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Oritavancin Activity against Vancomycin-Susceptible and Vancomycin-Resistant Enterococci with Molecularly Characterized Glycopeptide Resistance Genes Recovered from Bacteremic Patients, 2009-2010

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Oritavancin exhibited potent activity against vancomycin-susceptible (MIC_{50} and MIC_{90}, 0.015/0.03 μg/ml) and *vanB*-carrying *E. faecalis* isolates (MIC_{50} and MIC_{90}, 0.015 and 0.015 μg/ml). Higher (16- to 32-fold) MIC_{50}s and MIC_{90}s for *vanA*-harboring *E. faecalis* were noted (MIC_{50} and MIC_{90}, 0.25 and 0.5 μg/ml), although oritavancin inhibited all strains at ≤0.5 μg/ml. Vancomycin-susceptible and *vanB*-carrying *E. faecium* strains (MIC_{50} and MIC_{90}, ≤0.008 and ≤0.008 μg/ml for both) were very susceptible to oritavancin, as were VanA-producing isolates (MIC_{50} and MIC_{90}, 0.03 and 0.06 μg/ml). Oritavancin exhibited good *in vitro* potency against this collection of organisms, including vancomycin-resistant enterococci.

Enterococcus species have become important nosocomial pathogens and currently represent the third most frequent pathogens responsible for health care-associated infections in the United States (10), with *Enterococcus faecium* isolates eliciting greater concern, as they are often resistant to commonly used antimicrobial agents, such as ampicillin, aminoglycosides, and glycopeptides (14). *Enterococcus faecalis* and *E. faecium* account for approximately 90% of nosocomial enterococcal infections and may acquire various types of glycopeptide resistance determinants (*vanA*, *-B*, *-D*, *-E*, *-G*, and *-N*) (5, 12). Among these elements, *vanA* and *vanB* are the most prevalent in clinically relevant species (9, 21). Although the rate of vancomycin resistance among *E. faecalis* strains causing bloodstream infections (BSI) has been stable over the last decade, the rate in *E. faecium* has escalated to 30% and 79% in Europe and the United States, respectively (10, 19).

There is growing evidence demonstrating that nosocomial enterococci possess specific characteristics, such as the presence of antimicrobial resistance determinants and pathogenicity traits, which enable them to rapidly adapt to the hospital environment and cause a broad range of invasive infections (11, 16, 17). The ability to acquire, retain, and express genetic elements further enhances the propensity of enterococci to sustain selective pressure (9). As infections caused by resistant bacteria usually begin with colonization of mucosal surfaces, in particular the intestinal epithelium, broad-spectrum antimicrobial therapy depletes the intestinal microbiota, which plays an important role in the production of antimicrobial proteins by stimulating the immune system (2). An individual with a compromised mucosal innate immune defense is more prone to bacterial colonization, which eventually progresses to an infectious episode (2). In fact, it has been demonstrated that intestinal domination by vancomycin-resistant enterococci (VRE) precedes bloodstream infections (18).

Oritavancin is a semisynthetic bactericidal lipoglycopeptide in late stage of clinical development for treatment of severe infections caused by Gram-positive organisms. This drug has demonstrated potent activity against Gram-positive pathogens, including multidrug-resistant (MDR) enterococci (VRE), staphylococci, and streptococci (1). This study describes the activity of oritavancin compared to other antimicrobial agents against a contemporary (2009-2010) collection of enterococcal clinical isolates causing BSI in U.S. and European hospitals, including VRE strains with molecularly characterized glycopeptide resistance determinants.

A total of 2,260 enterococci (1,312 *E. faecalis*, 869 *E. faecium*, 24 *E. gallinarum*, and 15 *E. casseliflavus* isolates) were collected from 29 medical institutions in the United States and 27 centers in 13 European countries, including Turkey and Israel. Strains included in this study were those recovered from blood in a prevalence mode design following established protocols as part of the SENTRY Antimicrobial Surveillance Program (i.e., the first 20 unique and consecutive blood isolates collected each month for 12 months per medical site). Isolates were submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, IA) and had the bacterial species identification confirmed by an automated system (Vitek2; bioMérieux, Hazelwood, MO) or conventional biochemical algorithms, as required.

Isolates were tested for susceptibility by broth microdilution following Clinical and Laboratory Standards Institute (CLSI) recommendations (3). Oritavancin susceptibility testing was performed using dry-form panels (TREK Diagnostic Systems, Cleveland, OH), which provide results equivalent to those of the CLSI-approved broth microdilution method supplemented with 0.002% polysorbate-80 (3). Quality assurance was performed by concurrent testing of the CLSI-recommended strains *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 (4). Interpretation of comparator MICs was in accordance with published CLSI (4) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (7). Isolates with vancomycin MICs of ≥8 μg/ml were screened for *vanA* and *vanB* in a multiplex PCR

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TABLE 1 Antimicrobial activity of oritavancin against vancomycin-susceptible and vancomycin-resistant enterococcal clinical isolates causing bloodstream infections in U.S. and European hospitals

Organism and resistance (no. tested)	MIC (μg/ml)		Number[a] (cumulative %) inhibited at MIC (μg/ml) of[a]:						
	50%	90%	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5
E. faecalis (1,312)									
Vancomycin susceptible (1,275)	0.015	0.03	435 (34.1)	**575 (79.2)**	211 (95.8)	43 (99.1)	7 (99.7)	3 (99.9)	1 (100.0)
vanA (27)	0.25	0.5	0 (0.0)	1 (3.7)	3 (14.8)	3 (25.9)	0 (25.9)	**15 (81.5)**	5 (100.0)
vanB (10)	0.015	0.015	1 (10.0)	**8 (90.0)**	0 (90.0)	1 (100.0)	–	–	–
E. faecium (869)									
Vancomycin susceptible (383)	≤0.008	≤0.008	**374 (97.7)**	7 (99.5)	2 (100.0)	–	–	–	–
vanA (470)	0.03	0.06	76 (16.2)	74 (31.9)	**146 (63.0)**	133 (91.3)	37 (99.1)	4 (100.0)	–
vanB (16)	≤0.008	≤0.008	**16 (100.0)**	–	–	–	–	–	–
E. casseliflavus (15) and *E. gallinarum* (24)									
vanC (39)	≤0.008	0.015	**34 (87.2)**	5 (100.0)	–	–	–	–	–

[a] Modal MICs are in bold.

assay (6). The identification of *E. casseliflavus* and *E. gallinarum* was confirmed by PCR for the presence of *vanC1*, *-2*, and *-3* (6).

Of the 2,260 enterococcal strains recovered from blood, the majority were *E. faecalis* (1,312; 58.1%), followed by *E. faecium* (869; 38.5%). *E. faecium* represented the vast majority (486/523; 93.0%) of vancomycin-resistant strains (Table 1). The *vanA* genotype was by far the most prevalent, accounting for 73.0% (27/37) and 96.7% (470/486) of *E. faecalis* and *E. faecium* strains, respectively. All *vanA*-carrying enterococci showed a VanA phenotype (i.e., vancomycin and teicoplanin MICs, >16 and >8 μg/ml, respectively), except for two U.S. *E. faecium* isolates that exhibited teicoplanin MICs of ≤1 and 4 μg/ml (Table 2). Enterococcal strains carrying the *vanB* gene demonstrated vancomycin and teicoplanin MICs of ≥8 and ≤2 μg/ml, respectively.

Oritavancin inhibited all tested enterococci at ≤0.5 μg/ml, with potent MIC_{50}s and MIC_{90}s against vancomycin-susceptible *E. faecalis* (MIC_{50} and MIC_{90}, 0.015 and 0.03 μg/ml) and *E. faecium* (MIC_{50} and MIC_{90}, ≤0.008 and ≤0.008 μg/ml) (Tables 1 and 2). MIC_{50}s of oritavancin against *vanB*-carrying *E. faecalis* and *E. faecium* were equivalent to those obtained with the respective vancomycin-susceptible strains. Vancomycin-resistant (*vanA*) *E. faecalis* exhibited oritavancin MICs (MIC_{50} and MIC_{90}, 0.25 and 0.5 μg/ml) 16-fold higher than those for vancomycin-susceptible isolates (MIC_{50} and MIC_{90}, 0.015 and 0.03 μg/ml). Similarly, *vanA*-carrying *E. faecium* (MIC_{50} and MIC_{90}, 0.03 and 0.06 μg/ml) exhibited higher (≥4-fold) oritavancin MICs than vancomycin-susceptible strains and *vanB*-harboring strains. Enterococcal isolates harboring intrinsic vancomycin resistance determinants (i.e., *E. casseliflavus* and *E. gallinarum*) showed variable vancomycin MICs (0.25 to 8 μg/ml; MIC_{50} and MIC_{90}, 4 and 8 μg/ml); nevertheless, these strains were very susceptible to oritavancin (MIC_{50} and MIC_{90}, ≤0.008 and 0.015 μg/ml) (Tables 1 and 2).

Ampicillin (MIC_{90}, 2 μg/ml; VanA- and VanB-producing strains were 96.3% and 100% susceptible, respectively), daptomycin (MIC_{90}, 1 to 2 μg/ml; 100% susceptible), and linezolid (MIC_{90}, 1 μg/ml; 100% susceptible) were active against vancomycin-resistant *E. faecalis* isolates (Table 2). Oritavancin demonstrated *in vitro* MIC_{90}s 2- to 4-fold and 64- to 128-fold lower than these comparator agents against *vanA*- and *vanB*-carrying *E. faecalis* strains, respectively. Among comparators, activity against vancomycin-resistant *E. faecium* was noted for daptomycin (MIC_{50} and MIC_{90}, 2 and 2 μg/ml; 100% susceptible) and linezolid (MIC_{50} and MIC_{90}, 1 and 2 μg/ml; 98.1 to 100% susceptible). Quinupristin-dalfopristin (MIC_{50} and MIC_{90}, ≤0.5 and 1 μg/ml; 96.6% susceptible) demonstrated activity against *vanA*-carrying *E. faecium* (Table 2), while marginal coverage was noted against vancomycin-susceptible and vancomycin-resistant (*vanB*) strains (MIC_{50} and MIC_{90}, ≤0.5 and >2 μg/ml; 72.1 to 87.5% susceptible). *E. casseliflavus* and *E. gallinarum* isolates were very susceptible (97.4 to 100%) to ampicillin (MIC_{50} and MIC_{90}, ≤1 and 2 μg/ml), teicoplanin (MIC_{50} and MIC_{90}, ≤2 and ≤2 μg/ml), daptomycin (MIC_{50} and MIC_{90}, 1 and 2 μg/ml), and linezolid (MIC_{50} and MIC_{90}, 1 and 2 μg/ml) (Table 2).

This report provides an update on the distribution of *van* genes among vancomycin-resistant *E. faecalis* and *E. faecium* strains causing bacteremia in U.S. and European hospitals. A study conducted in the United States from 1995 through 2002 reported vancomycin resistance rates among *E. faecalis* (2%) and *E. faecium* (60%) strains responsible for BSI similar to those observed in this study (2.8 and 55.9%, respectively) (20). However, the present study highlights the emergence of *E. faecium* (38.5% of all enterococci) as an important pathogen causing BSI, which is of great concern given the higher antimicrobial resistance and mortality rate associated with this species (10). Interestingly, two *E. faecium* strains from U.S. hospitals demonstrated the combination of a VanB phenotype and *vanA* genotype. Strains displaying these characteristics have been reported in the East Asia region (Japan, China, South Korea, and Taiwan) (8); however, this appears to be the first report of such strains in the United States.

The clinical options for treating serious infections caused by VRE are very restricted and often present limitations (13). Oritavancin has demonstrated *in vitro* concentration-dependent bactericidal activity against VRE at the predicted free peak concentration derived from administering a human dose of 800 mg (~16 μg/ml) (15). In this report, the comparison of the *in vitro* activity of oritavancin and other agents revealed pronounced oritavancin activity against this collection of clinical isolates. Moreover, oritavancin demonstrated 4- to 128-fold-greater potency than the active comparator antimicrobial agents, particularly against VRE. These *in vitro* activity data suggest oritavancin as a promising agent for treating serious infections caused by vancomycin-

TABLE 2 Antimicrobial activity of oritavancin and comparator agents against vancomycin-susceptible and vancomycin-resistant enterococcal clinical isolates causing bloodstream infections in U.S. and European hospitals

Organism (no. tested) and antimicrobial agent	MIC (μg/ml)			% Susceptible/% resistant[a]	
	Range	50%	90%	CLSI	EUCAST
Vancomycin-susceptible *E. faecalis* (1,275)					
Oritavancin	≤0.008–0.5	0.015	0.03	−/−	−/−
Ampicillin	≤1–8	≤1	2	100.0/0.0	99.8/0.0
Vancomycin	0.25–4	1	2	100.0/0.0	100.0/0.0
Teicoplanin	≤2–4	≤2	≤2	100.0/0.0	99.9/0.1
Daptomycin	0.12–4	1	2	100.0/−	−/−
Linezolid	0.25–>8	1	2	99.9/0.1	99.9/0.1
Quinupristin-dalfopristin	≤0.5–>2	>2	>2	0.5/95.0	0.5/89.0
Levofloxacin	≤0.5–>4	1	>4	69.0/30.4	−/−
Tetracycline	≤2–>8	>8	>8	23.2/76.5	−/−
vanA E. faecalis (27)					
Oritavancin	0.015–0.5	0.25	0.5	−/−	−/−
Ampicillin	≤1–>16	≤1	2	96.3/3.7	96.3/3.7
Vancomycin	>16	>16	>16	0.0/100.0	0.0/100.0
Teicoplanin	>8	>8	>8	3.7/96.3	0.0/100.0
Daptomycin	0.5–2	1	2	100.0/−	−/−
Linezolid	1–2	1	1	100.0/0.0	100.0/0.0
Quinupristin-dalfopristin	2–>2	>2	>2	0.0/96.3	0.0/96.3
Levofloxacin	2–>4	>4	>4	3.7/96.3	−/−
Tetracycline	≤2–>8	>8	>8	3.7/96.3	−/−
vanB E. faecalis (10)					
Oritavancin	≤0.008–0.06	0.015	0.015	−/−	−/−
Ampicillin	≤1–2	≤1	2	100.0/0.0	100.0/0.0
Vancomycin	8–>16	>16	>16	0.0/80.0	0.0/100.0
Teicoplanin	≤2	≤2	≤2	100.0/0.0	100.0/0.0
Daptomycin	≤0.06–2	0.5	1	100.0/−	−/−
Linezolid	0.5–2	1	1	100.0/0.0	100.0/0.0
Quinupristin-dalfopristin	>2	>2	>2	0.0/100.0	0.0/100.0
Levofloxacin	>4	>4	>4	0.0/100.0	−/−
Tetracycline	≤2–>8	≤2	>8	50.0/50.0	−/−
Vancomycin-susceptible *E. faecium* (383)					
Oritavancin	≤0.008–0.03	≤0.008	≤0.008	−/−	−/−
Ampicillin	≤1–>8	>8	>8	14.4/85.6	14.1/85.6
Vancomycin	0.25–4	1	1	100.0/0.0	100.0/0.0
Teicoplanin	≤2–4	≤2	≤2	100.0/0.0	99.7/0.3
Daptomycin	0.12–>8	2	4	99.7/−	−/−
Linezolid	0.5–>8	1	2	99.2/0.8	99.2/0.8
Quinupristin-dalfopristin	≤0.5–>2	≤0.5	>2	72.1/15.7	72.1/11.7
Levofloxacin	≤0.5–>4	>4	>4	15.4/77.5	−/−
Tetracycline	≤2–>8	≤2	>8	56.7/42.8	−/−
vanA E. faecium (470)					
Oritavancin	≤0.008–0.25	0.03	0.06	−/−	−/−
Ampicillin	>8	>8	>8	0.0/100.0	0.0/100.0
Vancomycin	>16	>16	>16	0.0/99.6	0.0/100.0
Teicoplanin	≤1–>8	>8	>8	0.6/96.2	0.2/99.8
Daptomycin	0.12–4	2	2	100.0/−	−/−
Linezolid	0.5–>8	1	2	98.1/1.3	98.7/1.3
Quinupristin-dalfopristin	≤0.5–>2	≤0.5	1	96.6/1.3	96.6/1.3
Levofloxacin	2–>4	>4	>4	0.2/99.8	−/−
Tetracycline	≤2–>8	>8	>8	36.8/62.3	−/−
vanB E. faecium (16)					
Oritavancin	≤0.008	≤0.008	≤0.008	−/−	−/−
Ampicillin	>8	>8	>8	0.0/100.0	0.0/100.0

(Continued on following page)

TABLE 2 (Continued)

Organism (no. tested) and antimicrobial agent	MIC (μg/ml)			% Susceptible/% resistant[a]	
	Range	50%	90%	CLSI	EUCAST
Vancomycin	8–>16	>16	>16	0.0/75.0	0.0/100.0
Teicoplanin	≤2	≤2	≤2	100.0/0.0	100.0/0.0
Daptomycin	0.5–4	2	2	100.0/−	−/−
Linezolid	0.5–4	1	2	93.8/0.0	100.0/0.0
Quinupristin-dalfopristin	≤0.5–>2	≤0.5	>2	87.5/12.5	87.5/12.5
Levofloxacin	>4	>4	>4	0.0/100.0	−/−
Tetracycline	≤2–>8	>8	>8	37.5/62.5	−/−
vanC enterococci (39)[b]					
Oritavancin	≤0.008–0.015	≤0.008	0.015	−/−	−/−
Ampicillin	≤1–>16	≤1	2	97.4/2.6	97.4/2.6
Vancomycin	0.25–8	4	8	82.1/0.0	82.1/17.9
Teicoplanin	≤2	≤2	≤2	100.0/0.0	100.0/0.0
Daptomycin	≤0.06–4	1	2	100.0/−	−/−
Linezolid	0.5–2	1	2	100.0/0.0	100.0/0.0
Quinupristin-dalfopristin	≤0.5–>2	2	>2	7.7/48.7	7.7/30.8
Levofloxacin	≤0.5–>4	2	4	84.6/5.1	−/−
Tetracycline	≤2–>8	≤2	>8	74.4/25.6	−/−

[a] Criteria for susceptibility as published by the CLSI (4) and EUCAST (7) recommendations. −, no breakpoint available.
[b] Includes *E. casseliflavus* (15 isolates) and *E. gallinarum* (24 isolates).

susceptible and -resistant enterococci, as monotherapy or as part of combination regimens with other agents, contingent on further studies.

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