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Activity of Retapamulin (SB-275833), a Novel Pleuromutilin, against Selected Resistant Gram-Positive Cocci

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Retapamulin (SB-275833), the first pleuromutilin to be developed for human topical use, was tested against a selected population of staphylococci and β-hemolytic streptococci. The MIC_{90} results for retapamulin were 0.12 μg/ml for *Staphylococcus aureus* and ≤0.03 μg/ml for *Streptococcus pyogenes*; no cross-resistance was observed for organism subsets resistant to oxacillin, erythromycin, or mupirocin.

A class of antimicrobial agents that has remained largely undeveloped for human clinical use is the pleuromutilins (5). These antimicrobials are derivatives of the naturally occurring pleuromutilin produced by *Pleurotus mutilus*, an edible mushroom. The pleuromutilin class has a unique mode of action, which involves inhibition of bacterial protein synthesis by binding to the prokaryotic ribosome (5, 14). Agents in this class have been described as binding to domain V of 23S rRNA, thereby blocking peptide formation directly by interfering with substrate binding (13). This binding site is unique compared to other ribosomally targeted inhibitors. Pleuromutilins have no target-specific cross-resistance to other antibacterials, which makes them appealing for human clinical development (1). However, mutations in the genes encoding 23S rRNA have led to reduced susceptibility to tiamulin (11). In veterinary practice, tiamulin and valnemulin (two semisynthetic pleuromutilin analogs) are used for the control and treatment of serious infections in swine (1, 5). Tiamulin has exceptional activity (MIC, ≤1 μg/ml) against anaerobic bacteria, *Mycoplasma* spp., and intestinal spirochetes (1, 5, 6). Tiamulin has also shown potent activity against some staphylococci from human sources (MIC_{50}, ≤0.5 μg/ml) (5).

Retapamulin (formerly called SB-275833) (Fig. 1) is a novel pleuromutilin antimicrobial being developed for topical treatment of skin infections. According to a recent global surveillance report (4), the most common bacteria found in skin and soft tissue infections (SSTI) include the gram-positive organisms *Staphylococcus aureus* (55.2%), β-hemolytic streptococci (5.0%), and coagulase-negative staphylococci (CoNS) (4.9%). Escalating numbers of these bacteria causing SSTI have become resistant to the leading topical antimicrobials used in human clinical practice. Mupirocin, a topical agent, has shown resistance rates ranging from 1.3% in Latin America to 8.7% in Europe among *S. aureus* isolates (3). Mupirocin resistance has increased in CoNS, ranging from 12.7% in Europe to 38.8% in the United States (3). Deshpande et al. showed that mupirocin resistance is higher among oxacillin-resistant staphylococcal strains than oxacillin-susceptible organisms (*S. aureus*, 4.6 to 17.8%; CoNS, 14.0 to 43.1%) (3).

This study examines retapamulin activity against staphylococci and β-hemolytic streptococci that possess phenotypic/genotypic resistance to oxacillin, mupirocin, or erythromycin compared to that against wild-type susceptible organisms of the same species.

A total of 604 recent (2002 to the present) clinical isolates were identified and confirmed by a reference laboratory (JMI Laboratories, North Liberty, IA), using colonial characteristics on standard media, rapid tests (catalase test, latex agglutination kits, coagulase test, pyrrolidonyl arylamidase, etc.), the use of an automated identification system (Vitek; bioMerieux, Hazelwood, MO), and other tests, as necessary. The list of tested isolates included *S. aureus* (281 strains), CoNS (232 strains), and *Streptococcus pyogenes* (91 strains). The results were analyzed according to susceptibility pattern subgroups, with the number of strains varying from 56 to 212 for each group.

Determination of MICs was performed by reference M7-A6 methods in the broth microdilution format (8). Antimicrobials used for comparison included tiamulin (veterinary pleuromutilin), mupirocin (topical agent used for skin infections) (10), penicillin (active against gram-positive organisms), oxacillin (active against gram-positive organisms), erythromycin (topical, oral, and parenteral agent used for SSTI), clindamycin (topical, oral, and parenteral agent used for SSTI), ofloxacin (oral agent indicated for treatment of SSTI), gentamicin (topical and parenteral agent indicated for treatment of SSTI), cephalothin (a cephalosporin used as a drug class susceptibility comparator), bacitracin (topical agent used for SSTI as a component of triple antibiotic ointment [polymyxin B, neomycin,

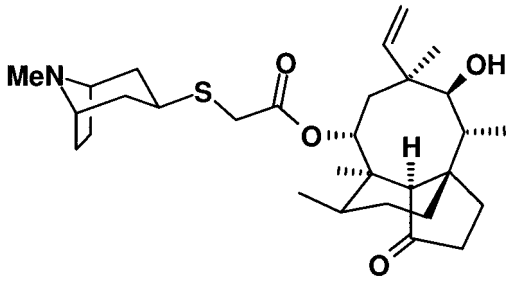

FIG. 1. Chemical structure of retapamulin, or mutilin 14-(*exo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-sulfanyl-acetate.

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TABLE 1. Activities of retapamulin and selected comparator agents against gram-positive coccus subsets, determined by reference MIC methods (8)

Test organism (*n*) and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range	% Susceptible/ % resistant[a]
S. aureus				
Erythromycin-resistant strains (212)				
Retapamulin	0.06	0.12	≤0.03–0.12	—
Tiamulin	0.5	1	≤0.25–1	—
Mupirocin	≤2	>256	≤2–>256	56.1/43.9
Bacitracin	>4	>4	2–>4	—
Cephalothin	4	>32	≤0.25–>32	52.8/40.1
Clindamycin	>100	>100	≤0.12–>100	34.0/65.6
Gentamicin	0.25	30	≤0.12–>30	87.7/12.3
Linezolid	2	2	0.25–4	100.0/—
Ofloxacin	30	>30	≤0.12–>30	19.8/79.2
Oxacillin	>2	>2	≤0.25–>2	18.4/81.6
Penicillin	>2	>2	≤0.03–>2	3.3/96.7
Mupirocin-resistant strains (100)				
Retapamulin	0.06	0.12	≤0.03–0.12	—
Tiamulin	0.5	1	≤0.25–1	—
Bacitracin	>4	>4	2–>4	—
Cephalothin	16	>32	≤0.25–>32	43.0/49.0
Clindamycin	>100	>100	≤0.12–>100	23.0/76.0
Erythromycin	>200	>200	≤0.12–>200	6.0/93.0
Gentamicin	0.25	30	≤0.12–>30	81.0/19.0
Linezolid	2	2	0.25–4	100.0/—
Ofloxacin	30	>30	0.25–>30	9.0/89.0
Oxacillin	>2	>2	≤0.25–>2	17.0/83.0
Penicillin	>2	>2	≤0.03–>2	6.0/94.0
Oxacillin-resistant strains (181)				
Retapamulin	0.06	0.12	≤0.03–0.12	—
Tiamulin	0.5	1	≤0.25–2	—
Mupirocin	≤2	>256	≤2–>256	54.1/45.9
Bacitracin	>4	>4	2–>4	—
Cephalothin	16	>32	≤0.25–>32	44.2/47.5
Clindamycin	>100	>100	≤0.12–>100	26.0/74.0
Erythromycin	>200	>200	≤0.12–>200	3.9/95.6
Gentamicin	0.25	30	≤0.12–>30	87.3/12.7
Linezolid	2	2	0.25–2	100.0/—
Ofloxacin	30	>30	0.25–>30	10.5/89.0
Penicillin	>2	>2	0.12–>2	0.6/99.4
Erythromycin-, mupirocin-, and oxacillin-susceptible strains (56)				
Retapamulin	0.06	0.12	≤0.03–0.12	—
Tiamulin	0.5	1	≤0.25–2	—
Mupirocin	≤2	≤2	≤2	100.0/0.0
Bacitracin	>4	>4	≤0.03–>4	—
Cephalothin	≤0.25	≤0.25	≤0.25–>32	98.2/1.8
Clindamycin	≤0.12	≤0.12	≤0.12	100.0/0.0
Erythromycin	≤0.12	0.25	≤0.12–0.25	100.0/0.0
Gentamicin	0.25	0.5	≤0.12–1	100.0/0.0
Linezolid	1	2	1–2	100.0/0.0
Ofloxacin	0.25	0.5	≤0.12–1	100.0/—
Oxacillin	≤0.25	0.5	≤0.25–1	100.0/0.0
Penicillin	>2	>2	≤0.03–>2	16.1/83.9
CoNS				
Mupirocin-resistant strains[b] (117)				
Retapamulin	≤0.03	0.06	≤0.03–0.12	—
Tiamulin	≤0.25	0.5	≤0.25–1	—
Bacitracin	>4	>4	0.25–>4	—
Cephalothin	0.5	2	≤0.25–>32	96.6/1.7
Clindamycin	>100	>100	≤0.12–>100	46.2/53.8
Erythromycin	>200	>200	≤0.12–>200	15.4/83.8
Gentamicin	2	30	≤0.12–>30	72.6/14.5
Linezolid	0.5	1	0.25–2	100.0/—
Ofloxacin	30	>30	≤0.12–>30	12.8/80.3
Oxacillin	>2	>2	≤0.25–>2	6.8/93.2
Penicillin	>2	>2	≤0.03–>2	7.7/92.3

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TABLE 1. *Continued*

Test organism (*n*) and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range	% Susceptible/ % resistant[a]
Oxacillin-resistant strains[c] (167)				
Retapamulin	≤0.03	0.06	≤0.03–0.12	—
Tiamulin	≤0.25	0.5	≤0.25–1	—
Mupirocin	32	>256	≤2–>256	34.7/65.3
Bacitracin	>4	>4	0.25–>4	—
Cephalothin	0.5	4	≤0.25–>32	92.8/5.4
Clindamycin	>100	>100	≤0.12–>100	49.1/50.9
Erythromycin	>200	>200	≤0.12–>200	15.0/84.4
Gentamicin	0.5	30	≤0.12–>30	76.6/12.0
Linezolid	0.5	1	0.25–2	100.0/0.0
Ofloxacin	30	>30	≤0.12–>30	21.6/71.9
Penicillin	>2	>2	0.06–>2	3.6/96.4
Mupirocin- and oxacillin-susceptible strains[d] (57)				
Retapamulin	≤0.03	0.06	≤0.03–0.25	—
Tiamulin	≤0.25	0.5	≤0.25–2	—
Mupirocin	≤2	≤2	≤2	100.0/0.0
Bacitracin	>4	>4	4–>4	—
Cephalothin	≤0.25	≤0.25	≤0.25–0.5	100.0/0.0
Clindamycin	≤0.12	≤0.12	≤0.12–>100	96.5/3.5
Erythromycin	≤0.12	>200	≤0.12–>200	73.7/26.3
Gentamicin	≤0.12	0.25	≤0.12–4	100.0/0.0
Linezolid	1	2	0.25–2	100.0/—
Ofloxacin	0.25	0.5	≤0.12–8	96.5/1.8
Oxacillin	≤0.25	≤0.25	≤0.25	100.0/0.0
Penicillin	0.5	>2	≤0.03–>2	33.3/66.7
S. pyogenes				
Erythromycin-resistant strains (91)				
Retapamulin	≤0.03	≤0.03	≤0.03–0.06	—
Tiamulin	≤0.25	≤0.25	≤0.25	—
Mupirocin	≤2	≤2	≤2	100.0/0.0
Bacitracin	1	>4	0.25–>4	—
Cephalothin	≤0.25	≤0.25	≤0.25	100.0/0.0
Clindamycin	≤0.12	>100	≤0.12–>100	89.0/11.0
Gentamicin	8	8	4–30	8.8/8.8
Linezolid	1	1	0.5–2	100.0/—
Ofloxacin	1	1	0.5–2	100.0/0.0
Oxacillin	≤0.25	≤0.25	≤0.25	100.0/0.0
Penicillin	≤0.03	≤0.03	≤0.03	100.0/—

[a] —, no interpretive criteria are published by the CLSI.
[b] Includes CoNS not identified to the species level (84 strains), *Staphylococcus auricularis* (2 strains), *S. capitis* (2 strains), *S. epidermidis* (20 strains), *S. haemolyticus* (3 strains), *S. hominis* (3 strains), *S. saprophyticus* (1 strain), and *S. warnerii* (2 strains).
[c] Includes CoNS not identified to the species level (110 strains), *Staphylococcus auricularis* (3 strains), *S. capitis* (7 strains), *S. epidermidis* (28 strains), *S. haemolyticus* (6 strains), *S. hominis* (6 strains), *S. intermedius* (1 strain), *S. lugdunensis* (1 strain), *S. saprophyticus* (2 strains), *S. simulans* (1 strain), and *S. warnerii* (2 strains).
[d] Includes CoNS not identified to the species level (35 strains), *Staphylococcus auricularis* (1 strain), *S. capitis* (7 strains), *S. epidermidis* (8 strains), *S. hominis* (3 strains), *S. lugdunensis* (1 strain), *S. simulans* (1 strain), and *S. warnerii* (1 strain).

and bacitracin]), and linezolid (oral and parenteral oxazolidinone indicated for treatment of SSTI caused by *S. aureus* or *Streptococcus pyogenes*). The highest MIC used for each dilution series for the topical agents represents a 1:100 dilution of the approved topical formation. Retapamulin is insoluble in water but is soluble in dimethyl sulfoxide and methanol. Broth microdilution panels were prepared by TREK Diagnostics (Cleveland, OH) with cation-adjusted Mueller-Hinton medium and were used to test the *Staphylococcus* spp. For processing of *Streptococcus* spp., lysed horse blood (2 to 5%) was added to the medium (8). Quality control (QC) testing was performed based on a prior multilaboratory study of retapamulin (12). The following QC organisms and target retapamulin ranges were tested concurrently: *S. aureus* ATCC 29213 (QC range, 0.06 to 0.25 μg/ml) and *Streptococcus pneumoniae* ATCC 49619 (QC range, 0.06 to 0.5 μg/ml) (12). QC ranges for comparison agents were those recommended in Clinical and Laboratory Standards Institute documents (2, 7, 9, 12). Susceptibility breakpoint criteria for retapamulin or other pleuromutilins have yet to be established for these monitored species.

Retapamulin activity was analyzed with three organism groups and eight subsets of susceptibility phenotype (Table 1). *S. aureus* subset results for retapamulin showed consistent $MIC_{50/90}$ values of 0.06/0.12 μg/ml. Mupirocin-, oxacillin-, and erythromycin-resistant strains of *S. aureus* did not have differing retapamulin $MIC_{50/90}$ results. Retapamulin had the lowest MIC_{50} (0.12 μg/ml) for mupirocin-resistant *S. aureus* among the tested comparator agents. Other topical agents performed poorly against resistant *S. aureus*, with the following MIC_{90} values: bacitracin, >4 μg/ml; clindamycin, >100 μg/ml; erythromycin, >200 μg/ml; and mupirocin, >256 μg/ml. The only

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other pleuromutilin tested (tiamulin) showed $MIC_{50/90}$ results of 0.5/1 μg/ml for all *S. aureus* strains, which is an eightfold increase in MIC results compared to the retapamulin $MIC_{50/90}$ values.

CoNS showed similarly low $MIC_{50/90}$ results for retapamulin (≤0.03/0.06 μg/ml) in various antimicrobial-resistant and -susceptible subsets. The highest retapamulin MIC (0.25 μg/ml) occurred with a single susceptible CoNS strain. Mupirocin and oxacillin resistance in CoNS did not affect the retapamulin $MIC_{50/90}$ results. The tiamulin MIC_{90} result (0.5 μg/ml) was eightfold higher than that of retapamulin for all CoNS isolates tested.

One subset of erythromycin-resistant β-hemolytic streptococci (*S. pyogenes*) was evaluated and displayed retapamulin $MIC_{50/90}$ values of ≤0.03/≤0.03 μg/ml. Retapamulin showed excellent activity against these isolates, with only two requiring a MIC of 0.06 μg/ml. Against *S. pyogenes*, retapamulin was at least fourfold more potent than it was versus *S. aureus*.

Overall, the retapamulin MIC population distribution showed a mode at 0.06 μg/ml and $MIC_{50/90}$ results of 0.06/0.12 μg/ml. Other tested agents with potent activities were tiamulin (MIC_{90} range, ≤0.25 to 1 μg/ml), linezolid (MIC_{90} range, 1 to 2 μg/ml; 100.0% of organisms were susceptible), and gentamicin (MIC_{90} range, 0.25 to 30 μg/ml; 72.6 to 100.0% of staphylococci were susceptible). Tiamulin had its highest MIC at 2 μg/ml, observed among staphylococcal isolates. Mupirocin resistance rates were elevated among erythromycin-resistant and oxacillin-resistant *S. aureus* subsets (43.9 and 45.9%, respectively) and for the oxacillin-resistant CoNS subset (65.3%).

Retapamulin demonstrated potent in vitro activity ($MIC_{50/90}$, 0.06/0.12 μg/ml) and a broad spectrum against several antimicrobial-resistant organism subsets of *S. aureus*, CoNS, and β-hemolytic streptococci. Resistance to erythromycin, mupirocin, or oxacillin did not adversely affect the MICs of the pleuromutilins. Compared to tiamulin, retapamulin appears to be uniformly eightfold more potent. While this antimicrobial class has not been developed previously for human application, the use of related antimicrobial agents in veterinary medicine has been well established (6, 9). Given the declining efficacy of contemporary approved topical agents (mupirocin), the development of novel agents lacking cross-resistance to other antimicrobial classes appears warranted.

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