Quinupristin-Dalfopristin Resistance in Gram-Positive Bacteria: Mechanism of Resistance and Epidemiology

Ellie Hershberger,1 Susan Donabedian,1 Konstantinos Konstantinou,1 and Marcus J. Zervos1,2
1William Beaumont Hospital, Royal Oak, and 2Wayne State University School of Medicine, Detroit, Michigan

Antimicrobial resistance in gram-positive bacteria is a continuing problem resulting in significant morbidity, mortality, and cost. Because of this resistance, new antimicrobial agents have been needed. Quinupristin-dalfopristin is a recently approved agent for treatment of these infections. Shortly after its introduction into clinical medicine, resistance was reported. Resistance can occur by one or more of several mechanisms, including enzymatic modification, active transport of efflux mediated by an adenosine triphosphate–binding protein, and alteration of the target site. Resistance is rare in isolates of staphylococci and Enterococcus faecium from humans. Resistance is common in isolates recovered from food animals and is related to the use of virginiamycin as a feed additive. Considering the effect antimicrobial resistance has on human health, as well as its economic impact, measures to preserve the usefulness of these agents and delay the development of resistance are urgently needed.

During the past 2 decades, drug resistance among gram-positive bacteria has been increasing. These bacteria continue to emerge as important clinical pathogens, resulting in significant morbidity, mortality, and cost [1, 2]. During the 1990s, coagulase-negative staphylococci, Staphylococcus aureus, and enterococci became the 3 most common causes of bloodstream infections among hospitalized patients in the United States [3]. Increasing resistance of staphylococci to methicillin and the recent description of vancomycin resistance in S. aureus have caused considerable concern [4]. Many isolates of vancomycin-resistant enterococci from humans are now resistant to all clinically useful antimicrobial agents. As a result of this increase in antimicrobial resistance, attention has been directed toward the development of newer antimicrobial agents, including quinupristin-dalfopristin (Synercid; King Pharmaceuticals) and linezolid (Zyvox; Pfizer), the 2 approved agents for management of these infections. However, not long after these agents were approved, bacteria with resistance to both agents were reported [5, 6].

**STREPTOGRAPHIN ANTIBIOTICS**

The streptogramin antibiotics are naturally occurring compounds isolated from Streptomyces pristinaespiralis. The streptogramin family comprises several classes of antibiotics, including the mikamycins, pristinamycins, oestreomycins, and virginiamycins [7–11]. Oral and topical pristinamycins have been used in humans in France for many years, primarily for the management of staphylococcal infection [12].

The streptogramins are divided into 2 groups. Members of group A are polyunsaturated cyclic macrolactone compounds that include virginiamycin M, pristinamycin IIA, and dalfopristin, and group B compounds are cyclic hexadepsipeptides and include virginiamycin S, pristinamycin IA, and quinupristin. Quinupristin was produced by modifying pristinamycin IA to quinuclindynylthiomethyl pristinamycin IA. Dalfopristin is diethylaminoethyl-sulfonyl-pristinamycin IIA. Both components inhibit bacterial protein synthesis by interfering with different targets of 23S RNA in the 50S subunit of the ribosome. Quinupristin-dalfopristin consists of a combination of quinupristin and dalfopristin in a wt/wt ratio of 30:70 [10, 11, 13]. Molecular modifications of the natural compounds were required to increase their aqueous solubility, thus enabling the drug to be formulated as an injectable agent for use in the management of serious infections. Individual pristinamycin compounds exhibit bacteriostatic activity against gram-positive bacteria. However, combinations using a compound from each
Table 1. Genes encoding streptogramin resistance in staphylococci and enterococci.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Genus or species</th>
<th>Gene(s) encoding resistance</th>
<th>Resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptogramin A (dalfopristin)</td>
<td>Staphylococci</td>
<td>vgaA, vgaB</td>
<td>ATP-binding protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vatA, vatB, vatC*</td>
<td>Acetyl transferase</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td></td>
<td>vatD, vatE</td>
<td>Acetyl transferase</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td></td>
<td>vatE</td>
<td>Acetyl transferase</td>
</tr>
<tr>
<td>Streptogramin B (quinupristin)</td>
<td>Staphylococci</td>
<td>msrA, msrB</td>
<td>ATP-binding protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vgbA, vgbB*</td>
<td>Lyase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ermA, ermB, ermC</td>
<td>rRNA methylases</td>
</tr>
<tr>
<td>Enterococci</td>
<td></td>
<td>ermB</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>msrC</td>
<td></td>
</tr>
</tbody>
</table>

* vatA and vgbA have rarely been found in E. faecium.

**MECHANISMS OF RESISTANCE**

Resistance to quinupristin-dalfopristin can occur by one of several mechanisms, including enzymatic modification of the antibiotic, active transport or efflux mediated by an ATP-binding protein, and alteration of the target site. Presence of ≥1 streptogramin A resistance gene (vat or vga) is necessary for an organism to become resistant to quinupristin-dalfopristin. The genes that encode streptogramin resistance in staphylococci and enterococci are listed in table 1.

**IN VITRO SUSCEPTIBILITY STUDIES**

Numerous in vitro susceptibility studies have been performed with quinupristin-dalfopristin and other streptogramin antibiotics [7, 10, 13, 14]. In addition to activity against *E. faecium*, quinupristin-dalfopristin has excellent in vitro activity against *Enterococcus faecium*, including isolates of vancomycin-resistant *E. faecium* (VREF) that are resistant to other clinically available agents.

The streptogramin A resistance genes found in *E. faecium* are *vatD* and *vatE*, which encode acetyltransferases. *vatD* was initially found in an *E. faecium* isolate recovered from a hospitalized patient in Europe [16]. It has since been demonstrated in isolates of both animal and human origin outside of the hospital in Europe [17–23]. Transfer of *vatD* between isogenic *E. faecium* strains has also been demonstrated in vitro [19] and in vivo [24]. *vatE* is more commonly found in *E. faecium* and was originally found in an isolate from sewage in Germany [25]. Unlike *vatD*, *vatE* has also been found in *E. faecium* isolates from the United States; these have mainly been recovered from chicken and turkey farms and from retail poultry, but an isolate was also recovered from a human [26–28]. *vatE* has also been identified in quinupristin-dalfopristin-resistant *E. faecium* isolated from retail meat, animals and humans in the community, and hospitalized patients in Europe [17, 21, 22].

*Enterococcus faecalis* is resistant to quinupristin-dalfopristin because of the expression of the *lsa* gene in these organisms [29]. The mechanism of resistance has not been completely elucidated, but it is most likely due to active efflux of antibiotic [29]. A recent study showed that clinical isolates of *E. faecalis* with nonsense mutations in the *lsa* gene were susceptible to quinupristin-dalfopristin [30]. Study of *E. faecalis* isolates re-

group of the streptogramin family usually result in synergistic and bactericidal in vitro activity against pneumococci and against some staphylococci. In general, quinupristin-dalfopristin has been reported to possess inhibitory activity against *Enterococcus faecium*, including isolates of vancomycin-resistant *E. faecium* (VREF) that are resistant to other clinically available agents.

The combination of quinupristin-dalfopristin with other agents may provide additional synergistic inhibition or decrease or eliminate the development of resistance to quinupristin-dalfopristin. Synergistic activity was seen when quinupristin-dalfopristin was used in combination with cell-wall-active agents against some isolates of *E. faecium* and *S. aureus* [15]. The combination of quinupristin-dalfopristin with doxycycline also has been shown to prevent the development of resistance to quinupristin-dalfopristin in VREF [15]. These in vitro findings suggest that, for serious infections, quinupristin-dalfopristin used in combination with certain other antimicrobial agents may be useful. These observations need confirmation from experimental animal studies and additional clinical studies to determine the clinical importance of these findings.
covered from retail poultry have also been shown to contain vatE [31].

The most commonly known resistance to streptogramins in enterococci is the MLS\(_B\) resistance conferred by the \(erm\) genes [32]. These genes encode an enzyme that dimethylates an adenine residue in the 23S rRNA, which results in decreased binding of macrolides, lincosamides, and streptogramins B [32]. Because quinupristin-dalfopristin is a combination of a streptogramin A (dalfopristin) and a streptogramin B (quinupristin), one would expect that it would remain active against MLS\(_B\)-positive organisms. \(vga\), which encodes a streptogramin-inactivating enzyme (lyase), has also been found rarely in isolates of \(E. faecium\). \(msr\), which confers resistance to streptogramin B antibiotics by active transport, is commonly found in \(E. faecium\) [33]. To date, there have not been reports of combination of \(>1\) streptogramin A resistance gene in \(E. faecium\); however, combinations of \(vatD-vga\), \(vatD-erm\), and \(vatE-erm\) have been reported [20, 34–36].

**Staphylococci.** Resistance to quinupristin-dalfopristin among staphylococcal isolates has been reported in French hospitals where the natural mixture has been used orally and topically for many years [12, 37]. The first quinupristin-dalfopristin-resistant \(S. aureus\) isolate reported in France was encountered in 1975, and the prevalence of resistance to individual A and B compounds among clinical isolates of staphylococci has been found to vary from 0% to 44% [12]. Resistance to quinupristin-dalfopristin in staphylococci requires resistance to the streptogramin A component, and it generally requires resistance to the streptogramin B component as well. A recent study showed that acquisition of both streptogramin A and B components was necessary to select for mutants that were resistant to quinupristin-dalfopristin both in vitro and in an experimental rabbit model of endocarditis [38]. Constitutive and inducible strains of MLS\(_B\)-positive \(S. aureus\) are susceptible in vitro to quinupristin-dalfopristin [10, 39, 40].

In staphylococci, the genes responsible for resistance to streptogramin B antibiotics include \(erm\), \(vga\), and \(msr\). \(erm\), \(vga\), and \(msr\) encode enzymes, which methylate an adenine residue in the 23S rRNA [39, 41, 42]. \(vga\) and \(vga\), which are plasmid mediated, encode a streptogramin-inactivating enzyme (lyase) [43–45]. \(msr\) and \(msr\) confer resistance to streptogramin B antibiotics by active transport after induction with erythromycin [46]. In a recent study, a mutation in the \(1.22\) ribosomal protein of an \(S. aureus\) clinical isolate was responsible for resistance to quinupristin-dalfopristin in that strain through abolishment of synergy between the streptogramin A and B components [47].

Mechanisms of resistance to streptogramin A antibiotics include \(vat\) [48], \(vat\) [49], and \(vat\) [44], which encode acetyl transferases that inactivate the antibiotic. \(vga\) [50], \(vga\) [51], and \(vga\) [12] encode ATP-binding proteins, which are most likely involved in active transport of streptogramin A.

It is interesting to note that combinations of streptogramin resistance genes in isolates of staphylococci have been seen previously, including isolates with \(>1\) streptogramin A resistance gene. Combinations such as \(vat\)-\(vga\)-\(vga\)-\(vat\)-\(vga\)-\(vat\)-\(vga\)-\(vat\)-\(vga\)-\(vat\)-\(vga\) have been reported [12, 52].

The prevalence of these quinupristin-dalfopristin–resistant strains of staphylococci has been estimated to be <5% in France. These isolates were recovered from the skin after the topical use of streptogramins and were not considered clinically significant. Cross-resistance to macrolides may be seen, although this is probably a relatively rare and clinically insignificant occurrence.

**EPIDEMIOLOGY OF RESISTANCE TO QUINUPRISTIN-DALFOPRISTIN**

Although rare, resistance has been reported in \(E. faecium\) isolates recovered from humans. However, it is of concern that resistance has developed when patients have been treated with quinupristin-dalfopristin. In clinical trials of quinupristin-dalfopristin, the occurrence of resistance in \(E. faecium\) during therapy was observed in 5 patients in a multicenter, prospective study evaluating the safety and efficacy of quinupristin-dalfopristin in 396 patients with VREF infection. Four of these cases resulted in clinical failure and persistent VREF infection [5].

Other streptogramin-resistant \(E. faecium\) isolate recovered from humans have been reported from Europe and the United States [5, 13, 14, 17–22, 26–28, 34]. In data from a Centers for Disease Control and Prevention (CDC) multistate surveillance study, a 1%–2% rate of resistance to quinupristin-dalfopristin in \(E. faecium\) isolated from human stool samples was reported [18, 23]. Similar observations have been reported from other centers in the United States. Rates of \(E. faecium\) resistance to quinupristin-dalfopristin in stool cultures as high as 7.4% were reported among patients in German hospitals [21]. Resistance to quinupristin-dalfopristin is also common among \(E. faecium\) isolates recovered from animal sources, which may pose a threat to humans. Of importance, this resistance to quinupristin-dalfopristin in \(E. faecium\) was also demonstrated in stool samples obtained from outpatients before the drug’s approval in the United States and Europe [18, 20–23, 31]. These findings raise concerns about the potential for emergence and spread of quinupristin-dalfopristin–resistant \(E. faecium\).

The sources and reservoirs of quinupristin-dalfopristin resistance in \(E. faecium\) remain undetermined. There is little information on the amount, the regional differences in use, and the impact of streptogramins on resistance in animal husbandry and veterinary use. Virginiamycin is a mixture of virginiamycin
M (a group A streptogramin) and virginiamycin S (a group B streptogramin). Virginiamycin is used worldwide and has been approved by the US Food and Drug Administration (FDA) in the United States for use in chickens, turkeys, swine, and cattle (fed in confinement for slaughter). It is used in all of these species to promote weight gain. It is also used to prevent necrotic enteritis caused by Clostridium perfringens in chickens, to prevent coccidiosis in chickens and turkeys, to treat and control swine dysentery, and to reduce the incidence of liver abscesses in cattle.

Virginiamycin has been banned in Europe since 1999, pending further studies on the possible relationship of virginiamycin use and resistance to streptogramins used clinically to treat infection in humans. It has been postulated that the use of virginiamycin in animals can result in quinupristin-dalfopristin resistance in E. faecium and thereby jeopardize the utility of quinupristin-dalfopristin for treatment of human infections. Thus, the potential for spread of multiple resistance genes on a single element in enterococci from one animal to multiple other animals is high.

A previous study of different ecological sources in Germany discovered rates of resistance to quinupristin-dalfopristin of up to 100% among E. faecium isolates recovered from sewage, poultry manure, and pig manure [21]. The rates of quinupristin-dalfopristin resistance among broiler chickens, pork meat, human stool samples, and hospitalized patients were 46%, 10%, 14%, and 7.4%, respectively. Of the 112 different isolates evaluated, 36.6% had vatD and 60.7% had vatE. Some of the isolates evaluated from the human stool samples or hospitalized patients did not possess either gene. Streptogramin-resistant enterococci have also been isolated from pigs in The Netherlands [20, 22, 53], and pigs and broiler chickens fed virginiamycin in Denmark [54]. vatD and vatE were detected in E. faecium isolates recovered from broiler chickens and pigs in Denmark [19, 36].

In collaborative studies with the FDA–Center for Veterinary Medicine and the CDC, 322 quinupristin-dalfopristin–resistant E. faecium isolates (MIC, 4–32 μg/mL; determined by broth microdilution) recovered from humans, farm animals, and retail food samples from 1999–2002 were evaluated [26, 27]. Isolates include 29 isolates from humans in 6 states, 56 fecal isolates recovered from chicken farms, 105 isolates recovered from turkeys, 51 isolates recovered from dairy cattle, 59 isolates recovered from swine, and 3 isolates recovered from beef cattle farms in Michigan. Three clonally related isolates were found in retail pork from Georgia and Michigan, and 18 were recovered from retail chicken from Georgia, Maryland, Minnesota, and Oregon. Human isolates did not share PFGE strain groups with farm animals or retail food isolates. Two human isolates from Georgia were the determined to be the same strain by PFGE. Three isolates from retail pork from Oregon were found to be the same strain by PFGE. By PCR, all isolates were found to be negative for known resistance determinant, with the exception that 14 (26%) of the chicken isolates and 41 (39%) of the turkey farm isolates contained vatE. Of importance, 1 of the human isolates was positive for the vatE. Transfer of quinupristin–dalfopristin resistance occurred in 20 (42%) of the 48 poultry isolates and in 1 of the human isolates at a frequency ranging from $10^{-7}$ to $10^{-9}$ cases per recipient. Quinupristin-dalfopristin resistance was commonly seen in animal farms, and the mean resistance rates among E. faecium isolates were 3% (range, 0%–14%) for beef cattle, 8% (range, 0%–50%) for swine, 36% (range, 0%–100%) for turkey, 98% (range, 90%–100%) for chicken, and 1.5% (range, 0%–20%) for dairy cattle farms. Turkey farms using virginiamycin as a growth-promoting agent had higher rates of quinupristin-dalfopristin resistance (mean, 75% of isolates; range, 50%–100%) than did farms that did not use virginiamycin (mean for turkey farms, 28% [range, 0%–40%]; mean for beef farms, 3% [range, 0%–14%]; mean for dairy farms, 1.5% [0%–20%]; and mean for swine farms, 8% [range, 0%–50%]). All 7 chicken farms reported use of virginiamycin, and the rate of resistance to quinupristin-dalfopristin was 90%–100% (mean, 98%) among E. faecium isolates recovered from these farms. Virginiamycin use was reported in farms with the highest prevalence of quinupristin-dalfopristin–resistant E. faecium. These data suggest that quinupristin-dalfopristin resistance is more common in farms that use virginiamycin. These data also suggest diversity among quinupristin-dalfopristin–resistant enterococci recovered from humans, farm animals, and retail food, with the highest prevalence among E. faecium isolated from farm animals. Additional work is necessary to determine the molecular mechanism for quinupristin-dalfopristin–resistant E. faecium and the source of resistance in animal isolates.

With the ban on antimicrobial use in animal feed in Denmark and Europe, a significant decrease was seen in antimicrobial resistance during 1995–2000 [54]. Avoparcin was banned from use in animals in Denmark in 1995, followed by virginiamycin in 1998. The avoparcin ban resulted in a significant decrease (66.9%) in the rate of glycopeptide resistance among E. faecium from 1995 to 2000 among broiler chickens. It is interesting to note that this decrease was not seen in swine, and it was found to be due to tylosin use in pigs, resulting in glycopeptide cross-resistance. Glycopeptide-resistant E. faecium isolates recovered from pig herds all contained a plasmid that contained genes for both glycopeptide and macrolide resistance [54, 55]. Later, a decrease in the use of tylosin resulted in a significant decrease in vancomycin resistance among E. faecium isolates recovered from swine. Of importance, after avoparcin was banned, virginiamycin use increased until 1998. The pattern of virginiamycin resistance followed its consumption closely, with an increase of 38.9% from 1995 to 1997. The rate of virginiamycin
resistance then decreased from 66.2% in 1997 to 33.9% in 2000 after its ban in 1998 [54]. This study demonstrates that discontinuation of antimicrobial use in animal husbandry will reduce antimicrobial resistance among animal isolates and can decrease human exposure to resistance genes from animal sources.

Increasing antimicrobial resistance among gram-positive bacteria has presented a formidable treatment problem. Since the late 1980s, advances have been made in the development of antibiotics active against multidrug-resistant, gram-positive pathogens. However, resistance to these new agents, including quinupristin-dalfopristin, has already been reported. Resistance to quinupristin-dalfopristin developed even before its approval for use in humans. This of a great importance, because limited options are available for the management of infections due to multidrug-resistant, gram-positive bacteria.

Nonhuman sources have been increasingly suspected as reservoirs for some antibiotic-resistant bacteria. Although there is clear evidence that the increase in the consumption of antibiotics by animals has been accompanied by an increase in antibiotic resistance and in the isolation of novel resistance genes, the potential role that antibiotic use in veterinary medicine and animal husbandry plays in the transfer of antibiotic-resistant bacteria to humans remains controversial [53, 54, 56–59].

CONCLUSIONS

Considering the effect that antimicrobial resistance has on human health and also its economic impact, measures to preserve these agents and delay the development of resistance are urgently needed. This includes judicial use of antibiotics for infection in humans, control measures to prevent the spread of resistant pathogens in health care facilities, and the decrease of resistance in reservoirs such as the environment and animal husbandry.

References


