Evolving Resistance Among Gram-positive Pathogens

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Antimicrobial therapy is a key component of modern medical practice and a cornerstone for the development of complex clinical interventions in critically ill patients. Unfortunately, the increasing problem of antimicrobial resistance is now recognized as a major public health threat jeopardizing the care of thousands of patients worldwide. Gram-positive pathogens exhibit an immense genetic repertoire to adapt and develop resistance to virtually all antimicrobials clinically available. As more molecules become available to treat resistant gram-positive infections, resistance emerges as an evolutionary response. Thus, antimicrobial resistance has to be envisaged as an evolving phenomenon that demands constant surveillance and continuous efforts to identify emerging mechanisms of resistance to optimize the use of antibiotics and create strategies to circumvent this problem. Here, we will provide a broad perspective on the clinical aspects of antibiotic resistance in relevant gram-positive pathogens with emphasis on the mechanistic strategies used by these organisms to avoid being killed by commonly used antimicrobial agents.

**Keywords.** antimicrobial resistance; multidrug-resistant; methicillin-resistant; vancomycin-resistant; penicillin-resistant.

The discovery and commercialization of antimicrobials revolutionized modern medicine and became a major tool for the development of complex medical interventions, such as cancer therapy, organ transplantation, and management of the critically ill. Unfortunately, antimicrobial resistance is now threatening the care of such patients. Indeed, infections caused by multidrug-resistant (MDR) organisms are associated with significant mortality and carry an important economic burden, estimated at >$20 billion per year, just in the United States [1]. Furthermore, the World Health Organization has identified antimicrobial resistance as one of the most serious health threats worldwide and suggested that failure to tackle it could jeopardize modern medical achievements [2].

MDR gram-positive organisms are major human pathogens, causing both healthcare- and community-associated infections. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and drug-resistant *Streptococcus pneumoniae* have been designated as serious public threats by the US Centers for Disease Control and Prevention [1]. Indeed, MRSA and VRE are leading causes of healthcare-associated infections in the United States, with conservative estimates suggesting they cause >12,000 deaths per year. Similarly, infections due to drug-resistant *S. pneumoniae*, the main cause of bacterial pneumonia and meningitis in adults, are estimated to cause 19,000 excess admissions and 7000 deaths per year in the United States alone [1].

The genetic and biochemical bases of antimicrobial resistance in gram-positive bacteria are diverse and they frequently differ within genera and/or species. Further, evolution of bacterial resistance is tightly
influenced by the presence of environmental stressors, among which the use (and misuse) of antimicrobials is thought to play a major role. For instance, exposure of *S. pneumoniae* to antibiotics has been shown to activate the transcription of a gene regulon that results in increased genetic exchange with co-colonizing organisms and the subsequent development of resistant strains [3]. In this article, we will focus on the mechanisms of resistance in clinically important gram-positive bacteria, with emphasis on the most relevant antimicrobials used to manage severe infections caused by these organisms.

**β-LACTAM RESISTANCE**

β-lactams disrupt cell-wall synthesis by mostly inhibiting transpeptidase activity of penicillin-binding proteins (PBPs) that cross-link the nascent peptidoglycan. Penicillin resistance was described shortly after penicillin discovery, prompting the search for novel and more potent β-lactams. However, the discovery of each new β-lactam compound has been consistently followed by emergence of resistance, highlighting that antimicrobial pressure is a major driving force of bacterial evolution. There are 2 main mechanisms of β-lactam resistance in gram-positive pathogens, enzymatic degradation through the production of β-lactamases and decreased affinity of the antibiotic for its target, usually either by acquisition of exogenous DNA encoding a low-affinity PBP or through changes in the native *pbp* genes (Table 1).

**β-Lactam Resistance in *S. aureus***

Emergence and dissemination of β-lactam resistance in *S. aureus* occurred in several epidemic waves that could be summarized by (1) acquisition of a plasmid-encoded penicillinase in the 1950s (particularly the clone known as phage type 80/81), (2) a second wave that started after the introduction of methicillin (1959) and was characterized by the emergence of hospital-associated MRSA isolates that mainly circulated in European hospitals until the 1970s, (3) the development of several highly successful hospital-associated MRSA clones that spread in different parts of the world, and continue to circulate (third wave), and (4) the last wave of MRSA resistance to date, characterized by the emergence and dissemination of community-associated MRSA in the 1990s [4]. Nowadays, most *S. aureus* are penicillin-resistant through the production of a plasmid-borne β-lactamase (4 types are known, including A, B, C, and D).

Although the anti-staphylococcal penicillins (eg, nafcillin) are stable against these enzymes, staphylococcal-type A β-lactamase has higher rates of cefazolin hydrolysis, and some methicillin-susceptible *S. aureus* (MSSA) isolates harboring this enzyme exhibit the so-called cefazolin inoculum effect. This phenomenon involves increased minimum inhibitory concentrations (MICs) to cefazolin when susceptibility testing is performed using a higher inoculum (10^7 colony-forming units/mL) compared with the standard inoculum (10^5 colony-forming units/mL). Although the clinical relevance of this phenomenon is still controversial, some evidence suggests that this phenotype may have important clinical implications in high-inoculum infections, such as infective endocarditis [5–7].

The mechanism of methicillin resistance relies on acquisition of the staphylococcal chromosomal cassette *mec* (SCCmec) containing *meca*, which encodes PBP2a, a transpeptidase with low affinity for all β-lactams (except for last-generation cephalosporins). Expression of *meca* is tightly regulated and it often requires induction by a β-lactam. There are a number of different variants of the SCCmec DNA; however, in general, shorter elements that lack resistance determinants to other antimicrobials are present in community-associated MRSA (SCCmec IV or V) [8].

Cefaroline and ceftepiboprole are new additions to the β-lactam family with potent activity against MRSA because of their enhanced affinity for PBP2a. Cefaroline resistance (MIC ≥4 μg/mL) in clinical MRSA is uncommon to date, but reports of isolates with MICs between 4 and 8 μg/mL have been published [9–11], although the mechanisms leading to this phenotype are unclear. During 2014, an MRSA isolate with a high cefaroline MIC (>32 μg/mL; ST5) was recovered from the bloodstream of a patient previously treated with cefaroline in Houston, Texas. The mechanism of resistance was associated with 2 contiguous substitutions (Y446N and E447K) in the penicillin-binding pocket of the transpeptidase domain of PBP2a [12]. Interestingly, the E447K substitution had been previously selected in vitro by ceftepiboprole passage [13].

**β-Lactam Resistance in Enterococci***

Treatment of enterococcal infections has long been considered a therapeutic challenge. Indeed, the MICs of penicillin and ampicillin (the most active anti-enterococcal β-lactams) are much higher than for other common gram-positive organisms, probably owing to the expression of lower affinity PBPs (eg, PBP5), and enterococcal isolates are often tolerant to β-lactam antibiotics (ie, lack of bactericidal activity in vitro), complicating the treatment of serious infections [14]. Furthermore, high-level resistance to ampicillin (MICs ≥64 μg/mL) is a hallmark of healthcare-associated MDR isolates of *E. faecium*. Conversely, ampicillin resistance continues to be strikingly uncommon in *Enterococcus faecalis* isolates.

The mechanisms of high-level ampicillin resistance in enterococci are not completely understood but they have been most consistently associated with changes in the *pbp5* gene, encoding a PBP with low affinity for β-lactams [15]. A variety of mutations have been described and although the individual contribution to resistance of each genetic change is unclear, some of them (eg, M485A plus insertion of Ser466) have been directly linked to the resistance phenotype [16]. In addition, studies on...
sequence variation of the \( \text{pbp5} \) allele identified specific differences between ampicillin-susceptible and ampicillin-resistant isolates (harboring \( \text{pbp5-S} \) and \( \text{pbp5-R} \) alleles, respectively), suggesting evolutionary adaptation [17]. Indeed, \( \text{PBP5-R} \) and \( \text{PBP5-S} \) sequences differ in approximately 5%, at the amino acid level, although no particular change has been consistently described in enterococcal isolates. Initially found in \( \text{E. faecalis} \) in the early 1980s, the frequency of this mechanism has remained low over the years and has been described in only a few \( \text{E. faecium} \) isolates [18].

**\( \beta \)-Lactam Resistance in Pneumococci**

\( \text{Streptococcus pneumoniae} \) is naturally susceptible to most antimicrobials, including penicillins, long considered the first-line therapy for pneumococcal infections. Current Clinical and Laboratory Standards Institute break points for parenteral penicillin establish that isolates not causing meningitis are categorized as susceptible, intermediate, or resistant, with MICs of \( \leq 2, 4, \)
and ≥8 μg/mL, respectively [19]. In contrast, meningitis isolates are considered penicillin susceptible (MIC ≤0.06 μg/mL) or resistant (≥0.12 μg/mL) [19]. The first significant outbreak of penicillin-resistant S. pneumoniae was reported in the 1970s and, since then, their frequency has steadily increased worldwide [20]. Prevalences of β-lactam-resistant pneumococci vary widely within different regions and are largely shaped by the use patterns of conjugated pneumococcal vaccines [21].

The main mechanism of pneumococcal β-lactam resistance is through changes in the native PBPs by recombination with exogenous pBP genes, in a process that relies on the pneumococcal ability to incorporate naked DNA from the environment (transformation). Point mutations in pBP genes also play a role and may act synergistically to further increase the MICs. Recombinant PBPs of resistant isolates are highly variable and classically show mosaic blocks that derive from the recombination with external pBP genes (mostly from Streptococcus mitis and Streptococcus oralis) [22, 23]. Strikingly, the magnitude of the recombination can be such that it may change the serotype of the isolate. Mosaicism in PBP2x and PBP2b is the most frequent change associated with β-lactam resistance. Moreover, mutational changes in PBP1a can result in high-level resistance when they occur in the background of low-affinity mosaic PBP2x or PBP2b [24]. Alterations in the remaining PBPs have been occasionally related to resistance, but their frequency is lower. Finally, mutations in other genes, such as murM (involved in the biosynthesis of the murein), pdgA (encoding a GlcNAc deacetylase), and cpoA (that codes for a glycosyltransferase) have also been uncommonly associated with β-lactam resistance in pneumococci [24].

GLYCOPEPETIDE RESISTANCE

Glycopeptides prevent cross-linking of peptidoglycan by binding to the terminal D-alanine-D-alanine (D-Ala-D-Ala) of peptidoglycan precursors, inhibiting cell-wall synthesis. Vancomycin was first approved for clinical use in 1958, and for many years has been the “workhorse” antibiotic for the treatment of MRSA infections. Despite its heavy use, it took >40 years for the first clinical isolates with high-level resistance to vancomycin to emerge [25].

Vancomycin-Resistant Enterococci

VRE isolates were first described as community commensals in 1986 in Europe, and were associated with the use of avoparcin, a glycopeptide used in animal husbandry. Consequently, avoparcin use was banned, and the frequency of VRE decreased. Later, VRE emerged in the United States (where avoparcin was never approved), mainly in clinical isolates recovered from the hospital environment. Since then, VRE prevalence in the United States has steadily increased, becoming one of the most challenging causes of healthcare-associated infections. Importantly, there are considerable interspecies differences, with most vancomycin resistance occurring in E. faecium isolates [26]. The successful spread of E. faecium has been tracked to the dissemination of a hospital-associated genetic clade that is often MDR, also harboring ampicillin and high-level aminoglycoside resistance determinants [27].

The mechanism of VRE is acquisition of van gene clusters, probably from environmental organisms. The most frequent gene cluster is vanA, usually located in a Tn3-family transposon (Tn1546) that has been found in conjugative and nonconjugative plasmids. In addition, 8 other van clusters (vanBC-DEGLMN) have been characterized in enterococcal isolates. These gene clusters encode a complex enzymatic machinery that modifies the terminal D-Ala-D-Ala termini of peptidoglycan precursors and destroys the “normal” D-Ala-D-Ala ending precursors. Most frequently, D-Ala-D-Ala is replaced for D-Ala-D-lactate, decreasing the affinity of vancomycin for its target by approximately 1000-fold. D-Ala-D-Ala can also be replaced for D-Ala-D-serine, which affects the affinity of vancomycin for its target to a lesser degree (resulting in relatively low-level resistance, with MICs of 4–32 μg/mL).

In general, the van clusters harbor 3 classes of genes: those encoding a 2-component regulatory system that tightly controls the expression of the resistance genes, those involved in the biochemical pathway for synthesis of D-lactate or D-serine, and those involved in the destruction of normal D-Ala-D-Ala-ending peptidoglycan precursors, encoding proteins with dipeptidase and carboxypeptidase activities. The biochemical pathways of vancomycin resistance in enterococci have been extensively described [28], and a detailed explanation is beyond the scope of this review.

Vancomycin Resistance in Staphylococci

Reduced vancomycin susceptibility in staphylococci can be categorized by 2 distinct phenotypes: (1) most commonly, isolates with intermediate susceptibility to vancomycin (vancomycin-intermediate S. aureus [VISA]; MIC, 4–8 μg/mL), and (2) rarely, high-level resistance to vancomycin (vancomycin-resistant S. aureus [VRSA]; MIC ≥16 μg/mL) [29]. The first VISA isolate (Mu50), reported in 1997, was derived from a vancomycin-susceptible strain (Mu3), later found to have a small subpopulation with MICs >2 μg/mL, a condition now designated heterogeneous VISA or hetero-VISA (hVISA). The hVISA/VISA phenotype usually arises in vivo in patients with invasive staphylococcal infections who received a prolonged course of vancomycin [30]. Thus, although the overall prevalence of hVISA/VISA is low, it can reach up to 30% in selected clinical scenarios using refined assays to detect resistant subpopulations (eg, patients with MRSA and infective endocarditis) [31]. Outbreaks caused by hVISA/VISA strains have also been reported, but their occurrence...
continues to be sporadic [32]. Interestingly, the VISA phenotype has also been described in a minority of MSSA strains.

Although the genetic basis of hVISA/VISA seems to be complex and not completely understood, the available evidence suggests that it requires a number of ordered and sequential mutations that usually involve several regulatory systems controlling cell-envelope homeostasis [33]. Among them, the genes most consistently implicated are walkR (also known as yycFG), vraRS (homolog of liaFSR), and graRS [34, 35]. Mutations in rpoB (encoding the RNA polymerase subunit B) have also been linked with the VISA phenotype. These genetic changes produce important remodeling of the cell envelope, resulting in a thickened cell wall that seem to harbor increased amounts of free D-Ala-D-Ala dipeptides with reduced cross-linking [30]. Overall, these perturbations may “trap” vancomycin in outer layers of the peptidoglycan, preventing its ability to reach its target of peptidoglycan precursors emerging from the cytoplasm.

The first reported case of VRSA occurred in Michigan in 2002 and resulted from the acquisition of the vanA gene cluster from a vancomycin-resistant E. faecalis [36]. However, in contrast to VRE, VRSA remain rare, with only 13 isolates described in the United States and a total of 4 additional cases reported from Brazil, Portugal, Iran, and India [33, 37]. Most VRSA strains have been recovered as “colonizers” from the skin of diabetic patients; 12 of the 13 cases found in the United States correspond to MRSA belonging to CC5, the most common clonal complex associated with MRSA hospital dissemination in the country (the remaining isolate belongs to CC30). Importantly, a Brazilian MRSA isolate carrying the vanA gene cluster (ST8) was found to be related to a community-associated MRSA genetic lineage with high dissemination capacity in Latin America (USA300, Latin American variant). Most worrisome, the vanA gene cluster in this isolate was found in a highly transferable staphylococcal plasmid that was also acquired by a different bloodstream isolate of MSSA within the same patient [37].

Finally, 3 antibiotics from the lipoglycopeptide family have been approved by the US Food and Drug Administration for the treatment of gram-positive infections, namely, telavancin [38], dalbavancin [39] and oritavancin [40]. All of these compounds appear to be active in vitro against hVISA/VISA isolates. Of these 3 drugs, oritavancin retains some in vitro activity against enterococcal strains exhibiting the VanA phenotype [41]. Nonetheless, its clinical efficacy against these latter strains remains to be established, and initial studies in animal models suggest that selection of resistant mutants is likely when oritavancin is used as monotherapy [42].

**OXAZOLIDINONE RESISTANCE**

Two oxazolidinones are currently available for the treatment of gram-positive infections, namely linezolid and tedizolid. Both compounds inhibit protein synthesis through interactions with the A site of the 50S ribosomal subunit. Mechanisms of oxazolidinone resistance include (1) mutations in the genes encoding the 23S ribosomal RNA (rRNA), (2) changes in the L3/L4 ribosomal proteins, and (3) methylation of the 23S rRNA by a methylase designated as Cfr (chloramphenicol-florfenicol resistance) (Figure 1). Overall, mutations in the central loop of the domain V of the 23 rRNA are the most frequent determinants of linezolid resistance (LNZ-R). Although several mutations have been described, the most frequent change found in clinical isolates is G2576T (Escherichia coli numbering). Importantly, because bacteria carry multiple copies of the 23S rRNA genes, mutations need to accumulate in several copies to increase the MIC (gene-dose effect) [43]. Substitutions in the L3 and L4 ribosomal proteins are also associated with development of LNZ-R in vivo and in vitro, both alone and in combination with other resistance determinants. Interestingly, mutations in the L3/L4 seem to be particularly frequent in coagulase-negative staphylococci (see below).

Cfr encodes a methyltransferase whose target is position A2503 of the 23S rRNA. Initially reported in a Staphylococcus sciuri recovered from cattle, cfr was first described in humans in 2005 in a S. aureus isolated from a patient in Colombia [44]. Since then, it has been found in several bacterial species. Overall, cfr carriage continues to be sporadic, however, areas of endemicity have been reported [45]. Furthermore, cfr-positive isolates have been associated with outbreaks of LNZ-R S. aureus and coagulase-negative staphylococci in different clinical settings [46, 47]. Cfr is the only transmissible LNZ-R determinant and it has been associated with various mobile genetic elements. In addition, it is frequently cotransmitted with other resistance determinants that affect protein synthesis, and it seems to carry a low fitness cost [48]. Altogether, these characteristics suggest that the potential for dissemination of this MDR trait is high. It is also important to note that in contrast to mutational resistance, in vivo data suggested that cfr-mediated LNZ-R could be overcome with higher doses of the antibiotic [49]. Importantly, carriage of cfr does not seem to confer resistance to tedizolid, the other oxazolidinone approved in 2014 by the Food and Drug Administration for the treatment of skin and soft-tissue infections [50].

**LNZ-R in Staphylococci**

Two comprehensive surveillances collecting worldwide isolates over a 10-year-period showed that staphylococcal isolates have remained highly susceptible to linezolid [45]. As mentioned, changes in L3/L4 ribosomal proteins are particularly frequent in Staphylococcus epidermidis, with 1 study finding them in 89 of 122 LNZ-R isolates (73%). In terms of frequency, cfr was present in 13% of S. aureus and in 15% of 182 LNZ-R coagulase-negative staphylococci from postmarketing surveillance [45]. In
addition, several outbreaks of cfr-positive S. aureus and S. epidermidis have been reported, and a 2013 report implicated cfr in local endemicity of LNZ-R in a hospital in Madrid [51]. Tedizolid resistance has been described in vitro, and the main mechanism relates to changes in the 23S rRNA (including the presence of a dual-mutation T2571C plus G2576T) [52].

**LNZ-R Enterococci**
The first described LNZ-R clinical isolates were *E. faecium* recovered from patients receiving linezolid as part of a compassionate program. As seen in other bacteria, the prevalence of LNZ-R enterococci has remained low. Data from 6718 enterococcal isolates tested from 2004 to 2012 showed that the prevalence of LNZ-R consistently remained <0.9% [53]. Mutations in the 23S rRNA genes are the most frequently identified changes. In fact, of 48 LNZ-R *E. faecalis* and 121 *E. faecium*, these mutations were the only identified resistance trait in 73% and 88% of isolates, respectively [45]. Furthermore, only 3 *E. faecium* isolates harbored changes in the L3/L4 ribosomal proteins.

Although cfr has been reported in clinical enterococcal isolates [54], its overall frequency continues to be low.

**LNZ-R Streptococci**
Very few cases of LNZ-R streptococci have been described, including sporadic reports of LNZ-R *S. pneumoniae, Streptococcus sanguinis*, and *S. oralis*. In addition, the cfr gene was reported from a *Streptococcus suis* isolate of porcine origin [55]. In a 2014 report, no resistance was found in 6691 pneumococci, 2463 viridans streptococci, or 4153 β-hemolytic streptococci [53].

**DAPTOMYCIN RESISTANCE**
Daptomycin (DAP) is a bactericidal lipopeptide antibiotic active against a wide range of gram-positive clinical pathogens. It damages the cell membrane (CM) in a calcium-dependent manner, interacting with its target mostly at the bacterial division septum. Importantly, the presence of specific CM
phospholipids (eg, phosphatidylglycerol [PG]) is crucial for the antibacterial action of DAP [56, 57]. Development of DAP resistance (DAP-R) during therapy seems to be an important problem affecting the clinical efficacy of DAP. The exact mechanisms of DAP-R remain to be elucidated, but current knowledge suggests that they are complex, diverse, and multifactorial, arising from mutational changes (Table 1).

DAP-R in S. aureus

Most S. aureus remain DAP susceptible (DAP-S), but reports documenting the emergence of DAP-R continue to accumulate, particularly in the context of high-inoculum infections and/or after exposure to vancomycin [58, 59]. It has been postulated that the mechanism of DAP-R in S. aureus is mediated by electrostatic repulsion of the DAP calcium complex from the cell surface, mainly by increasing the positive charge of the bacterial surface [60] (Figure 2). The most common gene implicated in the phenotype is mprF, which encodes a bifunctional enzyme (MprF) that incorporates the positive charged amino acid lysine to PG. The resulting lysyl-PG, a positively charged phospholipid, is translocated from the inner to the outer leaflet of the CM by the same enzyme [61].

Several mprF mutations have been associated with DAP-R, most frequently producing a “gain of function” phenotype. The increased positive charge of the cell envelope is thought to impair the binding of DAP to the CM target. The DAP repulsion theory has also been supported by the finding of DAP-R strains overexpressing the dlt operon, responsible for the incorporation of D-alanine (positively charged amino acid) into cell-wall teichoic acids [62]. However, repulsion is not the only explanation for DAP-R staphylococci, because not all isolates with mprF mutations exhibit significant changes in surface charge, and DAP-R isolates with either no mutations in mprF or without gain in function in the enzyme have been described.

Other determinants implicated in DAP-R include genes encoding enzymes involved in phospholipid metabolism, such PG and cardiolipin synthetases (pgsA and cls, respectively) [63]. In addition, DAP-R isolates seem to exhibit important changes in

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Figure 2. Proposed mechanisms of action and resistance to daptomycin in Gram-positive organisms. A, The daptomycin-calcium complex interacts with the cell membrane mainly at septal areas. B, In Enterococcus faecalis, the antibiotic is diverted from the septum (black arrow) in a process associated with redistribution of anionic phospholipid microdomains (eg, cardiolipin) away from the septal plane. C, In Staphylococcus aureus and Enterococcus faecium, the positively charged daptomycin-calcium complex is “repelled” from the cell surface in a process associated with increase in the net positive charge of the cell envelope (not all strains). Abbreviations: DAP-R, daptomycin-resistant; DAP-S, daptomycin-susceptible.
diplococcal infections, similar to those observed in VISA isolates. The 2-component regulatory systems VraSR and YycFG (WalKR) are thought to play a particularly important role in development of DAP-R [59]. Indeed, mutations in yycFG have been directly implicated in DAP-R, and inactivation of vraSR in a DAP-R isolate caused reversion to DAP-S [64]. Interestingly, 1 study showed that up to 80% of VISA isolates were DAP-R [59, 65]. In addition, DAP-R emerged in a patient with MRSA endocarditis in whom vancomycin therapy failed after a VISA phenotype developed, although never exposed to DAP [35].

**DAP-R in Enterococci**

Enterococci are less susceptible than staphylococci to DAP (Clinical and Laboratory Standards Institute break point, 4-fold that of *S. aureus*). Most genes implicated in DAP-R in enterococci seem to be grouped into 2 broad categories: those encoding regulatory systems that orchestrate cell-envelope homeostasis and stress response and those coding for enzymes involved in CM phospholipid metabolism. Interestingly, the mechanisms of DAP-R seem to differ between *E. faecium* and *E. faecalis* (Figure 2).

Genomic analysis of a clinical strain pair of *E. faecalis* identified 3 genes responsible for DAP-R in vivo [66]. Two of them (*gdpD* and *cls* encoding a phosphodiesterase and cardiolipin synthase, respectively) were involved in phospholipid metabolism; the remaining locus (*liaF*) was part of a 3-component regulatory system (*LiaFSR*, a homologue of the *S. aureus* VraTSR system described above) that orchestrates the cell-envelope stress response in gram-positive organisms. Changes in *LiaFSR* are thought to be the initial and pivotal modifications contributing to the development of DAP-R in vivo. Furthermore, deletion of *liaR*, the response regulator of the *LiaFSR* system, resulted in complete reversal of the DAP-R phenotype [67]. Most importantly, the mechanism of resistance does not seem to be mediated by repulsion of the antibiotic from the cell surface; instead, the lack of killing by DAP has been best correlated with a “redistribution” of CM cardiolipin microdomains away from the septum mediated by the LiaFSR system, a change that seems to prevent the interaction of DAP with its main septal target (the “diversion” hypothesis; see Figure 2) [68]. In addition, changes in the CM phospholipid composition (mainly decreased PG and increased cardiolipin) seem to be paramount in DAP-R in *E. faecalis*.

Recent evidence suggests that the “repulsion” mechanism is more likely to mediate DAP-R in *E. faecium* (Figure 2), although the genetic basis of resistance appears to be similar to that of *E. faecalis*. Furthermore, changes in *LiaFSR* were also frequently found in DAP-S clinical *E. faecium* isolates with MICs close to the break point (3–4 μg/mL), and their presence was sufficient to abolish the bactericidal activity of DAP. These data suggested that such mutations could increase the risk of therapeutic failure, irrespective of the MIC [69]. Of note, a 2014 report of DAP failure in a patient with VRE bacteremia caused by a DAP-S isolate (MIC, 3 μg/mL) harboring *liaFSR* mutations further supports the role of these genes in the DAP-R phenotype [70].

**DAP-R in Streptococci**

Reports of DAP-R streptococcal strains are rare, and large surveillance programs consistently show that streptococcal isolates remain almost uniformly susceptible to DAP. Importantly, recent evidence suggests that high-level and durable DAP-R can rapidly arise in vivo and in vitro when viridans group streptococci are exposed to DAP [71, 72]. However, the actual mechanism of this form of DAP-R has not been elucidated.

Development of DAP-R seems to be correlated with an increased susceptibility to β-lactam antibiotics (“see-saw” effect) [73]. Indeed, several reports have suggested that the DAP-β-lactam combination is efficacious and bactericidal against DAP-R isolates [73–76]. Although the mechanism of this apparent synergy has not been fully elucidated, it seems to be dependent on specific PBPs (ie, PBP-1 in *S. aureus*) [77] and on the genetic pathway leading to the DAP-R phenotype [78].

**CONCLUSION**

Resistance to front-line antibiotics is an evolving phenomenon in gram-positive bacterial pathogens. Despite the availability of new molecules to treat infections caused by these organisms, the optimal therapeutic approaches remain to be established. The immense genetic plasticity of the most clinically relevant gram-positive bacteria poses important challenges for designing strategies to curtail development of resistance. As new compounds become available, the adaptive bacterial response will probably emerge in clinical settings. Therefore, efforts to optimize the use of antibiotics with gram-positive spectrum and identify emerging mechanisms of resistance should be clinical priorities.

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