Daptomycin Tested Against 915 Bloodstream Isolates of Viridans Group Streptococci (Eight Species) and *Streptococcus bovis*

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ABSTRACT

Background: To evaluate the activity of daptomycin tested against numerous species of viridans group streptococci (VgS) and *Streptococcus bovis* which are pathogens associated with wound infections, sepsis, cellulitis, endocarditis, abscesses and dental caries. The incidence of penicillin-resistant (R) or MLS_BR strains among VgS often varies by species, and large collections of strains may be required to assess an antimicrobial true clinical susceptibility (S).

Methods: The activity of daptomycin was compared to seven other antimicrobial classes using reference broth microdilution (NCCLS, M7-A6) and disk diffusion (M2-A8) methods tested against 915 streptococci (815 VgS strains, 66 to 107 per species; 100 *S. bovis*). Mueller-Hinton broth was supplemented with 2 - 5% LHB and up to 50 mg/L Ca^{++}.

Results: Among VgS and *S. bovis*, 99.9% of isolates were daptomycin-S (breakpoint at <= 1 mg/L; MIC_{90}, 0.06 - 1 mg/L). In contrast, penicillin (65.5 - 98.1% S), macrolides (48.6 - 88.7%) and tetracycline (35.0 - 93.9%) activity varied widely between species. Erythromycin was least active, in contrast linezolid (99.1 - 100.0% S), vancomycin (100.0%) and quinupristin/dalfopristin (99.0 - 100.0%) were equally active as daptomycin, but less potent. Intermethod categorical agreement between daptomycin and linezolid (comparison agent) disk and microdilution tests was very high, each showing near complete S (99.9%).

Conclusions: Daptomycin was demonstrated to be an active agent that has clinically usable potency against these nine streptococcal species. The highest recorded daptomycin MIC was only 2 mg/L (1 strain; 0.1%). These results show that daptomycin would be an excellent candidate for further clinical trials targeting serious systemic infections caused by VgS/*S. bovis*.

INTRODUCTION

Daptomycin (formerly LY146032) is a novel cyclic lipopeptide which has demonstrated bactericidal activity against most Gram-positive organisms. Daptomycin's mode of killing action includes inhibiting cell wall synthesis and establishing multiple ion gradients across the cytoplasmic membranes giving it a unique dual mechanism thus having no cross-resistance with glycopeptides (teicoplanin and vancomycin). The bactericidal activity of daptomycin is dependent upon physiological levels of free calcium ions, Ca^{++} (50 mg/L). Increasing calcium concentration in the Mueller-Hinton agar test media has been demonstrated to produce optimal daptomycin disk diffusion results.

The daptomycin spectrum of activity includes many multidrug-resistant (MDR) strains for which there are few therapeutic alternatives, including vancomycin-resistant enterococci, methicillin- or oxacillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant streptococci. The United States Food and Drug Administration (US-FDA) recently approved categorical breakpoints for *S. aureus*, ß-haemolytic streptococci and vancomycin-susceptible *Enterococcus faecalis* for daptomycin at ≤ 1, ≤ 1 and ≤ 4 mg/L, respectively; these criteria were added to National Committee for Clinical Laboratory Standards (NCCLS, currently CLSI) tables and modified to include *Streptococcus* spp. (not *S. pneumoniae*) at a breakpoint of ≤ 1 mg/L. Here we report on the susceptibility profiles using breakpoint criteria promulgated by the NCCLS (see Table 1) for each of eight species of viridans group streptococci and *Streptococcus bovis* tested against daptomycin.

MATERIALS AND METHODS

Bacterial isolates: The study collection of 915 streptococci (target ≥ 100 strains per species) recovered from bloodstream infections included strains of *S. anginosus* (106 strains), *S. constellatus* (103 strains), *S. intermedius* (103 strains), *S. mitis* (102 strains), *S. mutans* (66 strains), *S. oralis* (107 strains), *S. salivarius* (101 strains), *S. sanguis* (127 strains) and *S. bovis* (100 strains). *S. anginosus*, *S. constellatus* and *S. intermedius* are often placed in the *S. milleri* group. All isolates were non-duplicate, clinical isolates collected as part of international surveillance studies by JMI Laboratories (North Liberty, Iowa, USA). Twenty-one additional bacteremic strains of *S. mutans* were received as a gift from Dr. M.J. Ferraro.

Susceptibility testing: All strains were tested using Mueller-Hinton broth supplemented with 2 - 5% LHB and to 50 mg/L calcium for daptomycin according to the NCCLS M7-A6 reference broth microdilution method. Daptomycin and seven comparator agents including penicillin, erythromycin, clindamycin, vancomycin, linezolid, quinupristin/dalfopristin and tetracycline were tested in reference frozen-form broth microdilution panels manufactured by TREK Diagnostics Systems (Cleveland, Ohio, USA). Daptomycin and three comparator agents (penicillin, vancomycin, and linezolid) were also tested against all strains using the NCCLS M2-M8 disk diffusion method on 5% sheep blood agar. The Mueller-Hinton blood agar (MHA) was tested to confirm a satisfactory minimum calcium concentration of 25 mg/L; the one lot (Remel #416527) that was used for the disk diffusion studies had an average calcium concentration of 33 mg/L. Susceptibility to daptomycin was defined as a MIC of ≤ 1 mg/L.

Quality control (QC): An ATCC QC strain (*S. pneumoniae* ATCC 49619) was processed in parallel and all susceptibility test results were within published ranges for the MIC method. The modal MIC results for the QC strain were: daptomycin (0.12 mg/L), penicillin (0.25 mg/L), erythromycin (0.06 mg/L), clindamycin (0.06 mg/L), linezolid (1 mg/L), quinupristin/dalfopristin (0.25 mg/L), vancomycin (0.12 mg/L) and tetracycline (≤ 1 mg/L).

For disk diffusion QC, the same strain (*S. pneumoniae* ATCC 49619) was utilized and all zone diameters were within NCCLS specified ranges. The median zone diameters generated for *S. pneumoniae* ATCC 49619 during the study were: daptomycin (23 mm), penicillin (25 mm), linezolid (29 mm) and vancomycin (24 mm).

RESULTS

- The results of testing 915 streptococci by reference NCCLS MIC methods against daptomycin and seven comparison agents are summarized in Table 1. Daptomycin MIC values ranged from ≤ 0.016 - 2 mg/L with 99.9% of isolates inhibited by ≤ 1 mg/L. One isolate of *S. oralis* had a reproducible daptomycin MIC of 2 mg/L. The least daptomycin-susceptible species were *S. oralis* and *S. sanguis* (MIC_{90}, 1 mg/L); the most susceptible species (MIC_{90}, 0.06 mg/L) was *S. bovis* (group D) followed by viridans group species, *S. mutans* and *S. salivarius* (MIC_{90}, 0.25 mg/L).
- All strains of viridans group streptococci were observed to be susceptible to vancomycin at ≤ 1 mg/L. Penicillin MIC results ranged from ≤ 0.008 to > 16 mg/L, and susceptibility rates widely varied (65.5 - 98.1%) by species. *S. sanguis* isolates were least penicillin-susceptible (65.5%) and *S. constellatus* isolates were highly susceptible (98.1%) at current NCCLS breakpoints.
- Macrolide (erythromycin) susceptibility was lowest among *S. oralis* strains (48.6%) whereas *S. anginosus* strains were more susceptible (88.7%). Clindamycin susceptibility rates ranged from 74.0% for *S. bovis* to 97.0% for *S. mutans*. The streptogramin combination, quinupristin/dalfopristin was very potent (99.0 - 100.0% susceptible) against all species except *S. bovis*, (94.0% susceptible), where six strains had elevated MIC results of 2 (intermediate) or 4 mg/L (resistant).
- Tetracycline MIC results were usually bimodally distributed with the susceptibility rates ranging from only 35.0% (*S. bovis*) to 93.9% (*S. mutans*).
- Linezolid showed acceptable potency versus these streptococci. MIC_{50} and MIC_{90} results were all 1 mg/L and the linezolid-susceptible rate was 99.9%. Only one strain resistant to linezolid was observed (*S. oralis*, MIC at 16 mg/L).
- Disk diffusion test results were collected for daptomycin and three comparator agents (penicillin, vancomycin, linezolid). Zone diameter results were compared to daptomycin MIC values using scattergram analysis for all 915 isolates (Figure 1). While all strains were daptomycin-susceptible by the disk diffusion method (zone at ≤ 16 mm), one strain (*S. oralis*) had a reproducible daptomycin MIC at 2 mg/L (non-susceptible) and a zone of 21 mm. The overall inter-method categorical agreement was 99.9% for daptomycin.

Figure 1. Scattergram displaying the relationship of daptomycin MIC (mg/L) and the disk inhibitory zone diameter (mm) for 915 strains of viridans group streptoci and *S. bovis*.

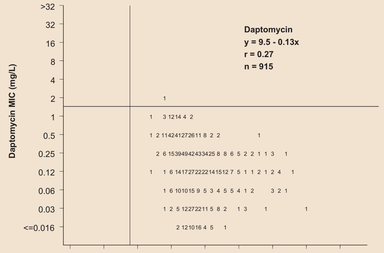

Table 1. In vitro activity of daptomycin and seven comparative antimicrobial agents tested against 815 viridans group streptococci and *S. bovis* (100 strains).

Organism/antimicrobial agent (no. tested)	MIC (mg/L) 50%	90%	Range	% susceptible[a]
S. anginosis (106)				
Daptomycin	0.25	0.5	≤0.016-1	100.0[b]
Vancomycin	0.5	0.5	0.25-1	100.0
Penicillin	0.03	0.12	≤0.008-2	93.4
Erythromycin	0.03	1	≤0.008->16	88.7
Clindamycin	0.03	0.03	≤0.008->16	94.3
Tetracycline	≤1	>8	≤1->8	72.6
Quinupristin/Dalfopristin	0.5	1	≤0.06-1	100.0
Linezolid	1	1	0.25-2	100.0
S. bovis (100)				
Daptomycin	0.03	0.06	0.03-0.25	100.0[b]
Vancomycin	0.25	0.5	0.12-0.5	100.0
Penicillin	0.03	0.06	≤0.008-1	97.0
Erythromycin	0.03	>16	≤0.008->16	54.0
Clindamycin	0.06	>16	≤0.008->16	74.0
Tetracycline	>8	>8	≤1->8	35.0
Quinupristin/Dalfopristin	0.5	1	0.12-4	94.0
Linezolid	1	1	0.5-2	100.0
S. constellatus (103)				
Daptomycin	0.25	0.5	0.03-1	100.0[b]
Vancomycin	0.5	1	0.25-1	100.0
Penicillin	0.03	0.06	≤0.008->16	98.1
Erythromycin	0.016	2	≤0.008->16	86.4
Clindamycin	0.03	0.06	≤0.008->16	93.2
Tetracycline	≤1	>8	≤1->8	79.6
Quinupristin/Dalfopristin	0.25	0.5	0.12-1	100.0
Linezolid	1	1	≤0.12-1	100.0
S. intermedius (103)				
Daptomycin	0.25	0.5	0.03-1	100.0[b]
Vancomycin	0.5	0.5	0.12-1	100.0
Penicillin	0.06	0.5	≤0.008-16	87.4
Erythromycin	0.03	2	≤0.008->16	79.6
Clindamycin	0.016	0.03	≤0.008->16	92.2
Tetracycline	≤1	>8	≤1->8	74.8
Quinupristin/Dalfopristin	0.5	0.5	≤0.06-2	99.0
Linezolid	1	1	≤0.12-2	100.0
S. mitis (102)				
Daptomycin	0.25	0.5	0.03-1	100.0[b]
Vancomycin	0.5	0.5	0.12-0.5	100.0
Penicillin	0.06	2	≤0.008->16	68.6
Erythromycin	0.25	4	≤0.008->16	52.9
Clindamycin	0.016	4	≤0.008->16	89.2
Tetracycline	≤1	>8	≤1->8	65.7
Quinupristin/Dalfopristin	0.25	0.5	≤0.06-2	99.0
Linezolid	1	1	≤0.12-2	100.0
S. mutans (66)				
Daptomycin	0.12	0.25	0.03-0.5	100.0[b]
Vancomycin	0.5	1	0.12-1	100.0
Penicillin	0.016	0.12	≤0.008-4	90.9
Erythromycin	0.016	2	≤0.008->16	83.3
Clindamycin	≤0.008	0.06	≤0.008->16	97.0
Tetracycline	≤1	≤1	≤1->8	93.9
Quinupristin/Dalfopristin	0.25	1	0.12-1	100.0
Linezolid	1	1	≤0.12-2	100.0
S. oralis (107)				
Daptomycin	0.5	1	0.03-2	99.1[b]
Vancomycin	0.5	0.5	0.25-0.5	100.0
Penicillin	0.06	4	≤0.008->16	66.4
Erythromycin	0.5	>16	≤0.008->16	48.6
Clindamycin	0.016	>16	≤0.008->16	84.1
Tetracycline	≤1	>8	≤1->8	69.2
Quinupristin/Dalfopristin	0.5	1	≤0.06-2	99.1
Linezolid	1	1	0.25-16	99.1
S. salivarius (101)				
Daptomycin	0.06	0.25	0.03-1	100.0[b]
Vancomycin	0.5	0.5	0.25-1	100.0
Penicillin	0.06	0.5	≤0.008->16	73.3
Erythromycin	0.016	8	≤0.008->16	65.3
Clindamycin	≤0.008	0.03	≤0.008->16	92.1
Tetracycline	≤1	>8	≤1->8	71.3
Quinupristin/Dalfopristin	0.5	1	0.12-2	99.0
Linezolid	1	1	0.25-2	100.0
S. sanguis (127)				
Daptomycin	0.25	1	0.03-1	100.0[b]
Vancomycin	0.5	0.5	0.25-1	100.0
Penicillin	0.06	1	≤0.008->16	65.5
Erythromycin	0.016	>16	≤0.008->16	64.6
Clindamycin	0.016	>16	≤0.008->16	89.0
Tetracycline	≤1	>8	≤1->8	71.7
Quinupristin/Dalfopristin	0.5	1	0.12-2	99.2
Linezolid	1	1	0.25-2	100.0

a. Susceptible percentages were established using NCCLS criteria (M100-S15).
b. A breakpoint of ≤ 1 mg/L was utilized to determine susceptibility rates for comparative purposes only. NCCLS interpretive criteria have not been established for the viridans group of streptococci, however, breakpoints used for staphylococci (≤ 1 mg/L), enterococci (≤ 4 mg/L) and ß-haemolytic streptococci (≤ 1 mg/L) have been approved for publication.

CONCLUSIONS

- Daptomycin, although described nearly a decade ago, has recently been approved for clinical use and continues to demonstrate significant in vitro bactericidal activity against a wide spectrum of Gram-positive pathogens. Furthermore, daptomycin has demonstrated a long serum half-life (nine hours) in Phase I studies and once daily dosing trials for 4, 6 and 8 mg/Kg have been conducted, displaying evidence that daptomycin remains a safe, efficacious and well-tolerated agent.
- Daptomycin was very active against all species of viridans group streptococci (eight species; 815 strains) and *S. bovis* (100 strains) tested (Table 1). The daptomycin MIC values with appropriate calcium concentrations (50 mg/L) ranged from ≤ 0.016 to 2 mg/L with 99.9% of results at ≤ 1 mg/L (MIC_{90}s, 0.06 - 1 mg/L).
- Comparable susceptibility testing results were achieved with the daptomycin disk diffusion test with only one false-susceptible error (MIC at 2 mg/L [non-susceptible]; zone at 20 mm [susceptible]) detected. Generally, both the reference broth microdilution (with 50 mg/L calcium) and the standardized disk diffusion method (with media containing at least 25 mg/L of calcium) produced high inter-method accuracy as confirmed here.
- Results from this comparison of two NCCLS in vitro susceptibility test methods using a large, comprehensive streptococcal organism collection demonstrates that, daptomycin is a potent antimicrobial agent active against viridans group streptococci, and is an excellent candidate for further clinical trials targeting serious infections produced by these species.

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