

Activity of a New Oral Streptogramin, XRP2868, against Gram-Positive Cocci Harboring Various Mechanisms of Resistance to Streptogramins

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The antibacterial activity of XRP2868, a new oral streptogramin composed of a combination of RPR132552 (streptogramin A) and RPR202868 (streptogramin B), was evaluated against a collection of clinical gram-positive isolates with characterized phenotypes and genotypes of streptogramin resistance. The effects of genes for resistance to streptogramin A or B on the activity of XRP2868 and its components were also tested by cloning these genes individually or in various combinations in gram-positive recipient strains susceptible to quinupristin-dalfopristin. The species tested included *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, and other species of streptococci. XRP2868 was generally fourfold more potent than quinupristin-dalfopristin against *S. aureus*, *E. faecium*, and streptococci and had activity against *E. faecalis* (MICs = 0.25 to 1 µg/ml). XRP2868 appeared to be affected by the same mechanisms of resistance as those to quinupristin-dalfopristin. Nevertheless, the strong activity of factor A of the oral streptogramin enabled the combination to be very potent against streptogramin-susceptible staphylococci, streptococci, and *E. faecium* (MICs = 0.03 to 0.25 µg/ml) and to retain low MICs against the strains harboring a mechanism of resistance to factor A or factor B of the streptogramin. However, the combination of mechanisms of resistance to factors A and B caused an increase in the MICs of XRP2868, which reached 1 to 4 µg/ml. As with the other streptogramins, there was a reduction in the bactericidal effect of XRP2868 when the staphylococcal strains acquired a constitutively expressed *erm* gene.

Multidrug resistance in gram-positive cocci has increased at an alarming rate in clinical settings worldwide, and overcoming bacterial resistance has become more important than ever. Several new drugs have been proposed as alternatives for the treatment of severe infections caused by multiply resistant gram-positive organisms, i.e., linezolid, daptomycin, tigecycline, and quinupristin-dalfopristin. Quinupristin-dalfopristin, an injectable streptogramin, was among the first to be developed internationally (3). Streptogramins have the advantage of bactericidal activity against pneumococci and staphylococci, provided that they are susceptible to clindamycin (11). However, quinupristin-dalfopristin can be administered only parenterally; and except for pristinamycin, which is available in France, oral streptogramins are not available (5). In addition, the administration of quinupristin-dalfopristin requires the presence of a deep-vein catheter because of venous toxicity (21). Therefore, the value of the international development of a new oral streptogramin that can be used as a replacement or as the treatment of first choice has become obvious.

XRP2868 is a new investigational oral streptogramin that is composed of a mixture of 70% RPR132552 (streptogramin group A) and 30% RPR202868 (streptogramin group B) (18). The new compound was shown to inhibit staphylococci, *Streptococcus pneumoniae*, nonpneumococcal streptococci, *Haemophilus influenzae*, and anaerobes at concentrations of 1

µg/ml or less (10, 12, 18). XRP2868 was approximately fourfold more potent than quinupristin-dalfopristin against *Staphylococcus aureus* and *Enterococcus faecium* (10).

Streptogramins cause inhibition of protein synthesis by binding of the streptogramin A (S_A) and streptogramin B (S_B) components to the 50S ribosomal subunit. Each streptogramin factor binds to a different site on the peptidyltransferase domain of the ribosome, but the binding of S_A increases the affinity of S_B for its target (5). Since S_A and S_B are chemically unrelated and have different binding sites, the mechanisms of resistance to these two streptogramin types are different (13). In *E. faecium* and staphylococci, acetyltransferases encoded by genes belonging to the *vat* family inactivate S_A . Other staphylococcal genes, such as *vga(A)* and *vga(B)*, mediate resistance to S_A by a putative efflux mechanism. Resistance to S_B is due either to lyases encoded by the *vgb* genes, initially reported in *S. aureus* and then in enterococci, or to modification of the ribosomal target by a 23S rRNA methylase encoded by the *erm* genes widely distributed in numerous species of gram-positive organisms (14). In addition, in staphylococci, the *msr(A)* gene encodes a protein which probably participates in the active efflux of macrolides and S_B (20). Generally, because of the synergism displayed by the two streptogramin types, acquisition of isolated resistance to S_A or S_B has only a partial negative impact on the antimicrobial activity of the combination (4).

The aim of this study was to investigate the behavior of XRP2868 and those of the two factors that compose it against strains exhibiting characterized mechanisms of resistance to

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TABLE 1. Activities of streptogramins against clinical isolates of staphylococci

Bacterial species (no. of strains)	MLS resistance phenotype ^a	Relevant genotype	MIC range (μg/ml)			
			Q-D ^b	RPR202868	RPR132552	XRP2868
<i>S. aureus</i> (6)	Susceptible	None	0.12–0.25	8	0.12–0.25	0.06
<i>S. aureus</i> (2)	MLS _B inducible	<i>erm(A)</i> or <i>erm(C)</i>	0.25–0.5	16	0.5	0.06–0.12
<i>S. aureus</i> (6)	MLS _B constitutive	<i>erm(A)</i> or <i>erm(C)</i>	0.25	>128	0.12–0.25	0.12
<i>S. aureus</i> (1)	LS _A	Unknown determinant	2	4	2	1
<i>S. aureus</i> (2)	S _A + S _B	<i>vat</i> + <i>vgb</i>	4–8	64–>128	1–4	1
<i>S. aureus</i> (4)	S _A + S _B	<i>erm</i> + <i>vat</i> + <i>vga</i> + <i>vgb</i>	4–8	>128	8–64	1–4
<i>S. aureus</i> (1)	S _A + S _B	<i>vat</i> + <i>vga</i> + <i>vgb</i>	16	32	16	4
<i>S. aureus</i> (1)	M + S _B	<i>rpIV</i> (L22 protein) mutation	2	64	0.5	0.5
Coagulase-negative staphylococci (8)	Susceptible	None	0.12	4–8	0.06–0.12	0.03–0.06
Coagulase-negative staphylococci (2)	MLS _B constitutive	<i>erm(A)</i> or <i>erm(C)</i>	0.125	>128	0.06–0.12	0.06
Coagulase-negative staphylococci (6)	MLS _B constitutive + S _A	<i>erm(A)</i> or <i>erm(C)</i> + unknown mechanism	4–32	>128	2–16	1–16

^a LS_A, resistance to lincosamides and streptogramin A; M, macrolides; MLS_B constitutive, constitutive resistance to macrolides-lincosamides-streptogramin B; MLS_B inducible, inducible resistance to macrolides-lincosamides-streptogramin B; S_A, resistance to streptogramin A; S_B, resistance to streptogramin B.

^b Q-D, quinupristin-dalfopristin.

factor A or factor B of the streptogramins and to compare their behaviors to that of quinupristin-dalfopristin.

MATERIALS AND METHODS

Bacterial strains. We used one collection of laboratory strains and one collection of clinical isolates. The set of laboratory strains was composed of isogenic pairs of *S. aureus*, *E. faecium*, *Enterococcus faecalis*, *S. pneumoniae*, and oral streptococci. Thirty-seven constructs containing resistance genes for S_A or S_B were tested and were compared with their susceptible counterparts. Some streptogramin-resistant strains were described previously, and others were constructed for this study. They were obtained either by cloning determinants of resistance on multicopy vectors pAT392 and pJIM2246 (2, 4) or by in vitro mutation of susceptible *S. aureus*, *S. pneumoniae*, and *Streptococcus gordonii* strains or transformation of *S. pneumoniae* CP1000 or *S. gordonii* strain Challis (4, 6, 15, 23). In some constructs, resistance genes were combined.

One hundred forty-seven clinical isolates obtained between 2000 and 2005 from various clinical samples and selected as presenting various phenotypes of resistance to macrolides and/or streptogramins were also tested. This collection included 23 *S. aureus* isolates, 16 coagulase-negative staphylococci, 18 *E. faecium* isolates, 11 *E. faecalis* isolates, 32 *S. pneumoniae* isolates, 23 oral streptococci, and 24 group A and group B streptococci.

S. aureus ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619 and ATCC 6303 were used as controls for determination of the MICs of the antibiotics.

Susceptibility to streptogramins. The MICs of XRP2868, each of its two components (RPR132552 [factor A] and RPR202868 [factor B]), quinupristin-dalfopristin, quinupristin, and dalfopristin were determined by the agar dilution method with Mueller-Hinton (MH) medium (bioMérieux, La-Balme-les-Grottes, France) not supplemented or supplemented with 5% sheep blood for streptococci, according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (8). All antibiotics were supplied by Aventis-Pharma (Romainville, France). Inocula of approximately 10⁴ CFU per spot were applied with a replicator, and the plates were examined for growth after overnight incubation at 37°C.

Killing experiments. Time-kill curves were used to test the bactericidal activity of XRP2868 against *S. aureus* strains according to a proposed CLSI (formerly NCCLS) guideline (17). Three clinical isolates were tested. One was susceptible to macrolides and streptogramins, and two contained an *erm(A)* or an *erm(C)* gene and were constitutively resistant to macrolides-lincosamides-streptogramin B (MLS_B phenotype). Strains of *S. aureus* RN4220 containing the vector plasmid pAT392 (2) and derivatives with the *erm(A)* or *erm(C)* gene cloned on pAT392 were also tested. Briefly, overnight Trypticase soy broth cultures were diluted in MH broth. After 3 h of incubation at 37°C under aeration, cells were added to fresh prewarmed MH broth in 40-ml glass tubes to yield an inoculum of ca. 5 × 10⁶ CFU/ml. Immediately after inoculation, XRP2868 was added at a final concentration of 2 and 10 times the MIC of the compound against the strains.

Cultures were further incubated at 37°C with aeration. Just before and at 3, 6,

and 18 h after the addition of antibiotics, 0.1-ml samples were removed from the flasks; serially diluted; and subcultured onto MH agar plates by using a spiral plater (Spiral System Inc., Intersciences, Cincinnati, OH), which prevents carryover effects. They were then incubated for 48 h at 37°C before the numbers of CFU were counted. Bactericidal activity was defined as a reduction of 3 log₁₀ of the initial inoculum. An antibiotic-free control was included on each occasion.

Characterization of resistance genes by PCR. Clinical isolates resistant to antimicrobials were tested for the presence of resistance genes by PCR. Specific oligonucleotide primers described previously were used to amplify the major *erm* genes [*erm(A)*, *erm(A)* subset *ermTR*, *erm(B)*, and *erm(C)*] encoding ribosomal methylases; the *mef(A)*, *msr(A)*, *vga(A)*, and *vga(B)* genes encoding efflux proteins; the *vat(A)*, *vat(B)*, *vat(D)*, and *vat(E)* genes encoding streptogramin A acetyltransferases; and the *vgb(A)* and *vgb(B)* genes encoding a streptogramin B lyase (1, 4, 19).

RESULTS

Activity of XRP2868 against clinical isolates. XRP2868 was generally four times more potent than quinupristin-dalfopristin against the S_A- and S_B-susceptible isolates, with MICs ranging from 0.008 to 0.06 μg/ml for staphylococci, *E. faecium*, and streptococci (Tables 1 to 3). The oral streptogramin displayed activity against *E. faecalis*, with MICs equal to or less than 1 μg/ml, although this species is considered intrinsically resistant to streptogramins.

In *S. aureus* and coagulase-negative staphylococci expressing the MLS_B phenotype, the presence of *erm(A)* or *erm(C)* genes raised the MIC of RPR202868 (S_B) when the genes were constitutively expressed (Table 1). However, the synergism was maintained between the A and the B factors, and the MICs of XRP2868 were similar to or 1 dilution greater than those for the susceptible isolates. Similarly, the presence of the *erm(B)* or the *ermTR* genes in enterococci and streptococci weakly affected the activity of the streptogramin combination (Tables 2 and 3). Streptococci resistant to erythromycin by macrolide efflux encoded by the *mef(A)* class of genes remained fully susceptible to RPR202868, RPR132552, and XRP2868 (Table 3).

Significant increases in the MICs of XRP2868 (0.5 to 8 μg/ml) were found in *S. aureus* (Table 1) and *E. faecium* (Table 2) when resistance to factor A was expressed by the isolates, either alone in the case of the LS_A phenotype (resistance to

TABLE 2. Activities of streptogramins against clinical isolates of enterococci

Bacterial species (no. of strains)	MLS resistance phenotype ^a	Relevant genotype	MIC range (µg/ml)			
			Q-D ^b	RPR202868	RPR132552	XRP2868
<i>E. faecium</i> (1)	Susceptible	None	0.25	2	0.12	0.06
<i>E. faecium</i> (3)	MLS _B	<i>erm</i> (B)	0.25–0.5	32–>128	0.12	0.06–0.12
<i>E. faecium</i> (9)	MLS _B + S _A	<i>erm</i> (B) + unknown determinant	1–8	2–>128	8–64	0.5–8
<i>E. faecium</i> (5)	LS _A	Unknown determinant	1	1–8	32	0.5
<i>E. faecalis</i> (6)	Susceptible	None	1–2	2–4	8–64	0.25–0.5
<i>E. faecalis</i> (5)	MLS _B	<i>erm</i> (B)	1–4	32–64	64	0.25–1

^a LS_A, resistance to lincosamides and streptogramin A; MLS_B, resistance to macrolides-lincosamides-streptogramin B; S_A, resistance to streptogramin A.

^b Q-D, quinupristin-dalfopristin.

lincomycin and streptogramin A) or combined with resistance to factor B of the streptogramins. In the latter case, S_A and S_B resistance was associated with the presence of the *vat* (S_A acetyltransferase), *vgb* (S_B lyase), and *erm* genes in various combinations. Interestingly, XRP2868 remained active against the group B streptococci with the LS_A phenotype, which were recently reported from New Zealand (16).

Again, XRP2868 was generally four times more active than quinupristin-dalfopristin against the clinical isolates with S_A or S_B resistance, regardless of the nature of the resistance determinants.

Activity of XRP2868 against laboratory strains. The MIC results for the laboratory strains of staphylococci, enterococci, and streptococci are listed in Tables 4, 5, and 6, respectively.

Against the susceptible control strains of *S. aureus*, enterococci, and streptococci, the MICs of XRP2868 were similar to those for the susceptible clinical isolates. The activity of the S_B component of XRP2868 (RPR202868) was less than (for *S. aureus*) or superior to (for enterococci) that of quinupristin. By contrast, the potency of the S_A component of XRP2868 (RPR132552) was always greater than that of dalfopristin.

The presence of the *erm*(A) or the *erm*(C) gene in *S. aureus* RN4220 (Table 4) and the presence of *erm*(B) from plasmid pAMβ1 in *E. faecalis* JH2-2 (Table 5), which confer the MLS_B phenotype with constitutive resistance to S_B, led to an increase

in the MIC of S_B (quinupristin and RPR202868). By contrast, the presence of an inducible *erm*(B) gene in *S. aureus* RN4220, enterococci, and streptococci did not affect or only moderately affected the activity of S_B. Indeed, as we have previously shown for quinupristin, inducible S_B resistance could be fully expressed only after previous induction with S_B (4). In all cases, as for the clinical isolates, there was a weak effect or no impact on the MICs of XRP2868 and quinupristin-dalfopristin since synergism was maintained between the A and the B factors.

Similarly, when other genes for resistance to either streptogramin factor were introduced individually into recipient strains, there was no impact or only a weak impact on the MIC of XRP2868, regardless of the mechanism of resistance (efflux or inactivation). By contrast, a significant increase in MICs of XRP2868 (in general, 8 or 16 times) was found when genes for resistance to S_A and S_B were combined. However, in *S. aureus*, the efflux of S_A [*vga*(A) gene] did not significantly contribute to streptogramin resistance. Also, superposition of inactivation of S_B (*vgb* gene) onto S_B target modification [*erm*(A) gene] did not amplify the level of resistance to quinupristin. By contrast, the coexistence of the *vat*(D) and *vgb* genes in the combinations of resistance genes was the most efficient at conferring resistance to streptogramins. The greatest impact on the MICs of XRP2868 was obtained when the gene combinations were introduced into *E. faecium* BM4107, showing that the LS_A

TABLE 3. Activities of streptogramins against clinical isolates of streptococci

Bacterial species (no. of strains)	MLS resistance phenotype ^a	Relevant genotype	MIC range (µg/ml)			
			Q-D ^b	RPR202868	RPR132552	XRP2868
<i>S. pneumoniae</i> (9)	Susceptible	None	0.25–0.5	1–4	1–4	0.03–0.12
<i>S. pneumoniae</i> (18)	MLS _B	<i>erm</i> (B)	0.25–1	8–>128	1–16	0.03–0.5
<i>S. pneumoniae</i> (1)	MLS _B	<i>erm</i> (A) subset <i>ermTR</i>	0.25	8	2	0.12
<i>S. pneumoniae</i> (3)	M	<i>mef</i> (A)	0.12–0.25	2–4	1–4	0.03–0.12
<i>S. pneumoniae</i> (1)	MLS _B + M	<i>erm</i> (B) + <i>mef</i> (A)	0.25	32	2	0.12
Group A and B streptococci (4)	Susceptible	None	0.03	2–4	0.03	0.008
Group A and B streptococci (7)	MLS _B	<i>erm</i> (B)	0.03–0.25	8–>128	0.03–0.12	0.008–0.03
Group A and B streptococci (7)	MLS _B	<i>erm</i> (A) subset <i>ermTR</i>	0.06	4	0.03	0.008
Group B streptococcus (1)	MLS _B	<i>erm</i> (B) + <i>erm</i> (A) subset <i>ermTR</i>	0.25	>128	0.125	0.03
Group A streptococcus (1)	M	<i>mef</i> (A)	0.06	4	0.03	0.008
Group B streptococci (2)	LS _A	Unknown	0.5	4–8	2–8	0.06–0.12
Group B streptococci (2)	MLS _B + M	<i>erm</i> (B) + <i>mef</i> (A)	0.25	>128	0.12	0.016–0.03
Oral streptococci (16)	Susceptible	None	0.12–0.5	4–8	0.25–4	0.016–0.12
Oral streptococci (4)	MLS _B	<i>erm</i> (B)	0.5	16–>128	0.12–4	0.06–0.12
Oral streptococci (3)	LS _A	Unknown determinant	0.5	4–8	16–64	0.06–0.12

^a LS_A, resistance to lincosamides and streptogramin A; M, resistance to 14- and 15-membered macrolides; MLS_B, resistance to macrolides-lincosamides-streptogramin B.

^b Q-D, quinupristin-dalfopristin.

TABLE 4. Activities of streptogramins against laboratory strains of *S. aureus*

<i>S. aureus</i> strain	Streptogramin resistance phenotype ^a	MIC ($\mu\text{g/ml}$) ^b						Reference or source
		Qui	Dal	Q-D	RPR202868	RPR132552	XRP2868	
RN4220/pJIM2246	Susceptible control	1	1	0.25	8	0.12	0.06	23
RN4220/pAT392	Susceptible control	2	2	0.25	8	0.12	0.06	7
HM1054	Susceptible control	8	4	0.5	8	0.25	0.06	23
RN4220/pAT392 Ω erm(A)	S _B (constitutive)	>32	2	0.5 (2)	>128	0.12	0.12 (2)	7
RN4220/pAT392 Ω erm(C)	S _B (constitutive)	>32	2	0.5 (2)	>128	0.12	0.06 (1)	7
HM1054 Ω erm(C)	S _B (constitutive)	64	4	0.5 (1)	>128	0.25	0.12 (2)	23
RN4220/pAT392 Ω erm(B)	S _B (inducible)	32	2	0.5 (1)	16	0.12	0.06 (1)	7
RN4220/pJIM2246 Ω vgb(A)	S _B	8	1	1 (4)	8	0.12	0.06 (1)	4
RN4220/pJIM2246 Ω vat(D)	S _A	1	8	0.5 (2)	8	0.5	0.06 (1)	4
RN4220/pAT392 Ω vga(A)	S _A	NT	NT	0.25 (1)	8	0.12	0.06 (1)	This study
RN4220/pJIM2246 Ω erm(B), vgb	S _B	8	1	1 (4)	8	0.12	0.06 (1)	4
RN4220/pAT392 Ω erm(A), vga(A)	S _A + S _B	NT	NT	0.25 (1)	32	0.12	0.12 (2)	This study
RN4220/pJIM2246 Ω erm(B), vat(D)	S _A + S _B	4	16	1 (4)	8	0.25	0.06 (1)	4
RN4220/pJIM2246 Ω vga(D), vgb	S _A + S _B	8	16	4 (16)	8	2	0.25 (4)	4
RN4220/pJIM2246 Ω erm(B), vat(D), vgb	S _A + S _B	8	16	4 (16)	16	2	0.5 (8)	4
RN4220/pAT392 Ω erm(A), vga(A), vgb, vat(D)	S _A + S _B	NT	NT	4 (16)	32	0.5	0.5 (8)	This study
RN4220/pUL5054 Ω msr(A)	S _B (inducible)	NT	NT	0.25 (1)	8	0.12	0.06 (1)	20
RN4220/rplV/R (L22 mutation)	S _B	8	4	2 (8)	32	0.25	0.25 (4)	15

^a S_A, resistance to streptogramin A; S_B, resistance to streptogramin B.

^b Qui, quinupristin; Dal, dalfopristin; Q-D, quinupristin-dalfopristin; NT, not tested. Values in parentheses are the MIC multiplication factors. The MIC multiplication factor is the ratio of the MIC for the strain containing the streptogramin resistance gene(s) to the MIC for the parental susceptible strain.

background of this host, due to an unknown mechanism, played an important role in resistance to streptogramin combinations.

Mutation of ribosomal targets of streptogramins may also confer resistance to these antimicrobials. Susceptible strain *S. aureus* RN4220 was transformed with DNA encoding the L22 mutation, which resulted in transformants that were erythromycin, quinupristin, and quinupristin-dalfopristin resistant (15). In this study, the MICs of XRP2868 and RPR202868 (factor B) were multiplied fourfold for the transformant. Similarly, mutation of protein L22 yielded a 32-fold increase in the MIC of XRP2868 in *S. pneumoniae*. Mutation of the L4 pro-

tein also markedly altered the potency of XRP2868 in *S. gordonii*.

As for the clinical isolates, irrespective of the acquired mechanism of resistance, XRP2868 remained two to four times more potent than quinupristin-dalfopristin.

Bactericidal activity of XRP2868 against *S. aureus* with constitutively expressed *erm* genes. The bactericidal activity was demonstrated at 18 h for the laboratory strain of *S. aureus* RN4220/pAT392, which is susceptible to macrolides-lincosamides-streptogramins (MLS), at concentrations of 2 and 10 times the MIC of XRP2868 (0.06 $\mu\text{g/ml}$) (Fig. 1A). The MICs of XRP2868 were equal to 0.12 and 0.06 $\mu\text{g/ml}$ for the deriv-

TABLE 5. Activities of streptogramins against laboratory strains of enterococci

Bacterial strain	Streptogramin resistance phenotype ^a	MIC ($\mu\text{g/ml}$) ^b						Reference or source
		Qui	Dal	Q-D	RPR202868	RPR132552	XRP2868	
<i>E. faecium</i> HM1070/pJIM2246	Susceptible control	1	1	0.25	0.25	0.12	0.03	4
<i>E. faecium</i> BM4107/pJIM2246	Control with LS _A	2	>128	1	1	32	0.25	4
<i>E. faecium</i> HM1070/pJIM2246 Ω erm(B)	S _B (inducible)	2	1	0.25 (1)	0.5	0.12	0.03 (1)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω vgb	S _B	8	1	1 (4)	0.5	0.12	0.06 (2)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω vat(D)	S _A	1	8	0.5 (2)	0.5	0.12	0.03 (1)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω vgb, erm(B)	S _B	8	1	1 (4)	0.5	0.12	0.06 (2)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω erm(B), vat(D)	S _A + S _B	2	32	2 (8)	0.5	4	0.5 (16)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω vat(D), vgb	S _B	8	32	4 (16)	0.5	2	0.5 (16)	4
<i>E. faecium</i> HM1070/pJIM2246 Ω erm(B)/vgb/vat(D)	S _A + S _B	16	32	4 (16)	2	4	0.5 (16)	4
<i>E. faecium</i> BM4107/pJIM2246 Ω erm(B), vat(D)	S _A + S _B	4	>128	8 (8)	4	32	0.5 (2)	4
<i>E. faecium</i> BM4107/pJIM2246 Ω erm(B), vgb	S _B	8	128	8 (8)	2	64	2 (8)	4
<i>E. faecium</i> BM4107/pJIM2246 Ω vat(D), vgb	S _A + S _B	8	>128	16 (16)	2	64	4 (16)	4
<i>E. faecium</i> BM4107/pJIM2246 Ω erm(B), vgb, vat(D)	S _A + S _B	16	>128	16 (16)	8	64	4 (16)	4
<i>E. faecalis</i> JH2-2	Susceptible control	8	>128	4	2	64	0.5	4
<i>E. faecalis</i> JH2-2/pAM β 1 [<i>erm</i> (B)]	S _B (constitutive)	>128	>128	4 (1)	>128	64	1 (2)	This study

^a LS_A, resistance to lincosamide and streptogramin A; S_A, resistance to streptogramin A; S_B, resistance to streptogramin B.

^b Qui, quinupristin; Dal, dalfopristin; Q-D, quinupristin-dalfopristin. NT, not tested. Values in parentheses are the MIC multiplication factors. The MIC multiplication factor is the ratio of the MIC for the strain containing the streptogramin resistance gene(s) to the MIC for the parental susceptible strain.

TABLE 6. Activities of streptogramins against laboratory strains of streptococci

Bacterial strain	Streptogramin resistance phenotype ^a	MIC ($\mu\text{g/ml}$) ^b				Reference or source
		Q-D	RPR202868	RPR132552	XRP2868	
<i>S. pneumoniae</i> CP1000	Susceptible control	0.12	4	2	0.032	6
<i>S. pneumoniae</i> 1	Susceptible control	0.25	4	1	0.06	6
<i>S. pneumoniae</i> 3	Susceptible control	0.25	2	4	0.06	6
<i>S. pneumoniae</i> 4	Susceptible control	0.25	2	4	0.12	6
<i>S. pneumoniae</i> 5	Susceptible control	0.12	1	2	0.03	6
<i>S. pneumoniae</i> CP1000/ <i>erm</i> (B)	S _B	0.25 (1)	32	4	0.12 (4)	This study
<i>S. pneumoniae</i> CP1000/ <i>rplV</i> -R (mutated L22 protein)	S _B	0.5 (4)	16	4	0.25 (8)	This study
<i>S. pneumoniae</i> 1/C2611U mutation in 23S rRNA	S _B	0.5 (2)	32	2	0.25 (4)	6
<i>S. pneumoniae</i> 4/C2611U mutation in 23S rRNA	S _B	0.5 (2)	16	2	0.12 (2)	6
<i>S. pneumoniae</i> 3/C2610U mutation in 23S rRNA	S _B	0.5 (2)	16	2	0.12 (2)	6
<i>S. pneumoniae</i> 5/A2058G mutation in 23S rRNA	S _B	0.5 (4)	4	4	0.12 (4)	6
<i>S. pneumoniae</i> 3/A2058T mutation in 23S rRNA	S _B	0.5 (2)	32	2	0.12 (2)	6
<i>S. pneumoniae</i> 3/A2059G mutation in 23S rRNA	S _B	0.5 (2)	4	2	0.25 (4)	6
<i>S. pneumoniae</i> 1/ <i>rplV</i> mutation (protein L22)	S _B	2 (8)	16	2	2 (32)	6
<i>S. gordonii</i> Challis	Susceptible control	0.5	8	4	0.12	This study
<i>S. gordonii</i> Challis/ <i>rpID</i> mutation (mutation of L4 protein after transformation)	S _B	32 (64)	>128	16	8 (64)	This study

^a S_B, resistance to streptogramin B.

^b Q-D, quinupristin-dalfopristin. Values in parentheses are the MIC multiplication factors. The MIC multiplication factor is the ratio of the MIC for the strain containing the streptogramin resistance gene(s) to the MIC for the parental susceptible strain.

atives containing the *erm*(A) and *erm*(C) genes, respectively. However, XRP2868 was no longer bactericidal against both constructs at concentrations equal to 2 or 10 times the MIC (Fig. 1B).

Similarly, bactericidal activity was also achieved at 10 times the MIC of XRP2868 against the clinical isolate susceptible to

MLS but not against the two clinical isolates containing the constitutively expressed *erm*(A) or *erm*(C) gene (2.5 and 2 log₁₀ reductions in bacterial counts at 18 h, respectively) (data not shown).

DISCUSSION

The study confirmed previous data from Eliopoulos et al., who showed that the new oral streptogramin XRP2868 was approximately fourfold more potent than quinupristin-dalfopristin against *S. aureus*, *E. faecium*, and streptococci (10). The strong activity of RPR132552, the S_A component of XRP2868, seemed to be the driver for the greater potency of the oral streptogramin compared to that of quinupristin-dalfopristin.

Interestingly, the oral streptogramin displayed activity against *E. faecalis*. This species is considered intrinsically resistant to lincosamides (lincomycin and clindamycin), S_A, and streptogramin combinations. In *E. faecalis* OG1RF, resistance has been related to the expression of a chromosomal *lsa* gene, which appears to be species specific and which could possibly participate in the synthesis of an efflux pump (22). Resistance to factor A of streptogramins seems to explain the weak activities of streptogramins against *E. faecalis*, since clinical isolates in which the *lsa* gene was inactive were susceptible to quinupristin-dalfopristin (9). Both RPR132552 (S_A) and RPR202868 (S_B) had only weak activities against the *E. faecalis* strain, strain JH2-2, that we tested in our study. However, these activities were superior to those of dalfopristin and quinupristin, respectively, which can explain the relatively low MIC of XRP2868. The clinical potential of this enhanced potency of the oral streptogramin against *E. faecalis* remains to be demonstrated.

The use of clinical isolates and of constructs with known streptogramin resistance genes allowed the more accurate assessment of the impact of streptogramin resistance genes on the activity of XRP2868 and of its two components.

As expected, the mechanisms of resistance to factor A or

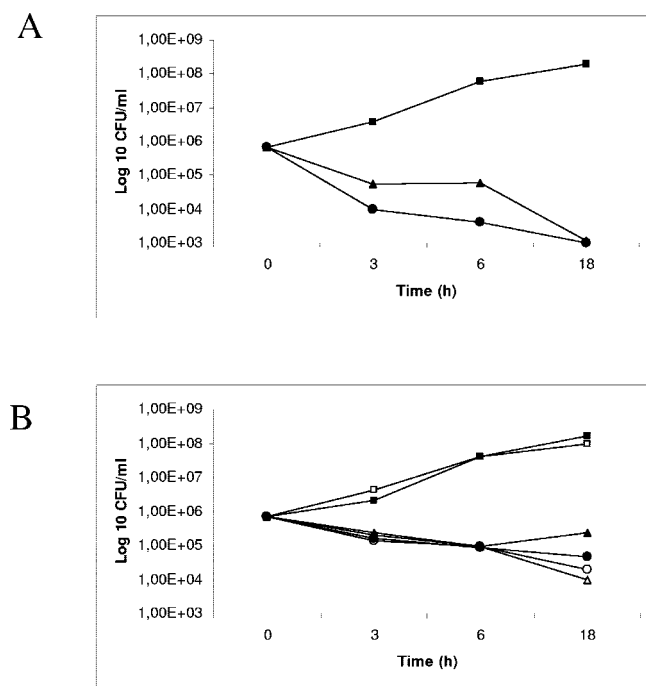


FIG. 1. Time-kill curves for (A) *Staphylococcus aureus* RN4220/pAT392, (B) *S. aureus* RN4220/pAT392 Ω *erm*(A) (open symbols), and *S. aureus* RN4220/pAT392 Ω *erm*(C) (closed symbols) grown in Mueller-Hinton broth in the absence of XRP2868 (controls; squares) or in the presence of XRP2868 at concentrations of 2 (triangles) and 10 (circles) times the MIC.

factor B of streptogramins affected the activities of the corresponding factors composing XRP2868 (Tables 1 to 3). Isolated or combined resistance to S_A or S_B multiplied the MIC of XRP2868 by a factor that resulted in an MIC similar to that for quinupristin-dalfopristin. However, due to the greater potency of XRP2868, the MICs of the new oral streptogramin remained lower than those of quinupristin-dalfopristin. The significant impact of the streptogramins on the MICs was observed when the mechanisms of resistance to both S_A and S_B were combined. The most efficient minimal combination was that of acetylation of S_A with inactivation by a lyase of S_B . The fact that clinical isolates with elevated MICs of XRP2868 or quinupristin-dalfopristin contained resistance gene combinations confirmed these conclusions. There were a few exceptions, such as isolated mutations of ribosomal proteins L22 and L4 in *S. aureus* and streptococci, which were sufficient to alter the potency of XRP2868. In particular, mutations of protein L22 have been reported in mutants of *S. aureus* selected during treatment with quinupristin-dalfopristin and in an in vivo model of aortic endocarditis in rabbits (15, 23). These mutations confer resistance to erythromycin and S_B and suppress in part the synergism between S_A and S_B (15). It remains to be established if the MIC of XRP2868 reached by the mutants is sufficient to be responsible for clinical failure.

From a clinical point of view, streptococcal or staphylococcal isolates resistant to streptogramin combinations are rare. The most frequent resistance is that to S_B due to ribosomal methylation, which has a weak impact on the MICs of the streptogramin combinations. However, this work confirmed that, as for the other streptogramins, the bactericidal activity of XRP2868 against staphylococcal strains expressing a constitutive MLS_B phenotype was altered.

In conclusion, the new oral streptogramin XRP2868 and its S_A and S_B components are affected by the same mechanisms of resistance as the other streptogramins, quinupristin-dalfopristin, pristinamycins IA and IIB, and virginiamycins S and M. However, the strong activity of compound A combined with the synergistic effect of the two factors, which is often maintained despite the acquisition of resistance, shows that it provides superior potency against a broad range of gram-positive organisms. The oral streptogramin XRP2868 might be useful as a substitute for parenteral antimicrobials and might also be attractive for the treatment of community-acquired infections due to pneumococci, streptococci, and community-associated strains of oxacillin-resistant *S. aureus*.

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