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Antimicrobial Activity of Lefamulin, an Investigational Pleuromutilin Antibiotic, against *Staphylococcus aureus* Strains with Decreased Susceptibility to Vancomycin

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ABSTRACT

Background: Lefamulin (BC-3781) is currently in late-stage development for intravenous and oral administration for the treatment of community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections.

Methods: Lefamulin and comparators were tested *in vitro* against 30 strains of *Staphylococcus aureus* with decreased vancomycin (VAN) susceptibility (S), including 10 VAN-resistant *S. aureus* (VRSA), 10 VAN-intermediate *S. aureus* (VISA) and 10 heterogeneous VISA (hVISA) strains. Isolates were tested by reference broth microdilution methods and quality control (QC) strains included *S. aureus* ATCC 29213, Mu3 and Mu50.

Results: Lefamulin and tigecycline were the most potent compounds tested ($MIC_{50/90}$, 0.06/0.25 µg/mL for both compounds). Lefamulin MIC distributions were very similar among the resistance subsets tested and the highest lefamulin MIC value was only 0.5 µg/mL (one VISA strain; see Table). Only two isolates (6.7%; one hVISA and one VISA) were oxacillin-S. S rates to daptomycin (DAP; $MIC_{50/90}$, 0.5/2 µg/mL) and ceftaroline (CPT; MIC_{50} and MIC_{90}, 1 µg/mL) were 70.0 and 90.0%, respectively; and all isolates were S to linezolid (MIC_{50} and MIC_{90}, 1 µg/mL), quinupristin-dalfopristin (MIC_{50} and MIC_{90}, 0.5 µg/mL) and tigecycline. Among hVISA, 90.0 and 80.0% of strains were S to DAP and CPT, respectively; whereas among VISA, S rates to DAP and CPT were 20.0 and 90.0%, respectively. All VRSA strains were S to DAP and CPT.

Conclusions: Lefamulin was highly active against hVISA, VISA and VRSA strains, and its activity was not affected by the mechanism or degree of VAN resistance.

Organism (no. tested)	No. of isolates (cumulative %) at lefamulin MIC (µg/mL) of: ≤0.03	0.06	0.12	0.25	0.5	% S DAP	% S CPT
S. aureus (30)	1 (3.3)	17 (60.0)	8 (86.7)	3 (96.7)	1 (100.0)	70.0	90.0
hVISA (10)	1 (10.0)	3 (40.0)	4 (80.0)	2 (100.0)		90.0	80.0
VISA (10)		6 (60.0)	3 (90.0)	0 (90.0)	1 (100.0)	20.0	90.0
VRSA (10)		8 (80.0)	1 (90.0)	1 (100.0)		100.0	100.0

Underline values indicate MIC_{50} and MIC_{90} values. DAP = daptomycin and CPT = ceftaroline.

INTRODUCTION

Lefamulin (BC-3781) is a novel antimicrobial agent belonging to the pleuromutilin class. Pleuromutilins inhibit bacterial protein synthesis of Gram-positive and Gram-negative organisms, as well as atypical respiratory pathogens, by selectively binding to the peptidyl transferase center of the bacterial ribosome. Lefamulin is the first representative of pleuromutilin class in clinical development for systemic administration. Phase 1 and 2 trials have demonstrated that IV and oral administration of lefamulin are well tolerated. In a Phase 2 trial in patients with acute bacterial skin and skin structure infections (ABSSSI) comparing lefamulin 100mg or 150mg IV q12 hours) to vancomycin, lefamulin administered daily for 5-14 days demonstrated comparable efficacy rates to vancomycin. Currently lefamulin is in late stage development for the treatment of community-acquired bacterial pneumonia (CABP).

The antibacterial profile of lefamulin covers all relevant bacterial pathogens causing CABP and ABSSSI, including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, β-hemolytic and viridans group streptococci, as well as organisms causing atypical pneumonia, such as *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*. No cross-resistance has been observed with macrolides, fluoroquinolones, tetracyclines, trimethoprim-sulfamethoxazole, mupirocin and β-lactam agents. In previous resistance development studies, lefamulin displayed very low spontaneous mutation frequencies and the *in vitro* resistance development by multi-passaging at subinhibitory concentrations appeared to be a slow process. Particularly for vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) strains, development of resistance to lefamulin appeared to be as slow as for the other *S. aureus* subsets, such as methicillin-susceptible *S. aureus* (MSSA), community-acquired (CA) methicillin-resistant *S. aureus* (MRSA) and hospital-acquired (HA) MRSA.

In the present study, we evaluated the *in vitro* activity of lefamulin against *S. aureus* isolates with reduced susceptibility to glycopeptides, including vancomycin-resistant *S. aureus* (VRSA), VISA and hVISA, which are related to different mechanisms of resistance such as *vanA* for VRSA and a variety of genes affecting the cell wall, autolytic activity and metabolism of the cell that is responsible for vancomycin non-susceptibility of VISA and hVISA.

MATERIALS AND METHODS

Susceptibility Test Methods: Organisms were tested by broth microdilution per Clinical and Laboratory Standards Institute (CLSI) M07-A10 [2015] using reference frozen-form panels produced by JMI Laboratories (North Liberty, Iowa, USA) with cation-adjusted Mueller-Hinton broth. Interpretive criteria for MIC results were those of the CLSI M100-S25 (2015) and EUCAST (2015), as published for comparison control agents, and concurrent quality control (QC) testing used *S. aureus* ATCC 29213, Mu3 (ATCC 700698) and Mu50 (ATCC 700699).

Organisms Tested: The collection included 10 VRSA strains from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) strain repository (all were tested positive for *vanA*; http://www.narsa.net/), 10 VISA strains and 10 hVISA strains confirmed by population analysis profiling.

RESULTS

- Lefamulin and tigecycline were the most potent compounds tested with $MIC_{50/90}$ values of 0.06/0.25 µg/mL (**Table 1**).
- Lefamulin MIC distributions were very similar among the resistance phenotypes including VRSA, VISA and hVISA. The highest lefamulin MIC value was 0.5 µg/mL (one VISA strain; see Abstract Table) and 80% of VRSA were inhibited at a lefamulin MIC of 0.06 µg/mL.
- Only two isolates (6.7%) were susceptible to oxacillin, one hVISA and one VISA (**Table 1**).
- Susceptibility rates to daptomycin ($MIC_{50/90}$, 0.5/2 µg/mL) and ceftaroline (MIC_{50} and MIC_{90}, 1 µg/mL) were 70.0 and 90.0%, respectively; and all isolates were susceptible to linezolid (MIC_{50} and MIC_{90}, 1 µg/mL), quinupristin-dalfopristin (MIC_{50} and MIC_{90}, 0.5 µg/mL) and tigecycline ($MIC_{50/90}$, 0.06/0.25 µg/mL; **Table 1**).
- Among hVISA, 90.0 and 80.0% of strains were susceptible to daptomycin and ceftaroline, respectively; whereas among VISA, susceptibility rates to daptomycin and ceftaroline were 20.0 and 90.0%, respectively. All VRSA strains were susceptible to daptomycin and ceftaroline (**Table 1**).

Table 1. Activity of lefamulin and comparator agents when tested against 30 isolates of *S. aureus* with decreased susceptibility to vancomycin.

Antimicrobial Agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S	CLSI[a] %I	CLSI[a] %R	EUCAST[a] %S	EUCAST[a] %I	EUCAST[a] %R
All (30)									
Lefamulin	**0.06**	**0.25**	**0.03 — 0.5**	-	-	-	-	-	-
Vancomycin	4	>32	1 — >32	33.3	33.3	33.3	33.3	-	66.7
Daptomycin	0.5	2	0.25 — 4	70.0	-	-	70.0	-	30.0
Linezolid	1	1	0.25 — 2	100.0	-	0.0	100.0	-	0.0
Q/D	0.5	0.5	0.12 — 1	100.0	0.0	0.0[b]	100.0	0.0	0.0
Tigecycline	0.06	0.25	≤0.03 — 0.25	100.0	-	-[c]	100.0	-	0.0
Ceftaroline	1	1	0.25 — 2	90.0	10.0	0.0	90.0	-	10.0
Oxacillin	>4	>4	0.06 — >4	6.7	-	93.7	6.7	-	93.7
hVISA (10)									
Lefamulin	**0.12**	**0.25**	**0.03 — 0.25**	-	-	-	-	-	-
Vancomycin	2	2	1 — 2	100.0	0.0	0.0	100.0	-	0.0
Daptomycin	0.5	1	0.5 — 4	90.0	-	-	90.0	-	10.0
Linezolid	1	1	0.5 — 2	100.0	-	0.0	100.0	-	0.0
Q/D	0.5	0.5	0.12 — 0.5	100.0	0.0	0.0[b]	100.0	0.0	0.0
Tigecycline	0.06	0.25	≤0.03 — 0.25	100.0	-	-[c]	100.0	-	0.0
Ceftaroline	1	2	0.5 — 2	80.0	20.0	0.0	80.0	-	20.0
Oxacillin	>4	>4	0.06 — >4	10.0	-	90.0	10.0	-	90.0
VISA (10)									
Lefamulin	**0.06**	**0.12**	**0.06 — 0.5**	-	-	-	-	-	-
Vancomycin	4	8	4 — 8	0.0	100.0	0.0	0.0	-	100.0
Daptomycin	2	2	1 — 2	20.0	-	-	20.0	-	80.0
Linezolid	1	1	0.5 — 2	100.0	-	0.0	100.0	-	0.0
Q/D	0.5	0.5	0.25 — 1	100.0	0.0	0.0[b]	100.0	0.0	0.0
Tigecycline	0.06	0.12	≤0.03 — 0.25	100.0	-	-[c]	100.0	-	0.0
Ceftaroline	1	1	0.25 — 2	90.0	10.0	0.0	90.0	-	10.0
Oxacillin	>4	>4	1 — >4	10.0	-	90.0	10.0	-	90.0
VRSA (10)									
Lefamulin	**0.06**	**0.12**	**0.06 — 0.25**	-	-	-	-	-	-
Vancomycin	>32	>32	16 — >32	0.0	0.0	100.0	0.0	-	100.0
Daptomycin	0.5	0.5	0.25 — 0.5	100.0	-	-	100.0	-	0.0
Linezolid	1	1	0.25 — 1	100.0	-	0.0	100.0	-	0.0
Q/D	0.25	0.5	0.25 — 0.5	100.0	0.0	0.0[b]	100.0	0.0	0.0
Tigecycline	0.06	0.12	≤0.03 — 0.25	100.0	-	-[c]	100.0	-	0.0
Ceftaroline	1	1	0.25 — 1	100.0	0.0	0.0	100.0	-	0.0
Oxacillin	>4	>4	>4 — >4	0.0	-	100.0	0.0	-	100.0

Abbreviation: Q/D = Quinupristin-dalfopristin.
a. Criteria as published by CLSI [2015] and EUCAST [2015]
b. CLSI breakpoints for reporting methicillin-susceptible *S. aureus* were applied for all strains.
c. Breakpoints from FDA Package Insert revised 12/2014.

CONCLUSIONS

- Lefamulin was highly active against hVISA, VISA and VRSA strains.
- Lefamulin's activity was not affected by the mechanism or degree of resistance to vancomycin.
- These data support the continued clinical development of lefamulin for the treatment *S. aureus* infections including CABP and ABSSSI.

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