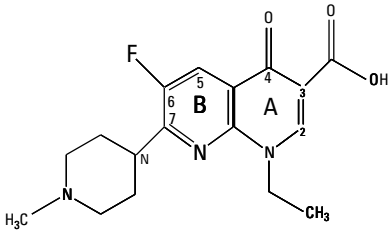


Flumequin

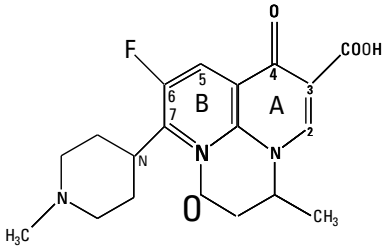


Pipemidic acid

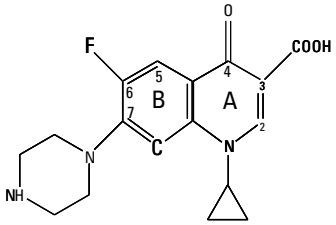
Fluoroquinolones



Pefloxacin (methylnorfloxacin)

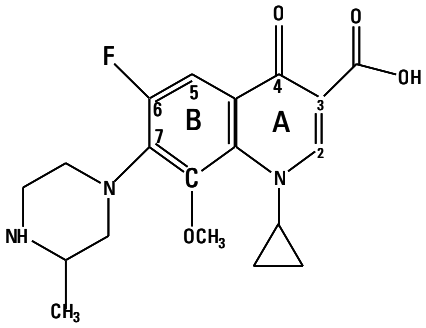


(Lev)-Orfloxacin

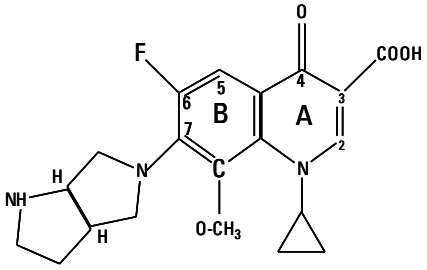


Ciprofloxacin

New fluoroquinolones

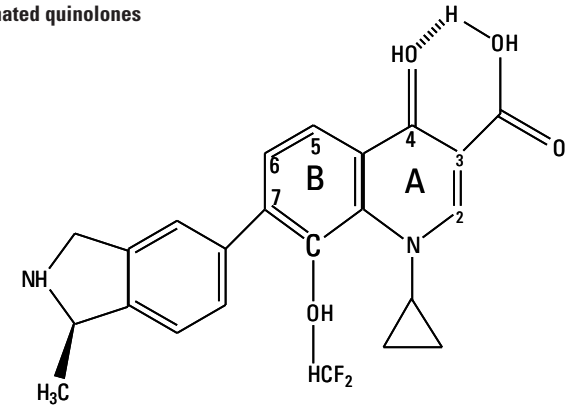


Gatifloxacin



Moxifloxacin

New non-fluorinated quinolones



Garenoxacine

Fig. 3
Structural classification of quinolones

fluoroquinolones to include streptococcus, in particular pneumococcus. Their antibacterial activity against anaerobic bacteria and mycobacteria differs according to the molecule used. Many of the molecules in this group were developed and then abandoned because of poor tolerance (sparfloxacin, trovafloxacin, grepafloxacin). There is a new group, similar to the new fluoroquinolones: the **new non-fluorinated quinolones**, such as garenoxacin, which, although it has a slightly different structure (no fluorine at C6), has an antimicrobial activity similar to that of the new fluoroquinolones, in particular against streptococcus.

Mechanism of action

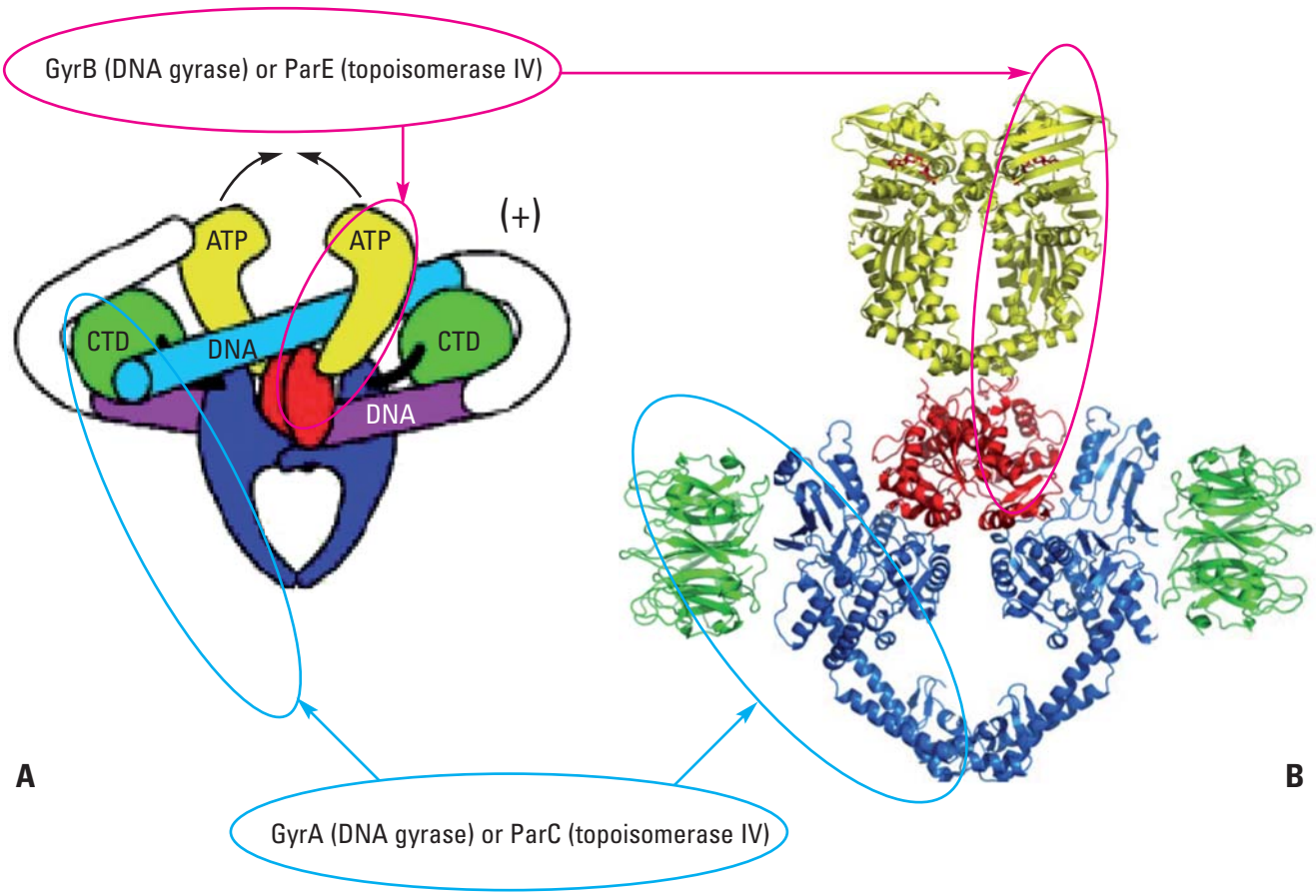
Quinolones penetrate Gram-negative bacteria using porines, especially as they are hydrophilic (norfloxacin and ciprofloxacin), while in hydrophobic molecules (e.g. ofloxacin, levofloxacin, moxifloxacin) penetration can also take place through the lipid bilayer. Gram-positive bacteria are penetrated by passive diffusion through the peptidoglycan.

Quinolones inhibit replication and transcription by inhibiting the functioning of bacterial type II topoisomerases, DNA gyrase (still called topoisomerase II) and topoisomerase IV. DNA gyrase is a tetrameric holoenzyme composed of the sub-units GyrA and GyrB in the form $GyrA^2GyrB^2$ (Fig. 4). Topoisomerase IV has a similar structure with sub-units ParC and ParE (13).

The quinolones attach to the DNA-topoisomerase complex. This complex becomes irreversible, leading to immobilisation of the enzymes that result in bacteriostasis and to the release of DNA double-strand breaks that activate the SOS system and produce the 'poison' effect that is responsible for the intense bactericidal action of quinolones. The details of the steps at cellular level leading to these effects are still poorly understood. The bactericidal effect varies depending on the molecule and the species of bacteria concerned.

Resistance mechanisms

Resistance mechanisms can be classified according to their function: reducing intra-cytoplasmic accumulation by



A: diagram
B: crystallographic structure
ATP: adenosine triphosphate
CDT: carboxyl-terminal domain

Fig. 4
The targets of quinolones; bacterial type II topoisomerases

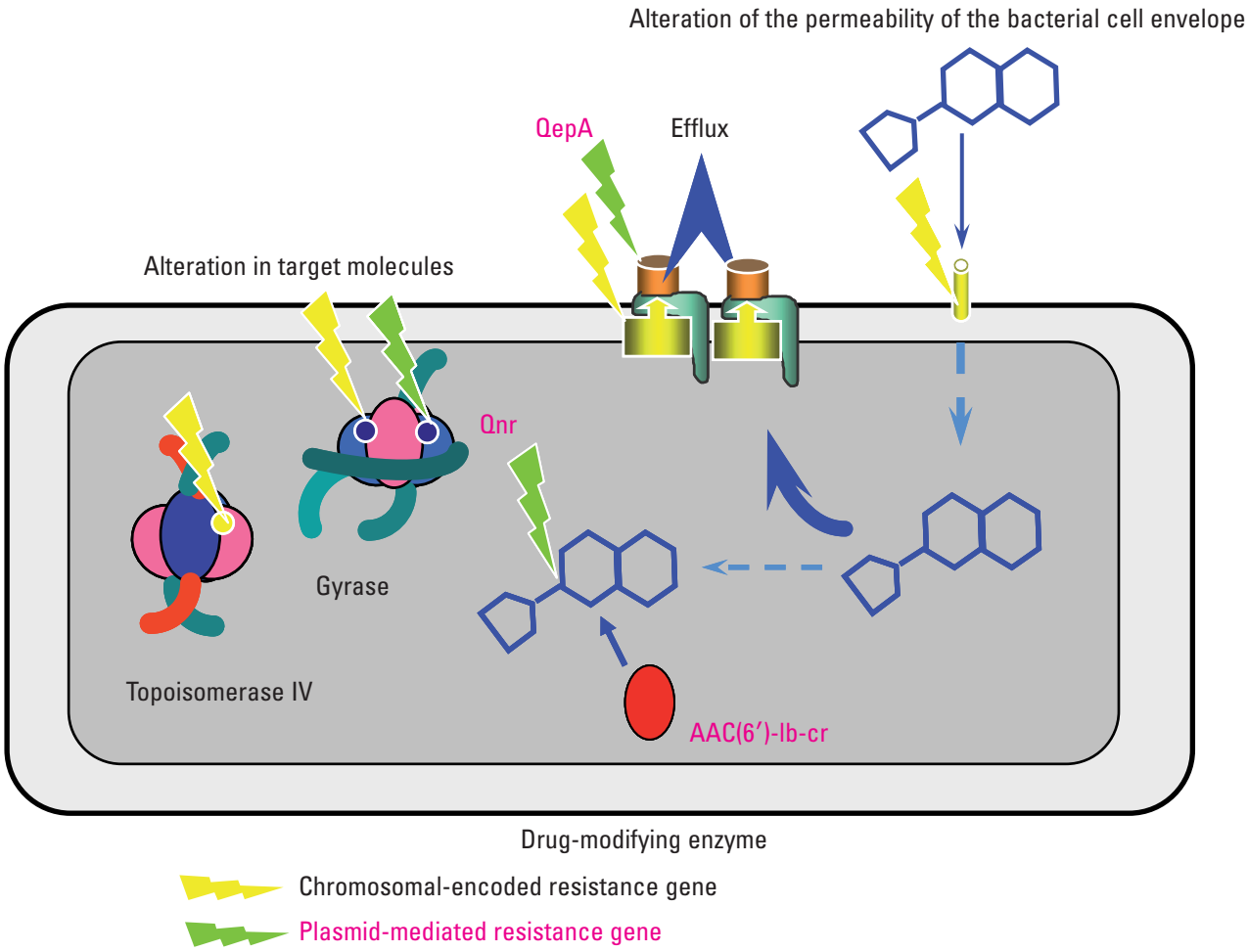


Fig. 5
Diagram of mechanisms of resistance to quinolones and fluoroquinolones

reducing the permeability of the wall or increasing the efflux, reducing the affinity of targets, inactivating enzymes or protecting targets (Fig. 5). Most of these mechanisms are chromosome-mediated, but plasmid-mediated genes have been described for a decade or so (4, 30).

Chromosomal resistance

The main mechanism of resistance is associated with mutations in type II topoisomerase structural genes, usually on the genes *gyrA* or *parC*, and more rarely on the genes *gyrB* or *parE*. These mutations are selected in the presence of quinolone with a frequency of around $1/10^8$. The mutations are found in a region called the quinolone resistance-determining region (QRDR) (12). We observe the phenomenon of step-resistance, with an increase in the minimum inhibitory concentration (MIC) with each new mutation acquired. The first mutation generally occurs in the topoisomerase for which the quinolone has the greatest affinity, which may vary according to the bacterial species and the quinolone (6, 7). The primary target of ciprofloxacin is sub-unit A of the DNA gyrase in Gram-negative bacilli, while it is sub-unit ParC of the topoisomerase IV in Gram-positive cocci (6). These mutations of type II topoisomerases confer a high level of resistance. The most frequently observed mutations in *E. coli* are on the codons 83 (Ser83Leu) and 87 (Asp87His) of GyrA, corresponding to the codons 80 and 84 of ParC.

There are two mechanisms that reduce quinolone accumulation in bacteria. These mechanisms are as described above for sulfamides and trimethoprim, with the addition of major facilitator superfamily (MFS) efflux pumps in Gram-positive bacteria that also result in resistance to quaternary ammoniums in some cases (22, 23).

Plasmid resistance

For many years, the only known mediation for resistance to quinolones was chromosomal, until the discovery of the gene *qnrA1* carried by a strain of *Klebsiella pneumoniae*, which was isolated in the United States in 1998 (30). Many other quinolone resistance (*qnr*) genes have been described since then: *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD*, each with several alleles (descriptions can be found online:

www.lahey.org) (15). The Qnr proteins act by protecting the topoisomerases against the action of quinolones. They belong to the family of pentapeptide repeat proteins (PRP), which are formed by a repeat series in tandem with five amino acids (33). Similar proteins are the protein McbG, which inhibits the microcin B17 in *E. coli* (MccB17) (12) and the protein MfpA discovered in mycobacteria (20, 30). The other mechanism with plasmid-mediation is the gene *qepA* encoding an efflux pump belonging to the MFS family. Another gene, *oqxAB,* encoding an efflux pump of the RND family, has been described in a strain of *E. coli* isolated in a pig (29). OqxAB confers resistance to olaquindox, a quinoline derivative used in veterinary medicine (29).

The mechanism for inactivating quinolones (Table I) did not exist prior to the description of the plasmid-mediated gene *aac(6′)-Ib-cr* encoding a bifunctional aminoglycoside 6′-N-acetyltransferase capable of acetylating both aminoglycosides and fluoroquinolones (26). This gene is a variant of the classic gene *aac(6′)-Ib* that confers resistance to certain aminoglycosides (amikacin, isepamicin, tobramycin). The new enzyme AAC(6′)-Ib-cr includes two mutations (Trp104Arg and Asp181Tyr) that reduce the aminoglycoside resistance but confer resistance to ciprofloxacin and norfloxacin, thanks to the N-acetylation of the secondary amine group of the piperazinyl cycle.

Acquisition of resistance to quinolones

Clinical resistance to quinolones generally results from a combination of several of the resistance mechanisms described above, with each mechanism acquired independently. For example, in *E. coli,* a level of resistance higher than critical clinical concentrations (0.5 mg/l for ciprofloxacin, for example) is achieved only by a combination of at least two mechanisms, most frequently a mutation of *gyrA* S83L and an efflux or impermeability. Only a combination of four or five mechanisms (mutations of *gyrA* and *parC* + efflux + qnr + AAC-(6′)-Ib-cr + impermeability) can achieve a high level of resistance in *E. coli* (ciprofloxacin MIC of 16 mg/l). For *Staphylococcus aureus*, two or three mechanisms are sufficient and for streptococci a single mutation often results in clinical resistance. Nevertheless, resistance is acquired in successive stages and it is therefore very important to be able to detect low levels of resistance, indicating the acquisition of a first resistance mechanism that could lead to the acquisition of clinical resistance.

Coumarins

Coumarin antibiotics include coumermycin and novobiocin, which are natural products from *Streptomyces* spp. These antibiotics also inhibit type II topoisomerases, DNA gyrase and topoisomerase IV but they use a different mechanism from that of quinolones. They act by competitive inhibition of the hydrolysis of adenosine-5′-triphosphate (ATP), which involves an N-terminal region of the sub-unit GyrB for DNA gyrase and the sub-unit ParE for topoisomerase IV (19). Since the sub-units are dimeric *in vivo*, we understand that coumermycin A, which is virtually the double of novobiocin, can block the two hydrolysis sites while novobiocin blocks only one.

As these molecules have rather a high molecular weight, penetration of the cell wall of Gram-negative bacteria is difficult, resulting in natural resistance to them. They are particularly active on streptococci and staphylococci (MIC from 0.003 to 0.02 mg/l), except for certain species such as *S. saprophyticus*.

The resistance mechanisms are associated with mutations in sub-unit B of DNA gyrase (codons 136 and 164) (8).

Transcription inhibitors: rifamycins

The rifamycins, still referred to as ansamycins, are natural products of *Streptomyces* spp. Their discovery was very important for the treatment of mycobacterial infections, including tuberculosis, caused by the tubercle bacillus (*Mycobacterium tuberculosis* complex), and leprosy, caused by *M. leprae* and *M. lepraemurium*.

The mechanism of these antibiotics inhibits transcription by binding to the beta sub-unit of the RNA polymerase, protein $\beta\beta'\alpha 2\omega$, coming between the RNA and the beta sub-unit (16). This prevents transcription from starting. These molecules are bactericidal antibiotics. They are among the few antibiotics that act on quiescent bacteria, i.e. not in the growth phase. This is why they are the preferred molecules for treating sub-acute and chronic infections.

Antibacterial activity

Rifampicin was discovered by P. Sensi and M.T. Timbal in 1959. It is produced by *Streptomyces mediterranei*. It acts mainly on Gram-positive bacteria such as streptococci, staphylococci and mycobacteria (Table II). Even though it also acts on Gram-negative cocci, the walls of Gram-negative enteric or aerobic *Pseudomonas* bacilli are often impermeable to them.

Rifabutin, rifapentin, rifaximin and rifamycin SV are other rifamycins that differ from rifampicin, either because of their pharmacokinetic properties or because of their antibacterial activity.

Resistance mechanisms

Modification of the target by mutations of the *rpoB* gene

The mutations are selected in the presence of rifamycin at a frequency of around $1/10^{-8}$. Several mutations can be combined to give a high level of resistance in streptococci and staphylococci (2).

One region of the gene ***rpoB*** is particularly well known in the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, *M. pinnipedii*, *M. canetti*) because it contains all the mutations associated with acquired resistance to rifampicin. This region extends from codon 511 to codon 533 (*E. coli* numbering system) (21). In strains resistant to rifampicin, in one case out of two we find the mutation Ser531Leu or Ser83Trp. These mutations are detectable by polymerase chain reaction (PCR) amplification followed by hybridisation or sequencing, and now using commercialised tests: GeneXpert®MTB/RIF (Cepheid, USA); GenoType®MTB-DR plus (Hain Lifescience, Germany); InnoLiPA® Rif-Tb (Innogenetics, Belgium).

Inactivation of rifamycins

This is caused by an ADP ribosylase encoded by the gene ***arr-3***. This gene is found in its natural state in the bacteria that produce it (chromosome-mediated), and has been transferred to Gram-negative bacteria where it is usually plasmid-mediated within the integron or between insertion sequences.

Other mechanisms

An efflux and aggravation of natural impermeability can also be responsible for resistance in Gram-negative bacteria.

Table II
Antimicrobial activity of rifampicin

Bacteria	MIC (mg/l)
Gram-positive species	
Mycobacterium tuberculosis	0.2
M. kansasii	0.5
M. leprae	S in mice
Staphylococcus aureus	0.005
Streptococcus pyogenes (group A)	0.002
Pneumocoque (*S. pneumoniae*)	0.001
Enterococcus faecalis	0.1
Corynebacterium diphtheriae	0.01
Clostridium perfringens	0.5
Gram-negative species	
Neisseria meningitidis	0.01
N. gonorrhoeae	0.01
Haemophilus spp.	0.02
Brucella spp.	0.01
Legionella spp.	0.03
Fusobacterium	0.1
Bacteroides	0.1
Escherichia coli	1 to 20
Pseudomonas	1 to 20

MIC: minimal inhibitory concentration

Toxic action on DNA

5-Nitroimidazoles

The 5-nitroimidazoles, including metronidazole, ornidazole and tinidazole, are antibiotics that act mainly against anaerobic bacteria because they require a prior reduction of ferredoxins (anaerobic pyruvate metabolism, $NO_2 \Rightarrow NH_2$) in the presence of DNA.

These antibiotics inhibit the synthesis of nucleic acids by binding to the DNA, especially in regions rich in thymidine and adenine. They are bactericidal but the mechanism is poorly understood.

The antibacterial spectrum is narrow and mainly concerns anaerobic bacteria, with Gram-negative bacteria being more susceptible than Gram-positive bacteria. Certain bacteria with a microaerophilic metabolism such as *Helicobacter pylori* are also susceptible.

The imidazoles are also effective on many parasites: *Trichomonas vaginalis*, *Lamblia* (giardia), and *Entamoeba histolytica* (amoeba).

Resistance mechanisms are rarely observed in anaerobic bacteria. We observe:

– a reduction in intra-bacterial accumulation by a reduction in the influx or efflux (22, 24)

– a reduction in nitro-reductase activity.

The ***nim*** genes (***nimA*** to ***nimF***) were found in anaerobic bacteria with acquired resistance (25, 28, 31). These genes encode reductases that cannot activate imidazoles. The genes are transferable because they are plasmid-mediated or transposon-mediated, but some of them can be found in chromosomes. The expression of ***nim*** genes is often subject to a promoter introduced upstream of the gene by an insertion sequence (32).

Resistance is frequently found in *H. pylori* (30% to 50%) but it is sometimes due to false resistance ***in vitro*** because of the difficulty of testing metronidazole in this slow-growing bacterium. While several genes encoding

reductases were blamed (*frxA*, *rdxA*), the main culprit was general modification of the oxide-reduction capacity of the bacteria (17).

Nitrofurans

The nitrofurans need to be transformed into active compounds by reduction, like the nitroimidazoles. The active compound causes lesions of the DNA that are poorly characterised.

Unlike the 5-nitroimidazoles, the reductases produced by aerobic–anaerobic bacteria such as enterobacteria are active and can transform the nitrofurans. That is why these molecules have a broad spectrum of antibacterial activity: Gram-negative bacteria such as enterobacteria (except *Proteae*) and Gram-positive bacteria such as staphylococci and streptococci.

The acquired resistance mechanisms are poorly understood:

– resistance by reducing the nitroreductase activity due to mutations in the genes *nfsA* and *nfsB*

– reduction in the accumulation in Gram-negative bacteria.

According to the current state of knowledge, these genes are chromosome-mediated.

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