Mechanisms of action of newer antibiotics for Gram-positive pathogens

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Certain Gram-positive bacteria, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and quinolone-resistant *Streptococcus pneumoniae* have achieved the status of "superbugs", in that there are few or no antibiotics available for therapy against these pathogens. Only a few classes of novel antibiotics have been introduced in the past 40 years, and all since 1999, including the streptogramin combination quinupristin/dalfopristin (Synercid), the oxazolidinone linezolid, and the lipopeptide daptomycin. This review discusses the mechanisms of antibiotic action against Gram-positive pathogens, and resistance counter-mechanisms developed by Gram-positive bacteria, with emphasis on the newer agents.

With the steady emergence and spread of antibiotic-resistant pathogens, and the necessary but slow development of new antibiotic classes, the landscape of clinical infectious diseases is continually changing. Choosing effective anti-infective therapies is becoming increasingly complex, and requires a working knowledge of the basic forces at play in the relation between microbe, host, and antibiotic intervention. Understanding mechanisms by which a microbe either succumbs to or evades an antibiotic is fundamental to optimal patient care, and, furthermore, is useful in such considerations as the need for bactericidal versus bacteriostatic agents, the synergistic or antagonistic potentials in antibiotic combinations, infection control, and resistance prevention. With the increasing prevalence of resistant Gram-positive infections, this review will focus on mechanisms of antibiotic action and counter-mechanisms of bacterial resistance among Gram-positive pathogens.

Bactericidal versus bacteriostatic action

The action of antibiotics can be classified as bactericidal (ie, causing death of bacteria) or bacteriostatic (ie, preventing bacterial growth). The implications of bactericidal action in serious Gram-positive infections that cause life-threatening disease in hospitalised patients[1] is the subject of much debate. Bactericidal antibiotics, such as the beta-lactams (including the cephalosporins, carbapenems, and cephems), glycopeptides (including vancomycin), fluoroquinolones, polymyxins, and the lipopeptide daptomycin, are often preferred for treatment of these diseases,[1] particularly for cases of febrile neutropenia, meningitis, and endocarditis.[2] However, there are important exceptions. Chloramphenicol has been used successfully in the treatment of meningitis despite being a bacteriostatic antibiotic.[2] Chloramphenicol and other bacteriostatic antibiotics (eg, the macrolides, tetracyclines, sulfonamides, trimethoprim, tigecycline, and clindamycin) have also shown efficacy against complicated skin and skin-structure infections and community-acquired pneumonia (CAP).[1] Furthermore, the bactericidal nature of an antibiotic is not an intrinsic property of a given antibiotic but may be influenced by the target species and/or the drug concentration.

Synergistic potential

Understanding the mechanisms of antibiotic action may influence the choice of antibiotic combinations that are used in an effort to avoid antagonism and especially to achieve synergy. For example, an antibiotic that acts to inhibit cell-wall synthesis might reasonably be expected to enhance the penetration of a drug acting intracellularly. Indeed, synergistic bactericidal effects against enterococcal infections have been consistently reported with concomitant administration of a cell-wall-active agent, such as penicillin, ampicillin, or vancomycin, and an

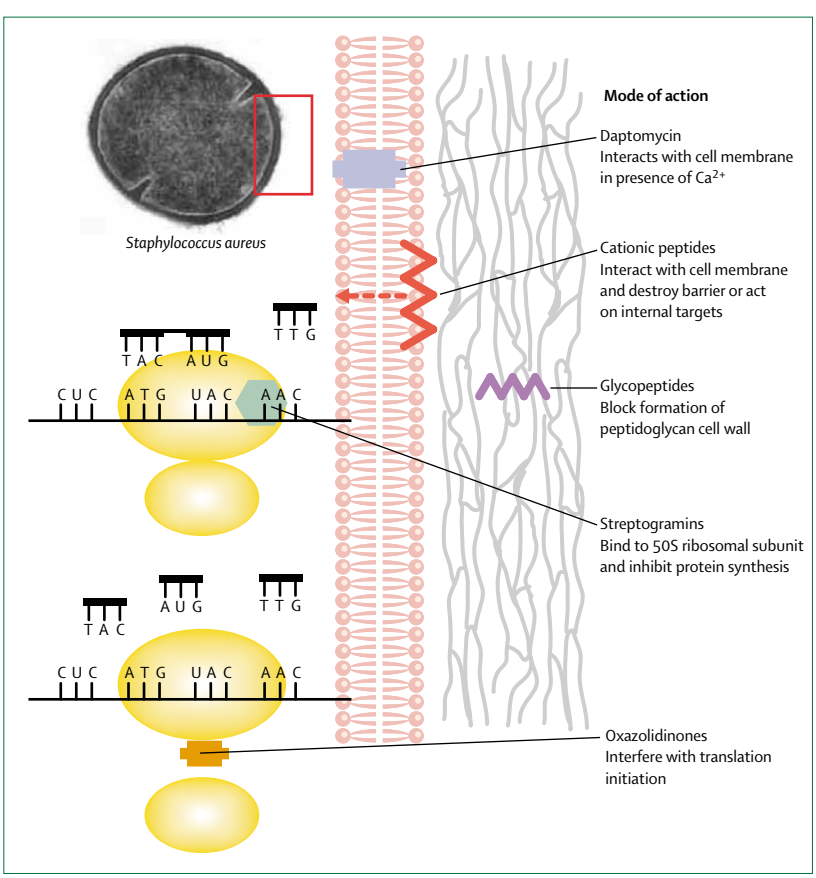

Figure 1: **The mechanisms of action of the newer antimicrobial agents introduced for use against Gram-positive bacteria.**

aminoglycoside antibiotic.[3] This observation is particularly important since enterococci are increasingly becoming antibiotic resistant, and because most antibiotics, used singly, are bacteriostatic against enterococci.[4]

Major targets

The major targets for the main classes of antibiotics include cell membranes (eg, mupirocin), cell-wall biosynthesis enzymes and substrates (eg, beta-lactams, vancomycin, and bacitracin), bacterial protein synthesis (eg, chloramphenicol, tetracyclines, macrolides, clindamycin, aminoglycosides, linezolid, mupirocin, and fusidic acid), and bacterial nucleic acid replication and repair (eg, co-trimoxazole [trimethoprim/sulfamethoxazole], which acts via an anti-metabolite mechanism, rifampicin, and quinolones). Many excellent reviews have covered the action of these agents and mechanisms of resistance against them in some detail.[1,5,6] This review concentrates on the action of newer agents against Gram-positive bacteria. Figure 1 presents an overview of the action of a selection of these newer agents on Gram-positive bacteria.

How antibiotic resistance develops

The widespread use of antibiotics, both for human consumption and animal feed, has fostered the development of resistance in a variety of pathogenic bacteria.[5] Unfortunately, the emergence of bacterial strains that exhibit resistance to a variety of antibiotics—ie, strains that are multiple-drug resistant—is becoming the major cause of treatment failure of infections worldwide. The treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) generally requires vancomycin as a last resort, while enterococcal strains that no longer respond to vancomycin have already been identified.[7]

Most antibiotics used in human beings originated from natural templates produced by particular species of bacteria or fungi as a mechanism of competition to ensure their own survival (eg, to gain a larger share of environmental food supplies by killing competitors). As the ability to produce lethal chemicals was developed by microorganisms, so was the counter-measure in this war for survival—namely antibiotic resistance. For example, in natural environments such as the soil, bacteria can develop resistance through mutation, or can exchange genetic information (including resistance genes) with great facility and relatively low species specificity, thus permitting the transmission of the molecular determinants of resistance to other microbes with great ease.[8] Mechanisms of resistance fall into three main categories: the inactivation of the antibiotic by modification of its active chemical moiety; the specific modification of the macromolecular target (ie, by mutagenesis of key residues); and the prevention of antibiotics from reaching their targets, through decreased uptake or, more commonly, active antibiotic efflux.[6]

Mechanisms of action of newer antibiotics

There is a growing need for novel antibiotics to treat diseases induced by Gram-positive pathogens. Many pathogens are developing resistance to the potent antibiotics used for treatment. Even more alarming, resistance is not restricted to a single agent but may involve resistance to multiple antibiotics. The ability of newer, directed-spectrum antibiotics to circumvent multidrug-resistance mechanisms is the result of their novel mechanisms of action.[9] Thus, these antibiotics provide a fresh face in antimicrobial chemotherapy and an invaluable tool in the fight to prevent overwhelming antibiotic-resistance issues.

Oxazolidinones: linezolid

Linezolid is a synthetic oxazolidinone antimicrobial agent that binds to the ribosome and inhibits microbial protein synthesis.[10,11] The antibiotic reversibly blocks the formation of protein synthesis initiation complexes by binding to the 23S ribosomal RNA (rRNA) of the 50S ribosomal subunit, near the interface formed with the 30S ribosomal subunit.[12] Linezolid binds near the chloramphenicol and lincomycin binding sites, since it competes with these agents for binding.[13,14] However, these antibiotics differ in the mechanism by which they act, with chloramphenicol inhibiting peptide bond formation, and linezolid inhibiting initiation complex formation. The result of this mechanistic difference is that there is only infrequent cross-resistance between linezolid and chloramphenicol or lincomycin.

Because linezolid has a novel mechanism of action, it has the same activity against many antibiotic-sensitive and antibiotic-resistant bacteria in vitro, and is active against pathogens resistant to meticillin and vancomycin.[15] In-vitro studies have confirmed that linezolid has good activity against most medically important Gram-positive bacteria.[16]

Streptogramins: quinupristin/dalfoprisitin

The streptogramin antibiotics were discovered in the 1960s, and one of them, virginiamycin, was used copiously as an animal feed additive.[17] In Europe, they have emerged as important agents for the treatment of infections caused by a variety of bacteria. The streptogramins consist of mixtures of two structurally distinct cyclic peptide antibiotics, type A and type B, that are separately bacteriostatic, but bactericidal in appropriate ratios. Quinupristin and dalfopristin (Synercid) were developed as human antibiotics with increased solubility compared with other streptogramins. The two components are generally bactericidal in combination, acting synergistically on the bacterial 50S ribosomal subunit to inhibit protein synthesis.[17,18] The molecular basis for this synergism is probably the result of the initial binding of dalfopristin to the ribosome enhancing the subsequent binding of quinupristin, and because these two antibiotic components bind to distinct

but overlapping regions of the ribosome peptidyl site.[19] Quinupristin binds to the same site as erythromycin and other macrolides. In-vitro synergism of the major metabolites with the complementary parent compound has been demonstrated,[17] thus the metabolites of these two streptogramins also contribute to Synercid's antimicrobial activity.

Synercid is bactericidal for most Gram-positive bacteria and most respiratory pathogens (including the pneumococci), as well as *Mycoplasma* spp, *Legionella* spp, and *Chlamydia pneumoniae*. More importantly, the combination is also active against 90% of *S aureus* and coagulase-negative staphylococci, including meticillin-resistant strains, as well as pencillin-resistant pneumococci. Quinupristin/dalfopristin is curiously bacteriostatic against *Enterococcus faecium* and is not active against *Enterococcus faecalis*, but is bactericidal against strains of staphylococci that are both susceptible and resistant to meticillin.[17] Nevertheless, Synercid has been approved by the US Food and Drug Administration (FDA) for the treatment of people with serious or life-threatening infections associated with vancomycin-resistant *E faecium* (VREF) and for the treatment of complications from skin and skin-structure infections caused by meticillin-susceptible *S aureus* (MSSA) and *Streptococcus pyogenes*.

Lipopeptides: daptomycin

Daptomycin is a member of a new class of bactericidal antibiotics called the lipopeptides, and has demonstrated an ability in vitro to rapidly kill virtually all clinically relevant Gram-positive bacteria via a mechanism of action distinct from those of other antibiotics on the market at present.[20] Daptomycin's mechanism of action involves the calcium-dependent insertion of the compound into the bacterial cytoplasmic membrane. Solving the structure of daptomycin (figure 2) with and without calcium ions has permitted a better understanding of this process.[21] Calcium binding between two of the aspartate residues on daptomycin decreases its net negative charge and increases the area of its hydrophobic surface, permitting it to interact better with membranes. In addition, calcium ions promote deeper insertion of daptomycin into the membrane by bridging the residual negatively charged aminoacids on daptomycin and the negatively charged phospholipids that are typically found in the cytoplasmic membrane of Gram-positive bacteria. The actual mechanism of bacterial killing subsequent to deep insertion into the cytoplasmic membrane is somewhat more controversial.

Daptomycin has been shown to cause ion movements across cell membranes (as evidenced by the induction of potassium efflux in *S aureus* and *Bacillus megaterium*)[22] and to interact in a calcium-dependent fashion with planar bilayer membranes[23] and phospholipid vesicles.[24] Thus, the effects of daptomycin have been proposed to result from a calcium-dependent action on the cytoplasmic membrane that dissipates the trans-membrane electrical potential gradient, a phenomenon termed depolarisation.[25] The maintenance of an appropriately energised cytoplasmic membrane is fundamental to the survival and growth of bacterial cells,[26] but depolarisation is not in itself a lethal action: valinomycin, which causes depolarisation in the presence of potassium ions, is bacteriostatic. However, in the absence of a proton motive force, the main component of which is the transmembrane electrical potential gradient, cells cannot synthesise ATP or take up the nutrients needed for growth. The collapse of the electrochemical gradient may explain the disparate effects produced by daptomycin (eg, inhibition of protein, RNA, DNA, peptidoglycan, lipoteichoic acid, and lipid biosynthesis) in *S aureus*,[27] or these events may be independent consequences of daptomycin action.

My colleagues and I demonstrated that, in non-growing cells, cytoplasmic membrane depolarisation occurred after cell death, indicating that under these conditions it may not be the direct cause of cell death.[21] Thus, the mechanism of action of daptomycin may involve multiple activities. Included among these would be effects on membrane integrity, rapid inhibition of protein, DNA, and RNA synthesis, and inhibition of lipoteichoic acid synthesis. Unlike cell-wall active agents, daptomycin causes rapid bactericidal activity without cell lysis.[28] Scanning electron micrographs of daptomycin-treated, killed MSSA show ultrastructural changes, or blebs, on

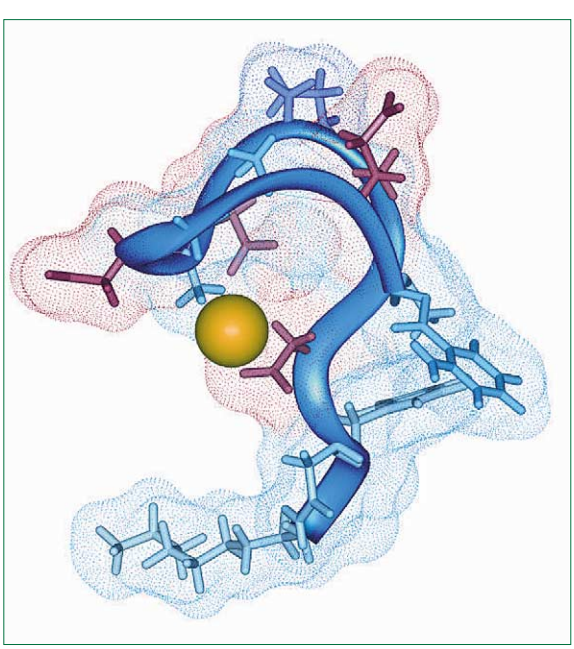

Figure 2: **Structure of daptomycin (backbone ribbon representation) with a bound calcium ion.**
The daptomycin structure was based on two-dimensional nuclear magnetic resonance and modelling of the best fit structures in Insight II version 97.2.[21] Hydrophilic, negatively charged side-chains are shown in red; hydrophobic side-chains, including the fatty acid chain, are shown in blue. The binding of the calcium ion (gold) causes a conformational shift such that daptomycin becomes more amphipathic and thus able to insert into the membrane.

the cell surface of otherwise intact bacterial cells. After exposure to daptomycin 8 μg/mL (four times the minimum inhibitory concentration [MIC]) for 1 hour, the surface of many bacteria appeared roughened with occasional protuberances, and after 4 hours most of the bacteria exhibited these processes.[29] Bacterial killing without lysis provides the advantage of a lower potential for sepsis and generally less inflammation by reducing the release of bacterial molecules—eg, teichoic acids, lipoteichoic acids, peptidoglycan, and bacterial DNA—that trigger such responses.[30]

An interesting property of daptomycin that seems to arise from its unique action is that, unlike most antibiotics that target only growing cells (eg, beta-lactams), daptomycin is effective at all growth phases, including the stationary phase. This property may be particularly useful in the treatment of indolent, deep-seated infections, such as endocarditis and osteomyelitis, in which bacteria spend a substantial amount of time in the stationary phase. Time-kill studies in an in-vitro model of simulated endocardial vegetations, using high bacterial inocula, permitted the comparison of the activity of daptomycin, nafcillin, linezolid, and vancomycin against MSSA. At high inoculum (10^9 colony-forming units/g), only daptomycin was bactericidal.[31]

Daptomycin is effective against clinically important Gram-positive organisms and may have the ability to circumvent existing resistance mechanisms.[9] In-vitro studies attempting to generate spontaneous daptomycin-resistant mutants of *S aureus, Staphylococcus epidermidis, E faecalis*, and *E faecium* have proven unsuccessful.[32] This lack of success may stem from the distinct mechanism of action of daptomycin in targeting the bacterial cytoplasmic membrane and subsequently action in a complex, multipronged fashion.[26,32] Thus, resistance should be slower to develop for this antibiotic class. The unique mechanism of action of this class may also help to avoid the development of cross-resistance that has plagued other antibiotic classes.

Cationic antimicrobial peptides

Cationic peptides are ubiquitous in nature, with more than 700 known natural peptides in all species, and are a component of the first line of defence against infectious agents. These agents, in addition to their antimicrobial activity, are able to modulate immune responses, including the boosting of innate immunity and the suppression of inflammatory responses/endotoxaemia.[33] Such peptides are, at present, in advanced development as topical agents for the prevention of catheter colonisation and as anti-acne drugs. There is a great heterogeneity in the secondary structures of these peptides but they fold in three dimensions into amphipathic molecules—with a hydrophobic face and a positively charged face—when they interact with, and insert into, membranes. The mechanism by which these peptides kill bacteria is obligately linked to the interaction with the cytoplasmic membrane of Gram-positive bacteria.[34] However, their killing mechanism is more complex than just membrane disruption. Indeed, one set of studies concluded that the mechanism of action of peptides on Gram-positive bacteria followed a multiple-hit model with several potential targets, including cell division, macromolecular synthesis, the cell wall, and the cytoplasmic membrane.[35,36]

Ketolides

Ketolides are semisynthetic derivatives of the first macrolide, erythromycin. They have a 14-membered macrolactone ring, but have a keto group instead of an L-cladinose sugar appended at position 3 (figure 3). In addition, hydroxyl groups in positions 11 and 12 are replaced by a cyclic carbamate. Telithromycin has an alkyl-aryl extension that is bound to its cyclic carbamate, whereas ABT-773 (cethromycin) has a quinolylallyl arm at the O-6 position. The crystal structure of telithromycin bound to the large ribosomal subunit of *Deinococcus radiodurans* indicated that telithromycin interacts with domain V (via the 3-keto group and additional hydrophobic interactions) and domain II (via the carbamate extension) of the 23S rRNA.[37] Domain V is the peptidyl transferase centre that catalyses peptide bond formation. Telithromycin blocks the ribosomal exit tunnel, thus terminating peptide synthesis. Cethromycin binds in a similar way. Although ketolides bind to a similar region of the 50S ribosomal subunit as does erythromycin, they tend to have substantially higher binding affinity and thus can still bind to erythromycin-resistant ribosomes.

The modification of the parent macrolide molecule leads to increased potency against many Gram-positive bacteria, particularly those that have acquired resistance to macrolides—eg, the ketolides have reasonable activity against macrolide-resistant *S pneumoniae*. Because they have activity against many Gram-positive organisms and some Gram-negative respiratory pathogens, they are often used for respiratory-tract infections, including CAP, acute exacerbations of chronic bronchitis, and sinusitis, as well as streptococcal pharyngitis. However they are not useful in treating MRSA or resistant *Enterococcus* spp.

Glycylcyclines: tigecycline

Tigecycline (9-[*t*-butylglycylamido]-minocycline) is a broad-spectrum glycylcycline derivative, structurally related to the tetracyclines, and is efficacious against highly resistant Gram-positive bacteria,[38] including MRSA and penicillin-resistant *S pneumoniae*, as well as having activity against a variety of Gram-negative and anaerobic pathogens. However, the compound is less active against clinically problematic Gram-negative opportunistic pathogens, such as *Pseudomonas aeruginosa* and *Proteus mirabilis*. By probing 70S ribosomes of *Escherichia coli* with the chemical modifying agent dimethyl sulfate and

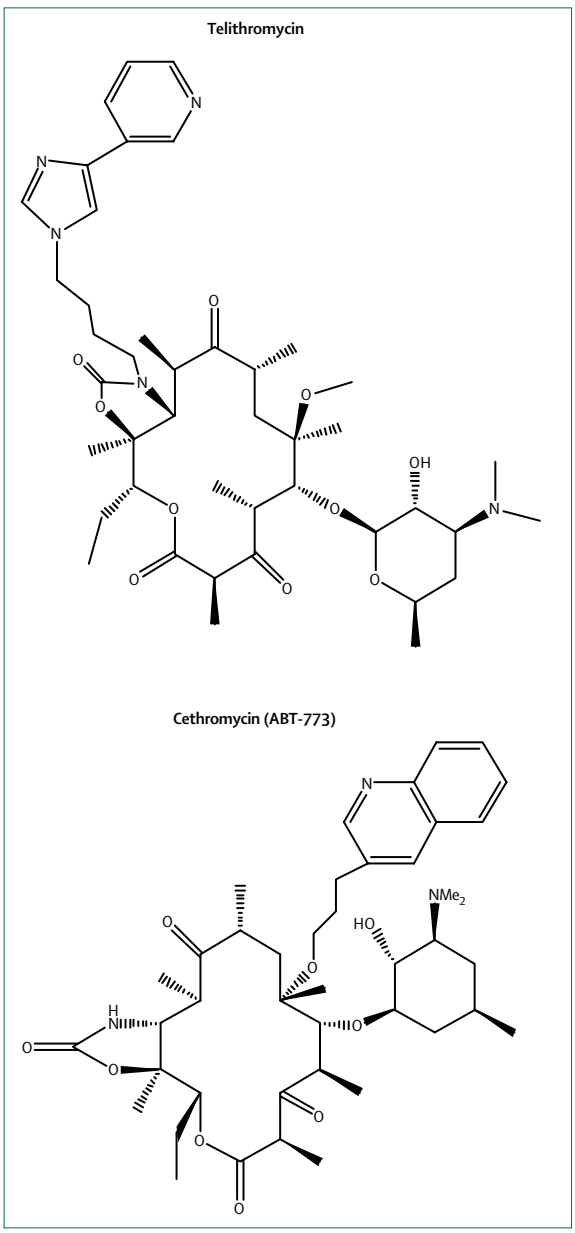

Figure 3: **Structures of the ketolides telithromycin and cethromycin.**

assessing Fe^{2+}-mediated cleavage by the iron-substituted forms of tetracycline and tigecycline, it was determined that these antibiotics share identical or overlapping binding sites on ribosomes, and thus probably have the same basic mode of action.[39] Tetracycline inhibits the binding of incoming aminoacyl transfer RNAs to the ribosomal acceptor site, thus interrupting the elongation step of protein synthesis. Crystal structure data show that there are as many as six tetracycline-binding sites on the 30S subunit of the ribosome.[40] Of these sites, one is composed of a binding pocket formed by helices 31 and 34 of the 16S rRNA, and exhibits the highest degree of tetracycline occupancy.

Oritavancin and dalbavancin

Oritavancin (LY333328) is a glycopeptide with a biphenyl side-chain. Studies show that this antibiotic, and related alkyl glycopeptides, inhibits bacterial cell-wall formation by blocking the transglycosylation step in peptidoglycan biosynthesis by direct interaction with the terminal D-Ala–D-Ala dipeptidyl residues of peptidoglycan precursors.[41] However, unlike vancomycin, oritavancin dimerises strongly and can anchor to the cytoplasmic membrane, by virtue of its alkyl side-chain. Cooperative interactions derived from dimerisation and membrane anchoring permit this antibiotic to bind to the terminal peptidoglycan residues of both vancomycin-susceptible (dipeptide, D-Ala–D-Ala) and vancomycin-resistant (didepsipeptide, D-Ala–D-Lac) enterococci, extending the spectrum of this antibiotic to VREF, as well as having excellent activity versus MRSA.

Another glycopeptide with activity against resistant organisms is dalbavancin. Dalbavancin is a semisynthetic derivative of the teichoplanin-like glycopeptide A40926, and shows potent activity against *S aureus*, including MRSA, as well as resistant Gram-positive species, including certain vancomycin resistant and intermediate resistant isolates.[42] Dalbavancin has a long half-life (9–12 days), and offers the opportunity of a once-weekly dosing regimen.

Ramoplanin

Ramoplanin is a lipoglycodepsipeptide antibiotic that is highly active against most Gram-positive bacteria, including MRSA, VREF, and strains resistant to ampicillin and erythromycin. Ramoplanin arrests cell-wall biosynthesis at a late stage in peptidoglycan biosynthesis—at a step where the enzyme MurG catalyses the conversion of an undecaprenyl-linked intermediate (lipid I, the target of glycopeptides) to lipid II.[43] Since ramoplanin alters the chromatographic migration profiles of both lipid I and lipid II, this antibiotic might also interfere with transglycosylation by capturing lipid II, as confirmed directly.[44]

Resistance mechanisms against the new antibiotics

Ozazoladinones: linezolid

With a very low spontaneous resistance rate (less than 1×10^{-9}) it is difficult to induce in-vitro resistance to linezolid,[45] and full activity is retained against Gram-positive cocci resistant to other antibiotics, including MRSA and VREF.[46] However, production of linezolid-resistant *S aureus* and *E faecalis* mutants is possible by serial passage on sub-MIC levels of linezolid, using spiral gradient plates.[45] The specific point mutations causing resistance in these Gram-positive pathogens have been mapped to several different locations in domain V of the 23S rRNA of the 50S ribosomal subunit.[47–49] Studies of resistant Gram-positive clinical isolates showed similar mutations in the 23S rRNA.[46,50,51] However, oxazolidinone

resistance based on inactivation has not been demonstrated in any bacterial species examined. Most organisms have multiple copies of the genes for rRNA species—eg, there are usually six copies in *E faecium* and four copies in *E faecalis*. By pyrosequencing each of these copies in mutants, a good correlation was evident between linezolid MICs in *Enterococcus* spp and the number of 23S rRNA gene copies carrying the G2576T mutation.[52] Indeed, MICs ranged from 2–4 μg/mL for organisms with no resistant rRNA gene allele, to 32–128 μg/mL for organisms with more than half of the rRNA gene copies carrying a resistance mutation. Therefore, higher levels of linezolid resistance may be achieved over time by recombination between resistant and susceptible alleles within the organism.

Reports to date indicate that most of the people who developed linezolid-resistant infections during therapy had indwelling prosthetic devices and were receiving extended antibiotic therapy.[50] *E faecium* is usually the organism that develops resistance in this setting, but a single clinical isolate of linezolid-resistant MRSA was recovered from a patient treated with linezolid for dialysis-associated peritonitis.[50] Linezolid-resistant VREF strains have been isolated from seven people at a liver, kidney, and pancreas transplantation unit.[52] During 2001–2002, eight resistant strains were identified by reference broth microdilution methods as arising in bloodstream, respiratory, skin and soft tissue, and urinary-tract infection isolates of *E faecalis*, *E faecium*, *S epidermidis*, and *Streptococcus oralis*.[53]

Streptogramins: quinupristin/dalfopristin

The potential of quinupristin/dalfopristin to select for resistant strains of bacteria may be reduced because its mode of action involves the synergistic action of two structurally unrelated compounds.[54] Indeed, overall resistance appears to occur at quite modest levels. However, acquired resistance to one or the other component by target modification, enzymatic degradation, and active efflux of the drug has been observed.[54] This resistance may be due in part to the widespread use of virginiamycin in agriculture. Resistance to the quinupristin component is mediated by 23S rRNA target methylation by members of the erythromycin-resistance methylase (*erm*) gene class, producing a macrolide–lincosamide–streptogramin B resistance (MLS_B) phenotype.[54] However, quinupristin/dalfopristin retains bacteriostatic action against MLS_B strains.[55,56] Quinupristin resistance in staphylococci can also be rarely achieved by linearisation of the hexadepsipeptide ring by a specific plasmid-mediated lyase.[57]

Resistance to the dalfopristin component alone is sufficient to dramatically reduce efficacy of the combined antibiotic. Enzymatic inactivation of dalfopristin can occur because of the plasmid-mediated dissemination of genes encoding a series of virginiamycin acetyltransferases. These enzymes use acetyl-coenzyme A to acetylate the sole hydroxyl group of dalfopristin.[58] Plasmid-mediated acetyltransferases confer resistance to streptogramins and threaten to limit the medical utility of the quinupristin/dalfopristin combination.[58]

Quinupristin/dalfopristin is almost always inactive against *E faecalis* because an efflux pump conferring resistance to dalfopristin seems to be intrinsic in this species.[59] However, most isolates of VREF are susceptible to the antibiotic.[54] Resistance to quinupristin/dalfopristin developed in five of 338 people with VREF infection, four of whom had therapy failure.[60] There is a low frequency (1–10%) of resistance in *S epidermidis* due to the VgaA/VgaB ATP-binding-cassette efflux proteins, although MICs to quinupristin/dalfopristin are only increased by four-fold to 1 μg/mL. There have been case reports of quinupristin/dalfopristin resistance in people with *S aureus*[61] and VREF bacteraemia.[62]

Lipopeptides: daptomycin

Spontaneous acquisition of resistance to daptomycin is rare in Gram-positive organisms.[20] No spontaneously resistant mutants have been found for any bacteria when challenged at concentrations eight-fold above the MIC.[20] Furthermore, in an in-vitro resistance study, there were no resistant isolates detected when multiple clinical and laboratory isolates were tested with detection limits of less than 10^{-10} for *S aureus*, and 10^{-9} for *S epidermidis*, *E faecalis*, and *E faecium*.[32] Thus, despite multiple passages of these pathogens in liquid media and following chemical mutagenesis, no substantial increase in resistance was observed.

The daptomycin MICs for the least-susceptible isolates are eight–32-fold higher than for most isolates.[32] Population analysis demonstrated that bacterial susceptibility to daptomycin was heterogeneous. In-vivo studies testing selected mutants for virulence in an acute murine intra-peritoneal infection model indicated that some daptomycin-resistant mutants were substantially less virulent. At least two different classes of mutant isolates were discovered: some isolates grew at normal rates and were virulent in a mouse infection model, whereas other isolates had substantial growth defects in vitro. The low spontaneous resistance rates, limited increases in MICs during serial passage, and ease of treatment of resistant isolates indicate that infections with some daptomycin-resistant organisms may still be easily treated.

Development of resistance is unlikely when therapeutically effective serum concentrations of daptomycin are maintained.[63] Indeed, emergence of resistance in clinical trials of daptomycin was rare, occurring in only two of more than 1000 infected people treated with daptomycin during phase II and III clinical trials, a rate of less than 0·2%.[64] In one case, a resistant *S aureus* strain was isolated from a patient in a phase II study who received daptomycin at a dose lower than specified by the protocol for the first 5 days of therapy. In the second case, a resistant *E faecalis* isolate was recovered

from a patient enrolled in a salvage trial with an infected chronic decubitus ulcer.

Other agents

Ketolides, such as telithromycin, were developed for their activity against resistant Gram-positive organisms.[65] They do not induce MLS_B resistance, are active against most *erm*-carrying Gram-positive cocci, and maintain activity against efflux mutants. However, a point mutation (U754A) in hairpin 35 of domain II of the 23S rRNA can give rise to resistance to lower concentrations of teilithromycin, and other mutants are starting to be observed in several bacteria.

Making cationic peptide-resistant mutants in the laboratory is difficult, but, in my experience, not impossible. However, the paucity of clinical experience with these agents, and other agents mentioned, render any discussion of resistance premature.

Factors contributing to resistance development

Failure to use narrow-spectrum drugs when able

The widespread and often inappropriate use of broad-spectrum antibiotics in the outpatient setting is recognised as an important contributing factor to the spread of resistance. Optimal and judicious selection of antibiotics for the therapy of infectious diseases requires clinical judgment and detailed knowledge of pharmacological and microbiological factors. When the infecting microorganism has been identified, it seems appropriate to institute definitive antibiotic therapy with a narrow-spectrum, low toxicity agent as an antiresistance measure.

Several investigations indicate that some infections, such as CAP and urinary-tract infections, can usually be successfully treated with narrow-spectrum antibiotics, especially if the infections are not life threatening.[66–68] Likewise, the avoidance of broad-spectrum antibiotics (eg, cephalosporins) and the re-introduction of narrower-spectrum drugs (eg, penicillin, trimethoprim, and gentamicin), when combined with infection-control practices, have been effective in reducing the occurrence of *Clostridium difficile* infections.[69] Reductions in antibiotic resistance have been associated with hospital-instituted programmes aimed at combining judicious overall use of antibiotics with the use of narrow-spectrum antibiotics.[70,71]

A meta-analysis of randomised clinical trials of people with acute sinusitis showed that in two-thirds of the cases there was spontaneous improvement or cure without antibiotic treatment.[72] Furthermore, reductions in the rates of antimicrobial resistance have been clearly demonstrated after policy changes or other interventions leading to reduced rates of antibiotic usage.[71, 73]

Colonisation pressure in hospitals

The risk of acquisition of a particular infection as a function of the proportion of people colonised has been called "colonisation pressure", and has been described as a major variable affecting the spread of VREF[74] and MRSA.[75] The widespread adoption of antibiotic-control measures and promotion of strict adherence to infection-control procedures are necessary to prevent the colonisation pressure observed in hospitals, especially intensive care units (ICUs).[76] Quantitative analysis of VREF transmission in an ICU indicates that staffing levels have a critical role in transmission, and that a productive alliance between patients and staff is a very effective means of decreasing transmission, such that the level of adherence to hand hygiene is an inverse function of the endemic level of VREF colonisation.[77]

Although alcohol-based hand rubs seem to be promising as hand-disinfectant agents, maintaining compliance may require continuous educational reinforcement, monitoring, and feedback to health-care workers.[78] With such aggressive operations, hand-hygiene rates of 60–80% can be accomplished.[78] Whether this measure is sufficient is open to question. For uncommon pathogens that may colonise or infect only a small percentage of patients, indirect patient-to-patient cross transmission via the hands of health-care workers may be effectively interrupted by high compliance rates. However, when colonisation pressure is greater because of a large number of colonised patients, such measures may not be sufficient. For example, when 30–50% of patients are colonised with VREF, even occasional interruptions in hand hygiene may be sufficient to sustain cross transmission.[74,77] An alternative approach to the colonisation pressure problem is to encourage the use of disposable examination gloves during contacts with patients and their environment.[78,79]

Length of hospital and ICU stays

Prolonged length of hospital stay appears to predispose people to infection with antibiotic-resistant bacteria.[74,80] This predisposition may result, in part, from the greater likelihood over time of becoming colonised with such bacteria or the generally poorer underlying immune status of the most seriously ill patients. In addition, the use of invasive devices, such as endotracheal tubes, intravascular catheters, and urinary catheters, seems to encourage such infections.[81,82] The rising presence of antibiotic-resistant infections among people in long-term treatment facilities can also be an important source for the entry of resistant bacteria into the ICU.[83] Furthermore, outbreaks of antibiotic-resistant bacterial infections resulting from inadequate infection-control practices, failure to recognise the presence of antibiotic resistance, or use of contaminated equipment are also key factors promoting the spread of resistance.[84–86] A reduction in the duration of mechanical ventilation could decrease the incidence of ventilator-associated pneumonia and consequently reduce the length of hospital or ICU stay.[80] Formalised weaning protocols for patients requiring mechanical ventilation have been shown to reduce the duration of mechanical ventilation and the length of ICU stay.[87]

Search strategy and selection criteria

Data for this review were identified by searches of Medline and the references from current articles. Search terms included "Gram-positive", the names of the major antibiotic-resistant Gram-positive bacteria, including "*Staphylococcus aureus*", "*Enterococcus* spp", and "*Streptococcus pneumoniae*", paired with the words "antibiotic", "therapy", or "antibiotic resistance". For the newer agents, "quinupristin", "dalfopristin", "Synercid", "linezolid", "daptomycin", etc, were used as search terms.

Antibiotic misuse in agriculture

One of the most fundamental measures that could be taken to minimise antibiotic resistance is to eliminate supplementation of animal feeds with antibiotics, including tetracycline, macrolide, and quinolone derivatives.[2] Resistant strains arising from this source can enter the human population through infection of farm workers, contamination of the ground water, or consumption of colonised animal and poultry products. For years far greater quantities of the glycopeptide avoparcin were used in European commercial animal husbandry than of the related vancomycin in human beings. Because strains resistant to avoparcin are cross-resistant to vancomycin, it is thought that avoparcin usage provided the selective pressure that permitted the development of vancomycin-resistant enterococci that are now found in hospitals,[87] and the belated banning of avoparcin in Europe may be too late.

Virginiamycin was also used as a feed additive in commercial animal husbandry in Europe for more than 20 years, creating reservoirs of streptogramin-resistant *E faecium* (SREF)[88] that are cross-resistant to quinupristin/dalfopristin. In Germany in 1998–1999, SREF could be isolated from the waste water of sewage treatment plants, from faecal samples and meat products of animals that were fed virginiamycin, from human stools in the community, and from clinical samples. These isolations of SREF occurred before quinupristin/dalfopristin was introduced for therapeutic purposes in German hospitals in May 2000. Thus, streptogramin resistance possibly originated from sources outside of the hospital setting.

Many other examples exist, and farm practices involving the use of antibiotics as feed additives and prophylactics should be carefully reviewed to eliminate the use of those agents that give rise to cross-resistance to antibiotics used in human medicine. Some countries have banned feed additive use and the WHO has recommended the discontinuation of the use of antibiotics as growth promoters because of the evidence of health risks in human beings.[89] Surveillance to give early warning of emerging problems would allow more time to evaluate prevention and control. Better education of practitioners, both in the community and in hospitals, and the phasing out of the use of antibiotics in animal husbandry and agriculture would be important steps towards limiting resistance.

The FAAIR initiative

The aim of the Facts about Antibiotics in Animals and Their Impact on Resistance (FAAIR) initiative, developed by the Alliance for the Prudent Use of Antibiotics (APUA), is to introduce scientific evidence to the policy debate on antimicrobial use in agriculture and the risk it poses to human, animal, and ecological health.[90] APUA convened an expert scientific advisory panel from a variety of fields in research and medicine. Panel members analysed relevant data from the scientific literature and developed consensus conclusions and policy recommendations. The committee concluded that the elimination of non-therapeutic use of antimicrobials in food animals and in agriculture would lower the burden of antibiotic resistance in the environment, with consequent benefits to human and animal health.[91] All uses of antimicrobials in animals, agriculture, and human beings contribute to the global pool of antimicrobial-resistance genes in the environment.

Conclusions

The use of antibiotics by physicians in hospitals and elsewhere requires an acute awareness of the increasing problems with resistant organisms. This awareness is especially important given the limited availability of fundamentally new antibiotics. Thus, unnecessary use of an antibiotic has public-health implications. Such use may serve to select for resistant organisms that may be carried to other, more vulnerable patients, and produce serious, difficult-to-treat infections. Antibiotic-control programmes can be an effective means to prevent inappropriate use of antibiotics in hospitals. Newer antibiotics should be included in such programmes to delay the emergence of resistant strains by limiting unnecessary use of such drugs.

Conflicts of interest

I have, in the past, received funding from Cubist for work on daptomycin, and am currently a member of the scientific advisory board of Vicuron Pharmaceuticals.

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