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Research Article

Antimicrobial Activity of Telavancin and Comparator Agents against Methicillin-Resistant Staphylococcus Aureus Isolates from a Community Hospital

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Keywords

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- Susceptibility testing
- Time-kill assay

Abstract

The antimicrobial activity of telavancin (TLV) and four comparative agents were determined using updated testing procedures. These antibiotics were tested against 103 recent (2015) clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from blood, sputum and skin/soft tissues. Minimum inhibitory concentrations (MICs) were determined for TLV, daptomycin (DAP), vancomycin (VAN), and linezolid (LZD) by broth microdilution using Sensititre CMP2STA panels. The MICs for these agents and ceftaroline (CPT) were again determined using E-test strips. In addition, time-kill experiments were performed using clinical concentrations of these drugs in serum against two randomly selected MRSA isolates from blood cultures. The MIC90s (90% of strains) were 0.03mg/L and 0.06mg/L (TLV), 0.5mg/L and 1.0mg/L (VAN), 0.25mg/L and 0.75mg/L (DAP) and 1.0mg/L and 2.0mg/L (LZD) for Sensititre panels and E-test strips, respectively. CPT had a MIC90 = 0.38mg/L via E-test. In time-kill experiments, TLV and DAP exhibited >1.5 log cfu/mL decrease at 2 hours whereas the other agents produced ≤ 0.6 log cfu/mL diminution at this time period. Each drug, except LZD, produced bactericidal activity by 6 hours of incubation. In summary, these clinical isolates of MRSA were highly susceptible to the study antibiotics and no MIC creep was observed. TLV was the most potent agent tested.

ABBREVIATIONS

MRSA: Methicillin-resistant *Staphylococcus aureus*; TLV: Telavancin; DAP: Daptomycin; VAN: Vancomycin; LZD: Linezolid; CPT: Ceftaroline MICs: Minimum inhibitory concentrations; CAMHB: Cation-adjusted Mueller-Hinton Broth 2

INTRODUCTION

The introduction of newer lipopeptide antibiotics has dramatically altered our therapeutic approach in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Daptomycin, a cyclic lipopeptide, has activity against MRSA with decreased susceptibility or resistance to vancomycin and can be dosed once daily [1]. Telavancin, the first lipoglycopeptide, has similar pharmacokinetics as daptomycin, a dual mechanism of action and activity against daptomycin-nonsusceptible MRSA [2]. The long-acting lipoglycopeptides (oritavancin and dalbavancin) are also highly active against MRSA and allow for single-dose treatment of acute bacterial skin/soft tissue infections [3].

The antibacterial potency of telavancin and its role in the treatment of MRSA infections has been further defined since its approval in 2009 for complicated skin and skin-structure infections (cSSSI). Updated testing procedures have demonstrated that this lipoglycopeptide is significantly more active against staphylococci than originally reported [4]. Moreover, telavancin is now FDA-approved for the treatment of hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP) including patients with concurrent bacteremia [5].

To further investigate the in vitro activity of telavancin and comparative agents against MRSA, we analyzed their antibacterial activity utilizing several techniques. These studies were performed to further update the inhibitory potency of these antimicrobials using current methodologies against recent clinical isolates. In addition, we evaluated the bactericidal activity of these antibiotics employing time-kill experiments.

MATERIALS AND METHODS

Bacterial isolates and antimicrobials

Unique clinical isolates of MRSA from blood, sputum and skin/soft tissues were obtained via charcoal swabs from the microbiology laboratory of a 700 bed community hospital over a 6 month period (January – July 2015). Telavancin (TLV) powder was obtained from Theravance Biopharma and ceftaroline (CPT)

powder was obtained from Actavis (now Allergan). Vancomycin (VAN), daptomycin (DAP) and linezolid (LZD) powders were purchased from Sigma Aldrich (St. Louis, MO).

Medium

Cation-adjusted Mueller-Hinton Broth 2 (CAMHB) (Sigma Aldrich, St. Louis, MO) was used for vancomycin, linezolid and ceftaroline microbroth dilution susceptibility testing and time-kill experiments. Telavancin powder was dissolved and diluted in DMSO, following Clinical and Laboratory Standards Institute (CLSI) recommendations for water-insoluble agents (Table 8B) (CLSI M100-24) [6]. All telavancin experiments were performed in CAMHB with 0.002% Polysorbate-80 (Sigma Aldrich, St.Louis, MO). All daptomycin experiments were performed in CAMHB with calcium adjusted to 50mg/L. Pooled normal human serum (Innovative Research, Sarasota, FL) IPLA-SER was mixed 1:1 (v/v) with CAMHB for the time-kill experiments.

Susceptibility testing

Isolates were tested in CAMHB for susceptibility (minimum inhibitory concentrations, MICs) to these antibiotics, except ceftaroline, according to CLSI M07-A9 for broth microdilution with dry Sensititre CMP2STA panels (Thermo Scientific, Oakwood Village, OH) [7]. Interpretations were determined by the CLSI M100-S24 breakpoint criteria. Validation of the MIC values was performed by concurrent testing of American Type Culture Collection (ATCC) QC strain, S. aureus ATCC 29213.

The isolates were also analyzed utilizing Etest strips, including ceftaroline, according to the manufacturer's instruction (bioMerieux, Durham, NC). The E-test method was completed by using Mueller-Hinton agar, overnight incubation at 35°C, and an inoculum of 0.5 McFarland.

Time-kill curves

Time-kill curves were generated against two random MRSA blood isolates [8]. Clinical peak serum levels of daptomycin (100mg/L), vancomycin (40mg/L), linezolid (20mg/L) and ceftaroline (20mg/L) were used for these experiments to maximize their killing potential. Telavancin was studied at 50mg/L (50% serum peak level) in order to keep the DMSO concentration of the sample well ≤ 3% to minimally affect antimicrobial activity [9]. Pooled normal human serum/CAMHB 50% (v/v), antibiotic, Polysorbate 80 to 0.002% (for telavancin only) and calcium to 50mg/L (for daptomycin only) were mixed in a total volume of 225µL in microtiter plates and inoculated with bacterial strains. To prepare the inoculum, Staphylococcus colonies were suspended to 5x106 cfu/mL in CAMHB. Twenty-five µL of inoculum were added to inoculate each sample. Viability counts of each culture were carried out at 0, 2, 6 and 24 hours after inoculation. Sampling was done by removing a 5-µL aliquot from each sample and serially diluting it 10-fold in media to minimize antibiotic carryover. Ten µL aliquots of both the undiluted and diluted samples were placed on Mueller-Hinton plates and colonies counted after 24 hours of incubation at 35°C. Time-kill assays were analyzed by determining the number of bacteria (log10 cfu/mL) at 2, 6 and 24 hours, compared with counts at 0 hour. The range of quantification was 20-200 cfu/mL. Growth controls were included in each experiment. Time-kill curves were constructed by plotting mean colony counts (log10 cfu/mL) versus time. Bactericidal activity was defined as a reduction of the original inoculum by ≥ 3 log cfu/mL (99.9%) at 24h.

RESULTS AND DISCUSSION

Isolate collection

A total of 103 clinical isolates of MRSA were obtained from blood (23), skin/soft tissue (66) and the respiratory tract (14) during the collection period.

Susceptibility testing

Telavancin exhibited the greatest potency against the collected isolates compared to the other tested antibiotics. All the isolates were susceptible to these compounds using Sensititre CMP2STA panels with the exception of one skin/soft tissue isolate which showed resistance to linezolid (Table 1).

Similar results were found following susceptibility testing of these isolates using E-test strips (Table 2). One blood isolate exhibited resistance to daptomycin but not the other drugs tested. The MIC_{90} (90% of strains) was found to be ~ 1-dilution lower for Sensititre CMP2STA panels compared to E-test strips.

Isolates from various sites had similar MIC patterns and MIC90s for both techniques.

Time-kill curves

Time-kill experiments in human serum were performed for 2 randomly chosen blood isolates. These studies produced similar results for both MRSA strains. Telavancin and daptomycin exhibited the most rapid (2h) decrease in bacterial colony counts (Table 3). All agents, except linezolid, exhibited bactericidal activity by the 6h time point. No regrowth was observed for these antibiotics at 24h.

DISCUSSION

Over the past decade there have been numerous reports of a rising incidence in MRSA with reduced susceptibility to vancomycin [10,11]. These isolates with MICs > 1mg/L are of therapeutic concern due to their association with higher rates of treatment failure [12]. In this study, we found that vancomycin exhibited potent in vitro activity against MRSA isolates from various body sites. None of these isolates had MICs > 1mg/L to vancomycin using broth microdilution susceptibility testing. Furthermore, only 7 (7%) of isolates had MICs > 1.0 mg/L using E-test strips. These findings are in contrast to our previous study of MRSA isolates, collected from the same hospital in 2007, where we discovered that 65% of strains had vancomycin MICs > 1.0 mg/L [13]. Our current findings are similar to other recent surveillance studies of MRSA isolates [14,15].

In comparison, the other agents studied exhibited in vitro activity that was similar or superior to vancomycin. Telavancin was found to be the most potent antibiotic tested. This enhanced antibacterial activity was not recognized when telavancin was initially analyzed [16]. This lipoglycopeptide is poorly soluble in water and has a propensity to bind to plastic surfaces. Procedural modifications have been introduced to improve telavancin

Table 1: *In vitro* susceptibility using Sensititre CMP2STA panels.
No. (cumulative %) of isolates inhibited at MIC (mg/L)

	≤0.015	0.03	0.06	0.12	0.25	0.5	1.0	2.0	4.0	≥4.0
TLV	5 (15)	91 (93)	7 (100)							
VAN						93 (90)	10 (100)			
DAP			4 (4)	85 (86)	12 (98)	2 (100)				
LZD						1 (1)	101 (99)			1 (100)

Susceptibility Breakpoints: TLV (0.12 mg/L), VAN (2.0 mg/L), DAP (1.0 mg/L), and LZD (4.0 mg/L)
Abbreviations: MIC: Minimum Inhibitory Concentration; TLV: Telavancin; VAN: Vancomycin; DAP: Daptomycin; LZD: Linezolid

Table 2: *In vitro* susceptibility using E-test strips
No. of isolates at each MIC

MIC (mg/L)	TLV	VAN	DAP	LZD	CPT
0.016	1				
0.023	12				
0.032	38				
0.047	51				
0.064	2				
0.094			1		
0.125			3		1
0.19			9		3
0.25			38	1	29
0.38			37	1	61
0.5		3	11	1	8
0.75		21	3	18	1
1.0		72		28	
1.5		7		33	
2.0			1	11	
3.0				1	
MIC_{50}	0.032	1.0	0.25	1.5	0.38
MIC_{90}	0.047	1.0	0.75	2.0	0.38

MIC, minimum inhibitory concentration; MIC50/90, MICs for 50% and 90% of the isolates, respectively
Abbreviations: TLV: Telavancin; VAN: Vancomycin; DAP: Daptomycin; LZD: Linezolid; CPT: Ceftaroline

Table 3: Time-Kill analysis of two blood isolates
Change from baseline (log_{10}cfu/mL)

	MIC*	MRSA (#78)			MIC*	MRSA (#82)		
	(mg/L)	2h	6h	24h	(mg/L)	2h	6h	24h
Telavancin	0.06	-1.92	-5.15	-5.15	0.05	-1.80	-5.10	-5.10
Vancomycin	1.50	-0.44	-5.14	-5.14	1.50	-0.36	-5.06	-5.06
Daptomycin	0.38	-4.97	-4.97	-4.97	0.25	-4.72	-4.72	-4.72
Linezolid	1.50	0	-0.36	-1.76	1.50	-0.08	-0.37	-1.98
Ceftaroline	0.50	-0.38	-5.10	-5.10	0.50	-0.60	-5.06	-5.06

*E-test methodology

solubilization with DMSO and to minimize binding to plastic microtiter panels with the inclusion of the nonionic surfactant P-80 [17]. This revised broth microdilution method for telavancin provides more accurate results and produces lower MICs than previously reported [18]. Our MIC results for telavancin by both broth microdilution and E-tests were found to be similar to those reported from a contemporary (2011-2013) U.S. collection of MRSA using these revised procedures [19]. The MIC_{90} observed in our investigation is approximately 3-fold lower than reported using previously established broth microdilution techniques. The MIC results for the other antibiotics included in this study did not exhibit any MIC creep and are comparable to previously reported in vitro studies [20,21].

Time-kill experiments are also useful to assess antibacterial activity because these assays can follow microbial killing and growth as a function of both time and antibiotic concentration [22]. The use of serum in this model is important because only non-protein bound drug is biologically active. The antimicrobial agents in this study vary considerably in extent of protein binding: daptomycin and telavancin>vancomycin> linezolid and ceftaroline [23]. These differences in protein binding provides a

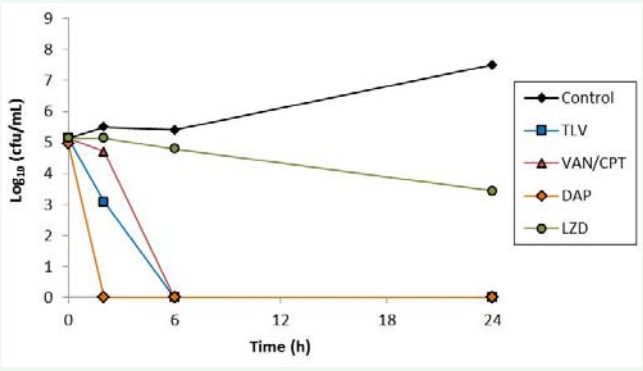

Figure 1 Time-kill curves against MRSA isolate #78
Abbrevations: TLV: Telavancin; VAN: Vancomycin; CPT: Ceftaroline; DAP: Daptomycin; LZD: Linezolid

narrow range of free peak serum drug concentrations (fC max, 10-20 mg/L) for these antibiotics. Even at 50% of its peak serum concentration, telavancin has the highest fCmax/MIC ratio against MRSA [24]. Although each of these agents provides fCmax/MIC ratios >10, they produce significant variations in time-kill curves against MRSA. In our time-kill experiments, the lipopeptides, daptomycin and telavancin, exhibited >1.5 log cfu/mL decrease at 2h whereas the other tested compounds produced ≤ 0.6 log cfu/mL diminution at this time period (Figure 1). Each antibiotic, except linezolid, produced bactericidal activity (≥ 3 log cfu/mL reduction) by 6h of incubation. These findings are in concordance with observations from other time-kill models [25,26].

CONCLUSION

In summary, all of the tested antibiotics were highly active against MRSA with telavancin providing the greatest potency. No MIC creep was observed in these clinical isolates to these agents. Moreover, all of these drugs, except linezolid, produced bactericidal activity in serum in time-kill assays against two MRSA strains isolated from blood cultures.

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