

Antimicrobial susceptibility studies

Dalbavancin activity against selected populations of
antimicrobial-resistant Gram-positive pathogensJennifer M. Streit^a, Helio S. Sader^{a,*}, Thomas R. Fritsche^a, Ronald N. Jones^{a,b}^aThe JONES Group/JMI Laboratories, North Liberty, IA 52317, USA^bTufts University School of Medicine, Boston, MA 02111, USA

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Abstract

Dalbavancin, a dimethylaminepropyl amide derivative of the lipoglycopeptide A40926, was tested against 375 antimicrobial-resistant Gram-positive pathogens collected worldwide during 2001–2003. The isolates were tested by reference and Clinical Laboratory Standards Institute broth microdilution susceptibility methods, and dalbavancin was compared with over 20 other antimicrobials. Vancomycin resistance determinants among enterococci were identified using PCR primer sets for *vanA* and *vanB*. Dalbavancin was generally more potent than vancomycin or teicoplanin. Dalbavancin was highly active against penicillin- and ceftriaxone-resistant *Streptococcus pneumoniae* strains (MIC₉₀, ≤ 0.016 µg/mL). Dalbavancin was also very active against teicoplanin-nonsusceptible coagulase-negative staphylococci (CoNS; MIC range, 0.03–0.25 µg/mL), but dalbavancin MIC results were slightly elevated compared with wild type strains. Dalbavancin inhibited *vanB* enterococci (MIC range, 0.03–0.12 µg/mL) and was active against other resistant, non-*vanA* enterococcal species. However, *vanA* enterococcal strains were not as susceptible to dalbavancin (MIC₅₀, 16 µg/mL). In summary, dalbavancin was very active against a wide spectrum of resistant Gram-positive isolates and demonstrated greater potency than vancomycin or teicoplanin.

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1. Introduction

Novel and increasing resistance mechanisms among Gram-positive pathogens are presenting challenges to existing antimicrobials (Chang et al., 2003; Meka and Gold, 2004). Oxacillin and glycopeptide resistance in staphylococci and glycopeptide resistance in enterococci have accelerated the need for the development of new antimicrobial agents to treat these Gram-positive infections. Dalbavancin is a second-generation lipoglycopeptide, synthesized by dimethylaminepropyl amide derivatization of the natural glycopeptide A40926, which recently completed phase III clinical trials in skin and skin structure infections. Results of Phase II studies in skin and skin structure infections and catheter-related bacteremia were recently published (Raad et al., 2005; Seltzer et al., 2003). Dalbavancin has exhibited a wide spectrum of activity against Gram-positive bacteria, including methicillin-resistant

staphylococci and multidrug-resistant organisms (Candiani et al., 1999; Jones et al., 2001; Lefort et al., 2004).

Dalbavancin's mechanism of action consists of inhibiting cell-wall peptidoglycan cross-linking, similar to that of other glycopeptides, but the agent is not completely cross-resistant to vancomycin (Raad et al., 2005). Dalbavancin has been shown to be more potent than vancomycin and teicoplanin and is active against *vanB* and *vanC* vancomycin-resistant enterococcal strains (Candiani et al., 1999; Jones et al., 2001). Dalbavancin has also been reported to be active against *Staphylococcus* spp. strains with decreased susceptibility to glycopeptides (Lefort et al., 2004). The extended half-life of dalbavancin (approximately 8 days) (Leighton et al., 2004) permits once-weekly dosing, making it a more advantageous therapeutic option than other similar acting glycopeptides (Candiani et al., 1999; Lefort et al., 2004; Seltzer et al., 2003). In the present study, we expanded previous studies (Gales et al., 2005; Streit et al., 2004) by evaluating the in vitro activity of dalbavancin against a contemporary worldwide collection of multidrug-resistant Gram-positive strains displaying the most problematic resistance phenotypes.

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2. Materials and methods

2.1. Bacterial isolates

A total of 375 Gram-positive isolates with antimicrobial-resistant phenotypes were selected from a worldwide collection (2001–2003). The resistant subsets included *Staphylococcus aureus* (4 quinupristin/dalfopristin-resistant

Table 1

Activity of dalbavancin and selected antimicrobial agents tested against resistant Gram-positive strains collected worldwide

Organism (no. tested)/ antimicrobial agent	MIC (μg/mL)			Percent susceptible
	Range	50%	90%	
<i>Streptococcus pneumoniae</i> , penicillin-resistant (202)				
Dalbavancin	≤0.016–0.03	≤0.016	≤0.016	— ^a
Amoxicillin/clavulanate	≤2–16	2	8	79.2
Ceftriaxone	≤0.25–8	1	1	91.6
Clindamycin	≤0.06–>8	≤0.06	>8	64.0
Erythromycin	≤0.25–>32	4	>32	25.6
Linezolid	≤0.25–2	1	1	100.0
Quinupristin/dalfopristin	0.12–1	0.25	0.5	100.0
Vancomycin	0.12–0.5	0.25	0.5	100.0
<i>E. faecium</i> , quinupristin/dalfopristin-resistant (vancomycin-susceptible; 29)				
Dalbavancin	≤0.016–0.12	0.06	0.12	— ^a
Ampicillin	≤2–>16	4	>16	44.8
Chloramphenicol	≤2–>16	8	16	82.8
Doxycycline	≤0.5–>4	50.5	>4	69.0
Linezolid	1–2	2	2	100.0
<i>E. faecium</i> , vancomycin-resistant (<i>vanA</i> -positive strains; 44)				
Dalbavancin	0.03–>32	16	32	— ^a
Ampicillin	2–>32	>32	>32	2.0
Chloramphenicol	≤2–>16	8	8	95.4
Quinupristin/dalfopristin	0.25–8	1	1	97.7
Linezolid	0.5–>16	2	2	97.7
Teicoplanin	>16	>16	>16	0.0
<i>E. faecalis</i> , vancomycin-resistant (<i>vanA</i> ; 14)				
Dalbavancin	0.12–>32	32	32	— ^a
Ampicillin	4–32	8	16	71.4
Chloramphenicol	4–>16	8	>16	57.1
Quinupristin/dalfopristin	≥8	>8	>8	0.0
Linezolid	0.5–2	1	2	100.0
Teicoplanin	>16	>16	>16	0.0
Enterococci, vancomycin-resistant (<i>vanB</i> ; 11) ^b				
Dalbavancin	0.03–0.12	0.03	0.12	— ^a
Ampicillin	2–>32	>32	>32	45.4
Chloramphenicol	4–>16	8	16	72.7
Quinupristin/dalfopristin	0.25–>8	4	8	45.5
Linezolid	1–2	1	2	100.0
Teicoplanin	≤2	≤2	≤2	100.0
Coagulase-negative staphylococci, teicoplanin-resistant (15)				
Dalbavancin	0.03–0.25	0.12	0.25	— ^a
Chloramphenicol	≤2–>16	4	>16	66.7
Linezolid	0.5–1	1	1	100.0
Oxacillin	0.12–>8	>8	>8	13.3
Quinupristin/dalfopristin	0.12–1	0.25	1	100.0
Vancomycin	1–4	2	2	100.0

^a No interpretive breakpoint has been established by CLSI (2005).

^b Includes *E. faecium* (5 strains) and *E. faecalis* (6 strains).

Table 2

Dalbavancin activity tested against other resistant subsets of Gram-positive cocci

Organism (no. tested)	MIC (µg/mL)		
	Range	50%	90%
<i>Streptococcus pneumoniae</i> , ceftriaxone-resistant (16)	≤0.016–0.03	≤0.016	≤0.016
viridans-group streptococci, penicillin-resistant (5) ^a	≤0.016–0.03	≤0.016	–
Enterococci, vancomycin-resistant (<i>vanA</i> ; 58) ^b	0.03–>32	16	32
Enterococci, vancomycin-resistant (<i>vanB</i> ; 11) ^c	0.03–0.12	0.03	0.12
Staphylococci, quinupristin/dalfopristin-resistant ^d (8)	0.03–0.06	0.06	–
Staphylococci, vancomycin-intermediate (10)	0.06–2	0.06	1
CoNS, vancomycin MIC at 4 µg/mL (3)	0.12–0.5	0.12	–
Gram-positive cocci, linezolid-resistant (14) ^e	≤0.016–>32	0.06	32

^a Includes *Streptococcus mitis* (1 strain), *Streptococcus oralis* (1 strain), and *Streptococcus viridans* or α-hemolytic streptococci (3 strains were not identified to species level).

^b Includes *E. faecalis* (14 strains) and *E. faecium* (44 strains).

^c Includes *E. faecalis* (6 strains) and *E. faecium* (5 strains).

^d Includes *Staphylococcus aureus* (4 strains), *Staphylococcus xylois* (1 strain), *Staphylococcus epidermidis* (1 strain), *Staphylococcus sciuri* (1 strain), and CoNS (1 strain was not identified to species level).

^e Includes *Staphylococcus aureus* (3 strains) and *E. faecalis* (3 strains), *Staphylococcus epidermidis* (1 strain), *Staphylococcus oralis* (1 strain), and *E. faecium* (6 strains).

strains; 10 vancomycin-intermediate strains; 3 linezolid-resistant strains), coagulase-negative staphylococci (CoNS; 3 strains, vancomycin MIC at 4 µg/mL; 4 quinupristin/dalfopristin-resistant strains; 15 teicoplanin-nonsusceptible strains, 1 linezolid-resistant strain), *Enterococcus faecium* (49 strains vancomycin-resistant; 33 strains quinupristin/dalfopristin-resistant; 6 strains linezolid-resistant), *Enterococcus faecalis* (20 strains vancomycin-resistant; 3 strains linezolid-resistant), *Streptococcus pneumoniae* (202 strains penicillin-resistant; 16 strains ceftriaxone-resistant), and viridans-group streptococci (5 strains penicillin-resistant).

2.2. Susceptibility testing

All tests were performed by reference Clinical and Laboratory Standards Institute (CLSI; formerly, National Committee for Clinical Laboratory Standards [NCCLS]) methods (M7-A6) using Mueller-Hinton broth, supplemented with 2–5% lysed horse blood for testing streptococci (CLSI, 2005; NCCLS, 2003). Validated dry-form Sensititre® panels for susceptibility testing were manufactured by TREK Diagnostics (Cleveland, OH) (Jones et al., 2004). Dalbavancin (Vicuron Pharmaceuticals, King of Prussia, PA) was compared with over 20 other antimicrobial agents, including the 13 drugs reported here: vancomycin, teicoplanin, linezolid, ampicillin, oxacillin, amoxicillin/clavulanate, ceftriaxone, clindamycin, erythromycin, quinupristin/dalfopristin, penicillin, chloramphenicol, and doxy-

cycline. Where needed, dalbavancin results were also compared with susceptible subsets (wild-type) to assess potency variations against linezolid-, quinupristin/dalfopristin-, penicillin-, ceftriaxone-, or vancomycin-resistant strains. The following quality control organisms were concurrently tested and were within published limits: *Streptococcus pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 (Anderegg et al., 2003).

2.3. Molecular methods

Vancomycin resistance determinants among enterococci were identified using PCR primer sets specific for *vanA* and *vanB*. The primers and testing conditions used were those described by Dutka-Malen et al. (1995).

3. Results and discussion

The in vitro activity of dalbavancin in comparison with several other antimicrobial agents tested against Gram-positive organisms is presented in Table 1. Penicillin- and ceftriaxone-resistant *Streptococcus pneumoniae* (Tables 1 and 2) strains were inhibited by very low concentrations of dalbavancin. Its MIC₉₀ was ≤ 0.016 $\mu\text{g/mL}$ and all strains were inhibited at ≤ 0.03 $\mu\text{g/mL}$ of dalbavancin. These strains also had diminished susceptibility rates to erythromycin (25.6%) and clindamycin (64.0%), but linezolid (MIC₉₀, 1 $\mu\text{g/mL}$), quinupristin/dalfopristin (MIC₉₀, 0.5 $\mu\text{g/mL}$), and vancomycin (MIC₉₀, 0.5 $\mu\text{g/mL}$) were completely active against the 202 *Streptococcus pneumoniae* strains tested.

Against 33 quinupristin/dalfopristin-resistant strains of *E. faecium*, including 4 *vanA* isolates, dalbavancin MIC_{50/90} were 0.06 and 8 $\mu\text{g/mL}$; among 29 isolates that were not *vanA*, all were inhibited by ≤ 0.12 $\mu\text{g/mL}$ of dalbavancin (Table 1). Only linezolid was active against all of these quinupristin/dalfopristin-resistant enterococcal strains, regardless of susceptibility to vancomycin. All teicoplanin-nonsusceptible CoNS strains were inhibited by low concentrations of dalbavancin (15 strains; MIC range, 0.03–0.25 $\mu\text{g/mL}$). Vancomycin (MIC₉₀, 2 $\mu\text{g/mL}$), quinupristin/dalfopristin (MIC₉₀, 1 $\mu\text{g/mL}$), and linezolid (MIC₉₀, 1 $\mu\text{g/mL}$) were also active against all CoNS strains tested (Table 1).

Decreased susceptibility to vancomycin, linezolid, or quinupristin/dalfopristin (Table 2) did not markedly affect dalbavancin activity against staphylococci. Dalbavancin was active (MIC range, 0.06–2 $\mu\text{g/mL}$) against vancomycin-intermediate staphylococci and CoNS strains with a vancomycin MIC value of 4 $\mu\text{g/mL}$; approximately 8- to 16-fold more potent than vancomycin. Dalbavancin had potent activity (all MICs ≤ 0.06 $\mu\text{g/mL}$) against quinupristin/dalfopristin-resistant staphylococci, linezolid-resistant staphylococci (4 strains), and penicillin-resistant viridans-group streptococcal strains.

Dalbavancin (MIC₉₀, 0.12 $\mu\text{g/mL}$) was active against all 11 vancomycin-resistant enterococci showing the *vanB* resistance pattern. Against enterococci with the *vanA* type of resistance, the dalbavancin MIC range was broader (Table 2; MIC₉₀, 32 $\mu\text{g/mL}$). Linezolid (MIC₅₀ and MIC₉₀ at 2 $\mu\text{g/mL}$) and quinupristin/dalfopristin (MIC₅₀ and MIC₉₀ at 1 $\mu\text{g/mL}$) were active against nearly all (98.0%) vancomycin-resistant *E. faecium* (49 strains). Among the agents tested, only linezolid was active against all 20 strains of vancomycin-resistant *E. faecalis* (Table 1).

In summary, the data presented here expand the results of our previous studies (Gales et al., 2005; Streit et al., 2004) by showing that dalbavancin was very active against a wide spectrum of resistant Gram-positive isolates. Dalbavancin showed greater potency than vancomycin and teicoplanin and all antibiotic-resistant organisms tested, with the exception of *vanA* enterococci (*vanA* positive), were inhibited by ≤ 2 $\mu\text{g/mL}$ of dalbavancin. Dalbavancin was active against most of the resistant organisms including *vanB* enterococci, non-*vanA* enterococcal species, all resistant staphylococcal species, and all penicillin-resistant *Streptococcus pneumoniae*. The enhanced spectrum of dalbavancin against resistant Gram-positive organisms (Candiani et al., 1999; Gales et al., 2005; Jones et al., 2001; Lefort et al., 2004; Streit et al., 2004) and favorable pharmacokinetics (long half-life permitting once-weekly dosing) (Leighton et al., 2004; Seltzer et al., 2003) favor continued clinical development of this compound.

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