

One enzyme inactivates two antibiotics

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A single enzyme is responsible for antibiotic resistance to two very different drugs (pages 83–88).

The fluoroquinolone antibiotics, such as ciprofloxacin, are used to treat a range of infections, from sexually transmitted diseases and typhoid fever to respiratory and urinary tract infections. Resistance to this class of antibiotics is mediated by drug efflux or by mutations in the genes encoding access to their target enzymes: DNA topoisomerases and gyrases.

In this issue, Robicsek *et al.* describe a third mechanism of inherited fluoroquinolone resistance in bacteria—enzymatic inactivation of fluoroquinolones¹. Their findings also show how mutations in a single enzyme-encoding gene can confer resistance to more than one class of antibiotic.

The authors found that mutations in the gene *aac(6′)-Ib* augment the level of resistance to fluoroquinolones¹. *aac(6′)-Ib-cr* increases the level of resistance to ciprofloxacin, as measured by the minimum inhibitory concentration in *Escherichia coli*. *aac(6′)-Ib-cr* does this either on its own or when paired with a second gene, *qnrA*, which encodes a protein that confers low-level resistance to fluoroquinolones². These observations emerged from clever experiments with transmissible plasmids responsible for disseminating resistance to ciprofloxacin in Gram-negative bacteria.

Intriguingly, *aac(6′)-Ib* encodes aminoglycoside acetyltransferase, a previously identified enzyme responsible for resistance to aminoglycosides, a class of antibiotics chemically different from fluoroquinolones. Moreover, the mutations that enable this enzyme to inhibit the effects of ciprofloxacin do so with only a modest decrease in their ability to inactivate aminoglycosides.

This is not the only case of a single gene conferring simultaneous resistance to antibiotics of different classes. Genes encoding efflux pumps mediate resistance to multiple antibiotics and compounds³. Recently, a single mutation in a ribosomal protein was shown to confer resistance to antibiotics of

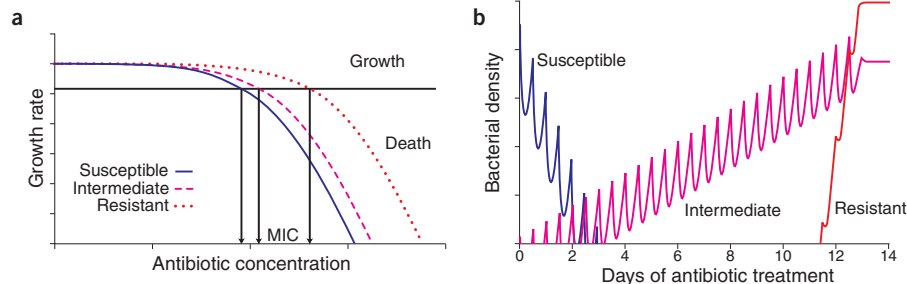


Figure 1 Intermediate and low levels of resistance can serve as stepping-stones for the evolution of bacteria with inherited resistance to clinical concentrations of antibiotics. **(a)** With a bactericidal antibiotic like a fluoroquinolone, the rate at which bacteria grow declines and the rate at which they die increases with the concentration of the antibiotic. The minimum inhibitory concentration (MIC) is the lowest concentration of the antibiotic at which the bacteria stop growing. The MICs of resistant bacteria are greater than those of susceptible bacteria. **(b)** When antibiotics are administered at periodic intervals, their effective concentrations usually decline between doses. As a result, during the course of treatment there are periods where even susceptible populations of bacteria can grow, although the net rate of growth of their population is negative and they are cleared. Although they are also killed at times by the antibiotic, bacteria with intermediate levels of resistance can have a net positive growth rate and slowly increase. Eventually, the number of replications in this intermediate population becomes sufficient for mutants with resistance to clinical concentrations of the antibiotic to be generated and ascend. For more information see www.eclif.net.

three disparate classes in *Streptococcus pneumoniae*, a community-acquired respiratory pathogen⁴.

The aminoglycoside acetyltransferase variant isolated by Robicsek *et al.* is not the first enzyme found to degrade fluoroquinolones⁵. But the mutant reported by Robicsek *et al.* is the first example in which a single gene variant is responsible for enzymatically inactivating multiple classes of antibiotics.

Fortunately, this recently evolved aminoglycoside acetyltransferase does not inactivate all fluoroquinolones, as some of these antibiotics have substitutions at the inactivation site that prevents resistance. Presumably as use of ciprofloxacin declines and other fluoroquinolones are used instead, these aminoglycoside acetyltransferase mutations in bacteria will be less favored or the bacteria will have to evolve again to keep up with the times.

Bacteria carrying either the *aac(6′)-Ib-cr* or *qnrA* mutations alone would pass as susceptible to ciprofloxacin by Clinical Laboratory Standards Institute breakpoint criteria for resistance (www.clsi.org). These susceptible mutations, however, can be stepping stones in the generation of strains that surpass the official breakpoint criteria for resistance and that, more importantly, cause

untreatable infections^{1,6,7}.

In this 'stepping stone' model (Fig. 1), bacteria are of three genetic states with respect to resistance to a hypothetical antibiotic: susceptible, intermediate and resistant. Susceptible strains can mutate to intermediate and intermediate to resistant. In the absence of mutations, a susceptible bacterial infection is readily cleared. But if intermediately resistant mutants emerge and are not cleared by the host's defenses, the bacteria slowly increase to sufficiently high numbers that allow evolution of fully resistant mutants. Resistance genes could be borne on plasmids and acquired by horizontal gene transfer rather than by mutation.

On first consideration, the results Robicsek *et al.* may seem as surprising, as they are disturbing. Antibiotic-inactivating enzymes are thought to evolve in bacteria exposed for eons to naturally occurring antibiotics; and the inactivating enzymes probably originated in the antibiotic-producing organisms⁸. The new findings provide two twists to this tale. First, fluoroquinolones are synthetic and presumably bacteria never encountered compounds of their ilk before they were first introduced for treatment in the 1960s. Second, the gene responsible is a variant of one that encodes an enzyme with

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a chemically different, but clinically related, substrate—antibiotics of another class.

Although this observation may be unprecedented for resistance to fluoroquinolones, this kind of enzymatic chicanery is not unanticipated evolutionarily. As new as the fluoroquinolones may be, the atoms they are composed of and the bonds that tie them together are not original. Were they so, they probably would not be biologically active. Moreover, as experimental evolutionists showed more than two decades ago⁹, enzymes are not that finicky about their substrates and if present in sufficient concentrations—possibly because of mutations that upregulate their synthesis—they catalyze reactions with substrates that are different from those for which they are named.

Initially these enzymes may not be very efficient, but if they enable the bacteria to

survive and replicate, and if selective pressure is maintained, subsequent evolution is likely to improve the catabolic activity on the new substrate. The evolution of genes with new functions is not too difficult when genes already exist. And, if a bacterium does not have the appropriate genes in its own genome, some other microbe or maybe even a eukaryote may generously provide one.

Is there a way to thwart this evolutionary trickery? To be sure, just because selective pressure exists—provided by the profligate use of antibiotics—resistance to multiple antibiotics is not a necessary outcome of evolution. Someday, effective antimicrobials against which bacteria can't evolve resistance may be produced, but prudence, reinforced by experience, suggests we shouldn't count on that.

At this time, the best alternative is to

employ antibiotic treatment regimes that reduce the likelihood of laying the stepping stones and generating resistance, for instance by increasing the dose of the antibiotic, reducing the time between treatments (Fig. 1), or by using more active compounds from within the antibiotic class.

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