

Antistaphylococcal Activity of Oritavancin and Its Synergistic Effect in Combination with Other Antimicrobial Agents

Gengrong Lin, Glenn Pankuch, Peter C. Appelbaum, Klaudia Kosowska-Shick*

Hershey Medical Center, Hershey, Pennsylvania, USA

Oritavancin exhibited *in vitro* activity against 169 strains of vancomycin-susceptible, methicillin-resistant *Staphylococcus aureus* (MRSA) with MICs ranging from 0.03 to 1 µg/ml and against vancomycin-intermediate MRSA (VISA; *n* = 29), heterogeneous vancomycin-intermediate MRSA (hVISA; *n* = 5), and vancomycin-resistant MRSA (*n* = 5) strains, with MICs ranging from 0.12 to 4 µg/ml. For 10 MRSA isolates comprising 5 VISA and 5 hVISA strains, synergy between oritavancin and gentamicin, linezolid, or rifampin was observed against most of the strains tested using a time-kill method.

Methicillin-resistant *S. aureus* (MRSA) is a global concern, with the majority of strains (and some methicillin-susceptible [MSSA] strains) also exhibiting resistance to currently available quinolones. Furthermore, the appearance and rapid spread of community-associated MRSA in the United States pose clinical challenges. Treatment of MRSA strains in certain types of infections, such as osteomyelitis and bacteremia, with glycopeptides has undoubtedly increased selective pressure, leading to nonsusceptible organisms such as heterogeneous vancomycin-intermediate *S. aureus* (hVISA), vancomycin-intermediate *S. aureus* (VISA) (1, 2), and vancomycin-resistant *S. aureus* (VRSA) (3).

The mechanism of glycopeptide resistance in VISA strains is due to cell wall thickening; some of these strains also exhibit reduced susceptibility to daptomycin (4, 5). Among Hershey MRSA strains isolated between August 2006 and February 2007 from patients that had received prior vancomycin therapy, we determined the incidence of hVISA and VISA to be approximately 1% (1).

Oritavancin, a lipoglycopeptide in late clinical development, has potent *in vitro* activity against MRSA (6, 7). Synergy between oritavancin and gentamicin, linezolid, or rifampin against 1 methicillin-susceptible strain and 2 MRSA strains (1 VISA and 1 VRSA strain) has been described (8). In this study, we examined the *in vitro* activities of oritavancin and comparators against 200

recent MRSA isolates. Furthermore, we used time-kill studies to assess synergy between oritavancin and gentamicin, linezolid, or rifampin against 5 VISA and 5 hVISA isolates.

Two hundred three recent MRSA isolates were used in this study, including 127 community-associated organisms (MRSA is defined as community-associated if the MRSA-positive specimen was obtained outside a hospital setting or within 2 days of hospital admission) and 42 hospital-associated organisms isolated from 25 geographically diverse regions in the United States. Two hVISA, 4 VISA, and 1 VRSA isolate were from the Hershey Medical Center. Four VRSA, one hVISA, and 22 VISA isolates were obtained from

Received 31 March 2014 Returned for modification 3 May 2014

Accepted 8 July 2014

Published ahead of print 14 July 2014

Address correspondence to Klaudia Kosowska-Shick, KlaudiaShick@eurofinsUS.com.

* Present address: Klaudia Kosowska-Shick, Eurofins Lancaster Laboratories, Lancaster, Pennsylvania, USA.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.02932-14

TABLE 1 Microdilution MIC ranges, MIC₅₀s, and MIC₉₀s for tested antimicrobials^a

Drug	MIC (µg/ml) for (no. of isolates):							
	Community-acquired MRSA (127)		Hospital-acquired MRSA (42)		VISA (24) + hVISA (5)		VRSA (5) ^b	
	50%	90%	50%	90%	50%	90%		Range
Oritavancin	0.12	0.25	0.12	0.25	0.5	1	0.12–1	
Vancomycin	1	1	1	1	4	8	128–>256	
Teicoplanin	0.5	0.5	0.5	1	2	8	2–8	
Linezolid	2	2	2	4	2	4	2	
Daptomycin	0.5	0.5	0.5	1	2	4	0.25–1	
Tigecycline	0.25	0.5	0.25	0.5	0.25	1	0.12–0.5	
Quinu/dalfo	0.5	0.5	0.5	1	0.5	1	0.5–1	
Minocycline	0.12	0.12	0.06	0.12	0.12	8	0.06–1	
Rifampin	0.008	0.016	0.008	0.016	1	>4	≤0.004–>4	
Trimeth/sulf	0.12	0.25	0.12	0.5	0.5	>4	0.12–>4	
Fusidic acid	0.12	0.25	0.12	0.25	0.12	0.5	≤0.06–0.25	

^a MRSA, methicillin-resistant *S. aureus*; VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; VRSA, vancomycin-resistant *S. aureus*; Quinu/dalfo, quinupristin-dalfopristin; Trimeth/sulf, trimethoprim-sulfamethoxazole.

^b MIC ranges instead of MIC₅₀ and MIC₉₀ are presented for VRSA isolates, as the number of strains is less than 10.

TABLE 2 MICs for strains tested in time-kill and time-kill synergy assays^a

Strain	Type	MIC ($\mu\text{g/ml}$) ^b											
		ORI	GEN	LZD	RIF	VAN	DAP	MIN	Q/D	TEI	T/S	FA	
<i>S. aureus</i>													
504	VISA	2 (2)	>16 (256)	2 (2)	>4 (128)	4	4	16	0.5	8	0.12	≤ 0.06	
506	VISA	2 (1)	0.5 (1)	2 (2)	>4 (256)	4	4	0.12	0.5	8	0.12	≤ 0.06	
507	VISA	1 (0.5)	>16 (>1,024)	2 (2)	2 (4)	2	4	4	1	4	>4	≤ 0.06	
508	VISA	1 (1)	>16 (>1,024)	2 (2)	0.008 (0.016)	4	2	4	0.25	8	>4	>0.5	
770	VISA	0.5 (0.5)	0.5 (0.5)	4 (2)	0.008 (0.016)	2 ^c	1	0.25	1	4	0.25	0.25	
710	hVISA	0.5 (0.5)	>16 (512)	2 (2)	0.008 (0.016)	1	2	16	1	4	0.12	0.12	
618	hVISA	0.25 (0.25)	≤ 0.25 (0.25)	4 (4)	0.008 (0.016)	0.5	2	0.25	1	1	0.5	0.25	
873	hVISA	0.25 (0.25)	≤ 0.25 (0.25)	2 (2)	0.008 (0.03)	1	1	0.25	1	1	0.25	0.12	
2222	hVISA	0.12 (0.12)	0.5 (1)	4 (4)	≤ 0.004 (0.008)	0.5	1	0.25	0.5	0.5	0.5	0.12	
2223	hVISA	0.25 (0.25)	16 (8)	2 (2)	>4 (>1,024)	1	2	4	1	1	>4	0.12	
ATCC 29213	QC	0.12 (0.12)	1 (0.5)	4 (4)	≤ 0.004 (0.016)	0.5	1	0.5	0.25	0.5	0.12	0.12	
<i>Enterococcus faecalis</i> ATCC 29212	QC	0.03 (0.03)	16 (8)	2 (2)	0.5 (1)	2	8	8	>4	8	0.06	>0.5	

^a QC, quality control; ORI, oritavancin; GEN, gentamicin; LZD, linezolid; RIF, rifampin; VAN, vancomycin; DAP, daptomycin; MIN, minocycline; Q/D, quinupristin-dalfopristin; TEI, teicoplanin; T/S, trimethoprim-sulfamethoxazole; FA, fusidic acid.

^b Values are broth microdilution MICs; values in parentheses are macrobroth MICs.

^c Initial vancomycin susceptibility determination and Etest showed MICs of $\geq 4 \mu\text{g/ml}$, and macro-Etest showed vancomycin-teicoplanin MICs of 6/24 $\mu\text{g/ml}$.

the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) (2). Two hVISA and 127 community-associated MRSA isolates were obtained from JMI Laboratories (Liberty City, IA).

Ten *S. aureus* strains (5 VISA and 5 hVISA strains) were tested in the time-kill studies. For synergy time-kill testing, which was performed according to CLSI guidelines and as previously described (4, 9, 10), oritavancin was combined with gentamicin, linezolid, or rifampin. Kill kinetics of each drug alone were determined by incubating an initial inoculum of 5×10^5 to 5×10^6 CFU/ml with drug concentrations at the MIC (see Table 2 for isolates' initial MICs used for time-kill testing) and 2 dilutions above ($2\times$ and $4\times$ MIC) and 3 dilutions below the MIC ($1/2\times$, $1/4\times$, and $1/8\times$ MIC). Viability counts were performed at 0, 3, 6, 12, and 24 h of incubation at 35°C in a shaking water bath by plating undiluted and 10-fold serially diluted samples. Concentrations of drugs used in synergy time-kill studies were selected such that one of the two drugs yielded a growth curve similar to that of the drug-free control, while the other drug may have been more active. To prevent drug carryover, aliquots were added to an equal volume of a 25-mg/ml sterile activated charcoal suspension (in 0.9% saline) and then serially diluted (11). Synergy was defined as a $\geq 2\text{-log}_{10}$ decrease in CFU/ml between the combination and its most active constituent after 3, 6, 12, and 24 h, with a decrease of

$\geq 2\text{-log}_{10}$ CFU/ml between the number of surviving organisms and the starting inoculum. Bacteriostatic and bactericidal activities were defined as $<3\text{-log}_{10}$ and $\geq 3\text{-log}_{10}$ reductions in CFU/ml, respectively, relative to the starting inoculum. The definitions of synergy and bacteriostatic and bactericidal activities were from the *Antimicrobial Agents and Chemotherapy* instructions to authors regarding *in vitro* susceptibility tests (12).

Drug panels in microtiter trays for susceptibility tests were provided by The Medicines Company (Parsippany, NJ). Broth microdilution and macrodilution MICs were determined in cation-adjusted Muller-Hinton broth (CAMHB; BD Diagnostics, Sparks, MD) according to CLSI guidelines (13, 14); MICs of quality control strains were verified during each determination. For all testing involving oritavancin, CAMHB was supplemented with 0.002% polysorbate-80 (Sigma-Aldrich).

The MIC ranges and MIC₅₀ and MIC₉₀ for all strains tested are presented in Table 1. Oritavancin showed potent activity against 127 community- and 42 hospital-associated vancomycin-susceptible MRSA strains with MICs ranging from 0.03 to 1 $\mu\text{g/ml}$, and MIC₅₀ and MIC₉₀ values of 0.12 and 0.25 $\mu\text{g/ml}$, respectively. An identical MIC₉₀ for oritavancin (0.25 $\mu\text{g/ml}$) was obtained in a prior study of community-associated MRSA from the New York/New Jersey area (7).

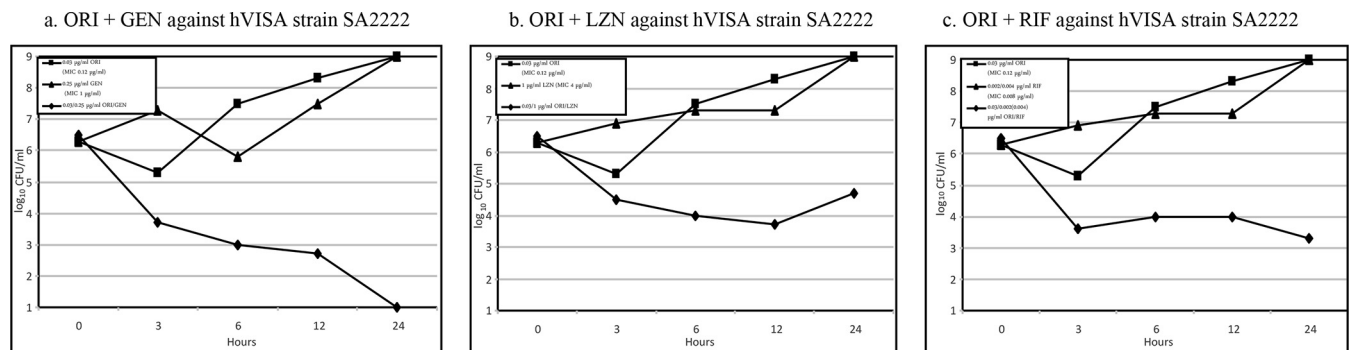


FIG 1 Time-kill curves of synergy at subinhibitory concentrations of oritavancin when combined with each antimicrobial tested for hVISA strain 2222. Abbreviations: ORI, oritavancin; LZD, linezolid; RIF, rifampin.

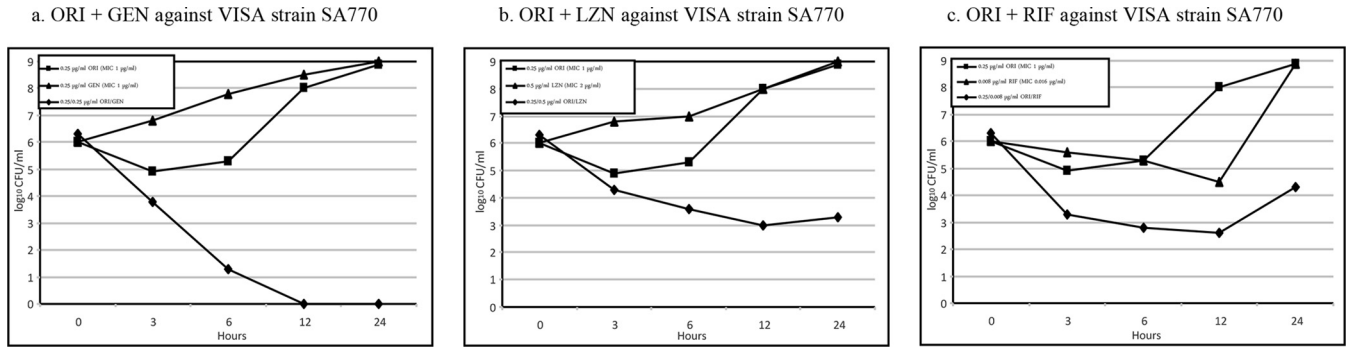


FIG 2 Time-kill curves of synergy at subinhibitory concentrations of oritavancin when combined with each antimicrobial tested for VISA strain 770. Abbreviations: ORI, oritavancin; LZD, linezolid; RIF, rifampin.

Against 5 hVISA and 24 VISA strains, oritavancin had an MIC range of 0.12 to 2 µg/ml, with MIC₅₀ and MIC₉₀ values of 0.5 and 1 µg/ml. When tested against five VRSA strains, oritavancin had an MIC range of 0.12 to 1 µg/ml. Seventeen of the 24 VISA strains were nonsusceptible to daptomycin (MIC > 1 µg/ml). Previous reports (5, 15) confirmed higher daptomycin MICs against VISA strains and the propensity of daptomycin therapy to select for such phenotypes. Using CLSI breakpoints (14), 13 (6%), 2 (1.0%), and 12 (6%) of all tested strains were resistant to trimethoprim-sulfamethoxazole, minocycline, and rifampin, respectively.

MIC results by broth micro- and macrodilutions are presented in Table 2. Broth microdilution MICs were similar to those obtained by broth microdilution. Two strains with gentamicin MICs of >1,024 µg/ml and one strain with a rifampin MIC of >1,024 µg/ml were not tested in synergy time-kill assays in combination with these agents. Also, no VISA isolate from an endocarditis patient treated with daptomycin was tested in the synergy study.

Figures 1 and 2 are graphic representations of synergy observed when oritavancin was combined with each of the tested antimicrobials at subinhibitory concentrations against an hVISA and a VISA strain, respectively. With the combination of oritavancin and gentamicin, synergy was observed against 2 of the 8 strains tested by 3 h (Table 3). By 6 h, synergy was observed against 7 strains. Although synergy was observed for all 8 strains by 12 and 24 h, for 3 of these strains it required concentrations of gentamicin considered too high to be clinically achievable (64 to 128 µg/ml).

The combination of oritavancin and linezolid yielded synergy against all 10 strains tested; synergy was observed in 2 strains by 6 h, and the other 8 strains by 12 h (Table 3). For all 10 strains, the

linezolid concentration used in the combination was below the CLSI susceptibility breakpoint (≤4 µg/ml) (14).

Oritavancin and rifampin in combination exhibited synergy against 7 of 9 strains tested by 24 h, with synergy observed against 2 and 4 strains by 3 h and 6 h, respectively (Table 3). Against the 7 strains, the rifampin concentration used in the combination was below the CLSI susceptibility breakpoint (≤1 µg/ml) (14).

In summary, oritavancin showed excellent *in vitro* potency by MIC against all MRSA strains tested, including community- and hospital-associated and vancomycin-susceptible and non-vancomycin-susceptible strains. Only 3 of all 203 strains tested had oritavancin MICs of >1 µg/µl, and all 3 were VISA isolates. Oritavancin also exhibited bactericidal activity against the 5 hVISA and 5 VISA (oritavancin MICs ranging from 0.12 to 2 µg/µl) strains tested by time-kill assays in this study. A similar bactericidal activity of oritavancin against VISA strains has been described previously (8, 16). Synergistic activity of oritavancin in combination with gentamicin or linezolid was observed in all the tested strains. For the combination of oritavancin and rifampin, synergy was observed in 7 of the 9 strains tested. No antagonism was found with any drug combination. Oritavancin concentrations observed in this synergy study were within concentrations predicted to be clinically achievable following a single 1,200-mg dose (17, 18). The marked cross-resistance between oritavancin and daptomycin (19, 20) in non-daptomycin-susceptible VISA isolates was not evaluated in this study.

Antimicrobial combinations that include oritavancin have potential to improve antimicrobial activity against VISA and hVISA isolates.

TABLE 3 Synergy time-kill results of oritavancin combined with gentamicin, linezolid, and rifampin^a

Strain	Type	MIC (µg/ml) ^b				Concn µg/ml at which synergy was observed											
		ORI	GEN	LZD	RIF	Oritavancin + gentamicin				Oritavancin + linezolid				Oritavancin + rifampin			
						3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h
504	VISA	2	256	2	128	NS	1/128	0.5/64	0.5/64	NS	NS	0.5/0.5	0.5/0.5	NS	NS	NS	NS
506	VISA	1	1	2	256	NS	0.25/0.25	0.25/0.25	0.25/0.25	NS	NS	NS	0.25/1	NS	NS	NS	NS
507	VISA	0.5	>1,024	2	4	NT	NT	NT	NT	NS	NS	0.25/1	0.25/1	NS	NS	0.25/1	0.12/2
508	VISA	1	>1,024	2	0.016	NT	NT	NT	NT	NS	NS	0.5/1	0.5/1	NS	NS	0.5/0.004	0.5/0.008
770	VISA	1	1	2	0.016	NS	0.25/0.25	0.25/0.25	0.25/0.25	NS	NS	0.25/0.5	0.25/0.5	NS	0.25/0.008	NS	0.25/0.008
710	hVISA	0.5	512	2	0.016	NS	NS	0.125/128	0.125/128	NS	NS	NS	0.25/0.5	NS	NS	0.12/0.004	0.12/0.008
618	hVISA	0.25	1	4	0.016	0.06/0.25	0.06/0.25	0.06/0.25	0.06/0.25	NS	NS	0.12/1	0.12/1	0.06/0.008	0.06/0.004	0.06/0.004	0.06/0.008
873	hVISA	0.5	1	2	0.03	0.125/0.5	0.125/0.25	0.125/0.25	0.125/0.25	NS	NS	0.12/0.5	0.12/0.5	0.12/0.008	0.12/0.008	0.12/0.008	0.12/0.008
2222	hVISA	0.12	1	4	0.008	NS	0.03/0.25	0.03/0.25	0.03/0.25	NS	0.03/1	0.03/1	0.03/2	NS	0.03/0.002	0.03/0.002	0.03/0.004
2223	hVISA	0.5	32	4	>1,024	NS	0.12/8	0.12/8	0.12/8	NS	0.12/1	0.12/1	0.12/1	NT	NT	NT	NT

^a ORI, oritavancin; GEN, gentamicin; LZD, linezolid; RIF, rifampin; Syn, synergy; VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; NS, no synergy; NT, not tested in combination because either the gentamicin or rifampin MIC for the isolate was >1,024 µg/ml.

^b MIC reading at 24 h of single agents directly from the deep-well plate under conditions used in time-kill assays. These values correspond to 1 × MIC in the synergy time-kill experiments.

ACKNOWLEDGMENT

This study was supported by a research grant from The Medicine Company (Parsippany, NJ).

REFERENCES

- Kosowska-Shick K, Ednie LM, McGhee P, Smith K, Todd CD, Wehler A, Appelbaum PC. 2008. Incidence and characteristics of vancomycin nonsusceptible strains of methicillin-resistant *Staphylococcus aureus* at Hershey Medical Center. *Antimicrob. Agents Chemother.* 52:4510–4513. <http://dx.doi.org/10.1128/AAC.01073-08>.
- BEI Resources. 2014. Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). <http://www.narsa.net/control/member/allapprovedisolates>.
- Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. 2010. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin. Microbiol. Rev.* 23:99–139. <http://dx.doi.org/10.1128/CMR.00042-09>.
- Credito K, Lin G, Appelbaum PC. 2007. Activity of daptomycin alone and in combination with rifampin and gentamicin against *Staphylococcus aureus* assessed by time-kill methodology. *Antimicrob. Agents Chemother.* 51:1504–1507. <http://dx.doi.org/10.1128/AAC.01455-06>.
- Julian K, Kosowska-Shick K, Whitener C, Roos M, Labischinski H, Rubio A, Parent L, Ednie L, Koeth L, Bogdanovich T, Appelbaum PC. 2007. Characterization of a daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* strain in a patient with endocarditis. *Antimicrob. Agents Chemother.* 51:3445–3448. <http://dx.doi.org/10.1128/AAC.00559-07>.
- Arhin FF, Draghi DC, Pillar CM, Parr TR, Jr, Moeck G, Sahn DF. 2009. Comparative in vitro activity profile of oritavancin against recent gram-positive clinical isolates. *Antimicrob. Agents Chemother.* 53:4762–4771. <http://dx.doi.org/10.1128/AAC.00952-09>.
- Arhin FF, Kurepina N, Sarmiento I, Parr TR, Jr, Moeck G, Kreiswirth B. 2010. Comparative in vitro activity of oritavancin against recent, genetically diverse, community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. *Int. J. Antimicrob. Agents* 35:93–94. <http://dx.doi.org/10.1016/j.ijantimicag.2009.07.012>.
- Belley A, Neesham-Grenon E, Arhin FF, McKay GA, Parr TR, Jr, Moeck G. 2008. Assessment by time-kill methodology of the synergistic effects of oritavancin in combination with other antimicrobial agents against *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 52:3820–3822. <http://dx.doi.org/10.1128/AAC.00361-08>.
- Lin G, Pankuch GA, Ednie LM, Appelbaum PC. 2010. Antistaphylococcal activities of telavancin tested alone and in combination by time-kill assay. *Antimicrob. Agents Chemother.* 54:2201–2205. <http://dx.doi.org/10.1128/AAC.01143-09>.
- National Committee for Clinical Laboratory Standards. 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26. NCCLS, Wayne, PA.
- Arhin FF, Belley A, McKay GA, Moeck G. 2010. Characterization of the in vitro activity of novel lipoglycopeptide antibiotics. *Curr. Protoc. Microbiol.* Chapter 17:Unit 17.1. <http://dx.doi.org/10.1002/9780471729259.mc1701s16>.
- American Society for Microbiology. 2014. Instructions to authors. American Society for Microbiology, Washington, DC.
- Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A9. Ninth ed. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing. Approved standard M100-S22. Twenty-second informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
- Kaatz GW, Lundstrom TS, Seo SM. 2006. Mechanisms of daptomycin resistance in *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* 28:280–287. <http://dx.doi.org/10.1016/j.ijantimicag.2006.05.030>.
- McKay GA, Beaulieu S, Arhin FF, Belley A, Sarmiento I, Parr T, Jr, Moeck G. 2009. Time-kill kinetics of oritavancin and comparator agents against *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*. *J. Antimicrob. Chemother.* 63:1191–1199. <http://dx.doi.org/10.1093/jac/dkp126>.
- Belley A, Arhin FF, Sarmiento I, Deng H, Rose W, Moeck G. 2013. Pharmacodynamics of a simulated single 1200 mg dose of oritavancin in an in vitro pharmacokinetic/pharmacodynamic model of methicillin-resistant *Staphylococcus aureus* infection. *Antimicrob. Agents Chemother.* 57:205–211. <http://dx.doi.org/10.1128/AAC.01428-12>.
- Ambrose PG, Drusano GL, Craig WA. 2012. In vivo activity of oritavancin in animal infection models and rationale for a new dosing regimen in humans. *Clin. Infect. Dis.* 54(Suppl 3):S220–S228. <http://dx.doi.org/10.1093/cid/cis001>.
- Saravolatz LD, Pawlak J, Johnson LB. 2010. In vitro activity of oritavancin against community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA) and daptomycin-non-susceptible *S. aureus* (DNSSA). *Int. J. Antimicrob. Agents* 36:69–72. <http://dx.doi.org/10.1016/j.ijantimicag.2010.02.023>.
- Arhin FF, Sarmiento I, Parr TR, Jr, Moeck G. 2009. Comparative in vitro activity of oritavancin against *Staphylococcus aureus* strains that are resistant, intermediate or heteroresistant to vancomycin. *J. Antimicrob. Chemother.* 64:868–870. <http://dx.doi.org/10.1093/jac/dkp286>.