C-018

Comparative Results of Oxacillin and Cefoxitin Disk Diffusion Staphylococcal Tests and the Occurrence of Inducible Clindamycin Resistance in Various Gram-Positive Species: Results from a 52-Center Trial of a Novel Lipoglycopeptide

DJ BIEDENBACH, RN JONES, HS SADER, TR FRITSCHE, MG STILWELL

JMI Laboratories, North Liberty, IA, USA

ASM 2007
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT

Background: The CLSI has modified staphylococcal testing guidelines to enhance the detection of *mec*A-mediated and inducible clidamycin (CL) resistance (R) by disk diffusion (DD) tests. Cefoxitin (CFOX) DD results best predict *mec*A presence compared to oxacillin (OXA) DD tests. In the absence of D test results for inducible clindamycin resistance, varying numbers of CL-susceptible (S), erythromycin (ER)-R strains harbor an unrecognized inducibly R pattern. The contemporary rates of these R were assessed in a 52-center study (USA; 2005-2006) of a new long half-life lipoglycopeptide, dalbavancin (DAL).

Methods: Participant sites processed a target number of 40 staphylococci and 10 ß-haemolytic streptococci (BST) by DD or an MIC method (DAL and vancomycin [VANC]). All ER-R, CL-S strains were tested by CLSI D-test; and OXA and CFOX DD results were compared for *S. aureus* (SA; 1,771) and coagulase-negative staphylococci (CoNS; 240). The potencies of DAL and VANC (µg/ml; 15 dilutions) were quantified by Etest (AB BIODISK, Solna, Sweden) and rates of S were determined by CLSI criteria (2006), where available.

Results:

Organism (no.)[a]	ER-R (%)	CL-R rate (%)		
		Baseline	Inducible	Total
MRSA (1,009)	94.1	41.7	13.7	55.4
MSSA (762)	30.6	5.8	13.9	19.7
MR-CoNS (182)	81.4	53.6	8.8	62.4
MS-CoNS (58)	51.6	21.0	10.4	31.4
BST (479)	20.2	6.0	8.8	14.8

a. MR = methicillin- or OXA-R; MS = methicillin- or OXA-R

Inducible CL-R ranged from 25.8% (MRSA) to 61.8% (BST) of ER-R, CL-S strains; but, highest total CL-R was among the MR-staphylococci (55.4-62.4%). Using CFOX DD results as a reference for MR, the OXA DD false-S rates were 2.1 and 1.2% for SA and CONS, respectively (false-R at only 0.3%, excluding *S. lugdenensis*). DAL (MIC_{90} range, 0.047-0.19 µg/ml) was 8- to 32-fold more active than VANC against all staphylococci and BST tested. The highest DAL MIC was only 0.5 µg/ml.

Conclusions: Using the recently recommended CLSI D-test and CFOX DD for *mec*A, the detection of significant CL-R and MR has been improved. These simple DD tests should be routinely applied to isolates from serious Gram-positive infections (bloodstream, endocarditis, pneumonia, and osteomyelitis) to best direct treatment, supplementing other test methods. DAL demonstrated a spectrum similar to VANC, but with significantly greater potency.

INTRODUCTION

It is very important to correctly characterize oxacillin and macrolide, lincosamide and type B streptogramin (MLS_B) susceptibility patterns among *Staphylococcus* spp. The resistance rates to these agents within a medical center or a community are very helpful to physicians who must decide upon appropriate antimicrobial therapy. Recently, the Clinical and Laboratory Standards Institute (CLSI) has modified the disk diffusion breakpoint guidelines for the enhanced detection of *mec*A-mediated ß-lactam resistance (CLSI, M100-S17, 2007). Specifically, in 2004 the cefoxitin disk test was stated as being the preferred surrogate for reporting resistance to oxacillin and all other ß-lactam agents. Additionally, the CLSI standard also recommends utilizing the disk approximation test (D-test) for the detection of inducible clindamycin resistance by erythromycin for both staphylococci and streptococci.

A multi-center trial for determining the activity of dalbavancin, a novel lipoglycopeptide, was completed during 2005-2006. This study provided a comparison between the activity of dalbavancin and vancomycin using the Etest (AB BIODISK, Solna, Sweden) method. In addition, other agents including oxacillin, cefoxitin, erythromycin and clindamycin were tested by the disk diffusion method. Using the recently updated CLSI disk diffusion recommendations for oxacillin and cefoxitin, the current study compared these two agents tested against *S. aureus* and coagulase-negative staphylococci (CoNS). A calculation of the rate of inducible clindamycin resistance was also made using this contemporary collection of staphylococci and streptococci.

MATERIALS AND METHODS

Fifty-two medical centers geographically dispersed across the United States were recruited to select 50 locally collected isolates of dalbavancin-indicated species, staphylococci and ß-haemolytic streptococci (ßHS). Participants were asked to test MRSA (20 isolates), MSSA or CoNS (20 isolates) and 10 strains of ßHS from significant patient infections. The sites were instructed to test the isolates using Etest (AB BIODISK) for dalbavancin and vancomycin. Additional antimicrobial agents were tested by disk diffusion which included oxacillin, cefoxitin, erythromycin, clindamycin, levofloxacin, gentamicin, tetracycline and linezolid for staphylococci. Penicillin, ceftriaxone, erythromycin, clindamycin, levofloxacin and linezolid were tested against the streptococci.

The D-test was also performed on all strains to determine the rate of inducible resistance at each medical center. Susceptibility test methods followed the recommendations of the CLSI (disk diffusion/D-test) or the manufacturer (Etest).

Quality control strains were tested concurrently during each day of testing and included *S. aureus* ATCC 25922 and 29213 and *S. pneumoniae* ATCC 49619. After quality control results were evaluated, a total of 2,490 validated strain results were available for analysis which included: MRSA (1,009), MSSA (762), MR-CoNS (182), MS-CoNS (58) and ßHS (479).

RESULTS

- Potency of dalbavancin was eight to 16-fold greater than that of vancomycin (MIC_{90}; 1.5 – 2 µg/ml) when tested against oxacillin-susceptible and -resistant staphylococci with MIC_{90} values of 0.12 - 0.19 µg/ml.
- Dalbavancin (MIC_{90}; 0.047 µg/ml) was 16-fold more active than vancomycin against ßHS and was more potent against *S. pyogenes* (MIC_{90}; 0.016 µg/ml) compared to the other serogroups tested (data not shown).
- Dalbavancin, vancomycin and linezolid were very active (>99%) against all tested isolates (Table 1).
- Higher co-resistances were noted among the oxacillin-resistant staphylococci with 80 to 95% resistance to erythromycin and 65 to 70% resistance to levofloxacin (Table 1). Resistance to gentamicin (35.6%) and tetracycline (16.4%) was highest among the oxacillin-resistant CoNS. A 20.2% erythromycin resistance rate was observed among ßHS.
- Clindamycin resistance was detected in 55.4 and 19.7% of the oxacillin-resistant and oxacillin-susceptible *S. aureus* isolates, respectively (Table 2). The proportion of clindamycin resistant isolates with inducible resistance was approximately 30% for CoNS and 60% for ßHS.

Table 1. 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-S17, 2007) 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. warneri* (four strains) and unspeciated CoNS (164 strains).
d. ß-haemolytic serotype of streptococci were group A (303 strains), group B (101 strains), group C (26 strains), group F (eight strains), and group G (42 strains).

- Oxacillin disk diffusion results produced 2.1% (*S. aureus*) and 1.2% (CoNS) false-susceptibile results using cefoxitin as the accurate surrogate for reporting oxacillin susceptibility (Figures 1 and 2). The overall false resistant rate for staphylococci was only 0.3%, disregarding four isolates of *S. lugdenensis* circled in Figure 2.

Figure 1. Comparison of cefoxitin and oxacillin disk diffusion zone diameter results tested against *S. aureus* isolates (1,761 strains).

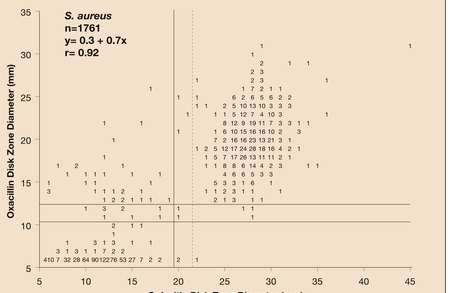

Figure 2. Comparison of cefoxitin and oxacillin disk diffusion zone diameter results tested against coagulase-negative staphylococcal isolates (243 strains).

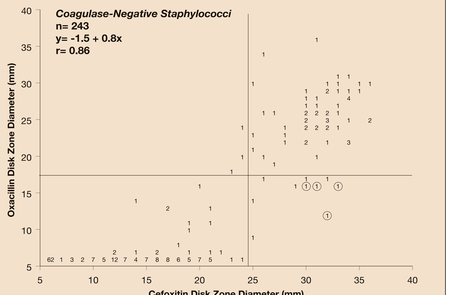

Table 2. Results of D-tests for inducible clindamycin resistance in the dalbavancin surveillance study. Only erythromycin-resistant, clindamycin-susceptible strains of each organism group were tested.

Organism group (no. tested)	D-test results (%)[a]	
	Positive	Negative
S. aureus		
Oxacillin-susceptible (192)	55.2	44.8
Oxacillin-resistant (535)	25.8	74.2
CoNS[b]		
Oxacillin-susceptible (19)	31.6	68.4
Oxacillin-resistant (50)	32.0	68.0
ß-haemolytic streptococci (68)	61.8	38.2

a. D-test performed as directed in the CLSI M100-S17 (2007).
b. CoNS = coagulase-negative staphylococci.

CONCLUSIONS

- Dalbavancin showed excellent potency and in vitro activity compared to vancomycin when tested against the indicated Gram-positive isolates from 52 US medical centers.
- Among erythromycin-susceptible and cindamycin-resistant isolates, inducible clindamycin resistance was proportionately highest for oxacillin-susceptible *S. aureus* and ßHS. However, constiutive and inducible clindamycin resistance within an entire population of tabulated Gram-positive pathogens was highest for oxacillin-resistant *S. aureus* and CoNS.
- Cefoxitin disk diffusion breakpoints to predict oxacillin susceptibility produced a relatively small number of primarily false-susceptible errors when compared to oxacillin disk results. This finding suggests that cefoxitin is a better predictor of *mec*A than the oxacillin disk.

SELECTED REFERENCES

1. Clinical and Laboratory Standards Institute. (2007). *M100-S17, Performance standards for antimicrobial susceptibility testing, 17th informational supplement*. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute. (2006). *M2-A9. Performance standards for antimicrobial disk susceptibility tests; approved standard - ninth edition.* Wayne, PA: CLSI.
3. Fritsche TR, Rennie RP, Goldstein BP, Jones RN (2006). Comparison of dalbavancin MIC values determined by Etest (AB BIODISK) and reference dilution methods using Gram-positive organisms. *J Clin Microbiol* 44: 2988-2990.
4. Seltzer E, Dorr MB, Goldstein BP, Perry M, Dowell JA, Henkel T (2003). Once-weekly dalbavancin versus standard-of-care antimicrobial regimens for treatment of skin and soft-tissue infections. *Clin Infect Dis* 37: 1298-1303.
5. Streit JM, Fritsche TR, Sader HS, Jones RN (2004). Worldwide assessment of dalbavancin activity and spectrum against over 6,000 clinical isolates. *Diagn Microbiol Infect Dis* 48: 137-143.
6. Swenson JM, Skov R, Patel JB (2007). The cefoxitin disk test - What a clinical microbiologist needs to know. *Clinical Microbiology Newsletter* 29: 33-40.