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In vitro Activity of Dalbavancin Susceptibility Tested Against *Staphylococcus* spp. and ß-haemolytic *Streptococcus* spp. Isolated in USA Medical Centers

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AMENDED ABSTRACT

Background: Dalbavancin is a novel lipoglycopeptide developed for the treatment of skin and soft tissue infections (SSTI) caused by Gram-positive pathogens. This study was designed to generate susceptibility (S) data for dalbavancin and vancomycin along with the S profiles of several drug classes each tested against staphylococci (STAPH) and ß-haemolytic streptococci (BHS) from a multicenter USA study.

Methods: 52 laboratories were recruited to locally test a total of 50 isolates including MRSA, MSSA, CoNS and BHS against dalbavancin and vancomycin using Etest (AB BIODISK) strips, and disk diffusion reagents for levofloxacin, erythromycin and clindamycin. STAPH were also tested against oxacillin, cefoxitin, gentamicin and tetracycline, and BHS against penicillin and ceftriaxone. Inducible resistance (R) to clindamycin was determined using the D-test. A total of 2,490 isolates are included in this report.

Results: Macrolide-R was 30.6%, 94.1%, 73.9% and 20.3% for MSSA, MRSA, CoNS and BHS, respectively. D-test results for inducible CL-R were 25.8% (MRSA [535]) to ≥55% (MSSA [192] and BHS [68]) among ER-R and CL-S strains. MRSA were more R to gentamicin, levofloxacin, and tetracycline compared to MSSA. Strains of BHS non-S to levofloxacin, penicillin and ceftriaxone were rarely reported.

Organism (no. tested)	Antimicrobial	MIC (µg/ml) 50%	90%	Range
MSSA (762)	Dalbavancin	0.06	0.12	0.03-0.5
	Vancomycin	1	2	0.5-8
MRSA (1,009)	Dalbavancin	0.06	0.25	0.03-0.5
	Vancomycin	1	2	0.5-8
MS-CoNS (58)	Dalbavancin	0.06	0.12	0.016-0.25
MR-CoNS (182)	Vancomycin	2	2	0.5-2
BHS (379)	Dalbavancin	0.016	0.06	≤0.002-0.25
	Vancomycin	0.5	1	0.25-1

Conclusions: Dalbavancin has a marked potency advantage (8-16X) compared to vancomycin against indicated species tested in this 2006 sample. All isolated species tested were inhibited by ≤0.5 µg/ml of dalbavancin. Dalbavancin represents an important, once weekly alternative therapy for SSTI caused by common pathogens which are increasingly becoming more R to other antimicrobial classes.

INTRODUCTION

Dalbavancin is a novel, second-generation glycopeptide antimicrobial agent indicated for the treatment of moderate to severe skin and skin structure infections (SSTI) caused by Gram-positive organisms. *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* are important pathogens associated with skin and soft tissue infections. Dalbavancin demonstrates excellent in vitro activity against these species including staphylococci resistant to oxacillin. Dalbavancin also shows excellent activity against strains resistant to other drug classes including MLS_B and multidrug-resistant strains. Dalbavancin has an extremely long elimination half-life and is administered intravenously at 1 gram on day 1 followed by a 500 mg dose on day 8.

The objective of this study was to generate quantitative (MIC) susceptibility testing data for dalbavancin and vancomycin (comparator agent) along with the susceptibility profiles of several drug classes against staphylococci including oxacillin-susceptible (MS) and -resistant (MR) strains and ß-haemolytic streptococci (βHS). This multicenter study identified the current susceptibility profiles of these important pathogens and determined the comparative activity of dalbavancin tested against nearly 2,500 isolates collected from over fifty leading medical institutions geographically dispersed throughout the United States (USA; Figure 1).

MATERIALS AND METHODS

Each study site was recruited to test 50 locally collected clinical isolates including: MRSA (20), MSSA or CoNS (20) and βHS (10). A total of 52 sites from 30 states participated in the study. Sites forwarded between 10 and 59 isolates of staphylococci and ß-haemolytic streptococci contributing a total of 2,538 isolates of which 2,490 were acceptable for analysis. These included MRSA (1,009), MSSA (762), MR-CoNS (182), MS-CoNS (58) and βHS (479). Isolates were collected from the following specimen sources: skin/skin structure (41%), bloodstream (28%), respiratory tract (21%), and unknown sources (10%).

Isolates were tested for susceptibility using Etest (AB BIODISK, Solna, Sweden) for dalbavancin and vancomycin. Six to eight additional antimicrobial agents were also tested on the same Mueller-Hinton agar plate by using disk diffusion test reagents (see Table 2). Inducible clindamycin resistance was determined by disk approximation with erythromycin using the D-test method (CLSI, M100-S16). Reported D-test results were determined only on those isolates observed to be erythromycin-resistant and susceptible to clindamycin (ER-CS phenotype). Susceptibility test methods followed recommendations from CLSI M2-A9 (disk diffusion) and the manufacturer's package insert (Etest).

Concurrent quality control (QC) was performed by each center using *S. aureus* ATCC 25922, *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619 for disk diffusion and Etest reagents. Sites performed QC on each day of testing and QC failures resulted in the elimination of susceptibility data for that day and for the affected antimicrobial agent(s).

RESULTS

- Overall, oxacillin-resistant staphylococci had slightly higher dalbavancin MIC values as compared to the susceptible strains with MIC_{90} values of 0.125 µg/ml and 0.19 µg/ml, respectively (only appreciated by using the 15-dilution Etest method; Table 1). This potency was eight- to 16-fold greater than vancomycin (MIC_{90}, 1.5-2 µg/ml).
- A total of 165 strains (9.3%) of *S. aureus* had dalbavancin MIC values of ≥0.19 µg/ml. The vast majority of these (80%) were recorded by 8 of 53 participant laboratories located in geographically diverse areas within the USA (Figure 1; red dots). The diffusion characteristics of dalbavancin (high molecular weight compound) in agar media was compromised which leads to narrow ellipses and erroneously elevated MIC values that were read by these cited centers.
- βHS were more susceptible to dalbavancin (MIC_{90}, 0.047 µg/ml) compared to vancomycin which had a significantly higher MIC_{90} (0.75 µg/ml) as shown in Table 1. Some variation in the antimicrobial activity of dalbavancin was demonstrated for different serotypes of βHS (Figure 2). The MIC_{90} for Group A (*S. pyogenes*) was 0.016 µg/ml compared to Group B (*S. agalactiae*) which was 0.064 µg/ml. The remaining serogroups (C, G, and F) had MIC_{90} values ranging from 0.023-0.047 µg/ml.

Table 1. Dalbavancin activity directly compared to vancomycin when tested against 2,490 recent Gram-positive isolates in the United States (2005-2006).

Organism group (no. tested)/Antimicrobial	Cumulative % inhibited at MIC (µg/ml):[a] ≤0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	MIC (µg/ml)[b] 50%	90%
S. aureus															
Oxacillin-susceptible (762)															
Dalbavancin	0.0	0.0	0.0	0.0	2.2	57.5	92.0	99.0	100.0	-	-	-	-	0.064	0.125
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	65.9	100.0	-	-	1.0	1.5
Oxacillin-resistant (1,009)															
Dalbavancin	0.0	0.0	0.0	0.0	2.4	59.0	89.7	98.8	100.0	-	-	-	-	0.064	0.19
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	61.4	99.8	99.9	100.0	1.0	1.5
Coagulase-negative staphylococci[c]															
Oxacillin-susceptible (58)															
Dalbavancin	0.0	0.0	0.0	12.1	31.0	82.8	93.1	100.0	-	-	-	-	-	0.047	0.125
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.1	38.7	100.0	-	-	1.5	2.0
Oxacillin-resistant (182)															
Dalbavancin	0.0	0.0	0.0	0.5	13.7	67.0	89.6	100.0	-	-	-	-	-	0.064	0.19
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.1	13.1	90.2	100.0	-	2.0	2.0
ß-haemolytic streptococci (479)[d]															
Dalbavancin	0.2	1.3	8.1	73.5	86.6	98.7	99.8	100.0	-	-	-	-	-	0.016	0.047
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.0	86.9	100.0	-	-	-	0.38	0.75

a. Etest results (AB BIODISK, Solna, Sweden), results rounded to $\log_2$ scale.
b. Etest results unrounded to the $\log_2$ scale allowing MIC precision at the one-half $\log_2$ scale (15 total dilution steps).
c. CoNS included *S. capitis* (three strains), *S. epidermidis* (59 strains), *S. haemolyticus* (four strains), *S. hominis* (four strains), *S. lugdunensis* (four strains), *S. saprophyticus* (one strain), *S. simulans* (two strains), *S. warnerii* (four strains) and unspeciated CoNS (159 strains).
d. ß-haemolytic serotype of streptococci were group A (302 strains), group B (101 strains), group C (26 strains), group F (eight strains), and group G (42 strains).

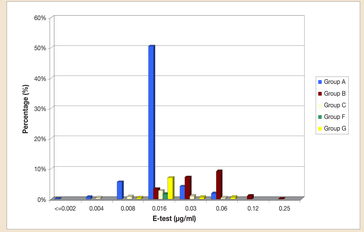
Table 2. Dalbavancin activity compared to seven other agents when tested against 2,490 Gram-positive cocci in 52 laboratories by Etest and disk diffusion methods (USA, 2005-2006).

Organism group (no. tested)	Antimicrobial agent	% Susceptible[a]	% Resistant
S. aureus			
Oxacillin-resistant (1,009)	Dalbavancin	98.8	-[b]
	Vancomycin	99.8	0.0
	Erythromycin	5.4	94.1
	Clindamycin	55.2	41.7
	Levofloxacin	27.7	70.3
	Gentamicin	92.0	7.9
	Tetracycline	91.8	8.0
	Linezolid	99.7	-
Oxacillin-susceptible (762)	Dalbavancin	99.0	-
	Vancomycin	100.0	0.0
	Erythromycin	65.8	30.6
	Clindamycin	92.8	5.8
	Levofloxacin	91.1	7.6
	Gentamicin	98.7	1.3
	Tetracycline	95.1	4.4
	Linezolid	99.9	-
Coagulase-negative staphylococci[c]			
Oxacillin-resistant (182)	Dalbavancin	100.0	-
	Vancomycin	100.0	0.0
	Erythromycin	18.6	81.4
	Clindamycin	41.5	53.6
	Levofloxacin	31.9	64.3
	Gentamicin	58.2	35.6
	Tetracycline	82.4	16.4
	Linezolid	100.0	-
Oxacillin-susceptible (58)	Dalbavancin	100.0	-
	Vancomycin	100.0	0.0
	Erythromycin	45.2	51.6
	Clindamycin	79.0	21.0
	Levofloxacin	82.3	9.7
	Gentamicin	95.0	5.0
	Tetracycline	95.0	5.0
	Linezolid	98.4	-
ß-haemolytic streptococci (479)[d]	Dalbavancin	100.0	-
	Vancomycin	100.0	0.0
	Penicillin	98.3	-
	Ceftriaxone	98.9	-
	Erythromycin	76.9	20.2
	Clindamycin	87.7	6.0
	Levofloxacin	98.7	0.2
	Linezolid	99.8	-

a. Susceptibility criteria of the CLSI (M100-S16, 2006) were used where available. For dalbavancin, a susceptibility breakpoint of ≤0.25 µg/ml was used for comparisons with vancomycin, both drugs tested by Etest (AB BIODISK).
b. - = no CLSI breakpoint criteria are published.
c. CoNS included *S. capitis* (three strains), *S. epidermidis* (59 strains), *S. haemolyticus* (four strains), *S. hominis* (four strains), *S. lugdunensis* (four strains), *S. saprophyticus* (one strain), *S. simulans* (two strains), *S. warnerii* (four strains) and unspeciated CoNS (159 strains).
d. ß-haemolytic serotype of streptococci were group A (302 strains), group B (101 strains), group C (26 strains), group F (eight strains), and group G (42 strains).

Figure 1. Geographic distribution of laboratories contributing data for the dalbavancin pre-launch susceptibility study.

Figure 2. Variations in the MIC values (µg/ml) of dalbavancin tested against different serotypes of ß-haemolytic streptococci.



- The MRSA and CoNS were much more resistant to other tested agents with rates of >81.4, >64.3 and >7.9% for erythromycin, levofloxacin, and gentamicin or tetracycline, respectively (Table 2). Nearly all (>98.4%) staphylococcal isolates were susceptible to dalbavancin (≤0.25 µg/ml), vancomycin and linezolid.
- D-test results for inducible clindamycin resistance showed that 55.0 and 26.0% of the oxacillin-susceptible and -resistant *S. aureus* were positive, respectively. Approximately 32% of CoNS having the ER-CS phenotype were D-test positive, regardless of oxacillin susceptibility.
- The βHS were very susceptible to the tested agents (Table 2) although a 20% resistance rate for erythromycin, and among the ER-CS phenotypes, a 62% inducible clindamycin resistance rate was observed. Rare strains showed reproducible non-susceptible disk zone diameters to penicillin, ceftriaxone, levofloxacin, and linezolid. These strains were considered as the natural extreme of the wildtype susceptible zone diameter distributions (usually 1-2 mm smaller than the breakpoint zone).

CONCLUSIONS

- Dalbavancin showed excellent in vitro activity against indicated species of staphylococci and βHS, and was eight- to 16-fold more potent than vancomycin.
- Dalbavancin MIC values obtained with Etest may be falsely elevated due to interpretive errors in reading narrow ellipses often produced by dalbavancin and related peptide compounds.
- Inducible clindamycin resistance was commonly observed among ER-CS phenotype isolates, particularly for oxacillin-susceptible *S. aureus* and ß-haemolytic streptococci. Over 92.0% of participant sites in this study reported inducible clindamycin resistance among the strains tested.

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