The astonishing complexity of antibiotic resistance

G. Cornaglia, A. Mazzariol and R. Fontana

Institute of Microbiology, University of Verona, Italy

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Gram-negative bacteria face the assault on their integrity essentially by protecting the possible targets of antibiotic activity. This defence can consist of (i) obstructing access to the target; (ii) modifying either the target structure and affinity or the antibiotic molecular features; and, finally (iii) extruding (or increasing the extrusion rate of) the invading substances. Some of these mechanisms may be preferred by the different bacteria, but in many instances strains of the same taxa may use different mechanisms and, often, several mechanisms may be used at the same time. Furthermore, a number of recently described efflux mechanisms are capable of pumping compounds out of the cell that are only loosely related to one another and even belong to different classes of antibiotics [1–3].

The combination of more than one resistance mechanism usually accounts for higher levels of resistance, and attempts aimed at mathematically modeling this interplay have been made [4]. Owing to the interplay between outer membrane permeability and β-lactamase activity, even mild to moderate hydrolytic activities in the periplasm may be sufficient to counteract the impaired influx of antibiotic molecules through deficient porins, thus efficiently preventing adequate binding to the penicillin-binding proteins (PBPs). As a typical result of this mechanism, carbapenems may prove poorly effective against *Pseudomonas aeruginosa* even in the absence of metallo-enzymes – which are endowed with high specific activity – since the enzymatic barrier provided by serine β-lactamases is enhanced by the permeability barrier raised by a significant reduction in OprD porin expression [5]. Recently, attempts have also been made to clarify the interplay between β-lactamase activity, outer membrane permeability and the newly described efflux resistance mechanisms [6–8].

On the strength of these basic considerations, it is evident that when a molecular modification enables a given compound to escape different resistance mechanisms at the same time, this modified compound is also very likely to prove highly effective against bacteria with high-level resistance to the parent molecule. Major advances in the biological properties of molecules endowed with a distinctly greater ability to overcome the resistance mechanisms lead to the definition of a 'new generation' of compounds.

A 'new generation' of cephalosporins should encompass both Gram-positive and Gram-negative pathogens, including of course *Pseudomonas aeruginosa*, in their extended clinically useful spectrum, and their use should not be fraught with the problem of resistance, which hampers the use of earlier broad-spectrum cephalosporins active against Gram-negative pathogens [9]. This is the rationale for presenting cefepime and other compounds endowed with a novel C-3′ quaternary ammonium group as 'fourth-generation' cephalosporins (4-GCs). These molecules exhibit fast transit through the outer membrane porins, low affinity to many β-lactamases and high affinities to PBPs, thus accounting for their potent antibacterial activity, including antipseudomonal activity.

4-GCs, which are dipolar ionic compounds, are capable of penetrating the porin channels much more rapidly than the third-generation cephalosporins (3-GCs); thus, they very efficiently overcome the first resistance mechanism encountered obstructing access to the target, i.e. the permeability barrier represented by the bacterial outer membrane [10], and have a very pronounced advantage over the earlier cephalosporins owing to their ability to reach higher concentrations in the periplasmic space.

Moreover, as compared with 3-GCs, the molecular features of cefepime and the other 4-GCs also afford a greater ability to overcome the second barrier coming between the drug and the target, i.e. the enzymatic one. The newer compounds are more stable than 3-GCs to chromosomal, Bush's group 1 β-lactamases and may exhibit greater in vitro activity against *P. aeruginosa* as well as against those *Enterobacteriaceae* species that typically present inducible or derepressed hyperproduction of these enzymes – such as *Enterobacter* spp., *Citrobacter freundii*, *Serratia* spp., *Morganella morganii*, *Providencia stuartii* and *Providencia rettgeri*. Only total derepression noticeably compromises cefepime activity [11]. Owing to its reduced affinity for these enzymes, cefepime at the concentrations usually attainable in serum has also proved capable of virtually suppressing the emergence of the aforementioned derepressed *ampC* mutants [12].

4-GCs also show a greater stability to Bush's group 2be (ESBL) β-lactamases, although, in this instance, the clinical data would appear insufficient to suggest that 4-GCs can be effectively used to treat patients after a 3-GC treatment has failed.

Corresponding author and reprint requests: Giuseppe Cornaglia, Istituto di Microbiologia, Università degli studi di Verona, 37134 Verona, Italy
Fax: +39 045 584606

The combination of fast transit through the porins and low sensitivity to β-lactamase action results in high periplasmic concentrations of 4-GCs, but for these concentrations to exert an effective antibacterial activity, good affinity for PBPs is needed. 4-GCs have indeed maintained the high affinity for the essential PBP 3 typical of 3-GCs, and cefepime also exhibits high affinity for *Escherichia coli* PBP 2, which is a very unusual property among cephalosporins [13].

Compared with the information we have about the classic resistance mechanisms, little is known about 4-GCs as substrates of the recently described efflux pumps, whose activities seem to be coregulated with other resistance mechanisms at the genetic level, furnishing what may be regarded as an astonishingly sophisticated example of an integrated defence strategy.

The various energy-dependent efflux systems recently described in *P. aeruginosa* share a similar gene organization, in that an efflux operon codes for a periplasmic fusion protein, a cytoplasmic membrane efflux pump and an outer membrane protein. Typically, in some of these strains, the increase in resistance involves carbapenems, but not other β-lactams [1,8], and seems to be attributable to a concomitant reduction of the carbapenem-specific OprD porin protein. Co-regulation between antibiotic efflux and outer membrane porin synthesis has also been described in *E. coli mar* mutants [14].

The different resistance mechanisms used by a bacterial strain, the possibility of interplay with other mechanisms and the different contributions of the various mechanisms operating in a combined defence are all factors accounting for different resistance phenotypes within a given bacterial species and lead to therapeutic decisions that may vary greatly from one setting to another. Testing clinical isolates for their susceptibilities to all the available β-lactams can provide both microbiologists and clinicians with invaluable information about the phenotypes prevailing at that moment, local susceptibility patterns and the spread of clones endowed with peculiar resistance mechanisms.

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