Mechanisms of Resistance and Clinical Relevance of Resistance to β-Lactams, Glycopeptides, and Fluoroquinolones

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specialized units, resistance is becoming increasingly common in the community setting, leading to substantial changes in our typical prescribing practices. The use of β-lactam antibiotics to empirically treat soft tissue infections in many regions of the world has been compromised by the widespread isolation of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA). Similarly, the emergence and spread of Escherichia coli strains resistant to both fluoroquinolones and extended-spectrum cephalosporins has driven the greater use of carbapenems for the treatment of urinary tract infections. Perhaps as a consequence, the emergence of carbapenem resistance in previously susceptible species has been identified around the world.

For most of the antibiotic era (roughly from the mid-1940s forward), concerns about resistance were tempered by the knowledge that newer, more potent agents were being developed by the dozens of companies in the business of making antibiotics. We no longer have the luxury of anticipating the imminent introduction of the solution to our resistance problems. The number of large pharmaceutical corporations actively engaged in antibiotic discovery has dwindled to the single digits, and the number of new antimicrobial agents introduced has been reduced to a trickle over the past decade. Numerous explanations for the retreat from antimicrobial discovery have been proffered. Concern has been raised that the criteria for clinical development promoted by the US Food and Drug Administration is increasing the cost of drug development. Along the same lines, pharmaceutical companies preferentially develop drug classes with greater potential for profit (net asset value) than that obtained with antibiotics. These concerns can be addressed by public policy modifications. Perhaps the most problematic challenge to developing new antibiotics that are active against currently resistant pathogens is that, given the multiresistant nature of modern pathogens and the varied (sometimes nonspecific) mechanisms of resistance, identifying and developing safe new agents with broad activity is extremely difficult. As such, it has never been more important for practitioners to develop better strategies for using antibiotics to minimize the emergence and spread of resistance. This review focuses on resistance to 3 classes of antibiotics (β-lactams, glycopeptides, and fluoroquinolones), reviewing mechanisms and pointing out some of the challenges in employing antimicrobial usage strategies to curb growing resistance.

DEFINING RESISTANCE

Antimicrobial resistance and susceptibility in the clinical setting take many forms that are not predictable by in vitro susceptibility testing. For example, susceptible bacteria deep inside an abscess may not be accessible to antibiotics and therefore behave as if they are resistant. A fully susceptible organism may also act resistant if present in a biofilm attached to a foreign body. Conversely, species often considered resistant to specific antibiotics (eg, Pseudomonas aeruginosa and tetracycline) may be treated successfully if the infection occurs in the lower urinary tract, where the antibiotic can be concentrated heavily and the density of bacteria is generally low. Thus, the advisability of using an antimicrobial agent in a particular situation depends on a careful consideration of the in vitro susceptibility of the bacterial strain, the drug concentrations achievable at the site of infection, and the metabolic state of the infecting bacteria. Standard-setting organizations (the US Food and Drug Administration, the European Committee on Antimicrobial Susceptibility Testing, the Clinical and Laboratory Standards Institute) establish susceptible, intermediate, and resistance standards based on a careful analysis of achievable serum levels, susceptibilities of bacteria, results of animal experiments, and human clinical trials. Given the variability of individual clinical circumstances, it is clear that these designations must be considered educated guides rather than firm pronouncements.

Resistance can be achieved either through gene mutation or through the acquisition of exogenous resistance determinants. Mechanisms by which resistance genes are acquired vary. Transferable plasmids may be very large (>150 kb) and contain a variety of resistance gene. Plasmids may form cointegrates with transposons that incorporate one or more resistance genes. Some plasmids encode their own transfer machinery, whereas others can be mobilized by a corespondent transferable plasmid. Chromosomal elements may also transfer on their own or be mobilized by transferable plasmids. Superb recent work by Manson et al has shown that large chromosomal transfers among Enterococcus faecalis result from mobilization of segments of the chromosome by conjugative plasmids through cointegration across identical insertion sequences located on both replicons. These findings suggest that virtually any part of the genome can be mobilized, emphasizing the fluidity of many bacterial genomes.

RESISTANCE TO SPECIFIC ANTIMICROBIAL CLASSES

Resistance to β-Lactams

β-Lactam antibiotics act by binding to cell wall synthesis enzymes known as penicillin-binding proteins (PBPs), thereby inhibiting peptidoglycan syn-
thesis. Inhibition of PBPs weakens the cell wall, resulting in inhibition of cell growth and frequently in cell death. The 3 mechanisms of β-lactam resistance are reduced access to the PBPs, reduced PBP binding affinity, and destruction of the antibiotic through the expression of β-lactamases (enzymes that bind and hydrolyze β-lactams) (Table). In gram-positive bacteria, antibiotics have free access to the bacterial cytoplasmic membrane, where the PBPs are located. In gram-negative bacteria, the bacterial outer membrane (absent in gram-positive bacteria) can both restrict β-lactam entry and concentrate β-lactamase molecules. If β-lactam molecules are sufficiently excluded from this periplasmic space by either reduced entry or increased efflux, and if β-lactamase molecules are heavily concentrated, even a relatively weak β-lactamase can confer high levels of resistance.

### Resistance to β-Lactams in Gram-Positive Bacteria

With the exception of staphylococci (which produce a narrow-spectrum penicillinase), clinically important β-lactam resistance in gram-positive species occurs almost exclusively through the expression of PBPs that bind β-lactams with low affinity. In S. aureus, resistance to methicillin results from the expression of low-affinity PBP2a. Penicillin-binding protein 2a is encoded by the mec determinant, which is found exclusively in mobile chromosomal elements referred to as staphylococcal chromosomal cassette mec (SCCmec). It is presumed that the transfer of these elements between staphylococcal strains has contributed to the spread of MRSA, although such transfer has not been documented in vitro. The sizes of the SCCmec elements vary, with recent CA-MRSA isolates demonstrating a smaller, more compact element devoid of other resistance determinants (partially explaining the greater susceptibility of the community-acquired strains than their nosocomial counterparts). Genotyping data suggest that relatively few CA-MRSA clones are circulating around the world, although more recent data using genome-wide single nucleotide polymorphisms suggest that there may have been multiple transfers of the element into clinical strains with similar genotypes.

Expression of methicillin resistance in S. aureus is complex, involving the participation of several other loci that have become known as fem (factors essential for methicillin resistance) or aux (auxiliary) factors. Many of these loci encode functions involved in the development of precursors of the cell wall. Their inactivation generally results in reduced levels of resistance, suggesting that PBP2a is limited in its ability to process cell wall precursors differing from the norm. As a class B PBP, PBP2 does not have a functional glycosyltransferase region, so it must work in concert with the glycosyltransferase of PBP2 to make peptidoglycan. Consequently, deletion of PBP2 renders PBP2a unable to confer resistance to β-lactam antibiotics.

The chromosomal location of the SCCmec determinants and the small size of the more recently identified regions suggest that the metabolic costs of retaining these determinants may be insignificant. Moreover, β-lactam antibiotics do not achieve sufficient concentrations at sites of S. aureus colonization to convincingly select for colonization by resistant strains. As such, strategies to limit MRSA by reducing consumption of β-lactam antibiotics have had, at best, mixed results. Strategies designed to limit the spread of MRSA through infection control interventions have been more effective.

Compelling data suggest that β-lactam antibiotics are superior to vancomycin for the treatment of methicillin-susceptible S. aureus. Until the recent introduction of cefotibiprole and ceflaroline, expression of PBP2a was considered to confer resistance to all β-lactam antibiotics. Whether the superiority of β-lactams will extend to MRSA now that these agents are available remains to be determined.

The widespread resistance of Enterococcus faecium to ampicillin is attributable to the expression of low-affinity PBP5, which though apparently intrinsic to the species, is transferable between E. faecium strains. The spread of ampicillin-resistant E. faecium...
cium throughout the world has been attributed to clonal complex, a loosely associated group of strains that have undergone significant gene acquisition. Recent work suggests that Enterococcus faecium of this clonal complex has acquired lower-affinity PBPs on several occasions. The extent to which genetic exchange and recombination occur in E. faecium strains seriously complicates attempts to establish specific genetic lineages.

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hibitor combinations or carbapenems would result in reductions in their prevalence. Several institutions reported reductions in extended-spectrum β-lactamase prevalence in association with reduced cephalosporin use during this period.37-39

The third wave overlapped with the second and involved the emergence and spread of the CTX-M family of β-lactamases.40 Derived from the chromosomal enzyme of *Kluyvera* spp, these enzymes are natural cephalosporinases. They have penetrated widely into many different species, including *Kpneumoniae*41. In contrast to the first wave, CTX-M enzymes are also widely prevalent in pathogenic *Ecoli*.42 Strains expressing these enzymes have spread worldwide and are characteristically resistant to all β-lactam antibiotics as well as resistant to inhibition by currently available β-lactamase inhibitors. In vitro expression of resistance to carbapenems is variable and may be dependent on difficult-to-discern factors like plasmid copy number or porin reduction,46 making these strains difficult to detect at times. The CTX-M enzymes are often encoded on a mobile transposon designated Tn4401 and can be transferred to different species on transferable plasmids. Originally concentrated in *Kpneumoniae* in the United States, KPC genes have now been identified in a wide variety of different gram-negative species and have been found throughout the world.45

The fourth wave of β-lactamase–mediated resistance is the emergence and spread of carbapenemases.44 The carbapenems are broadly active precisely because they resist hydrolysis by a wide range of β-lactamases and are often a “last line” of effective therapy for serious infections caused by gram-negative bacteria. There are 3 broad categories of carbapenemases. The first is the KPC (*Kpneumoniae* carbapenemase) class, which is found primarily, but not exclusively, in *Kpneumoniae*.45 Strains expressing these enzymes have spread worldwide and are characteristically resistant to all β-lactam antibiotics as well as resistant to inhibition by currently available β-lactamase inhibitors. In vitro expression of resistance to carbapenems is variable and may be dependent on difficult-to-discern factors like plasmid copy number or porin reduction,46 making these strains difficult to detect at times. The KPC enzymes are often encoded on a mobile transposon designated Tn4401 and can be transferred to different species on transferable plasmids. Originally concentrated in *Kpneumoniae* in the United States, KPC genes have now been identified in a wide variety of different gram-negative species and have been found throughout the world.45

The second major class of carbapenemases are the metalloenzymes, so-named because they require the presence of a metal (usually zinc) as a
of metalloenzymes have been described. Originally concentrated in Japan (where the number of carbapenems and their use was greatest in the 1980s and 1990s), they have now been identified worldwide.\textsuperscript{45} The mechanism by which they hydrolyze \(\beta\)-lactams differs in important ways from the other types of \(\beta\)-lactamases, and therefore they are not subject to inhibition by any of the clinically available \(\beta\)-lactamase inhibitors.\textsuperscript{46} Although metalloenzymes in general hydrolyze aztreonam poorly, and therefore their presence can be suggested by carbapenem resistance in the setting of aztreonam susceptibility,\textsuperscript{49} the frequent co-occurrence can be suggested by carbapenem resistance in the drolyze aztreonam poorly, and therefore their presence can be suggested by carbapenem resistance in the setting of aztreonam susceptibility,\textsuperscript{49} the frequent co-expression of more common extended-spectrum \(\beta\)-lactamases in conjunction with metalloenzymes results in clinical strains that express resistance to both classes. Although a number of different types of metalloenzymes have been described, enzymes designated VIM-1 and NDM-1 have been implicated in recent international outbreaks and in rising endemicity in different regions.\textsuperscript{45}

The third major class of carbapenemases are the OXA-type enzymes. More than 100 such enzymes have been described in a number of gram-negative species. Only a few are associated with carbapenem resistance. These enzymes are responsible for most of the carbapenem resistance observed in \textit{Acinetobacter} species.\textsuperscript{50} Like the other carbapenemases, they are not susceptible to inhibition by currently available \(\beta\)-lactamase inhibitors.

In many instances, in vitro analysis of carbapenem hydrolysis by carbapenemases demonstrates relatively weak activity. Clinical resistance results from the frequent presence of auxiliary mechanisms that augment \(\beta\)-lactamase–mediated resistance, such as increased expression of \(\beta\)-lactamase (generally through the acquisition of stronger promoters) and reduced \(\beta\)-lactam access to the periplasmic space through porin mutations or pump activations.\textsuperscript{50}

The broad activity of carbapenemases compromises both therapy for individual patients and strategies designed to modify antimicrobial selective pressures. There are no classes of \(\beta\)-lactams that have predictable activity against strains producing carbapenemases, and many carbapenem-resistant strains also express resistance to fluoroquinolones and aminoglycosides. Thus, clinicians must frequently turn to second-line agents such as colistin or tigecycline. Given concerns about toxicity, efficacy, and spectrum that accompany use of these agents, it is hard to envision circumstances in which their use as empirical agents would be welcomed.

**Resistance to Glycopeptides**

Glycopeptides (vancomycin is the only glycopeptide licensed for use in the United States) are of sufficient size to preclude their passage through the porins that allow entry into the periplasmic space of gram-negative bacteria, so they are active only against gram-positive bacteria. Glycopeptides act by binding to the terminal D-alanine present on the pentapeptide stem of peptidoglycan precursors, thereby inhibiting the transpeptidation step required for peptidoglycan cross-linking. High-level resistance to glycopeptides results from the acquisition and expression of operons that substitute a terminal D-lactate or D-serine for the D-alanine, thereby reducing the vancomycin binding affinity.\textsuperscript{51} Expression of these operons is regulated by response regulators encoded by the same transposons that encode resistance, so the cost of having them is minimized. Vancomycin-resistant enterococci (VRE) (predominantly \textit{E faecium}) emerged in the 1980s, most likely in response to the widespread use of orally administered (and nonabsorbed) glycopeptides in humans with gastrointestinal infection due to \textit{Clostridium difficile} (in the United States) and to the use of orally administered glycopeptides to animals as a feed additive (in Europe). Although a large number of different glycopeptide resistance operons have now been described (VanA, B, C, D, E, F, G, L, M), VanA and VanB remain the most clinically relevant. Both have been identified on transposons that are presumed to be the mechanisms that facilitate their dissemination.\textsuperscript{52,53} For reasons that remain unexplained, the acquired glycopeptide resistance determinants have remained concentrated in \textit{E faecium}. Vancomycin-resistant \textit{E faecalis} generally represents a minority of isolates but may be particularly important in the rare transfer of these operons to \textit{S aureus}.\textsuperscript{54}

Of great concern more than a decade ago was the possibility that the glycopeptide resistance determinants prevalent in \textit{E faecium} would become widespread in MRSA, many strains of which were susceptible only to glycopeptides. The level of concern over this possibility has diminished in the intervening years for 2 reasons. The first is that several new agents (quinupristin-dalfopristin, linezolid, daptomycin, tigecycline, telavancin, ceftaroline) have been introduced that have activity against MRSA. The second reason is the \textit{S aureus} strains expressing the known vancomycin resistance determinants have been exceedingly rare.\textsuperscript{55} To date, roughly a dozen such strains have been reported, and there is no compelling evidence for any clonal spread.

A more common type of reduced vancomycin susceptibility observed in \textit{S aureus} is a stepwise, intermediate resistance that raises the minimum inhibitory concentration (MIC) marginally (to 4-8 \(\mu\)g/mL) but enough to compromise treatment of clinical staphylococcal infection by vancomycin.\textsuperscript{56} This type of intermediate resistance (vancomycin-intermediate \textit{S aureus}, or VISA) appears to be an intrinsic adapta-
tion of certain S. aureus strains that occurs under circumstances of persistent vancomycin selective pressure. Although the mechanisms underlying the intermediate phenotype remain unclear, most appear to involve a thickening of the cell wall in a way that contains increased numbers of unlinked peptidoglycan precursors. These unlinked precursors are thought to bind the glycopeptides before they can interact with peptidoglycan precursors at the cytoplasmic membrane, essentially soaking up the antibiotic before it can bind to the cell wall precursors. The intermediate phenotype is often unstable in the absence of persistent glycopeptide selective pressure, and clonal spread of these strains has not been observed.

A potentially more troublesome development in S. aureus has been what some have noted as “MIC creep.” Clinical failures have been observed more commonly with S. aureus strains having MICs of 2 μg/mL or higher. Consequently, the most recent recommendation from the Infectious Diseases Society of America for treatment of serious S. aureus infections is that alternative therapeutic agents should be used for patients whose isolate MICs are equal to or greater than 2 μg/mL. The extent to which S. aureus MIC creep truly exists is debatable. One recent study indicated that the creep identified over a 10-year period was methodology dependent and most pronounced with the use of E-test strips. It remains good advice to follow up patients closely and, if the clinical progress dictates, switch to a non-glycopeptide for the treatment of serious S. aureus infections. Some experts recommend targeting higher trough concentrations in dosing regimens. However, a recent multicenter study concluded that a 3-fold increased risk of renal dysfunction was associated with vancomycin regimens in which the intermediate phenotype remained.

The mutational evolution to vancomycin resistance in S. aureus appears to be a phenomenon that is only advantageous in the setting of continued vancomycin selective pressure, probably because thick-walled S. aureus coccii are not favored in the natural environment. But what of the vancomycin resistance operons? Is it likely that reduced vancomycin use will reduce their prevalence in E. faecium? Probably not. Associations between vancomycin use in the clinical setting and VRE colonization have often been tenuous. The potential connection between oral vancomycin use and VRE colonization was recognized early, leading to recommendations that metronidazole, rather than oral vancomycin, be used to treat C. difficile colitis. Unfortunately, it was soon recognized that exposure to potent antianaerobic antibiotics, including metronidazole, was a significant risk factor for VRE colonization. Recent data indicate that reductions of use of oral glycopeptides in European feed animals have dramatically decreased animal colonization with VRE. However, reduction of parenteral vancomycin use in humans is unlikely to have a major impact on hospital prevalence, since little vancomycin enters the gastrointestinal tract when it is administered parenterally. The strongest selective pressure for VRE colonization and infection most likely comes from the use of extended-spectrum cephalosporins, which select for ampicillin-resistant E. faecium (the vast majority of VRE are ampicillin-resistant E. faecium). Whether systematic efforts to reduce cephalosporin use will reduce VRE prevalence remains to be determined.

Resistance to Fluoroquinolones

High levels of resistance to fluoroquinolones in both gram-positive and gram-negative bacteria are attributable to the accumulation of point mutations in genes encoding cellular topoisomerases (enzymes that act to coil and uncoil complementary DNA strands) along with acquisition of auxiliary mechanisms that serve to augment the level of resistance expressed. These point mutations occur primarily in the quinolone resistance–determining regions, the areas of the topoisomerases involved in quinolone binding. The level of resistance to a specific fluoroquinolone associated with a mutation depends on the nature of the mutation and whether it is located in the gene encoding the primary target for that fluoroquinolone (gyrA or parC, for example). In general, single point mutations confer only modest levels of resistance. This observation has led to the idea that a “mutant prevention concentration” (MPC) can be identified that prevents the clinical emergence of resistant strains from susceptible populations. In other words, keeping the concentration of a fluoroquinolone persistently above the level of resistance expressed by a first-order mutant effectively suppresses that mutant from emerging. In vitro and some animal studies support the effectiveness of this strategy.

Unfortunately, in the clinical environment, the relationship between antimicrobial administration and the emergence of resistance is not simple. There are several mechanisms by which bacteria, especially gram-negative bacteria, can move closer to the breakpoint for resistance without actually becoming clinically resistant. Mechanisms facilitating such increases include the increased expression or acquisition of a number of efflux pumps, the acquisition of plasmids that encode “protection enzymes” (Qnr), or the acquisition of plasmids encoding enzymes that inactivate the fluoroquinolone [aac(6’)-Ib-cr] (Figure 2). In one study, the presence of qnrA in an E. coli strain increased the MPC 8-fold, from 1 to 8 μg/mL, pushing it beyond clinically achievable
Moreover, the concentrations of fluoroquinolone achieved throughout the body are not uniform. The concentration in the lung may be considerably higher than in the bowel (where many mutants are waiting to emerge). Finally, the MPC for moxifloxacin against *S pneumoniae* is considerably different than the moxifloxacin MPC against *P aeruginosa*. Consequently, concentrations that suppress the emergence of fluoroquinolone-resistant mutants in *S pneumoniae* may promote their emergence in *P aeruginosa* in the same patient. In the current environment, in which fluoroquinolone-resistant variants of common pathogens are commonplace, use of these agents invites colonization by resistant strains. It is therefore unlikely that any strategies designed to try to suppress the emergence of resistance by using higher concentrations of fluoroquinolones will be successful in the clinical setting.

**CONCLUSION**

Although the emergence of antimicrobial resistance is invariably associated with antimicrobial use, the multiple mechanisms of resistance, the frequency of gene exchange in the natural environment, and the nonspecific nature of many resistance mechanisms make developing resistance-specific strategies to reduce individual resistance phenotypes complicated and fraught with potential deleterious unintended consequences. Efforts to reduce overall antimicrobial exposure, for example, through organized efforts to identify appropriate minimal lengths of therapy, hold greater promise for reducing the burden of resistance.
resistance. Reductions in the use of antibiotics (eg, the fluoroquinolones) that promote the emergence of broad-spectrum mechanisms of resistance may have greater benefits in reducing the prevalence of resistance to a variety of troublesome nosocomial pathogens.

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