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Treatment Options for Vancomycin-Resistant Enterococcal Infections

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Abstract

Serious infection with vancomycin-resistant enterococci (VRE) usually occurs in patients with significantly compromised host defences and serious comorbidities, and this magnifies the importance of effective antimicrobial treatment. Assessments of antibacterial efficacy against VRE have been hampered by the lack of a comparator treatment arm(s), complex treatment requirements including surgery, and advanced illness-severity associated with a high crude mortality.

Treatment options include available agents which don't have a specific VRE approval (chloramphenicol, doxycycline, high-dose ampicillin or ampicillin/sulbactam), and nitrofurantoin (for lower urinary tract infection). The role of antimicrobial combinations that have shown *in vitro* or animal-model *in vivo* efficacy has yet to be established. Two novel antimicrobial agents (quinupristin/dalfopristin and linezolid) have emerged as approved therapeutic options for vancomycin-resistant *Enterococcus faecium* on the basis of *in vitro* susceptibility and clinical efficacy from multicentre, pharmaceutical company-sponsored clinical trials.

Quinupristin/dalfopristin is a streptogramin, which impairs bacterial protein synthesis at both early peptide chain elongation and late peptide chain extrusion

steps. It has bacteriostatic activity against vancomycin-resistant *E. faecium* [minimum concentration to inhibit growth of 90% of isolates (MIC_{90}) = 2 μg/ml] but is not active against *Enterococcus faecalis* (MIC_{90}= 16 μg/ml). In a noncomparative, nonblind, emergency-use programme in patients who were infected with Gram-positive isolates resistant or refractory to conventional therapy or who were intolerant of conventional therapy, quinupristin/dalfopristin was administered at 7.5 mg/kg every 8 hours. The clinical response rate in the bacteriologically evaluable subset was 70.5%, and a 65.8% overall response (favourable clinical and bacteriological outcome) was observed. Resistance to quinupristin/dalfopristin on therapy was observed in 6/338 (1.8%) of VRE strains. Myalgia/arthralgia was the most frequent treatment-limiting adverse effect. *In vitro* studies which combine quinupristin/dalfopristin with ampicillin or doxycyline have shown enhanced killing effects against VRE; however, the clinical use of combined therapy remains unestablished.

Linezolid, an oxazolidinone compound that acts by inhibiting the bacterial pre-translational initiation complex formation, has bacteriostatic activity against both vancomycin resistant *E. faecium* (MIC_{90} = 2 to 4 μg/ml) and *E. faecalis* (MIC_{90} = 2 to 4 μg/ml). This agent was studied in a similar emergency use protocol for multi-resistant Gram-positive infections. 55 of 133 evaluable patients were infected with VRE. Cure rates for the most common sites were complicated skin and soft tissue 87.5% (7/8), primary bacteraemia 90.9% (10/11), peritonitis 91.7% (11/12), other abdominal/pelvic infections 91.7% (11/12), and catheter-related bacteraemia 100% (9/9). There was an all-site response rate of 92.6% (50/54).

In a separate blinded, randomised, multicentre trial for VRE infection at a variety of sites, intravenous low dose linezolid (200mg every 12 hours) was compared to high dose therapy (600mg every 12 hours) with optional conversion to oral administration. A positive dose response (although statistically nonsignificant) was seen with a 67% (39/58) and 52% (24/46) cure rate in the high- and low-dose groups, respectively.

Adverse effects of linezolid therapy have been predominantly gastrointestinal (nausea, vomiting, diarrhoea), headache and taste alteration. Reports of thrombocytopenia appear to be limited to patients receiving somewhat longer courses of treatment (>14 to 21 days). Linezolid resistance (MIC ≥8 μg/ml) has been reported in a small number of *E. faecium* strains which appears to be secondary to a base-pair mutation in the genome encoding for the bacterial 23S ribosome binding site. At present a comparative study between the two approved agents for VRE (quinuprisin/dalfopristin and linezolid) has not been performed.

Several investigational agents are currently in phase II or III trials for VRE infection. This category includes daptomycin (an acidic lipopeptide), oritavancin (LY-333328; a glycopeptide), and tigilcycline (GAR-936; a novel analogue of minocycline). Finally, strategies to suppress or eradicate the VRE intestinal reservoir have been reported for the combination of oral doxycyline plus bacitracin and oral ramoplanin (a novel glycolipodepsipeptide). If successful, a likely application of such an approach is the reduction of VRE infection during high risk periods in high risk patient groups such as the post-chemotherapy neutropenic nadir or early post-solid abdominal organ transplantation.

The emergence of enterococci with high level resistance to vancomycin (VRE) in the US and some other parts of the world during the 1990s has severely constrained therapeutic options for the management of serious infection since enterococci already possess intrinsic and acquired resistance to most other antimicrobials.[1-3] Recent multicentre nosocomial surveillance studies in the US have demonstrated that 18 to 20% of enterococcal bloodstream isolates were resistant to vancomycin.[4,5] A significant divergence has been observed in the incidence of vancomycin-resistance between *Enterococcus faecium* and *Enterococcus faecalis*. Up to 51% of *E. faecium* strains isolated from the bloodstream exhibit vancomycin-resistance while only 3% of *E. faecalis* strains were vancomycin-resistant and the vast majority retain susceptibility to ampicillin.[5]

Serious VRE infection has disproportionately affected patients in the intensive care unit (ICU), immunosuppressed hosts, particularly liver and other solid organ recipients and patients with post-chemotherapy neutropenia, and patients with intravascular and bladder catheter devices.[6-11] Other dominant risk factors include prolonged hospital or ICU length of stay, previous antibacterial exposure (vancomycin, later generation cephalosporins, anti-anaerobic agents), and exposure to healthcare workers caring for other patients with VRE colonisation.

It is critical for the clinician to discriminate between colonisation with VRE versus true infection as this has a direct bearing on the intent-to-treat with VRE-directed antimicrobials. VRE isolates from superficial wounds, removed intravascular catheters without accompanying local or systemic signs of infection, nonpyuric urine, and intraperitoneal or biliary drains are common examples of VRE colonisation which do not merit therapy.

Some less serious VRE infections may respond to conservative management (wound debridement, catheter-removal) without VRE-directed antimicrobial therapy[12] (table I). Isolation of VRE from two or more blood culture sets and other sterile body sites, with accompanying local or systemic signs of infection are significant findings. Such deep-seated infections do require specific antimicrobial therapy either alone or as an adjunct to non-antimicrobial management (percutaneous- or surgical drainage, catheter removal, etc). The current review focuses on antimicrobial options for VRE infection, and the level of evidence which supports their efficacy and safety in these patients with complex conditions.

1. Definition of Vancomycin-Resistant Enterococci (VRE)

Enterococci are normal colonisers of the human gastrointestinal tract and are organisms with relatively low virulence.[1,2] *E. faecalis* and *E. faecium* comprise 80 to 85% and 15 to 20% of clinical isolates, respectively, followed by several less common species (*E. durans, E. avium, E. raffinosus, E. gallinarum, E. casselflavus, E. flavescens* and others).

These organisms possess a wide range of intrinsic resistance to a diverse range of antimicrobial agents including β-lactams, aminoglycosides, lincosamides and cotrimoxazole (trimethoprim/sulfamethoxazole).[1-3] High level vancomycin-resistance was first observed in France and England in 1986, and not in the US until 1989 in the New York City area.[13-15] Vancomycin-resistance

Table I. Principal and adjunctive non-antimicrobial measures for VRE infections

Principal measures
Intravascular catheter removal
Bladder catheter removal
Wound debridement
Percutaneous drainage of deep collection
Decompression of visceral obstruction
Surgical drainage/repair
Retransplantation
Allograft removal (kidney, intestines)
Adjunctive measures
Reducing iatrogenic immunosuppression
Eliminating VRE-selective antimicrobials
Shortening period of neutropenia (G-CSF, GM-CSF)

G-CSF = granulocyte colony-stimulating factor; **GM-CSF** = granulocyte-macrophage colony stimulating factor; **VRE** = vancomycin-resistant enterococci.

is one of several acquired resistance mechanisms observed amongst enterococci. Others include high level aminoglycoside (streptomycin and gentamicin) resistance (caused by hydrolase or phosphorylase modifying enzymes), which precludes the achievement of bactericidal activity when combined with a cell wall active agent, and high level ampicillin resistance (caused by altered penicillin-binding proteins which diminish affinity) or less commonly, penicillinase–producing strains of *E. faecalis*.[16-19]

Enterococci may express varying degrees of resistance to vancomycin and other glycopeptides that is conferred by one of several distinct gene-based mechanisms. There are five described phenotypes of glycopeptide resistance (table II).[20]

The VanA phenotype is characterised by inducible, high-level resistance to vancomycin [minimum inhibitory concentration (MIC) >64 μg/ml] and teicoplanin (MIC >8 μg/ml). The *vanA* gene a 7-gene complex located on a specific transposon (*Tn 1546*) encodes for a ligase that modifies the alanine-alanine peptide precursor terminus to either an alanine-lactate bond or single alanine residue for which vancomycin and teicoplanin have only a very low affinity. Thus, enterococcal cell-wall synthesis proceeds without impediment despite the presence of vancomycin. The VanA phenotype comprises the majority (70 to 80%) of VRE.

Enterococci which are moderate to highly resistant to vancomycin (MIC 4 to >1000 μg/ml) but retain susceptibility to teicoplanin (MIC 0.5 to 32 μg/ml) characterise the VanB phenotype. The *vanB* gene cluster is quite similar yet still has a partially distinct DNA homology from the *vanA* gene cluster and also results in cell wall modification to an alanine-lactate linkage.

Table II. Major phenotypes of vancomycin-resistant enterococci

Phenotype	Glycopeptide resistance profile	Enterococcal species
Van A	Vancomycin (HLR) Teicoplanin (R)	*E. faecium, E. faecalis*
Van B	Vancomycin (HLR) Teicoplanin (S)	*E. faecalis, E. faecium*
Van C	Vancomycin (LLR)	*E. gallinarum, E. casseliflavus*
Van D	Vancomycin (HLR) Teicoplanin (S)	*E. faecium*
Van E	Vancomycin (HLR) Teicoplanin (S)	*E. faecalis*

HLR = high level resistance; **LLR** = low level resistance; **R** = resistant; **S** = susceptible.

VanC resistance strains are featured by constitutive endogenous genes, express only low-level vancomycin-resistance (MIC 8 to 16 μg/ml) and usually present in less common species such as *E. gallinarum*, *E. casseliflavus* and *E. flavescens*. Conversion to an alanine-serine cell wall linkage is the product of Van C-mediated resistance.

Both the VanD and VanE phenotypes have only recently been described in *E. faecium* and *E. faecalis*, respectively, appear to be rare, culminate, respectively, in alanine-lactate and alanine-serine cell wall modifications, and demonstrate resistance patterns similar to the VanB phenotype.[21,22] For this review, the literature cited relates to the treatment of infection with the VanA or VanB phenotypes.

2. Experimental Data

2.1 *In Vitro* Combination Studies

A significant number of *in vitro* and animal studies have been conducted to examine the efficacy of various antimicrobial combinations against VRE. The combination of vancomycin, penicillin and gentamicin demonstrated a bactericidal level of killing (>2 $\log_{10}$) in time-killing curves against VanA and VanB enterococcal strains which were high-level gentamicin susceptible.[23] The theory behind this synergy is that vancomycin-induced expression of cell wall peptidases favourably channels the building cell wall to greater penicillin susceptibility. However, others have shown inconsistent synergism with this triple combination.[24,25]

In vitro synergism has also been shown for the combinations of vancomycin plus ciprofloxacin,[26] and ciprofloxacin plus ampicillin[27] amongst others. Although these observations point to potential unique therapeutic opportunities with approved

agents, the clinical use of any such combinations has not been proven.

2.2 Animal Endocarditis Studies

Bactericidal activity (5 $\log_{10}$ reduction) was observed in a rat aortic valve VRE endocarditis model using the combination of ciprofloxacin plus gentamicin with a ciprofloxacin-susceptible VRE strain;[28] and rabbit aortic valve endocarditis with ampicillin and imipenem/cilastatin,[29] ceftriaxone, vancomycin and gentamicin,[30] and penicillin, gentamicin and vancomycin.[31] The precise mechanism of these interactions has not been elucidated. Human clinical experience with such combinations has been either anecdotal or not yet reported in the literature, and thus they cannot be recommended as first-line therapy for serious VRE infections.

3. Clinical Studies

Therapy for VRE infection is presented in this section as approved conventional agents, recently approved novel agents, and compounds that are currently in pre-clinical or clinical trials (table III).

3.1 Conventional Approved Antimicrobials

3.1.1 Ampicillin and Ampicillin/Sulbactam

Ampicillin in conventional daily dosages (8 to 12 g/day) can often be administered effectively for infections caused by vancomycin-resistant *E. faecalis* strains of which the vast majority retain ampicillin susceptibility. Moreover, if such strains exhibit high level aminoglycoside susceptibility then the addition of gentamicin will achieve synergistic bactericidal activity. Most vancomycin-resistant *E. faecium* strains also express high level resistance to ampicillin as a result of increased expression of penicillin-binding protein (PBP-3) sites with low penicillin/ampicillin affinity. However, there are some select circumstances where ampicillin or ampicillin-sulbactam may be effectively used for VRE infections.

Ampicillin or ampicillin/sulbactam has been used successfully at high parenteral dosages (18 to 24 g/day) for serious VRE infections including endocarditis.[20,32]

Table III. Spectrum of antimicrobials for the therapy of vancomycin-resistant enterococcal infection

Conventional antimicrobials
High dose ampicillin or ampicillin-sulbactam
Teicoplanin
Doxycycline
Novobiocin
Bacitracin
Nitrofurantoin (UTI)
Recently approved novel antimicrobials
Quinupristin/dalfopristin
Linezolid
Investigational antimicrobials
Daptomycin
Oritavancin (LY-333328)
Tigilcycline (GAR-936)

UTI = urinary tract infections.

However, this approach appears limited to those uncommon VRE strains with MIC values of 32 to 64 μg/ml which, although above the ampicillin MIC breakpoint of 16 μg/ml, may still be inhibited by average plasma ampicillin concentrations of 100 to 150 μg/ml.[20]

Continuous infusions of high dose ampicillin (20 g/day) or ampicillin/sulbactam (30 g/day) combined with an aminoglycoside was reported to be successful in six patients with VRE bacteraemia,[33] and high dose ampicillin and streptomycin was effective in a patient with VRE bacteraemia from an intra-abdominal abscess.[34] Presumably, synergism as a result of the different sites of activity of ampicillin and gentamicin (cell wall and ribosome, respectively) is the basis for the favourable clinical and microbiological response in these patients. However, the mechanism of benefit from sulbactam remains unexplained, since β-lactamase inhibition is not required for activity against VRE. Its activity may be secondary to the penicillin-binding protein properties of sulbactam.

3.1.2 Glycopeptides

Serum vancomycin concentrations high enough to exceed the vancomycin MIC of enterococcal strains with high level vancomycin-resistance are not achievable. Teicoplanin, a glycopeptide, which

is marketed in Europe and other countries but not in the US, has been used for entercoccal infection. An 84.1% clinical response and 87.2% eradication rate was reported in a series of 63 patients with an enterococcal infection from Europe in the 1980s; however, no VRE strains were included.[35] Teicoplanin does have preserved activity against VanB phenotypic enterococci. Successful treatment of a 6-year old with post-neurosurgical meningitis caused by a VanB, high-level aminoglycoside-resistant strain of *E. faecium* has been reported with the combination of intrathecal teicoplanin 10 mg/day combined with systemic ampicillin, clindamycin and rifampin.[36] However, teicoplanin resistance amongst VanB *E. faecalis* strains has developed during teicoplanin therapy in both experimental endocarditis and *in vivo*.[37,38]

However, glycopeptide use may predispose to VRE superinfection. Oral vancomycin or teicoplanin has clearly been shown to select for the overgrowth of VRE within the intestinal reservoir which may enhance the risk for VRE superinfection.[39] However, a clear independent relationship between parenteral vancomycin and both the intestinal VRE inoculum or VRE infection was not demonstrated in two recent studies.[40,41]

3.1.3 Chloramphenicol

A number of investigators have reported clinical success with antimicrobials which inhibit protein-synthesis. Chloramphenicol has bacteriostatic activity against enterococci but has never been considered a 'first line' agent for this organism.

Norris and colleagues[42] reported the outcome of 16 patients treated with either chloramphenicol (n = 12) or chloramphenicol plus rifampin (4). A partial list of co-morbidities included prior organ transplant (4), neutropenia (2), mechanical ventilation (5) and renal disease (8).[42] Seven patients had VRE bloodstream infection. A clinical and microbiological response was observed in 8/14 (57%) and 8/11 (73%) evaluable patients, respectively. No chloramphenicol resistance was observed. In a larger study confined to just patients with VRE bacteraemia from the same centre a favourable clinical response was observed in 22/36 (61%) evaluable patients amongst a total of 80 patients with VRE bacteraemia; however, no significant mortality reduction was present in the chloramphenicol-treated subset.[43] A single case of clinical success in a paediatric patient with VRE meningitis who was treated with chloramphenicol has also been recently published.[44]

Considerations in the use of chloramphenicol include the need for chloramphenicol concentration monitoring to avoid concentration-dependent myelosuppression, and the extremely rare occurrence of aplastic anaemia.

3.1.4 Miscellaneous Antimicrobial Agents

There are multiple anecdotal reports of favourable clinical results with the use of tetracycline,[45] doxycyline[46] and oral novobiocin with either ciprofloxacin[47] or doxycycline.[48] However, such reports offer only anecdotal experience and no previous or ongoing controlled trials are available.

Nitrofurantoin may be effective in urinary tract infection[49] or in chronic VRE prostatitis (in conjunction with rifampin).[50] Nitrofurantoin is not indicated for the treatment of any VRE infection other than lower urinary tract infection as this agent is preferentially excreted only into the urine. Its use should be avoided in patients with a creatinine clearance < 30 ml/min as diminished urinary nitrofurantoin excretion with higher blood concentrations may be associated with serious sequelae including hepatic, pulmonary, haematological and other toxicities.

3.2 Recently Approved Agents

Two novel antimicrobials, quinupristin/dalfopristin and linezolid, have received regulatory agency approval in the US and Europe for the treatment of vancomycin-resistant *E. faecium* infection. Their major features, clinical studies conducted in patients with VRE infection, and efficacy by infection site are summarised in tables IV, V and VI, respectively.

3.2.1 Quinupristin/Dalfopristin

Quinupristin/dalfopristin is a parenteral, semi-synthetic antimicrobial derived from pristinamycin,

Table IV. Comparison of major features of quinupristin/dalfopristin and linezolid

Feature	Quinupristin/dalfopristin	Linezolid
Antimicrobial class	Streptogramin	Oxazolidinone
Peak serum concentrations (mg/L)	10-12	15.1
Elimination half-life (h)	0.8 (Q), 0.6 (D)	5.5
Major metabolic routes	Hepatobiliary	Peripheral non-oxidative
Major elimination routes	Faecal (70-75%)	Nonrenal (65%)
	Urinary (19%)	Urinary (30%)
Protein binding (%)	30 (Q) 70 (D)	31
Mechanism of action	Protein synthesis inhibition	Protein Synthesis inhibition
Site of action	50S Ribosome	70S Initiation Complex
Post-antibiotic effect (h)	6-8	1
Bactericidal (*vs* VRE)	No	No
Cytochrome P-450 inhibition	Yes	No
Formulations	Parenteral	Parenteral + Oral
Dose and administration	5-7.5 mg/kg q 8-12h	600mg q 12h
Dosage adjustment	None	None
Approved indications	VRE	VRE
	Complicated SSSI	Complicated SSSI
	Nosocomial pneumonia	Nosocomial pneumonia
Major adverse effects	Phlebitis (peripheral)	Myelosuppression
	Myalgia/arthralgia	
Cost ($US per day; 2000 values)	$300-350	$115 (parenteral)
		$80 (oral)

D = dalfopristin; **Q** = quinupristin; **qXh** = every X hours; **SSSI** = skin and skin structure infection; **VRE** = vancomycin-resistant enterococci.

a natural compound elaborated by *Streptomyces pristinaspiralis*.[56] This agent is in the streptogramin class which is related but chemically distinct from the macrolides and lincosamide compounds. Its mechanism of action is inhibition of early (peptide chain elongation) and late stages of bacterial protein synthesis.[57,58] It possesses a broad Gram-positive spectrum of activity, including methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci, *E.faecium*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and other major streptococci, *Corynebacteria* spp., and other less common Gram-positive species.[59,60]

At steady-state dosage administration of 7.5 mg/kg a peak composite (quinupristin + dalfopristin) serum concentration of 10 to 12 mg/L is reported.[61] Despite the short serum-half life of both components, this agent can be administered every 8 hours because of its transformation to active metabolites and a variable post-antibiotic effect of 0.2 to 3.5 hours against vancomycin-resistant *E. faecium*, which is shorter than that observed for vancomycin-susceptible *E. faecium* isolates.[62,63]

Quinupristin/dalfopristin is atypical for an anti-enterococcal agent as it has good *in vitro* activity against *E. faecium* (MIC_{90} = 1 to 2 μg/ml)[60,64] but very poor activity against the more prevalent species *E. faecalis* (MIC_{90} = 8 to 16 μg/ml).[64,65] The reason for this disparity is probably related to diminished 50S bacterial ribosomal binding for quinupristin/dalfopristin in *E. faecalis*.

Quinupristin/dalfopristin is internally synergistic (initial dalfopristin binding produces a permanent conformational change in the 50S ribosome which accelerates subsequent quinupristin binding) and this results in bactericidal activity. However, time-killing studies have shown that the majority of vancomycin-resistant and vancomycin-susceptible *E. faecium* strains are only inhibited by a quinupristin/dalfopristin concentration of 8 μg/ml or greater with less than a 3-log inoculum

Table V. Summary of clinical outcome of VRE clinical trials with quinupristin/dalfopristin and linezolid

Study	Comparator	Outcome
Quinupristin/dalfopristin (Q/D)		
Emergency use[51]	Noncomparative	CR = 70.5%; BR = 70.5%; OS = 65.4%
University of Pittsburgh[52]	Historical control	Associated mortality: Q/D = 25% Control = 40%
UCLA[53]	Noncomparative	CR = 83%; BR = 74%
Linezolid		
Dose-comparative trial[54]	High dose (600mg q 12h)	CR = 67%
	vs low dose (200mg q 12h)	CR = 52%
Emergency use[55]	Noncomparative	CR = 92.6%

BR = bacteriological response (eradication or presumed eradication); **CR** = clinical response (cure or improvement); **q12h** = every 12 hours; **OS** = overall success (favourable clinical- and bacteriological response); **VRE** = vancomycin-resistant enterococci.

reduction at 72 hours incubation.[66] The lack of bactericidal activity versus *E. faecium* is due to the expression of the (macrolide, lincosamide, streptogramin) MLS_B phenotype conferred by the *erm* (erythromycin methylase) gene which encodes for methylation of the 23S ribosomal binding site.[66] The MLS_B phenotype may be either inducible or constitutive and results in diminished quinupristin binding which leaves residual dalfopristin (and bacteriostatic) activity. *In vitro* resistance to erythromycin serves as an excellent surrogate marker for MLS_B expression amongst enterococci and staphylococci.[59,66]

Interest in the utilisation of quinupristin/dalfopristin for the treatment of serious VRE infection was initially based on *in vitro* susceptibility results, the unavailability of other approved agents for these pan-resistant strains, and the clear need for antimicrobial therapy in patients who were often critically ill and immunocompromised.

A multicentre, nonblind, noncomparative, emergency use programme was conducted in the US and Europe for patients with serious infection caused by Gram-positive organisms which were resistant (*in vitro*) or clinically refractory to conventional antimicrobials or patients who were intolerant of conventional therapy. This programme eventually enrolled over 7000 patients by the time of US regulatory approval in September, 1999. The majority of patients involved had infections caused by either vancomycin-resistant *E. faecium* or MRSA. Early reports of clinical success in this programme included successful treatment of continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis,[67] prosthetic valve endocarditis[68] clinically refractory to teicoplanin, prosthetic aortic graft infection,[69] and neonatal central nervous system shunt meningitis,[70] amongst others.

The largest reported experience in vancomycin-resistant *E. faecium* infection was by Moellering and colleagues[51] who reported a 70.5% clinical response rate and a 65.8% overall success rate (cure/improvement plus eradicated/presumed eradicated) amongst 396 evaluable patients. The five most common primary sites of infection amongst the bacteriogically evaluable subset and their respective response rates were intra-abdominal infection (33/56, 58.9%), bacteraemia of unknown origin (14/27, 51.9%), urinary tract infection (16/18, 88.9%), central catheter-related bacteraemia (10/12, 83.3%), and skin/skin-structure infection (13/18, 72.2%). The modest response rates must be viewed within the perspective of the high co-existing illness severity in such patients, which included high rates of mechanical ventilation, renal failure, iatrogenic immunosuppression and other co-morbidities. Six patients with VRE resistant strains (1.8%) were observed during or after quinupristin/dalfopristin therapy with a rise in the

Table VI. Reported efficacy of quinupristin/dalfopristin and linezolid by site of VRE infection

Site of VRE infection	Response rates (no. respond/no. treated)	
	Quinupristin/dalfopristin	Linezolid
Bloodstream		
catheter-related	10/12 (83%)	9/9 (100%)
primary	14/27 (52%)	10/11 (91%)
Urinary tract	16/18 (89%)	1/1 (100%)
Skin-skin structure	13/18 (72%)	7/8 (88%)
Intra-abdominal	33/56 (59%)	22/24 (92%)
Bone and joint	5/6 (83%)	NA
Endocarditis	1/4 (25%)	NA

NA = not available; **VRE** = vancomycin-resistant enterococci.

quinupristin/dalfopristin MIC to 16, 32 or 64 μg/ml from susceptible/intermediate pre-treatment baseline MICs of 2 to 4 μg/ml. Molecular strain typing studies were performed for four susceptible/resistant strain pairs and demonstrated that resistance evolved from the original infecting strain and not from a new superinfecting strain.

Resistance to quinupristin/dalfopristin amongst *E. faecium* has also been described in 3/150 (2%) tested strains in a European study and appears to require both the presence of the *erm* gene and the *satA* (streptogramin A acetyltransferase) gene that inactivates the dalfopristin component.[71] Superinfection with *E. faecalis* emerged in 16 patients during or after quinupristin/dalfopristin therapy and has also been described by others during quinupristin/dalfopristin therapy of vancomycin-resistant *E. faecium*.[72]

Several centres in the US have reported their own experience with the use of quinupristin/dalfopristin for this indication. At the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA, we compared the outcome of 20 liver transplant recipients with vancomycin-resistant *E. faecium* bacteraemia treated with quinupristin/dalfopristin to an historical cohort 42 liver recipients with bacteraemia prior to the quinupristin/dalfopristin emergency use programme.[52] Rates of recurrent bloodstream infection (5/20 *vs* 21/42, $p = 0.11$), persistent isolation of VRE at the primary site (6/14 *vs* 18/23, $p = 0.06$), and VRE-associated mortality (5/20 *vs* 17/42, $p = 0.05$) were lower in the quinupristin/dalfopristin-treated group. Crude mortality rates were very high in both groups (65% in the quinupristin/dalfopristin group and 52% in the control). A more recent report from the University of California in Los Angeles (UCLA), California, USA, involving 23 critically ill patients with documented VRE infection (19 with bacteraemia) observed a cure/improvement rate of 19/23 (83%) and a favourable bacteriological response (eradicated or presumed eradicated) of 17/23 (74%).[53]

Since most VRE strains express the constitutive MLS_B phenotype and are only inhibited by the remaining dalfopristin activity there has been a significant amount of investigation to examine the role of combining quinupristin/dalfopristin with other agents to enhance killing activity. Most *in vitro* tested combinations with quinupristin/ dalfopristin have shown additive or indifferent effects. However, a synergistic (≥ 2 $\log_{10}$ inoculum reduction at 24 hours), bactericidal combination (quinupristin/dalfopristin plus doxycycline, quinupristin/dalfopristin plus ampicillin/sulbactam) by time-killing curve was observed in 7 of 12 tested vancomycin-resistant *E. faecium* strains.[73] A second study also demonstrated more rapid killing with quinupristin/dalfopristin plus doxycyline than either agent alone in a simulated endocardial vegetation model.[74] The clinical role of such synergistic combinations for *E. faecium* infection is not yet established. In one report, quinupristin/dalfopristin was combined with doxycycline and rifampin to achieve a clinical cure of endocarditis.[75]

There are several important limitations in the use of quinupristin/dalfopristin for VRE infection. The incidence of associated myalgias and arthralgias which were only 1.3% in the earlier comparative studies[76] rose to 9.1% and 6.6% of patients in the multicentre emergency use VRE programme.[71] The reasons for this difference and the mechanism of the symptoms are still unclear. Even higher rates of myalgia/arthralgia (33 to 47%) have been reported from two US centres more recently.[53,77]

Both joint and muscle involvement are reversible and unaccompanied by local inflammatory changes (redness, warmth, induration), rheumatological markers (that is, depressed serum complement, measurable immune complexes) or evidence of rhabdomyolysis (creatine phosphokinase elevation).[76] These observations have raised the hypothesis that this syndrome may be neuropathic in origin. One retrospective study found a higher incidence amongst patients with liver disease[76] and another study only observed myalgia/arthralgia in patients receiving 7.5 mg/kg but not a 5 mg/kg dosage.[73] Thus, an accumulation of either

native drug or metabolites because of either higher dosage administration and/or diminished hepatic transformation appears to be one plausible reason why this syndrome is seen more often in critically ill patients.

Other observed adverse effects have been gastrointestinal (nausea, vomiting) and a reversible elevation of the conjugated bilirubin level.[78] When quinupristin/dalfopristin is administered by peripheral vein there was a high rate of phlebitis (pain with or without inflammation) which ranged from 34% in the VRE multicentre trial[71] to as high as 74% in a the complicated skin/skin structure trials,[79] although this usually did not progress to discontinuation of therapy. Phlebitis can be avoided by administering quinupristin/dalfopristin via a central venous catheter or a peripherally-inserted central catheter (PICC) access.

The overall incidence of resistance of *E. faecium* to quinupristin/dalfopristin has remained low as reported in large scale surveillance studies. A study of over 28 000 Gram-positive clinical isolates from centres in the US and Canada showed a quinupristin/dalfopristin MIC_{90} of 1 μg/ml amongst VRE strains with only 0.2% exhibiting resistance (MIC >2 μg/ml).[59]

An emerging threat to the efficacy of quinupristin/dalfopristin for VRE and other Gram-positive organisms is the commercial use of virginiamycin as an additive in domestic livestock feed with vertical food chain transmission as evidenced by the isolation of vancomycin-resistant enterococci from supermarket poultry and genetic similarity of human and animal VanA genomes.[71,80-82] A similar precedent was seen in Europe where the use of avoparicin (a glycopeptide) for similar purposes was linked to the presence of VRE in the general community.[83] This practice has since been banned by regulatory agencies within the European Union.

3.2.2 Linezolid

This novel antimicrobial belongs to the oxazolidinone family. These agents were first developed by DuPont Inc. in the late 1970s as a botanical agent directed at both bacterial and fungal diseases in plants. Two derivatives of the original compound (DuP-721, DuP-105) were later shown to have a broad Gram-positive spectrum of activity. However, further development of these compounds for human use was halted because of lethal effects in animal models.[84] Subsequent chemical modification of this original compound to two derivatives (eperzolid and linezolid) that demonstrated both preserved Gram-positive activity with no serious detectable toxicity in animal models and human phase I clinical trials. Linezolid was selected for further clinical development based upon its favourable pharmacological properties.

Linezolid acts at a pre-translational focus to prevent the synthesis of bacterial proteins by inhibiting the formation of the 70S-initiation complex comprised of the 50S and 30S ribosomes, mRNA, initiation factors 2 and 3 and fMet-tRNA.[85] This unique site of activity reduces the likelihood of cross-resistance amongst Gram-positive strains which have either intrinsic or acquired resistance to other inhibitors of protein synthesis such as chloramphenicol, clindamycin or the macrolides.

Linezolid has a comprehensive spectrum of activity against the major nosocomial-acquired Gram-positive pathogens, which is not diminished against strains with multi-resistant antimicrobial phenotypes. Susceptibility criteria have been established for linezolid by broth or agar dilution and disk diffusion methods with dilution breakpoint criteria of <2 μg/ml for *Enterococcus* spp., *S. pneumoniae* and other *Streptococcus* spp and <4 μg/ml for *Staphylococcus* spp.[86]

Linezolid has shown consistent activity against both vancomycin-susceptible and vancomycin-resistant strains of *E. faecium* and *E. faecalis* from several published series that tested a large number of contemporaneous enterococcal strains and species by microbroth or agar dilution methods.[87-89] Enterococci with high level resistance to vancomycin due to *vanA* or *vanB* genes demonstrate MIC_{90} values in the range of 2 to 4 μg/ml. Similar activity was present for both *E. faecalis* and *E. faecium* species. Time-kill studies have consistently demonstrated bacteriostatic activity (<3 log inoculum

reduction) against enterococci.[90] Checkerboard- and time-killing assays which have combined linezolid with a second antimicrobial (aminoglycosides, β-lactams, vancomycin) have generally shown an additive/indifferent and no synergistic against enterococci.

The simple pharmacokinetics of this compound facilitate dose administration strategies over a wide range of other morbid conditions, and with the co-administration of other medications and food. The peak and trough serum concentration and area under the concentration/time curve (AUC) parameters are generally similar whether this agent is administered as an oral or parenteral formulation in healthy volunteers. The mean peak (standard deviation) and trough plasma concentrations are 15.1 (±2.5) μg/ml and 3.68 (±2.36) mg/L after intravenous administration of 600mg every 12 hours, producing a concentration-time curve which exceeds the MIC for most or all of the administration interval for Gram-positive pathogens.[90] Oral administration produces plasma concentrations and AUC profiles comparable to parenteral administration.

The dominant metabolic pathway is non-enzymatic oxidation in both plasma and tissue forming two carboxylic acid inactive metabolites; aminoethoxyacetic acid (metabolite A) and hydoxyethyl glycine (metabolite B).[91] Both urinary excretion (30% unchanged, 10% as metabolite A, 40% as metabolite B) and faecal elimination (3% metabolite linezolid A and 6% metabolite B) are observed.[92] The cytochrome P450 enzymatic complex neither participates in linezolid metabolism nor undergoes induction/inhibition. Dose adjustments are not required for advanced age, renal or hepatic dysfunction (Childs class A or B). Haemodialysis patients should receive linezolid following the dialysis session or receive a supplemental dose because there is significant dialytic clearance of linezolid.[93]

Linezolid is a weak competitive reversible inhibitor of the enzyme monoamine oxidase (MAO) and has the potential to cause hypertension when co-administered with either adrenergic or serotonergic agents (including tyramine). General caution is recommended if linezolid is co-administered with over-the-counter sinus decongestants and other cold remedies, which may contain a sympathomimetic compound. Reversible bone marrow suppression affecting the erythrocyte and platelet cell lines has been reported in patients receiving greater than two weeks of linezolid treatment. Bone marrow analysis revealed changes similar to those observed in reversible chloramphenicol myelosuppression.[94]

Linezolid has been studied in two unique clinical trial formats for its efficacy in vancomycin-resistant enterococcal infection; a blinded, dose-randomised (high *vs* low dose) protocol and a nonblind emergency use programme. High-dose linezolid (600mg intravenously every 12 hours) was compared to low-dose linezolid (200mg intravenously every 12 hours) in patients with clinical and microbiological evidence of serious infection caused by vancomycin-resistant enterococci.[54] Concomitant aztreonam and/or an aminoglycoside were optional to cover suspected or documented co-infection with Gram negative organisms. The most frequent primary sites of VRE infection included skin/skin structure, urinary tract, bacteraemia of unknown origin and intra-abdominal infection. The vast majority of infecting strains were *E. faecium*, whereas only six were *E. faecalis*. A total of 79 patients were randomised to high-dose linezolid versus 66 patients in the low dose arm with 58 and 46 evaluable patients in the high and low dose groups, respectively. The clinical outcomes demonstrated a modest dose-response effect with a 67% (39/58) cure rate for high-dose linezolid and 52% (24/46) in the low-dose group, although this difference was not statistically significant ($p = 0.17$).

An emergency use protocol for multi-resistant Gram-positive infection was conducted from October, 1997 until US Food and Drug Administration (FDA)-approval of linezolid in April, 2000.[55] From the first 133 evaluable patients there were 55 VRE infections, which were treated with linezolid (600mg intravenously every 12 hours parenterally

followed by oral administration in some patients) for a duration of 10 to 28 days. Clinical cure rates ranged from 88 to 100% depending on the type of infection (see table IV). Successful therapy has been reported with linezolid or linezolid plus gentamicin treatment of patients with VRE bacteraemia who failed to respond to quinupristin/dalfopristin.[95,96] Five of six liver recipients with sepsis secondary to intra-abdominal VRE infection were cured at end of treatment, although a sixth patient relapsed with a linezolid-resistant *E. faecium* strain (MIC = 8 μg/ml).[97,98] A rise in the linezolid MIC from pre-treatment MIC values of 2 μg/ml to 16 μg/ml and 32 μg/ml occurred in two patients who had *E. faecium* bacteraemia arising from unremovable intravascular prosthetic devices.[99] In both patients, parenteral linezolid was administered for a duration exceeding 4 weeks. In a separate report five patients developed linezolid resistance, four of whom were transplant patients receiving prolonged linezolid therapy. Treatment failure was the outcome in three of these five patients.[100]

Linezolid resistance derives from an alteration of the 23S bacterial ribosomal binding target caused by one of several possible base-pair substitution mutations. Repeat linezolid susceptibility testing is prudent in those patients with persistence or recurrent VRE isolation. It should be noted that although linezolid has *in vitro* activity against vancomycin-resistant *E. faecalis*, it has only received approval for vancomycin-resistant *E. faecium* infection because of the paucity of patients with *E. faecalis* isolates in the phase III trials.

3.3 Investigational Compounds

The continuing need to develop new antimicrobials with Gram-positive activity has spurred ongoing clinical trials for several unique compounds with potential use in the therapy of VRE infections. Daptomycin, a cyclic lipopeptide with a broad Gram-positive spectrum, has unique cell-membrane targeted mechanisms of activity which theoretically reduces the likelihood of the development of cross-resistance.[99] This compound is rapidly bactericidal against most Gram-positive organisms including VRE (MIC_{90} = 2 μg/ml).[101,102] *In vitro* susceptability is 2- to 4-fold greater in the presence of supplemental calcium.[102] Development of this compound was suspended in the early 1990s because of dose-dependent toxicity (creatinine phospokinase elevation) when administered every 12 hours. However, recent investigation has shown preserved clinical efficacy and minimal toxicity when this compound is administered at 4 mg/kg every 24 hours.[103] Daptomycin is currently in phase III trials for a variety of Gram-positive infections.

A second promising agent is oritavancin (LY-333328), which is a true glycopeptide and a diphosphate derivative of vancomycin.[104] Oritavancin has excellent *in vitro* activity against all Gram-positive species including VRE, *S. aureus* (including MRSA and glycopeptide-intermediate strains), and the major streptococci.[105,106] Although oritavancin binds to the identical molecular target for vancomycin, it retains activity against VRE perhaps due in part to dimer formation at low concentrations and its affinity for the bacterial membrane. It is rapidly bactericidal as a single agent in VRE time-killing assays.[107] This agent is highly protein bound and has a very long half-life of 144 hours which enables once-daily administration. However, this attribute has raised concerns with respect to drug accumulation and the consequent potential for toxicity.[108] Oritavancin has shown excellent clinical efficacy in a dose-ranging protocol for bloodstream infection including VRE bacteraemia. Phase III trials are currently planned for a number of Gram-positive infections, including skin and skin structure infections and bacteraemia.

Finally, the glycylcyclines are derivatives of tetracycline with activity against VRE, and other Gram-positive and many Gram-negative bacteria.[109] Clinical development of the earliest glycylcycline compounds were limited by intractable nausea and vomiting.[108] Tigilcycline (GAR-936) is a minocycline derivative (terbutylminocycline), which has a more tolerable adverse

effect profile, is currently in early clinical trials for complicated urinary tract infection, intra-abdominal infection, skin structure infections and community-acquired pneumonia.

4. Suppression or Eradication of VRE Colonisation

The major precedent for 'decolonisation' in Gram-positive infection has been the use of topical intra-nasal mupirocin for the eradication of MRSA, which has shown long term benefit in successfully reducing subsequent MRSA infections in dialysis patients,[110] cardiothoracic patients[111] and other 'at-risk' hosts, although its benefit appears marginal in the endemic MRSA setting.[112] The intestinal VRE inoculum may be as high as $>10^8$ cfu/faecal gram, may persist for indefinite periods in some hosts and is the source for human skin colonisation.[113-115] An antecedent VRE colonisation state is the precursor for subsequent VRE colonisation when anatomic opportunities (i.e. catheterisation, surgical wounds, viscus perforation or obstruction) occur.

Thus, strategies which can decrease or eliminate the VRE inoculum in the intestinal colonisation reservoir could potentially reduce the incidence of serious VRE superinfection in susceptible hosts. Currently, pharmacological efforts to achieve VRE colonisation suppression or eradication have only examined outcome based upon on-therapy and follow-up stool or rectal swab cultures for the presence of VRE, but have not been designed to show diminished VRE infection. VRE intestinal carriage persisted for up to 15 days of therapy with oral novobiocin and doxycycline.[48] Variable rates of VRE colonisation suppression/eradication have been reported for oral bacitracin at 50 000 U/day or 100 000 U/day, although VRE colonisation relapses were common after several weeks.[116,117] A larger study examined the combination of oral doxycyline 100mg twice daily and bacitracin showed VRE suppression during but not after therapy. [118]

Ramoplanin, a glycolipodepsipeptide that inhibits cell-wall peptidoglycan synthesis, has bactericidal VRE activity but severe toxicity precluded its use as a parenteral agent.[119,120] However, since this agent does not undergo gastrointestinal absorption after oral administration, it was recently studied for its ability to eradicate or suppress intestinal VRE colonisation after oral administration in a controlled trial with three treatment arms (100mg twice daily, 400mg twice daily or placebo for 7 days) in patients with documented VRE intestinal colonisation.[121] Stool cultures on VRE-selective media were obtained at end-of-treatment (day 7) and at 14 and 21 days follow-up. The proportion (number VRE-free patients/number tested patients) who were free of intestinal VRE colonisation at day 7 were placebo (0/20), ramoplanin 100mg (17/21) and ramoplanin 400mg (18/20), and at day 14 were placebo (2/20), ramoplanin 100mg (6/21) and ramoplanin 400mg (7/17). A phase III multicentre, randomised trial of enteral ramoplanin VRE eradication in VRE-colonised oncological patients is currently ongoing. At present VRE decolonisation is an unproven concept whose ultimate value will need to be measured as a reduction in serious VRE infection for at risk patients and secondarily as the reduction in rates of VRE cross transmission.

5. Conclusion

The loss of conventional antimicrobial options for enterococcal infection coupled with the tendency for VRE strains to infect the sickest patients has presented the medical and surgical community with very challenging problems. The inherent complexities of such patients and their treatment requirements, and the paucity of available agents has been a relative impediment to the performance of 'gold standard' randomised clinical trials to validate the efficacy of anti-VRE therapies. Nevertheless, clinical data has supported the approval of two novel antimicrobials and promises to bring several new agents (daptomycin, oritavancin, tigilcycline) in the near future. The proliferation of these new compounds should permit randomised comparative trials to better determine the optimal agent(s) for use in VRE infections. These promising developments need to be tempered by the real-

isation that their utilisation should be reserved for those patients with the clear medical necessity for VRE-directed antimicrobial therapy. Such appropriate rationing is critical to preserving the effectiveness of our Gram-positive antimicrobials and diminishing iatrogenic causes of Gram-positive resistance.

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