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Influence of Inducible Cross-Resistance to Macrolides, Lincosamides, and Streptogramin B-Type Antibiotics in *Enterococcus faecium* on Activity of Quinupristin-Dalfopristin In Vitro and in Rabbits with Experimental Endocarditis

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The influence of inducible cross-resistance to macrolides, lincosamides, and streptogramin B (MLS_B) type antibiotics (inducible MLS_B phenotype) on the activity of quinupristin-dalfopristin was investigated against *Enterococcus faecium* in vitro and in rabbits with experimental endocarditis. In vitro, quinupristin-dalfopristin displayed bacteriostatic and bactericidal activities against a MLS_B-susceptible strain similar to those against two strains with the inducible MLS_B phenotype. In addition, induction of the two MLS_B-resistant strains with quinupristin (0.016 to 1 μg/ml) or quinupristin-dalfopristin (0.08 to 0.25 μg/ml) increased the MICs of quinupristin from 8 μg/ml to 32 to >128 μg/ml, but did not modify the MIC of dalfopristin (2 μg/ml) or quinupristin-dalfopristin (0.5 μg/ml). In a rabbit endocarditis model, quinupristin-dalfopristin was as active as amoxicillin against the MLS_B-susceptible *E. faecium* strain. In contrast, the activity of quinupristin-dalfopristin was significantly decreased in animals infected with either of the two inducible MLS_B-resistant strains ($P < 0.05$), although no mutants resistant to quinupristin-dalfopristin were detected. Against the clinical strain with the inducible MLS_B phenotype, quinupristin-dalfopristin was not effective and was less active than amoxicillin ($P < 0.001$); however, the activity of the combination of amoxicillin and dalfopristin-quinupristin was superior to that of amoxicillin ($P < 0.01$). The different impact of the inducible MLS_B phenotype in *E. faecium* on the activity of quinupristin-dalfopristin in vitro and in experimental endocarditis may be related to the reduced diffusion of dalfopristin compared with that of quinupristin into cardiac vegetations that we previously reported. This result emphasizes the importance of the constant presence of dalfopristin at the site of infection to ensure synergism with quinupristin.

Enterococci are now recognized as major nosocomial pathogens (4). Enterococci are the third most common cause of hospital-acquired bacteremia (23) and account for up to 50% of bacteremias at some centers (2). The major cause of concern with these microorganisms is the emergence in clinical settings of multidrug-resistant enterococci that belong, for the most part, to the species *Enterococcus faecium* which are resistant to penicillins, aminoglycosides, and glycopeptides (17). There is currently no uniformly effective antimicrobial therapy for patients infected with multidrug-resistant enterococci, emphasizing the need for new therapeutic options.

Quinupristin-dalfopristin is a new semisynthetic injectable streptogramin composed of two synergistic components, i.e., quinupristin, a peptide macrolactone classified as a streptogramin B antibiotic, and dalfopristin, a polyunsaturated macrolactone classified as a streptogramin A antibiotic, in a 30:70 ratio. Quinupristin-dalfopristin is active in vitro against gram-positive cocci, including multiresistant isolates, with an MIC at which 90% of isolates are inhibited of 2 μg/ml for vancomycin-resistant *E. faecium* isolates (24). Antibiotics belonging to the streptogramin family share with macrolides and lincosamides a comparable mode of action, inhibiting protein synthesis in bacteria by affecting ribosome function. Cross-resistance to macrolides, lincosamides, and streptogramin B (MLS_B)-type antibiotics (MLS_B phenotype), resulting from target modification by a methylase, is the most common mechanism of acquired resistance to these antibiotics, present in the majority of enterococci (15). Expression of MLS_B resistance may be inducible or constitutive. Expression of MLS_B resistance is inducible in the majority of enterococci (22). Whatever the expression of the resistance in enterococci, the strains are cross-resistant to all MLS_B-type antibiotics. Streptogramin A-type antibiotics, including dalfopristin, are not affected by this type of resistance.

Therefore, the aim of the present study was to investigate the influence of inducible MLS_B resistance in *E. faecium* on the activity of quinupristin-dalfopristin in vitro and in rabbits with experimental endocarditis.

MATERIALS AND METHODS

In vitro studies. (i) Organisms. Three strains of *E. faecium* were used for in vitro and in vivo experiments. *E. faecium* HM1070 was a laboratory mutant resistant to rifampin. The strain was derived from a clinical isolate from Henri Mondor Hospital and is susceptible to erythromycin, quinupristin, dalfopristin, penicillin, and vancomycin and is resistant to low levels of gentamicin. Two strains of *E. faecium* that were resistant to erythromycin, lincomycin, and quinupristin and susceptible to dalfopristin were investigated; *E. faecium* HM1080 was a clinical strain that was resistant to amoxicillin, that had low-level resistance to gentamicin, and that was susceptible to vancomycin, and *E. faecium* HM1070/ER was obtained after conjugative transfer by matings on filters, as described previously (6), of MLS_B resistance from *E. faecium* HM1080 to *E. faecium* HM1070. The resistance phenotypes displayed by the bacteria were characterized by the agar diffusion technique with discs of erythromycin, lincomycin, clindamycin, quinupristin, dalfopristin, and quinupristin-dalfopristin (16).

(ii) Media and antibiotics. Mueller-Hinton broth (MHB) and Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were used. All incubations were at 37°C. Drugs were supplied by their manufacturers: amoxicillin by SmithKline Beecham, Nanterre, France; gentamicin by Panpharma,

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TABLE 1. MICs of *E. faecium* HM1070, *E. faecium* HM1080, and *E. faecium* HM1070/ER before and after induction with quinupristin and quinupristin-dalfopristin

Drug (concn [μg/ml])	MIC (μg/ml) for the following strain before and after induction with the indicated agent:								
	E. faecium HM1070 (Ery^s)			*E. faecium* HM1080 (MLS_B inducible)			*E. faecium* HM1070/ER (MLS_B inducible)		
	Quinupristin-dalfopristin	Quinupristin	Dalfopristin	Quinupristin-dalfopristin	Quinupristin	Dalfopristin	Quinupristin-dalfopristin	Quinupristin	Dalfopristin
None	0.5	2	2	0.5	8	2	0.5	8	2
Quinupristin-dalfopristin									
0.008	0.5	2	4	0.5	32	4	0.5	32	2
0.03	0.5	2	4	0.5	128	4	0.5	32	2
0.12	0.5	2	4	0.5	>128	4	0.5	128	2
0.25	0.5	2	4	0.5	128	4	0.5	32	2
Quinupristin									
0.016	0.5	2	4	0.5	64	4	0.5	128	2
0.12	0.5	2	4	0.5	128	4	0.5	>128	2
1	0.25	1	1	0.5	>128	4	0.5	>128	2

Fougères, France; and quinupristin, dalfopristin, and quinupristin-dalfopristin by Rhône-Poulenc Rorer, Vitry sur Seine, France.

(iii) In vitro susceptibility to antibiotics. The MICs of quinupristin, dalfopristin, quinupristin-dalfopristin, amoxicillin, and gentamicin were determined by the agar dilution method.

(iv) In vitro tests of induction. Enterococcal strains were grown for 3 h in MHB with agitation without antibiotic or in the presence of subinhibitory concentrations of quinupristin (0.016, 0.12, and 1 μg/ml) or quinupristin-dalfopristin (0.008, 0.03, 0.12, and 0.25 μg/ml). The MICs of quinupristin, dalfopristin, and the combination were then determined for induced and noninduced cells.

(v) In vitro selection of mutants. A total of 10^9 CFU each of *E. faecium* HM1070 and HM1080 per ml was inoculated onto agar plates containing dalfopristin (8, 16, and 32 μg/ml) and quinupristin-dalfopristin (2, 4, 8 μg/ml); for HM1070, quinupristin (2, 4, and 8 μg/ml) was also used. After 48 h of incubation, 10 to 15 growing colonies were studied for their susceptibilities to streptogramins.

(vi) Study of bactericidal activity. Time-kill curves were used to test the bactericidal activities of quinupristin-dalfopristin alone against the three study strains and in combination with amoxicillin or gentamicin against *E. faecium* HM1080 (14). Overnight cultures were diluted in glass tubes containing 10 ml of fresh MHB to yield an inoculum of approximately 5×10^5 CFU/ml. The following concentrations were used: 0.5 and 2 μg/ml for quinupristin-dalfopristin (1× and 4× the MIC, respectively), 4 μg/ml for gentamicin, and 32 and 100 μg/ml for amoxicillin (1× and 3× the MIC, respectively). After 0, 6 and 24 h of incubation at 37°C, serial dilutions of 0.1-ml samples were subcultured onto agar plates by using a spiral plater (Spiral System Inc., Cincinnati, Ohio), and the plates were incubated for 24 h before the numbers of CFU were counted. In preliminary experiments, antibiotic carryover was ruled out by plating samples of bacterial suspensions containing 10^1 to 10^3 CFU/ml in the presence or absence of antibiotics alone or in combination (21).

Enterococcal experimental endocarditis. Investigations were performed with female New Zealand White rabbits (weight range, 2.2 to 2.8 kg). Aortic endocarditis was induced in rabbits by insertion of a polyethylene catheter through the right carotid artery into the left ventricle to induce the formation of vegetations. Twenty-four hours after catheter insertion, each rabbit was inoculated via the ear vein with 10^8 CFU of *E. faecium* in 1 ml of 0.9% NaCl. The catheter was left in place throughout the experiment. Forty-eight hours after inoculation, the animals were treated intramuscularly three times daily for 4 days with one of the following regimens: quinupristin-dalfopristin at 30 mg/kg of body weight, amoxicillin at 100 mg/kg, gentamicin at 2 mg/kg, quinupristin-dalfopristin plus amoxicillin, and quinupristin-dalfopristin plus gentamicin. The quinupristin-dalfopristin regimen produced levels in serum that covered the range of concentrations obtained in humans (8) and that was previously shown to be effective and well tolerated in this animal model (9). The amoxicillin and gentamicin regimens produced levels in serum comparable to those achieved in humans for the treatment of enterococcal infections. Control animals were left untreated; they were allowed to die or were sacrificed, if necessary, at the same time as the treated animals.

Animals were killed by intravenous injection of pentobarbital 12 h after the last antibiotic injection. All vegetations from each rabbit were excised, rinsed in saline, pooled, and weighed. They were homogenized in 1 ml of sterile saline, and 0.1-ml portions were quantitatively subcultured onto agar plates for 24 h. Colony counts were expressed as $\log_{10}$ CFU per gram of vegetation.

For each strain, portions (0.1 ml) from the undiluted suspension and the 1:10 suspension of vegetations from animals treated with quinupristin-dalfopristin were plated onto agar plates containing a final concentration of 2× and 4× the MIC of quinupristin-dalfopristin and 4× the MIC of dalfopristin, and the plates were incubated for 48 h at 37°C in order to detect the emergence of resistant derivative mutants. For the HM1070/ER and HM1080 strains, the absence of spontaneous curing of MLS_B resistance in the surviving bacteria was checked by placing a disc of erythromycin onto the agar.

Studies of pharmacokinetics in serum. Serum antibiotic levels were determined in three rabbits after single intramuscular injections of 30 mg of quinupristin-dalfopristin per kg, 100 mg of amoxicillin per kg, and 2 mg of gentamicin per kg. For quinupristin-dalfopristin, 1-ml samples of blood were obtained at 15, 30, 45, 60, 90, 120, 180, and 360 min after the injection and were immediately placed into a tube containing 0.5 ml of 0.25 N hydrochloric acid; the mixture was stirred strongly by hand and centrifuged (1,500 × *g* for 10 min) as described previously (10). The upper layer was stored at −70°C. For amoxicillin and gentamicin, blood samples were obtained 0.5, 1, 2, 4, 6, and 8 h after the injection.

The concentrations of quinupristin-dalfopristin in serum were measured by the agar diffusion method, as described previously (10) by using *Micrococcus luteus* ATCC9341 as the indicator organism and medium no. 2 (Difco). The concentrations of amoxicillin and gentamicin in serum were measured by high-performance liquid chromatography and fluorescence polarization immunoassay, respectively. The sensitivities of the assays were 0.1 μg/ml for quinupristin-dalfopristin and amoxicillin and 0.2 μg/ml for gentamicin.

Pharmacokinetic parameters were generated with the SIPHAR program (SIMED, Créteil, France) (11). The elimination half-life in serum was calculated by nonlinear regression from the terminal portion of the concentration-versus-time curve.

Statistics. All the results were expressed as means ± standard deviations. The comparisons of the treatment effect of quinupristin-dalfopristin for each strain of *E. faecium* in terms of reduction in $\log_{10}$ CFU per gram of vegetation and the comparisons of the treatment effect for a given strain were performed by analysis of variance followed by a multiple comparison of means by Fisher's least-significant-difference procedure (26).

RESULTS

In vitro data. (i) Bacteriostatic activity of quinupristin-dalfopristin. The MIC of quinupristin was 2 μg/ml for the MLS_B-susceptible strain and 8 μg/ml for the strains with the inducible MLS_B phenotype (Table 1). In contrast, the MIC of quinupristin-dalfopristin was similar for the three strains. When cells were grown in the presence of quinupristin or quinupristin-dalfopristin, no change in the MICs of quinupristin, dalfopristin, and quinupristin-dalfopristin for the susceptible strain *E. faecium* HM1070 was observed. However, when the same experiment was performed with the MLS_B-inducible strains *E. faecium* HM1080 and HM1070/ER, induction occurred, with a 4- to >16-fold increase in the MICs of quinupristin but no change in the MICs of dalfopristin and quinupristin-dalfopris-

TABLE 2. Serum antibiotic levels and elimination half-life in rabbits after a single injection[a]

Regimen (dose [mg/kg i.m.])[b]	Peak concn (μg/ml) (time [min] of sampling)	Trough concn (μg/ml) (8 h)	Half-life (min)
Quinupristin/dalfopristin (30)	3.0 ± 0.7 (15)	<0.1	91 ± 37
Amoxicillin (100)	48 ± 21 (30)	1.3 ± 0.5	101 ± 29
Gentamicin (2)	4.8 ± 0.9 (30)	<0.2	83 ± 20

[a] Values are means ± standard deviations.
[b] i.m., intramuscular.

tin (Table 1). The MICs of amoxicillin were 0.25 and 32 μg/ml for *E. faecium* HM1070 and HM1080, respectively, and those of gentamicin were 16 and 16 μg/ml, respectively.

(ii) In vitro selection of mutants. Mutants resistant to dalfopristin (MICs, 64 to 128 μg/ml) were selected in vitro by this antibiotic at a frequency of ca. 10^{-8} for *E. faecium* HM1070 and *E. faecium* HM1080. Synergism between quinupristin and dalfopristin was lost in part for these mutants (MICs of quinupristin-dalfopristin, 2 to 4 μg/ml, versus 0.5 μg/ml for the parent strains). No mutant of *E. faecium* HM1070 resistant to quinupristin or quinupristin-dalfopristin could be selected (frequency, $<10^{-9}$). However, mutants of *E. faecium* HM1080 resistant to quinupristin-dalfopristin were selected at very low frequencies ($<10^{-9}$ to 10^{-8}).

(iii) Bactericidal activity of quinupristin dalfopristin. Quinupristin-dalfopristin at 2 μg/ml (4× the MIC) produced a reduction of 1.6, 1.1, and 1.4 $\log_{10}$ CFU/ml after 24 h of incubation for *E. faecium* HM1070, HM1080, and HM1070/ER, respectively. This killing was comparable to that obtained with quinupristin-dalfopristin at 0.5 μg/ml (1× the MIC) (data not shown). Against *E. faecium* HM1080, the activity of quinupristin-dalfopristin at 0.5 and 2 μg/ml was not enhanced by the addition of gentamicin (4 μg/ml) or amoxicillin (32 and 100 μg/ml) (data not shown).

Experimental endocarditis. The peak and trough concentrations and serum elimination half-lives of quinupristin-dalfopristin, amoxicillin, and gentamicin are presented in Table 2. After 4 days of therapy, quinupristin-dalfopristin significantly reduced bacterial titers in vegetations from rabbits infected with the MLS_B-susceptible strain HM1070 compared with the titers in vegetations from control rabbits ($P < 0.001$) (Table 3). In contrast, no significant effect was observed against the two MLS_B-inducible strains HM1080 and HM1070/ER (Table 3). Quinupristin-dalfopristin was significantly more effective against the MLS_B-susceptible strain than against any of the two MLS_B-inducible strains ($P < 0.05$).

No resistant bacteria were detected on agar containing dalfopristin or quinupristin-dalfopristin for vegetations from animals treated with quinupristin-dalfopristin.

TABLE 3. Activity of quinupristin-dalfopristin against three strains of *E. faecium* with various phenotypes of resistance to MLS_B antibiotics after 4 days of therapy for experimental endocarditis in rabbits

Regimen	$\log_{10}$ CFU/g of vegetation (no. of sterile animals/no. of treated animals) for the following strain (phenotype):		
	HM1070 (Ery^s)	HM1080 (MLS_B inducible)	HM1070/ER (MLS_B inducible)
Control	8.1 ± 1.3 (0/7)	8.7 ± 1.0 (0/5)	7.4 ± 1.2 (0/5)
Quinupristin-dalfopristin	4.5 ± 2.3[a] (1/9)	8.5 ± 1.2 (0/14)	6.0 ± 1.0 (0/7)

[a] $P < 0.001$ versus controls.

TABLE 4. Activities of quinupristin-dalfopristin, amoxicillin, and gentamicin, alone or in combination, against *E. faecium* HM1080 (MLS_B inducible) after 4 days of therapy for experimental endocarditis in rabbits

Treatment	No. of survivors/ no. of treated animals	$\log_{10}$ CFU/g of vegetation (mean ± SD)
Control	0/5	8.7 ± 1.0
Quinupristin/dalfopristin	9/14	8.5 ± 1.2
Gentamicin	4/5	7.4 ± 0.7
Quinupristin/dalfopristin + gentamicin	8/8	7.1 ± 1.4[a]
Amoxicillin	6/6	5.6 ± 1.1[b]
Quinupristin/dalfopristin + amoxicillin	8/8	3.9 ± 0.5[b,c]

[a] $P < 0.05$ versus control.
[b] $P < 0.001$ versus control and quinupristin-dalfopristin.
[c] $P < 0.01$ versus amoxicillin.

(i) Activity of antibiotic regimens against *E. faecium* HM1070. Bacterial titers in vegetations from rabbits treated with amoxicillin (3.3 ± 0.9 $\log_{10}$ CFU/g of vegetation; $n = 7$) and quinupristin-dalfopristin (4.5 ± 2.2 $\log_{10}$ CFU/g of vegetation; $n = 9$) were significantly lower ($P < 0.001$) than those in vegetations from control animals (8.1 ± 1.3 $\log_{10}$ CFU/g of vegetation; $n = 7$). No significant difference was detected between the activity of amoxicillin and that of quinupristin-dalfopristin.

(ii) Activity of antibiotic regimens against *E. faecium* HM1080. The activity of quinupristin-dalfopristin, alone or in combination with gentamicin or amoxicillin, is shown in Table 4. Amoxicillin was the only single-drug regimen that significantly reduced bacterial titers in vegetations compared with the titers in vegetations from controls ($P < 0.001$). The combination of quinupristin-dalfopristin with gentamicin was more active than quinupristin-dalfopristin and controls ($P < 0.05$) but was not more active than gentamicin alone. In contrast, the combination of quinupristin-dalfopristin with amoxicillin was more active than quinupristin-dalfopristin or amoxicillin alone ($P < 0.01$).

DISCUSSION

Expression of inducible MLS_B resistance differs between streptococci-enterococci and staphylococci. Distinct induction patterns could result from differences in the regulatory regions preceding the structural *ermAM* genes of streptococci-enterococci and the *ermA* and *ermC* genes of staphylococci (12). In staphylococci, resistance is dissociated between 14- and 15-membered macrolides, which are inducers of the methylase, and 16-membered macrolides, lincosamides, and streptogramins A and B, which are not inducers (17, 28). In contrast, in streptococci and enterococci there is cross-resistance between the macrolides, lincosamides, and streptogramins B, which are efficient inducers (12, 17). Indeed, our in vitro studies indicated that quinupristin, a streptogramin B-type antibiotic, was a self-inducer of resistance in *E. faecium* HM1080 and HM1070/ER. However, even when bacteria were grown in the presence of quinupristin, the enhanced activity between quinupristin and dalfopristin was retained, and the MIC of quinupristin-dalfopristin for bacteria grown in the presence of quinupristin remained similar to that for noninduced cells. In addition, the bacteriostatic and bactericidal activities of quinupristin-dalfopristin in vitro were not reduced against the two strains that harbored the MLS_B-inducible phenotype compared with that against the susceptible strain. These results

outline the critical importance of the combination with a streptogramin A to achieve in vitro synergy, since these antibiotics are unaffected by MLS_B resistance (5). However, it is of major importance to emphasize that our three study strains were susceptible to dalfopristin, the streptogramin A-type antibiotic. Intrinsic resistance to this antibiotic in *Enterococcus faecalis* is responsible for the lower in vitro efficacy of quinupristin-dalfopristin compared with its efficacy against *E. faecium* (25).

The major finding of our in vivo experiments was the decreased activity of quinupristin-dalfopristin against experimental endocarditis caused by the two *E. faecium* strains harboring the MLS_B-inducible phenotype compared with that against the susceptible strain. This apparent discrepancy with the in vitro data may be explained by our previous findings on the patterns of distribution of [^{14}C]quinupristin and [^{14}C]dalfopristin into infected cardiac vegetations (9). Unlike [^{14}C]quinupristin, which was homogeneously distributed throughout the vegetation, [^{14}C]dalfopristin showed a decreasing gradient of cencentration between the periphery and the core of the vegetation. Therefore, bacteria located in the core of the vegetation might be exposed, at least transiently, only to quinupristin, which is an effective inducer of the resistance, leading to bacterial growth in the absence of dalfopristin. This result emphasizes the critical importance of the constant presence of dalfopristin at the site of infection to ensure synergism with quinupristin and explains why in vitro tests, performed with constant ratios and concentrations of quinupristin and dalfopristin, over time may not predict the in vivo outcome in some specific foci of infection. Whether or not our previous findings on the patterns of distribution of [^{14}C]quinupristin and [^{14}C]dalfopristin are characteristic of the cardiac vegetation or are also the same for other infected tissues remains unclear. In serum, other investigators reported that dalfopristin has a shorter life span than quinupristin in rats (7), but this may not be the case in humans (19). Therefore, bloodstream infections due to *E. faecium* with the inducible MLS_B phenotype treated with appropriate dosing regimens of quinupristin-dalfopristin may have a favorable outcome in humans.

The decreased activity of quinupristin-dalfopristin in experimental endocarditis against *E. faecium* with the inducible MLS_B phenotype is in contrast to our previous findings for *Staphylococcus aureus* (10). This difference is related to the fact that quinupristin is an effective inducer of the synthesis of methylase, which is responsible for the target modification, in enterococci but not in staphylococci. However, when the expression of MLS_B resistance is constitutive, a decreased activity of quinupristin-dalfopristin has been demonstrated in experimental endocarditis with *S. aureus* (7, 10) and may be anticipated in enterococci from the present study. In pneumococci, like in enterococci, quinupristin is an efficient inducer of the inducible cross-resistant MLS_B phenotype (17, 28). Nevertheless, quinupristin-dalfopristin demonstrated good in vitro and in vivo bactericidal activities against pneumococci harboring the MLS_B-inducible strains (3, 20). This may be related to the fact that in pneumococci, in contrast to enterococci, quinupristin-dalfopristin displays a rapid bactericidal effect, so that induction has no time to occur.

It is important that no resistant mutant was detected in vegetations from animals treated with quinupristin-dalfopristin and infected with the MLS_B-susceptible or inducibly resistant *E. faecium* strains, even when the treatment was not effective. This is in accordance with the very low frequencies of mutation observed in vitro ($\leq 10^{-8}$). This model previously allowed us to detect the in vivo selection of glycopeptide-resistant mutant enterococci, with corresponding in vitro rates of mutation of 10^{-7} to 10^{-8} (1). On the other hand, the selection of *S. aureus* mutants resistant to quinupristin-dalfopristin has been reported in an vitro model with *S. aureus*-infected fibrin clots characterized by a very high inoculum (ca. 10^{10} CFU/g) (13). Therefore, it can be anticipated that the emergence of *E. faecium*-resistant mutants during therapy with quinupristin-dalfopristin might occur only with heavy in vivo inocula.

When the in vivo activity of quinupristin-dalfopristin was compared to that of amoxicillin, a comparable level of efficacy was achieved against the MLS_B-susceptible strain of *E. faecium*. However, it should be noted that for quinupristin-dalfopristin-treated animals, the distribution of the bacterial titers in vegetations was extremely heterogeneous, implying that in some cases, the synergy between streptogramins A and B was not fully achieved, probably because of the different distribution of the two components of the combination into the core of the vegetation, as discussed above. Against the clinical isolate HM1080 with the inducible MLS_B phenotype that was also resistant to low levels of amoxicillin, amoxicillin alone was effective and was significantly more active than quinupristin-dalfopristin. Therefore, given the fact that quinupristin-dalfopristin was, in our experiments, at best as effective as amoxicillin alone and that amoxicillin alone is generally inadequate therapy for human enterococcal endocarditis, it seems unlikely that quinupristin-dalfopristin, as sole therapy for serious enterococcal infections such as endocarditis, will be efficacious clinically.

The in vivo activity of the combination of amoxicillin and quinupristin-dalfopristin was significantly superior to the activity of single-drug therapy against HM1080, although no benefit was observed in vitro. This result might be related to the homogeneous diffusion of [^{3}H]amoxicillin into the vegetation (18), allowing for the inhibition of growth of bacteria exposed to quinupristin alone. Some investigators reported an additive or synergistic effect in vitro between quinupristin-dalfopristin and amoxicillin against strains of *E. faecium* that were susceptible or resistant to vancomycin, according to the methods and the concentrations that they used (27). Additional in vivo studies with this combination against other strains of *E. faecium* are needed, in particular, against strains highly resistant to penicillins.

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