

## *In Vitro* Activities of Retapamulin and 16 Other Antimicrobial Agents against Recently Obtained *Streptococcus pyogenes* Isolates<sup>▽</sup>

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Received 1 December 2010/Returned for modification 13 February 2011/Accepted 15 February 2011

**Retapamulin *in vitro* activity against 400 *Streptococcus pyogenes* clinical isolates obtained from skin, pharynx, ear fluid, and blood samples recovered from 2007 to 2009 was studied. The isolates belonged to 26 different *emm* types, including isolates nonsusceptible to erythromycin ( $n = 187$ ), tetracycline ( $n = 99$ ), ciprofloxacin ( $n = 59$ ), and bacitracin ( $n = 43$ ). Results were compared to the activities of 16 other antibiotics for topical and systemic use. Retapamulin MICs ranged from  $\leq 0.015$  to  $0.12 \mu\text{g/ml}$ , showing the highest intrinsic activity among the topical antimicrobial drugs studied.**

*Streptococcus pyogenes* and *Staphylococcus aureus* are the most common pathogens involved in skin and skin structure infections (18). Despite penicillin's intensive use, *S. pyogenes* remains fully susceptible to penicillin. In contrast, the high rates of macrolide resistance observed in recent years are a matter of concern in many countries worldwide (3, 12, 14). Whereas systemic antibiotics are needed to treat complicated skin structure infections, mild cases of impetigo and other skin infections can be treated with topical agents, among which mupirocin and fusidic acid are the most common therapeutic options. The need for a new topical antimicrobial drug for skin infections was reinforced by recent reports of the development of resistance to mupirocin and fusidic acid, especially in *S. aureus* (1, 2, 6), and by the poor intrinsic activity on *S. pyogenes* of other topical antimicrobial drugs, such as neomycin and bacitracin. Retapamulin is a novel semisynthetic antimicrobial agent in the new class of pleuromutilins, approved for human use as a 1% ointment (Altanax, Altargo; GlaxoSmithKline) for the treatment of impetigo and infected small wounds. It also seems appropriate for the treatment of bacterial infections in atopic dermatitis (9). It acts by binding specifically to the peptidyl transferase center, inhibiting the development of the amino acid chain onto tRNA; it also blocks P-site interactions and prevents normal formation of 50S ribosomal subunits. Retapamulin has been approved by the European Medicine Agency and by the Food and Drug Administration in the United States.

The aim of this study was to determine the *in vitro* activity of retapamulin against recently obtained clinical isolates of *S. pyogenes*, including multidrug-resistant isolates, and to compare it with that of 16 other antimicrobial agents for topical and systemic use to treat acute bacterial skin infections.

This study included 400 *S. pyogenes* isolates obtained from the microbiology laboratory of Hospital Donostia (San Sebastián, Spain) between January 2007 and June 2009. The isolates were divided into those obtained from skin lesions ( $n = 144$ ), blood ( $n = 17$ ), and other body sites such as the pharynx or ear fluid ( $n = 239$ ). Patients of all ages were included (range, 0 to 96 years old). All isolates from skin lesions were included in this study, whereas isolates from other body sites were selected in order to include a representative assortment of different *emm* types and macrolide-, lincosamide-, tetracycline-, and ciprofloxacin-nonsusceptible isolates.

MICs were determined by the broth microdilution method using Sensititre Microtiter Trays (Trek Diagnostics Systems, United Kingdom) containing 16 antibiotics apart from retapamulin (Table 1) in accordance with CLSI guidelines (4). Susceptibility criteria for antimicrobials not included in the CLSI guidelines were arbitrarily chosen and are listed in Table 2 and later in the text. For retapamulin, a susceptibility breakpoint of  $\leq 0.25 \mu\text{g/ml}$  was established based on previous studies (10, 19). *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were used as controls.

(i) *emm* typing of isolates by PCR-restriction fragment length polymorphism assay and sequencing and (ii) detection of the *mef*, *erm*, and *tet* genes and mutations in the *parC* and *gyrA* genes were performed as previously reported (8, 13).

The MICs of antimicrobial agents required to suppress the growth of 50% (MIC<sub>50</sub>) and 90% (MIC<sub>90</sub>) of the *S. pyogenes* isolates tested are summarized in Table 1. Retapamulin showed potent *in vitro* activity against all clinical *S. pyogenes* isolates independently of the source of the sample and the resistance phenotype, including macrolide-, tetracycline-, fusidic acid (MIC,  $\geq 32 \mu\text{g/ml}$ )-, quinolone-, and bacitracin (MIC,  $\geq 16 \mu\text{g/ml}$ )-resistant isolates. The range of retapamulin susceptibility was between  $\leq 0.015$  and  $0.12 \mu\text{g/ml}$ , showing the highest intrinsic activity of the antimicrobial drugs often used topically (Table 2).

Overall, 26 different *emm* types of *S. pyogenes*, comprising 400 isolates, were included, of which 187 were nonsusceptible

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<sup>▽</sup> Published ahead of print on 22 February 2011.

TABLE 1. MIC<sub>50</sub>s and MIC<sub>90</sub>s of retapamulin and 16 other antimicrobial agents for 400 clinical *S. pyogenes* isolates obtained from skin and other body sites in Gipuzkoa from 2007 to 2009

Antimicrobial agent	Skin isolates (n = 144)			Isolates from other body sites (n = 256)			Total (n = 400)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
Mupirocin	≤0.12 <sup>a</sup>	0.25	≤0.12–1	≤0.12	0.25	≤0.12–2	≤0.12	0.25	≤0.12–1
Bacitracin	1	2	≤0.25–>32	1	32	≤0.25–>32	1	32	≤0.25–>32
Fusidic acid	4	8	2–16	4	8	0.25–32	4	8	0.25–32
Neomycin	32	>64	≤8–>64	32	>64	≤8–>64	32	>64	≤8–>64
Gentamicin	4	4	≤1–32	4	4	≤1–>32	4	4	≤1–>32
Erythromycin	≤0.25	>32	≤0.25–>32	8	>32	≤0.25–>32	≤0.25	>32	≤0.25–>32
Josamycin base	≤0.5	>4	≤0.5–>4	≤0.5	>4	≤0.5–>4	≤0.5	>4	≤0.5–>4
Clindamycin	≤0.25	>1	≤0.25–>1	≤0.25	>1	≤0.25–>1	≤0.25	>1	≤0.25–>1
Lincomycin	≤0.5	>4	≤0.5–>4	≤0.5	>4	≤0.5–>4	≤0.5	>4	≤0.5–>4
Norfloxacin	≤4	≤4	≤4–>32	≤4	16	≤4–>32	≤4	16	≤4–>32
Ciprofloxacin	≤1	≤1	≤1–32	≤1	2	≤1–4	≤1	2	≤1–32
Tetracycline	≤2	>8	≤2–>8	≤2	>8	≤2–>8	≤2	>8	≤2–>8
Tigecycline	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Penicillin	≤0.015	≤0.015	≤0.015–0.06	≤0.015	≤0.015	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.06
Amoxicillin	≤0.03	≤0.03	≤0.03–0.12	≤0.03	≤0.03	≤0.03–0.06	≤0.03	≤0.03	≤0.03–0.12
Cloxacillin	0.12	0.12	≤0.12–>0.5	0.12	0.12	≤0.06–>0.5	0.12	0.12	≤0.06–>0.5
Retapamulin	0.03	0.06	≤0.015–0.12	0.03	0.06	≤0.015–0.12	0.03	0.06	0.015–0.12

<sup>a</sup> Values are in micrograms per milliliter.

to erythromycin, 99 were nonsusceptible to tetracycline, 59 were nonsusceptible to ciprofloxacin, and 43 were nonsusceptible to bacitracin (Table 2). Among the erythromycin-nonsusceptible isolates, the *ermB* gene was detected in 102 isolates, *ermA* (TR) was detected in 12, and *mef* was detected in 73. Of 99 tetracycline-nonsusceptible isolates, 79 harbored the *tetM* gene; among the remaining isolates, the *tetO* gene was detected in 17. All ciprofloxacin-nonsusceptible isolates showed a mutation in the Ser79 codon in the *parC* gene, and one isolate also harbored the Ser81/Phe mutation within the *gyrA* gene, which confers a high level of ciprofloxacin resistance (MIC, ≥32 µg/ml).

Among the isolates obtained from streptococcal skin lesions during the entire period of this study, nonsusceptibility to erythromycin and clindamycin was present in 27.8% and 15.9%, respectively. Nonsusceptibility to tetracycline (tetracycline hydrochloride) was present in 19.4%, whereas nonsusceptibility to tigecycline (MIC, ≥1 µg/ml) was not detected.

Most of the nonsusceptible isolates belonged to a few *emm* types. Of the 40 erythromycin-nonsusceptible skin isolates, 70% were grouped into three *emm* types, i.e., *emm11* (n = 8), *emm12* (n = 9), and *emm28* (n = 11). Multiresistance, defined as nonsusceptibility to three or more antimicrobials, was present in 14.6% of the skin isolates, most of these belonging to the *emm11* (8/21, 38.1%) and *emm28* (10/21, 47.6%) types.

Full susceptibility to mupirocin (MIC, ≤1 µg/ml) was observed, and resistance to bacitracin was present in isolates obtained from nonskin lesions. All bacitracin-resistant isolates belonged to a single multiresistant clone (11) already detected in several countries in Europe and in the United States (7, 15). Neomycin had the lowest intrinsic activity of the antibiotics tested. Based on MIC<sub>90</sub> values, retapamulin was at least 4-fold, 533-fold, 133-fold, and 1,066-fold more active than the most frequently used topical drugs, mupirocin, bacitracin, fusidic acid, and neomycin, respectively.

TABLE 2. MIC<sub>90</sub>s of seven antimicrobial drugs often used topically for *S. pyogenes* clinical isolates according to their susceptibility phenotypes and most prevalent *emm* types

Susceptibility phenotype (nonsusceptibility criterion [concn in µg/ml])	No. of isolates	Prevalent <i>emm</i> type(s) (no. of isolates) <sup>a</sup>	MIC <sub>90</sub> (µg/ml) <sup>d</sup>						
			RET	MUP	FUS	BAC	NEO	GEN	CIP
Erythromycin (≥0.5)	48	<i>emm4</i> (12), <i>emm12</i> (22)	0.06	0.5	8	1	64	>4	≤1
Tetracycline (≥4)	34	<i>emm77</i> (16)	0.06	0.25	8	2	32	4	≤1
Ciprofloxacin (≥2) <sup>b</sup>	33	<i>emm6</i> (24), <i>emm75</i> (8)	0.06	0.121	8	2	32	4	2
Erythromycin (≥0.5), clindamycin (≥0.5)	7	<i>emm22</i> (2), <i>emm28</i> (2)	0.03	0.25	8	1	32	4	≤1
Erythromycin (≥0.5), tetracycline (≥4)	11	— <sup>c</sup>	0.06	0.5	8	1	64	32	≤1
Erythromycin (≥0.5), ciprofloxacin (≥2)	25	<i>emm6</i> (25)	0.06	0.25	16	2	64	4	4
Tetracycline (≥4), ciprofloxacin (≥2)	1	<i>emm77</i> (1)	0.03	0.121	4	1	32	4	>4
Erythromycin (≥0.5), clindamycin (≥0.5), tetracycline (≥4)	53	<i>emm11</i> (46)	0.06	0.25	8	1	>64	4	≤1
Erythromycin (≥0.5), clindamycin (≥0.5), bacitracin (≥16) <sup>b</sup>	43	<i>emm28</i> (43)	0.03	0.121	8	>32	>64	4	≤1
Full susceptibility	145	—	0.12	0.25	8	1	32	4	≤1

<sup>a</sup> *emm* type of isolates representing ≥20% of this phenotype.

<sup>b</sup> Non-CLSI criterion, arbitrary breakpoint.

<sup>c</sup> —, no predominant *emm* types were found.

<sup>d</sup> Bacitracin, BAC; ciprofloxacin, CIP; fusidic acid, FUS; gentamicin, GEN; mupirocin, MUP; neomycin, NEO; retapamulin, RET.

Increasing antibacterial resistance is a major problem in the treatment of skin and soft-tissue infections worldwide; the choice of empirical antibiotic treatment must include agents with activity against resistant isolates (17). Previous *in vitro* studies have demonstrated a wide spectrum of activity of retapamulin against most of the bacteria commonly found in skin infections (10, 16, 19), including methicillin-, fusidic acid-, and mupirocin-resistant *S. aureus* isolates. Retapamulin inhibits bacterial protein synthesis through a novel mechanism of action different from that found in other antibiotics, such as mupirocin, fusidic acid, or macrolides, minimizing the potential to develop cross-resistance (5, 16).

In conclusion, retapamulin demonstrated potent *in vitro* activity against *S. pyogenes* isolates comprising a large number of *emm* types and including multiresistant clinical isolates.

This work was supported in part by a grant from GlaxoSmithKline, Spain, and by grant PI 08/0808 from Fondo de Investigación Sanitaria, Ministerio de Sanidad y Política Social, Spain.

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