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In Vitro Activity of Retapamulin against *Staphylococcus aureus* Resistant to Various Antimicrobial Agents

Louis D. Saravolatz, Joan Pawlak, Stephanie N. Saravolatz, Leonard B. Johnson

St. John Hospital and Medical Center and Wayne State University School of Medicine, Detroit, Michigan, USA

Retapamulin and six other antimicrobial agents were evaluated against 155 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, including strains resistant to vancomycin, linezolid, daptomycin, and mupirocin by microdilution tests. Time-kill assays were performed against representative MRSA, vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA) isolates. Retapamulin and mupirocin demonstrated MIC_{90}s of 0.12 μg/ml and 8 μg/ml, respectively, with resistance seen in 2.6% and 10% of isolates, respectively. Retapamulin maintained good activity against 94% (15/16) of mupirocin-resistant isolates.

Retapamulin is a novel, semisynthetic antimicrobial agent in the class of pleuromutilins. It has a complex mode of action with inhibition of translation and 50S ribosomal subunit formation. This dual inhibitory effect differentiated retapamulin from other bacterial protein synthesis inhibitors, such as macrolides and ketolides (1). Retapamulin acts at a site distinct from other antimicrobial agents, preventing the development of cross-resistance (2).

Retapamulin ointment (1%) is the first approved pleuromutilin antimicrobial for the treatment of uncomplicated superficial skin infections caused by staphylococcal bacteria (3). Although at this time, retapamulin is not approved for methicillin-resistant *Staphylococcus aureus* (MRSA) infections, the recognized importance of this pathogen prompted us to evaluate retapamulin's *in vitro* activity against a select group of *S. aureus* isolates resistant to a variety of antimicrobial agents used in the topical or systemic treatment of this infection.

A collection of 155 strains of *Staphylococcus aureus* were selected for evaluation. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains ($n = 96$) were isolated from patients admitted to St. John Hospital and Medical Center, Detroit, MI, from sources including blood ($n = 30$), respiratory ($n = 36$), wound or tissue ($n = 28$), catheter tip ($n = 1$), and percutaneous endoscopic gastrostomy ($n = 1$) sources. Daptomycin-nonsusceptible *Staphylococcus aureus* (DNSSA) strains ($n = 7$) were obtained from blood isolates collected from patients at St. John Hospital and Medical Center, Detroit, MI. The St. John Hospital and Medical Center strains were collected from July 2002 to April 2008. Vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates ($n = 33$), vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates ($n = 13$), and linezolid-nonsusceptible *Staphylococcus aureus* (LNSSA) isolates ($n = 4$) were obtained through the Network on Antimicrobial Resistance in *S. aureus* (NARSA) program; these isolates were collected from 1996 to 2010. Two LNSSA blood isolates were obtained from Robinson Memorial Hospital in Ohio from April 2008 to May 2009. The VISA isolates were cultured from blood ($n = 12$), wound ($n = 5$), bile ($n = 2$), peritoneal fluid ($n = 1$), bone ($n = 1$), cerebrospinal fluid (CSF) ($n = 1$), respiratory ($n = 1$), urine ($n = 1$), and unknown ($n = 9$) sources. The VRSA isolates were cultured from wounds ($n = 8$), a catheter site ($n = 1$), urine ($n = 1$), a nephrostomy tube ($n = 1$), and prosthetic knee drainage ($n = 2$). The LNSSA isolates from NARSA were cultured from an unknown source ($n = 3$) and sputum ($n = 1$).

Microdilution tests using cation-adjusted Mueller-Hinton broth were used to determine the MICs of retapamulin (RETAP), mupirocin (MUP), vancomycin (VAN), linezolid (LZD), clindamycin (CLI), trimethoprim-sulfamethoxazole (SXT), and minocycline (MIN). MICs were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (4). MICs were read visually as the lowest drug concentration well with no visible bacterial growth. Susceptibility categories were determined according to CLSI breakpoints when available. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used to monitor quality control for the antibiotics. We used the following breakpoints as proposed by Traczewski and Brown for retapamulin: susceptible, ≤0.5; intermediate, 1.0; resistant, ≥2 (5). The minimal bactericidal concentrations (MBCs) for all the isolates were determined according to CLSI guidelines (6). The MBC was determined as the antibiotic concentration that reduced the number of viable cells by ≥99.9% as determined by colony counts (7).

Time-kill assays were performed on three isolates according to procedures previously described (8). The assays were performed in triplicate. The lower limit of detection was determined to be 100 CFU/ml, and bactericidal activity was defined as a ≥3-$\log_{10}$ decrease in numbers of CFU/ml compared to the time-zero count. Retapamulin and mupirocin were tested against one community-acquired MRSA (CA-MRSA), one VISA, and one VRSA isolate. The density of the starting cultures was approximately 10^6 CFU/ml. The antibiotics were tested at 64 times and 4,096 times the MIC, with colony counts taken at 0, 2, 4, 6, and 24 h. For the colony counts, aliquots of 0.1 ml were removed from the cultures and diluted in cold saline and plated onto blood agar plates. In order to minimize antibiotic carryover, the bacterial samples were

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Address correspondence to Louis D. Saravolatz, louis.saravolatz@stjohn.org.

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TABLE 1 MICs and MBCs for activities of all antimicrobials tested against MRSA, VISA, VRSA, and DNSSA isolates[a]

Isolate and agent	MIC (μg/ml)				MBC (μg/ml)		
	Range	MIC_{50}	MIC_{90}	% S	Range	MBC_{50}	MBC_{90}
MRSA ($n = 96$)							
RETAP	0.06–0.12	0.12	0.12	100	1–8	4	4
MUP	0.06–>512	0.12	0.25	94	4–>512	16	32
VAN	0.5–2	1	1	100	0.5–4	1	1
LZD	1–4	2	2	100	2–>8	>8	>8
CLI	0.06–>64	0.12	>64	61	1–>64	8	>64
SXT	0.06/1.2–>4/76	0.12/2.4	0.5/9.5	98	0.06/1.2–>4/76	0.12/2.4	1/19
MIN	0.06–16	0.12	0.5	96	0.5–>16	>16	>16
VISA ($n = 33$)							
RETAP	0.03–0.25	0.06	0.12	100	0.06–4	2	4
MUP	0.03–>512	0.25	>512	82	0.5–>512	8	>512
VAN	4–8	4	8	0	4–16	4	8
LZD	0.5–4	2	2	100	2–>8	8	>8
CLI	0.06–>64	>64	>64	30	0.12–>64	>64	>64
SXT	0.06/1.2–>4/76	0.5/9.5	>4/76	70	0.12/2.4–>4/76	2/38	>4/76
MIN	0.03–16	0.12	4	94	0.06–>16	>16	>16
VRSA ($n = 13$)							
RETAP	0.03–0.25	0.06	0.12	100	1–4	2	4
MUP	0.06–32	0.25	16	77	0.5–>512	4	>512
VAN	32–>64	>64	>64	0	64–>64	>64	>64
LZD	0.5–4	2	2	100	8–>8	>8	>8
CLI	>64	>64	>64	0	>64	>64	>64
SXT	0.06/1.2–>4/76	0.12	2/38	92	0.12/2.4–>4/76	>4/76	>4/76
MIN	0.03–2	0.12	2	100	8–>16	>16	>16
DNSSA ($n = 30$)							
RETAP	0.03–0.25	0.06	0.12	100	0.06–4	2	4
MUP	0.03–>512	0.25	0.5	93	0.5–>512	8	16
VAN	1–8	4	8	23	2–16	4	8
LZD	0.5–4	2	2	100	2–>8	8	>8
CLI	<0.03–>64	>64	>64	27	0.12–>64	>64	>64
SXT	0.06/1.2–>4/76	0.25/4.8	>4/76	73	0.12/2.4–>4/76	0.5/9.5	>4/76
MIN	0.03–16	0.12	4	93	0.06–>16	>16	>16

[a] Abbreviations: RETAP, retapamulin; MUP, mupirocin; VAN, vancomycin; LZD, linezolid; CLI, clindamycin; SXT, trimethoprim-sulfamethoxazole; MIN, minocycline; % S, percent susceptible.

centrifuged and reconstituted to their original volume with sterile saline (9).

The results of the MIC and MBC determinations are summarized in Tables 1 and 2. Retapamulin provided consistent results irrespective of the decreased susceptibility to vancomycin, daptomycin, clindamycin, trimethoprim-sulfamethoxazole, or minocycline. For all isolates reported in Table 1, the MIC_{90} was 0.12 μg/ml. MBCs were 16 to 32 times higher than the MICs, consistent with the bacteriostatic activity of retapamulin. The only isolates that demonstrated resistance to retapamulin were four strains of *S. aureus* that were linezolid nonsusceptible.

Retapamulin was active against all isolates irrespective of the

TABLE 2 MIC_{50} and MBC_{50} and geometric mean MICs and MBCs for all antimicrobials tested against LNSSA isolates[a]

Test agent	MIC (μg/ml)				MBC (μg/ml)		
	Range	MIC_{50}	Geometric mean	% S	Range	MBC_{50}	Geometric mean
RETAP	0.06–16	1	0.99	33	4–64	32	25.39
MUP	0.12–32	0.12	0.34	83	4–>512	16	25.39
VAN	1–2	1	1.12	100	1–2	1	1.26
LZD	16–64	16	28.5	0	32–>64	>64	90.5
CLI	0.03–>64	1	2.5	33	0.12–>64	4	7.9
SXT	0.06/1.2–>4/76	0.06/1.2	0.19/3.8	83	0.06/1.2–>4/76	0.12/2.4	0.3/6
MIN	0.12–4	0.5	0.7	100	1–>16	>16	17.96

[a] Abbreviations: RETAP, retapamulin; MUP, mupirocin; VAN, vancomycin; LZD, linezolid; CLI, clindamycin; SXT, trimethoprim-sulfamethoxazole; MIN, minocycline; % S, percent susceptible.

TABLE 3 Activity of all antimicrobial agents against mupirocin-susceptible and high-level- and low-level-resistant *S. aureus* isolates[a]

Isolate group and test agent	MIC (μg/ml)				MBC (μg/ml)			
	Range	MIC_{50}	MIC_{90}	Geometric mean	Range	MBC_{50}	MBC_{90}	Geometric mean
Mupirocin susceptible ($n = 139$)								
RETAP	0.03–16	0.06	0.12		0.06–64	4	4	
MUP	0.03–0.5	0.12	0.25		0.5–32	16	16	
VAN	0.5–>64	1	8		0.5–>64	1	8	
LZD	0.5–>8	2	2		2–>8	>8	>8	
CLI	<0.03–>64	1	>64		0.12–>64	8	>64	
SXT	0.06/1.2–>4/76	0.12/2.4	1/19		0.06/1.2–>4/76	0.12/2.4	>4/76	
MIN	0.03–16	0.12	4		0.06–>16	>16	>16	
Mupirocin high-level resistance ($n = 10$)								
RETAP	0.06–0.25	0.12	0.12		0.12–4	4	4	
MUP	>512	>512	>512		>512	>512	>512	
VAN	0.5–4	1	4		0.5–4	1	4	
LZD	1–2	2	2		2–>8	8	>8	
CLI	0.06–>64	>64	>64		0.12–>64	>64	>64	
SXT	0.12/2.4–>4/76	4/76	>4/76		0.12/2.4–>4/76	>4/76	>4/76	
MIN	0.06–4	0.12	4		4–>16	>16	>16	
Mupirocin low-level resistance ($n = 6$)								
RETAP	0.03–1	0.06		0.076	0.25–64	2		2.82
MUP	8–32	16		17.95	256–>512	>512		812.75
VAN	1–>64	4		17.95	1–>64	8		20.15
LZD	0.5–8	2		2	4–>8	>8		10.08
CLI	0.03–>64	>64		31.78	0.12–>64	>64		40.04
SXT	0.06/1.2–2/38	0.12/2.4		0.22/4.26	0.06/1.2–>4/76	0.5/9.5		12.5/24.02
MIN	0.03–0.5	0.12		0.12	1–>16	16		11.31

[a] Abbreviations: RETAP, retapamulin; MUP, mupirocin; VAN, vancomycin; LZD, linezolid; CLI, clindamycin; SXT, trimethoprim-sulfamethoxazole; MIN, minocycline.

mupirocin susceptibility (Table 3). Among the 155 individual isolates in this study, 6.45% (10/155) demonstrated high-level resistance and 3.87% (6/155) demonstrated low-level resistance to mupirocin.

Time-kill assays were performed on three selected isolates, CA-MRSA USA-300 (SA#2), VISA (NRS-22), and VRSA (VRS-9) (Table 4). For all three organisms, mupirocin demonstrated bacteriostatic activity with less than a 2.1-log reduction in growth after 24 h. In only one of the three isolates, VISA (NRS-22), retapamulin was found to be bactericidal with a 3.4-log reduction at 24 h when tested at 4,096 times the MIC.

Using established breakpoints, all the isolates in this study except the linezolid-nonsusceptible strains were susceptible to retapamulin. Mendes and Candel reported similar findings when testing isolates which were not susceptible to linezolid (10, 11). Retapamulin resistance was found in 3.7% (6/164) of all *S. aureus* isolates tested, compared to 2.6% (4/155) in our study, and was active against 68% (17/25) of *S. aureus* isolates resistant to mupirocin, compared to 94% (15/16) in our study.

In vitro work evaluating multipassage studies for up to 50 days compared retapamulin to mupirocin, fusidic acid, cephalexin, erythromycin, linezolid, vancomycin, and quinupristin-dalfopristin against *Staphylococcus aureus* isolates, including methicillin-resistant, vancomycin-intermediate, and vancomycin-resistant (VRSA) strains (2). Retapamulin had a lower frequency of spontaneous resistance against *S. aureus* than all other drugs

TABLE 4 Time-kill results for CA-MRSA, VISA, and VRSA isolates

Isolate and drug (concn)	Count reduction ($\Delta\log_{10}$ CFU/ml) at[a]:			
	2 h	4 h	6 h	24 h
CA-MRSA (USA-300)				
Retapamulin (64×)	0	0.1	0.1	1.2
Retapamulin (4,096×)	0.1	0.3	0.3	1.6
Mupirocin (64×)	0	0.1	0.1	1.2
Mupirocin (4,096×)	0	0.1	0.2	1.4
VISA sample (NRS-22)				
Retapamulin (64×)	0.1	0.3	0.6	1.4
Retapamulin (4,096×)	0.3	0.7	1	**3.4**
Mupirocin (64×)	0	0.4	0.8	1.7
Mupirocin (4,096×)	0.1	0.6	1.1	2.1
VRSA sample (VRS-9)				
Retapamulin (64×)	0	0.2	0.3	1.3
Retapamulin (4,096×)	0	0.2	0.2	1.4
Mupirocin (64×)	0	0.1	0.3	1.2
Mupirocin (4,096×)	0	0	0.2	1.2

[a] Shown is the $\Delta\log_{10}$-CFU/ml count reduction in relation to the total count of CFU/ml at time zero. Reductions of ≥3 log CFU are highlighted in bold.

tested except linezolid. Clones selected for prolonged selection yielded mutants with retapamulin MICs ranging from 4 to 16 μg/ml. This work suggests that resistance development in retapamulin is a slow, multistep process and that mutations accumulate gradually in the presence of drug pressure.

Our results involved *in vitro* studies from clinical isolates reflective of a diverse group of strains based on susceptibility to commonly used antistaphylococcal agents. The strains tested included all available VRSA isolates at the time of the study as well as a significant number of VISA and daptomycin-nonsusceptible isolates. In this study, retapamulin demonstrated lower resistance rates than mupirocin, both of which are commonly used for the treatment of uncomplicated *S. aureus* and *Streptococcus pyogenes* skin and soft tissue infections. Ongoing studies and surveillance will be needed to determine how these agents can be used most effectively.

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