ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1990, p. 681–684
0066-4804/90/050681-04$02.00/0

Vol. 34, No. 5

MINIREVIEW

Plasmid-Mediated 4-Quinolone Resistance: a Real or Apparent Absence?†

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Nalidixic acid, the first quinolone, was introduced into clinical practice in the early 1960s. The 6-fluorinated quinolones, which are significantly superior, in particular, in intrinsic activity and antibacterial spectrum, have been increasingly used since the 1970s. It is the purpose of this review to analyze why, in the late 1980s, bacterial plasmid-mediated resistance to quinolones has not yet been found.

APPARENT ABSENCE OF PLASMID-MEDIATED RESISTANCE TO QUINOLONES

It has generally been observed that, sooner or later after the introduction of a new antibiotic into medical practice, plasmid-mediated resistance to the compound emerges and subsequently spreads. Bacterial resistance of this type is likely to precede the clinical use of the new molecule, but use (often indiscriminate) of the antibiotic favors selection, dissemination, and hence detection of resistance. Also, and quite obviously, the availability of the drug is a prerequisite for screening and finding, by medical microbiologists, of the new resistance phenotype. As usual, there are a few exceptions to the rule; nitrofurans, novobiocin, polypeptides, quinolones, and rifampin have no known plasmid-mediated resistance.

It is noteworthy that novobiocin (coumermycin), which also inhibits DNA gyrase, belongs to this group. There has been a single report of plasmid-mediated resistance to nalidixic acid in bacteria (14). A *Shigella dysenteriae* type 1 isolate responsible for an epidemic in southern Bangladesh was found to harbor a 30-kilobase plasmid conferring resistance to nalidixic acid only. However, upon careful reinspection of the data, it appeared that nalidixic acid-resistant mutants of the recipient rather than authentic transconjugants were obtained. This observation, possibly due to the fact that the plasmid acts as a mutator factor specific for nalidixic acid resistance (Z. U. Ahmed, personal communication), confirms that chromosomal mutation is the mechanism so far responsible for bacterial resistance to quinolones.

IS THERE A (BACTERIAL) NEED FOR PLASMID-MEDIATED RESISTANCE TO QUINOLONES?

As already mentioned, bacteria may be resistant to quinolones, as well as to other groups of antibiotics (aminoglycosides, β-lactams, chloramphenicol, erythromycin, fosfomycin, fusidic acid, novobiocin, polypeptides, quinolones, and rifampin), following chromosomal mutations.

Certain mutations (e.g., *gyrA*) lead to cross resistance to all commercially available quinolones, and only to quinolones. The degree of cross resistance depends upon the intrinsic activity of the drug considered: the higher the activity, the lower the level of resistance. This observation implies that genetic and clinical resistances do not always correlate and that the correlation also depends upon the site of infection. Mutations of this class, which are distinct, although clustered (22), are stable and not deleterious for the host. Considering the inheritable character of chromosomal mutations, one can easily imagine that such mutants do not require transferable resistance to survive and disseminate. This mechanism would provide efficient protection to the progeny of mutants but not to other bacteria of the same or different species present in the ecosystem. Plasmid-mediated resistance to quinolones in these mutants is likely not to be detectable, since the host is already resistant.

Other mutations (e.g., *nfxB* and *norC*) confer resistance to quinolones and to structurally unrelated antibiotics such as β-lactams, chloramphenicol, tetracycline, and trimethoprim (10, 12). These permeability mutants are resistant to low levels of quinolones but can be selected by other groups of antibiotics. Even more strikingly, *marA* mutants resistant to fluoroquinolones can be isolated at a 1,000-fold-higher frequency by tetracycline and chloramphenicol than by norfloxacin (3). *marA* mutants can, in turn, lead to second-step mutants highly resistant to fluoroquinolones.

Taking these findings into account and considering the multiplicity of mutations leading to quinolone resistance (Table 1), it therefore seems that there is no need for the acquisition of extrachromosomal DNA for bacteria to cope with the impressingly increasing selective pressure exerted by quinolones. In this regard, it is of interest to note that, although this does not apply to fosfomycin, no plasmid-mediated resistance has developed towards antibiotics (quinolones, rifampin, and fusidic acid) in organisms exhibiting high frequencies of mutation leading to high-level resistance. Nevertheless, because of the efficacy of intra- or intergeneric conjugal transfer, the acquisition of a plasmid conferring high-level resistance to quinolones (and most likely to many other groups of antibiotics by different mechanisms) would give rise to quinolone-resistant cells at a much higher frequency than would chromosomal mutations, in particular, when two independent events are required for the latter. It would also provide recipient cells with a very efficient way to escape the bactericidal activity of combinations of quinolones with other antibiotics.

† This review is dedicated to the memory of Jean-Marc Costrejean.

TABLE 1. Quinolone resistance mutations in *Escherichia coli*

Selecting agent	Mutation(s)
Nalidixic acid	*gyrA*, *gyrB*, *nalB*, *nalD*, *crp*, *cya*, *icd*, *purB*, and *ctr*
Norfloxacin	*nfxB*, *norB*[a], and *norC*
Ciprofloxacin	*cfxB*[a]
Tetracycline or chloramphenicol	*marA*[a]

[a] Possibly allelic mutations.

POTENTIAL MECHANISMS OF PLASMID-MEDIATED RESISTANCE TO QUINOLONES

Some predictable mechanisms of plasmid-borne resistance to quinolones are listed in Table 2.

Bypass. A bypass mechanism is signified by the presence of an additional target insusceptible or much less susceptible to the antibiotic in the host bacterium. A prerequisite for this mechanism is that the plasmid-borne allele must be dominant over its chromosomal counterpart. The results of transcomplementation tests (Table 3) indicated that plasmid-mediated resistance to quinolones cannot be achieved by providing either one of the subunits of the altered DNA gyrase.

Recessiveness of the resistant mutant allele versus the wild-type (susceptible) chromosomal gene may also explain the lack of plasmid-mediated resistance to rifampin by altered transcriptase. However, on the basis of preliminary data (16) and despite the fact that the mechanism(s) of resistance to quinolones in naturally resistant bacterial genera or species is not known, one can envisage that a merodiploid encoding a susceptible DNA gyrase and a resistant (in both subunits) DNA gyrase might be resistant to quinolones. A similar mechanism accounts, in the vast majority of clinical isolates, for resistance to sulfonamide and trimethoprim which, like quinolones, are synthetic compounds.

Transfer of genetic material from gram-positive to gram-negative bacteria has been shown to occur under natural conditions (15). In addition, there is no barrier to the expression of genes from gram-positive microorganisms in gram-negative microorganisms (5), apparently including the structural gene(s) for both subunits of staphylococcal gyrase (16), whereas the reverse is generally not true. Considering the rate of high-level quinolone resistance in gram-positive cocci, in particular, staphylococci, it may well be that, as in other resistance systems (2, 23), gram-positive cocci will turn out to be the progenitors of plasmid-borne quinolone resistance genes in gram-negative bacteria. The fact that the genes for the two subunits of the gyrase may be contiguous in *Staphylococcus* spp. would facilitate the transfer of genetic material from gram-positive to gram-negative microorganisms.

Drug inactivation. Drug detoxification is the most common mechanism of resistance in bacterial pathogens. This is possibly due to dominance of resistance, level (high) of resistance, and cross resistance to structurally related antibiotics belonging to the same group. Enzymatic inactivation of quinolones may result from various reactions (Table 2). However, as mentioned above, quinolones are entirely synthetic molecules, and their presence in nature is therefore unlikely. Thus, it is difficult to conceive of bacteria developing resistance, by enzymatic modification, to an antibiotic to which they have never been exposed in nature.

There is increasing circumstantial evidence that antibiotic resistance plasmid genes in human pathogens originate in antibiotic-producing microorganisms. Conjugal transfer of genetic material from procaryotes to eucaryotes has recently been demonstrated (9). However, transfer in the opposite direction has not yet been reported. This apparent polarity in DNA transfer does not favor the acquisition by bacteria of quinolone resistance determinants from the producing organism, *Homo sapiens*.

Cellular impermeability. Decreased drug penetration can be achieved by several mechanisms (Table 2). It is unlikely that altered porins are plasmid borne. When chromosomally encoded, this mechanism confers only low-level resistance to quinolones. If plasmid mediated, the level of resistance would be even lower, since the additional gene would have to compete with the chromosomal wild-type allele. The presence of the incoming gene on a multicopy plasmid would tend to increase the resistance level by a gene dosage effect. However, in vitro cloning constructions of this sort indicate that the presence of large amounts of a cellular membrane structural component in a cell is often toxic for the host. Gene substitution, by homologous recombination, generally tends to be the final outcome of such unstable merodiploids. Finally, modified preexisting cellular structures have, to my knowledge, not been found to be infectious in nature.

Antibiotic resistance by impermeability is often associated with reduced growth yield and rate and, in competition studies with the parent in the absence of selective pressure, the resistant isolate is often rapidly outgrown (4). It would not be very tempting for a plasmid to pick up a gene conferring bacterial resistance to quinolones under these conditions. The new host would be rapidly selected against in the absence of the antibiotic, a situation which a bacterium can still experience.

Increased efflux, as for tetracycline resistance, is a more likely mechanism. This could be obtained by the modulation

TABLE 2. Conceivable mechanisms of plasmid-mediated resistance to quinolones

Type	Biochemical mechanism	Origin
Bypass	Both subunits of DNA gyrase altered	Gram-positive bacteria
	Insensitive DNA gyrase	Naturally resistant bacteria[a]
Drug detoxification	Oxidation, reduction, esterification, acetylation[b], adenylylation[b], and phosphorylation[b]	Organism possessing a biotransformation pathway
Impermeability	Porin reduction	Naturally resistant bacteria
	Decreased uptake and increased efflux	Chromosomal mutation

[a] e.g., *Acinetobacter baumannii*, *Clostridium difficile*, *Enterococcus* spp., *Pseudomonas cepacia*, and *P. maltophilia*.
[b] Although the product of the reaction would be chemically unstable.

TABLE 3. Relationship of dominance and recessiveness among *gyr* mutations

Genotype[a]	Phenotype
gyrA$^+$ *gyrA*	GyrA^s
gyrB$^+$ *gyrB*	GyrB^s
gyrA$^+$ *gyrB*$^+$ *gyrA gyrB*	GyrA^r GyrB^{rb}

[a] *gyrA*$^+$ and *gyrB*$^+$, Wild-type genes conferring susceptibility; *gyrA* and *gyrB*, mutant genes conferring resistance.
[b] Speculated on the basis of data in reference 16.

in *trans* of the preexisting machinery of the host at the level of gene expression.

QUINOLONES OPPOSE PLASMIDS

Although quinolones apparently are not themselves subject to plasmid-mediated resistance, they exert action against this category of replicons. Certain resistance plasmids increase the quinolone susceptibility of the host (6). Quinolones tend to eliminate plasmids (Table 4) (11); they inhibit both their replication (17) and their transfer (18). In addition, certain *gyrB* mutations decrease the ability of bacteria to act as donors or recipients or to stably maintain extrachromosomal DNA (8, 21).

PREVENTION OF PLASMID-MEDIATED RESISTANCE TO QUINOLONES: A SHAKESPEAREAN SITUATION

Apart from the classical approaches to minimizing the emergence and subsequent spread of bacterial resistance to antibiotics (20), a question specific to this group of antibiotics remains: to use or not to use? Clearly, intensive use of these very potent antibiotics leads to a substantial increase in the resistance rate (19). However, considering the above-mentioned activities of quinolones on plasmid physiology, one may conceive of situations in which the net result of the use of these drugs would be to decrease the incidence of resistance in the bacterial population. Unfortunately, plasmid curing and inhibition of transfer are never 100% efficient, in particular, in vivo (e.g., in animal models; Y. A. Chabbert, Proc. 6th Int. Congr. Chemother., p. 733, 1969), and such efficiency is rarely achieved under the usual regimens. Because of the above-mentioned high frequency of intergeneric plasmid transfer, the increase in the resistance rate is most often extremely rapid after the cessation of therapy; the in vivo efficacy of the most potent plasmid-curing agent can therefore only be most transient.

Antibiotic combinations may not constitute the best approach to the prevention of plasmid-mediated resistance to quinolones. As already pointed out, resistance plasmids often confer resistance to a large variety of structurally unrelated antibiotics. Combinations against strains harboring such replicons turn out to be, most of the time, monotherapy at best.

TABLE 4. Plasmid-curing agents

Type	Agent(s)
Intercalating dyes	Acridine orange, acriflavine, ethidium bromide, and quinacrine
Inhibitors of DNA gyrase	Quinolones and novobiocin
Inhibitors of transcriptase	Rifampin
Surfactants	Sodium dodecyl sulfate
Physical agents	UV light (254 nm) and temp (42°C)

CONCLUSION: UNE AUSSI LONGUE ABSENCE

Various factors can account for the apparent absence of plasmid-mediated resistance to quinolones in bacteria 30 years after the introduction of the first molecules into clinical settings—une aussi longue absence (7). However, because of the many advantages of this family of drugs, there is a tremendous selective pressure towards resistance. A similar evolution has recently been observed with glycopeptides and 5-nitroimidazoles which in both cases led, after a lag phase of several decades, to plasmid-mediated transferable resistance (1, 13).

ACKNOWLEDGMENTS

I thank D. C. Hooper and L. Mitcher for help with Tables 1 and 2, respectively, and D. R. Helinski for laboratory hospitality.

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