Journal of Antimicrobial Chemotherapy (1998) **42**, 711–719

JAC

Comparative activity of quinupristin/dalfopristin and RPR 106972 and the effect of medium on in-vitro test results

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Quinupristin/dalfopristin and RPR 106972 were active *in vitro* against a wide range of aerobic Gram-positive organisms including *Enterococcus faecium.* However, most isolates of *Enterococcus faecalis* were resistant or of intermediate sensitivity. Against *Staphylococcus aureus* quinupristin/dalfopristin was more active but for all other species the range of activity of the two drugs was the same or RPR 106972 was more active. RPR 106972 was also more active against the respiratory pathogens *Haemophilus influenzae* and *Moraxella catarrhalis*. Quinupristin/dalfopristin MICs for isolates of *H. influenzae* (1–8 mg/L) clustered around the breakpoint. There were differences in the quality of growth, but little difference in MICs or zone diameters was obtained on three different media: Mueller–Hinton (MHA), Iso-Sensitest (ISA), and Diagnostic Sensitivity Test (DST) agars. The addition of blood to the medium increased MICs 2- to 4-fold, with MHA showing the greatest increase, and reduced zone diameters around quinupristin/dalfopristin discs by 3–4 mm, with the greatest effect on ISA.

Introduction

The isolation rate of resistant Gram-positive organisms has increased over the past decade with major outbreaks of multi-resistant *Staphylococcus aureus*,[1,2] vancomycin-resistant enterococci[3,4] and penicillin-resistant pneumococci[5,6] reported worldwide. There have been recent reports of *S. aureus* with diminished susceptibility to vancomycin.[7] There has also been a steady increase in the isolation of coagulase-negative staphylococci associated with infection, particularly intravenous-line infections,[8] and these organisms are often multi-resistant. The emergence of these resistant strains of Gram-positive bacteria has changed the emphasis in the development of antimicrobial drugs from those with activity directed mainly at Gram-negative isolates (although many have had a broad-spectrum) to those with activity against Gram-positive bacteria, including those resistant to the agents commonly used for treating infections caused by them.

We report on the in-vitro activity of two new streptogramins, both of which are composed of two pristinamycin derivatives. Like the naturally occurring pristinamycin components, neither derivative has good antibacterial activity alone but together they have a synergic bactericidal effect[9] against Gram-positive but not Gram-negative bacteria, with a few exceptions.[10] RPR 59500 (Synercid) is a mixture of quinupristin (derived from pristinamycin IA) and dalfopristin (derived from pristinamycin IIA) in a 30:70 w/w ratio.[10] RPR 106972 is a mixture of RPR 112808 (derived from pristinamycin IB) and RPR 106950 (derived from pristinamycin IIB) in a 45:55 w/w ratio (personal communication, Rhône-Poulenc Rorer, Collegeville, PA, USA). All derivatives bind to the ribosome to form a stable complex with a long half-life, and induce a conformational modification that inhibits peptidyl transferase activity.[9] Quinupristin/dalfopristin is a water-soluble injectable agent and RPR 106972 is an oral agent.

There have been few published studies of direct comparisons of different media in susceptibility testing. We have shown that for many organisms there is little difference in the results determined using Mueller–Hinton (MHA), Iso-Sensitest (ISA) or Diagnostic Sensitivity Test (DST) agars, but for some organisms and some antibiotics there are differences.[11]

Materials and methods

Antimicrobial agents

The agents tested, gifts of the manufacturers and supplied as powders of known potency, were quinupristin/dalfopristin and RP 106972 (Rhône-Poulenc Rorer, College-

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ville, PA, USA), vancomycin and erythromycin (Eli Lily & Co. Ltd, Basingstoke, UK), penicillin, ampicillin and methicillin (SmithKline Beecham, Harlow, UK), tetracycline (Wyeth Laboratories, Maidenhead, UK), clindamycin (Pharmacia & Upjohn Ltd, Milton Keynes, UK) and gentamicin (Sigma-Aldrich Co. Ltd, Poole, UK).

Organisms

The 598 organisms included in the study (listed in the Table) were mostly clinical isolates from Guy's and St Thomas's Hospitals and were selected to include isolates known to be resistant to one or more of the comparator antimicrobials. *Lactobacillus* spp. other than *Lactobacillus rhamnosus* were isolated from faecal specimens in a screening programme for vancomycin-resistant enterococci.

Susceptibility testing

MICs were determined either by an agar dilution method (quinupristin/dalfopristin, RPR 106972, tetracycline and clindamycin) or a broth microdilution method.[12] Quinupristin/dalfopristin and RPR 106972 were tested on Mueller–Hinton agar (MHA; Oxoid Ltd, Basingstoke, UK), Iso-Sensitest agar (ISA; Oxoid), and Diagnostic Sensitivity Test agar (DST; Oxoid) and the comparators were tested either on ISA or in Iso-Sensitest broth (Oxoid). The media were supplemented with 5% saponin-lysed horse blood for fastidious organisms (and also 10 mg/L nicotinamide adenine dinucleotide for *Haemophilus influenzae*). The inoculum size was 10^4–10^5 cfu and plates were incubated for 18 h at 35°C in air (with added CO_2 for fastidious organisms). Tentative breakpoints of $\leq$1 mg/L for sensitive and $\geq$4 mg/L for resistant were used for quinupristin/dalfopristin (as suggested by Barry *et al.*[13] and based on clinical evidence) and for RPR 106972 (for comparison, since no clinical evidence is yet available) and breakpoints recommended by the BSAC Working Party[14] were used for all other agents.

S. aureus NCTC 6571 and a clinical isolate of *Enterococcus faecalis* (number STH35401) were included in all tests as controls.

Disc diffusion tests for quinupristin/dalfopristin were performed by the comparative method[14] on MHA, ISA and DST with 15 μg discs supplied by Rhône-Poulenc Rorer.

The effect of added blood on MICs and zone diameters was determined for all three agars against 68 randomly selected isolates of staphylococci and enterococci.

Analysis of results

Results for MICs and zone diameters were analysed with a computer program developed from that described by Shannon & Phillips.[15]

Results

The ranges of MIC, MIC_{50} and MIC_{90} for the compounds tested are listed in the Table, in which results for quinupristin/dalfopristin and RPR 106972 are those obtained with ISA. No isolate had a quinupristin/dalfopristin MIC >8 mg/L or an RPR 106972 MIC > 4 mg/L.

Of the 100 isolates of *S. aureus*, 94% were resistant to methicillin (including seven isolates with low-level resistance because of hyperproduction of staphylococcal β-lactamase), 90% were resistant to erythromycin, 56% were resistant to gentamicin and 47% were resistant to tetracycline. Of the *S. aureus* isolates, 99% were susceptible to quinupristin/dalfopristin (MIC ≤1 mg/L) and 77% were susceptible to RPR 106972; three isolates, with RPR 106972 MICs of 4 mg/L, were resistant. The 50 isolates of coagulase-negative staphylococci (species listed in the Table), all from blood, had a variety of susceptibility patterns, with many multi-resistant isolates although none was resistant to vancomycin. All but one were sensitive to quinupristin/dalfopristin and RPR 106972.

Of the 200 isolates of *Enterococcus faecium*, 40% were resistant to vancomycin, 35% had high-level resistance to gentamicin (MIC >1000 mg/L) and 65% were resistant to tetracycline. All but 2% of isolates were sensitive to quinupristin/dalfopristin, three isolates were of intermediate sensitivity and one was resistant (MIC 4 mg/L). All isolates had RPR 106972 MICs <1 mg/L. In contrast the 36 isolates of *E. faecalis*—of which 53% were resistant to vancomycin, 62% had high-level resistance to gentamicin and 85% were resistant to tetracycline—were mostly resistant (70%) or of intermediate sensitivity (22%) to quinupristin/dalfopristin, and 3% were resistant and 70% of intermediate sensitivity to RPR 106972, with MICs clustered around the breakpoints. The eight other enterococci were sensitive to RPR 106972 but quinupristin/dalfopristin was 2- to 4-fold less active and one isolate of *Enterococcus avium* was resistant.

All 50 isolates of *Streptococcus pneumoniae* were sensitive to both quinupristin/dalfopristin and RPR 106972. Fifty-two per cent were moderately resistant to penicillin (MICs 0.12–1 mg/L), 48% were resistant to erythromycin and 34% were resistant to tetracycline. RPR 106972 was 2- to 4-fold more active than quinupristin/dalfopristin.

All the isolates of *Streptococcus pyogenes* and *Streptococcus agalactiae* (including tetracycline-resistant and erythromycin-resistant strains) were susceptible to quinupristin/dalfopristin and RPR 10692. The α-haemolytic streptococci (species listed in the Table), all from patients with endocarditis, were susceptible to RPR 106972 but only 70% were sensitive to quinupristin/dalfopristin. A further 28% were of intermediate sensitivity and one isolate of *Streptococcus sanguis* was resistant, with a quinupristin/dalfopristin MIC of 8 mg/L.

Table. Comparative in-vitro activity of quinupristin/dalfopristin, RPR 106972, and other agents against aerobic Gram-positive bacteria, *H. influenzae* and *M. catarrhalis*

Organism	Number	Compound	Range	MIC_{50}	MIC_{90}
S. aureus	94	quinu/dalfo	0.25–2	0.5	1
methicillin resistant		RPR 106972	0.25–4	1	2
		tetracycline	0.125–>128	1	>128
		erythromycin	0.125–>32	>32	>32
		gentamicin	<1–32	8	>32
		penicillin	1–>8	>8	>8
		methicillin	8–>32	>32	>32
		clindamycin	0.03–>16	0.25	>16
		vancomycin	0.5 -2	1	1
S. aureus	6	quinu/dalfo	0.25–1	0.5	1
methicillin sensitive		RPR 106972	0.5–1	0.5	1
		tetracycline	0.125–32	0.25	32
		erythromycin	0.125–>32	0.25	>32
		gentamicin	<1–32	<1	32
		penicillin	0.06–>8	>8	>8
		methicillin	1–2	1	2
		clindamycin	0.03–0.06	0.06	0.06
		vancomycin	1	1	1
Coagulase-negative	35	quinu/dalfo	0.06–2	0.25	0.5
staphylococci[a]		RPR 106972	0.06–2	0.25	0.5
methicillin resistant		tetracycline	0.125–>128	1	64
		erythromycin	0.125–>32	>32	>32
		gentamicin	<1–>32	>32	>32
		penicillin	0.25–>8	>8	>8
		methicillin	8–>32	>32	>32
		clindamycin	0.03–>16	0.125	>16
		vancomycin	1–4	2	2
Coagulase-negative	15	quinu/dalfo	0.125–0.5	0.25	0.5
staphylococci[b]		RPR 106972	0.06–0.5	0.125	0.25
methicillin sensitive		tetracycline	0.125–32	2	32
		erythromycin	0.03–>32	0.5	>32
		gentamicin	<1–>32	<1	>32
		penicillin	0.016–>8	2	>8
		methicillin	0.06–4	2	4
		clindamycin	0.03–0.125	0.06	0.125
		vancomycin	0.5–2	2	2
E. faecium	121	quinu/dalfo	0.125–4	0.5	1
vancomycin sensitive		RPR 106972	0.06–1	0.25	0.5
		tetracycline	0.125–>128	16	128
		erythromycin	0.06–>128	>128	>128
		clindamycin	0.06–>16	16	>16
		gentamicin	4–>2048	32	>2048
		ampicillin	1–>128	32	64
		vancomycin	0.5–2	1	1
E. faecium	79	quinu/dalfo	0.125–2	0.5	0.5
vancomycin resistant		RPR 106972	0.06–0.5	0.125	0.25
		tetracycline	0.125–128	8	64
		erythromycin	0.5–>128	>128	>128
		clindamycin	0.03–>16	4	>16
		gentamicin	4–>2048	32	>2048
		ampicillin	1–>128	64	128
		vancomycin	8–>128	>128	>128

Table. *Continued*

Organism	Number	Compound	Range	MIC_{50}	MIC_{90}
E. faecalis vancomycin sensitive	17	quinu/dalfo	1–4	4	4
		RPR 106972	0.5–4	2	2
		tetracycline	0.25–128	32	128
		erythromycin	1–>128	4	>128
		clindamycin	4–>16	16	>16
		gentamicin	4–>2048	64	>2048
		ampicillin	0.5–2	0.5	1
		vancomycin	1–4	1	4
E. faecalis vancomycin resistant	19	quinu/dalfo	1–4	4	4
		RPR 106972	0.25–2	2	2
		tetracycline	0.06–>128	32	64
		erythromycin	0.25–>128	>128	>128
		clindamycin	8–>16	>16	>16
		gentamicin	4–>2048	>2048	>2048
		ampicillin	0.25–2	0.5	2
		vancomycin	8–>128	>128	>128
Other enterococci[c]	8	quinu/dalfo	1–4	2	4
		RPR 106972	0.5–1	0.5	1
		tetracycline	0.5–64	64	64
		erythromycin	0.25–>128	0.5	>128
		clindamycin	1–8	4	8
		gentamicin	8–>2048	16	>2048
		ampicillin	0.25–64	0.5	64
		vancomycin	1–>128	4	>128
S. pneumoniae penicillin sensitive	23	quinu/dalfo	0.5–1	1	1
		RPR 106972	0.06–0.125	0.125	0.125
		tetracycline	0.06–32	0.25	0.25
		erythromycin	0.03–32	0.125	16
		clindamycin	0.06–>16	0.125	0.125
		penicillin	0.004–0.03	0.016	0.016
		vancomycin	0.06–0.5	0.25	0.5
S. pneumoniae penicillin resistant	27	quinu/dalfo	0.25–1	1	1
		RPR 106972	0.06–0.25	0.125	0.125
		tetracycline	0.03–32	16	32
		erythromycin	0.06–>128	4	>128
		clindamycin	0.06–>16	0.125	>16
		penicillin	0.06–1	0.5	1
		vancomycin	0.25–0.5	0.5	0.5
S. pyogenes	20	quinu/dalfo	0.125–1	0.125	0.125
		RPR 106972	0.06–0.125	0.06	0.125
		tetracycline	0.25–64	32	32
		erythromycin	0.008–4	0.016	0.03
		clindamycin	0.016–0.06	0.016	0.03
		penicillin	0.002–0.008	0.004	0.008
		vancomycin	0.25–0.5	0.5	0.5
S. agalactiae	20	quinu/dalfo	0.25–0.5	0.5	0.5
		RPR 106972	0.06–0.125	0.06	0.125
		teracycline	0.25–64	32	32
		erythromycin	0.016–0.03	0.03	0.03
		clindamycin	0.03–0.06	0.06	0.06
		penicillin	0.008–0.03	0.03	0.03
		vancomycin	0.5–1	0.5	1

Table. *Continued*

Organism	Number	Compound	Range	MIC_{50}	MIC_{90}
α- and non-haemolytic streptococci[d]	51	quinu/dalfo	0.25–8	1	2
		RPR 106972	0.06–1	0.125	0.25
		tetracycline	0.125–64	0.25	32
		erythromycin	0.008–>128	0.03	0.125
		clindamycin	0.016–>16	0.06	0.06
		penicillin	0.008–0.25	0.06	0.06
		vancomycin	0.5–1	1	1
Other Gram-positive[e] organisms	23	quinu/dalfo	0.5–4	2	2
		RPR 106972	0.06–1	0.25	0.25
		tetracycline	0.25–16	1	8
		erythromycin	0.016–1	0.03	0.06
		ampicillin	0.25–8	2	4
		vancomycin	32–>128	>128	>128
H. influenzae	20	quinu/dalfo	1–8	4	8
		RPR 106972	0.5–2	1	2
		tetracycline	0.25–16	0.5	8
		erythromycin	1–64	4	8
		ampicillin	0.25–128	1	128
M. catarrhalis	20	quinu/dalfo	0.125–0.5	0.5	0.5
		RPR 106972	0.06–0.25	0.125	0.25
		tetracycline	0.25		
		erythromycin	0.06–0.5	0.125	0.125
		ampicillin	0.03–8	1	4

[a] *Staphylococcus epidermidis* (25 isolates), *Staphylococcus haemolyticus* (3), *Staphylococcus hominis* (4), *Staphylococcus sciuri* (2), *Staphylococcus capitis* (1).
[b] *S. epidermidis* (10 isolates), *S. haemolyticus* (2), *S. hominis* (2), *Staphylococcus simulans* (1).
[c] *Enterococcus avium* (4 isolates), *Enterococcus gallinarum* (3), *Enterococcus casseliflavus* (1).
[d] *Streptococcus anginosus* (6 isolates), *Streptococcus bovis* (5), *Streptococcus constellatus* (1), *Streptococcus gordonii* (3), *S. mitis* (6), *Streptococcus mutans* (6), *Streptococcus oralis* (9), *Streptococcus salivarius* (3), *Streptococcus sanguis* (10), *Streptococcus vestibularis* (1), *Aerococcus viridans* (1).
[e] *Lactococcus lactis* (1), *Lactobacillus brevis* (1), *Lactobacillus curvatus* (1), *Lactobacillus coprophilus* (2), *Lactobacillus paracasei* (6), *Lactobacillus rhamnosus* (5), *Lactobacillus* spp. (1), *Leuconostoc* spp. (3) *Pediococcus pentosaceus* (3).

The group of 22 miscellaneous vancomycin-resistant Gram-positive organisms (listed in the Table) were all sensitive to RPR 106972. Eight isolates were sensitive to quinupristin/dalfopristin, including the three *Leuconostoc* spp., 12 isolates were of intermediate sensitivity, and two, both *Pediococcus* spp., were resistant (quinupristin/dalfopristin MIC 4 mg/L).

Both quinupristin/dalfopristin and RPR 106972 were active against the 20 isolates of *Moraxella catarrhalis* but RPR 106972 was four-fold more active than quinupristin/dalfopristin against the 20 *H. influenzae* isolates, about half of which were resistant to the latter, with MICs clustered around the breakpoint.

There was little difference in MIC results on the three agars for quinupristin/dalfopristin or RPR 106972 (a typical regression is shown in Figure 1a). Correlation coefficients for quinupristin/dalfopristin were 0.88 or 0.89, and for RPR 106972 they were 0.94 or 0.95; virtually all results for pairs of media were within one doubling dilution. The mean MIC of quinupristin/dalfopristin for all 598 isolates was highest on MHA (0.613 mg/L) and lowest on ISA (0.602 mg/L). The mode MIC for all three agars was 0.5 mg/L. The difference was slightly greater for RPR 106972, with a mean MIC of 0.291 mg/L (mode 0.25 mg/L) on MHA, 0.272 mg/L on ISA and 0.268 mg/L on DST (mode 0.125 mg/L for both). The difference with MHA was largely due to a group of *S. aureus* isolates with RPR 106972 MICs of 2 mg/L on MHA and 1 mg/L on ISA and DST. There were no major differences in categorization on the three media although for some isolates there were minor changes from sensitive to intermediate and intermediate to resistant.

Correlation of zone sizes for quinupristin/dalfopristin on the three media was good, with a correlation coefficient of 0.93 for all comparisons: a typical result is shown in Figure 1b. There were differences among the three media in the quality of growth—which was generally best on DST, followed by ISA and then MHA—and zone sizes tended to be slightly smaller on DST (mean zone size 26.8 mm) than on MHA (mean 27.4 mm) or ISA (mean

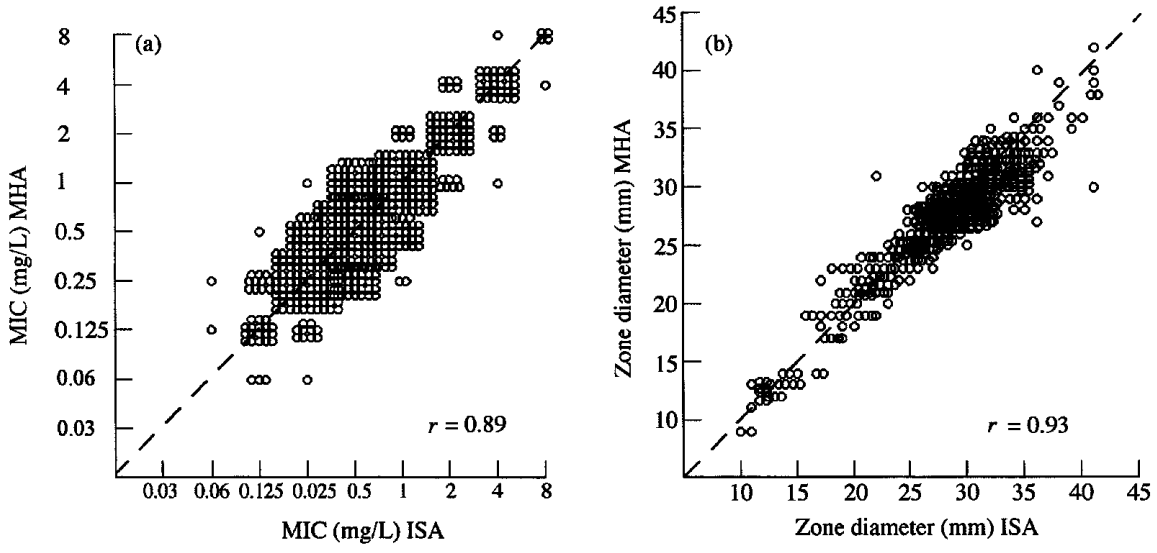

Figure 1. Correlation of quinupristin/dalfopristin (a) MICs and (b) zone diameters for 598 isolates on ISA and MHA. The broken line represents equivalent MICs or zone diameters on the two media.

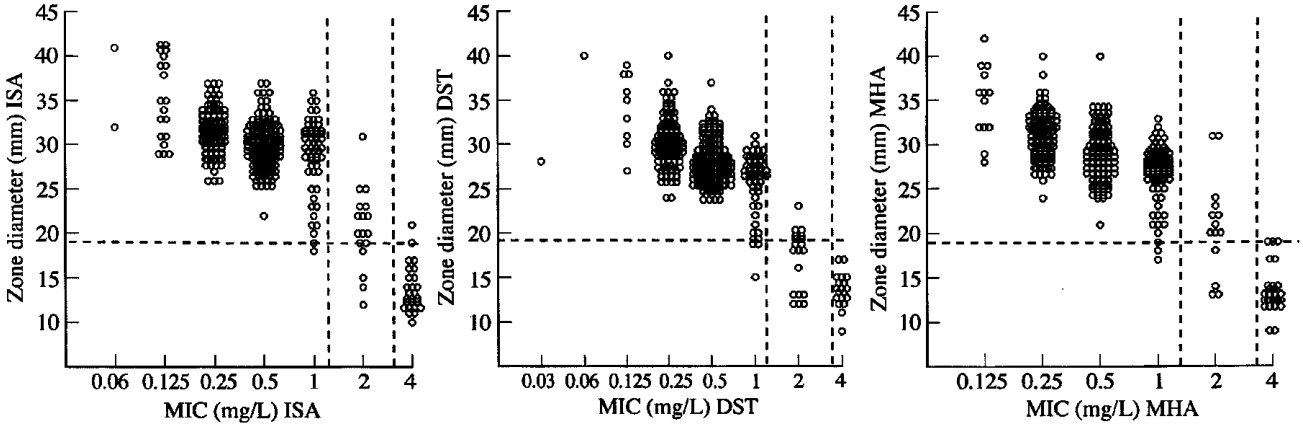

Figure 2. Correlation of quinupristin/dalfopristin MICs and zone diameters for 388 isolates on ISA, DST and MHA with no added blood. Broken lines represents zone breakpoints.

28.2 mm). However, there were individual isolates of coagulase-negative staphylococci and enterococci which failed to grow (four), or grew poorly (three), on one or other of the three media; poor growth resulted in differences in zone sizes of up to 8 mm. Thus there were isolates that were resistant on one medium and sensitive on another, all with quinupristin/dalfopristin MICs close to the breakpoint.

For the 388 isolates tested with no additives and incubated in air (staphylococci and enterococci) the correlation of zone size with MIC for quinupristin/dalfopristin (Figure 2) was similar on all three agars (r = 0.79 for DST and MHA, and 0.77 for ISA) and a zone size of ⩾19 mm gave good discrimination between sensitive and resistant organisms. The exception was one isolate of *E. faecalis* which had quinupristin/dalfopristin MICs of 1 mg/L and zone diameters <19 mm on all three media, as did one further isolate of *E. faecalis* on MHA. Isolates with quinupristin/dalfopristin MICs of 2 mg/L, and thus of intermediate sensitivity, had zone sizes ranging from 13 to 31 mm but all isolates with zones <19 mm were *E. faecalis* and those with zones >19 mm were other species of enterococci and staphylococci.

For the 205 isolates tested with added blood (Figure 3), the correlation of zone size with MIC was not good for any of the media but was best on DST (r = 0.67), followed by ISA (r = 0.56) and then MHA (r = 0.53). Despite this poor correlation a zone size of ⩾19 mm was discriminatory between sensitive and resistant isolates, with the exception of *H. influenzae*, which had quinupristin/dalfopristin MICs of 1–8 mg/L and zones ranging from 18 to 35 mm. For these organisms a zone diameter of 25 mm was more appropriate for discriminating between sensitive and resistant isolates. The correlations were also affected by

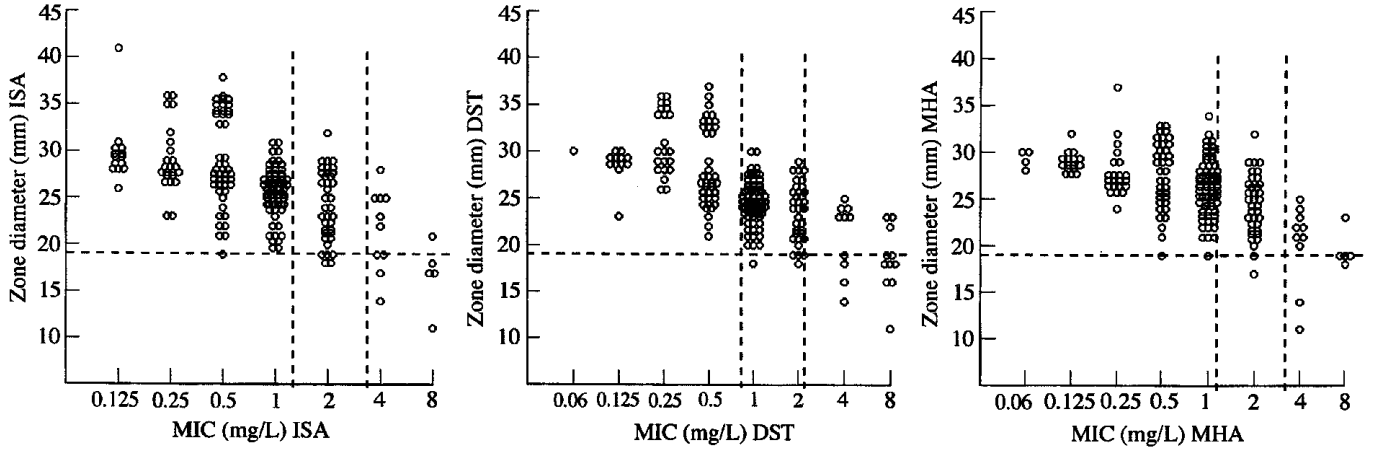

Figure 3. Correlation of quinupristin/dalfopristin MICs and zone diameters for 205 isolates on ISA, DST and MHA with added blood. Broken lines represents zone and MIC breakpoints.

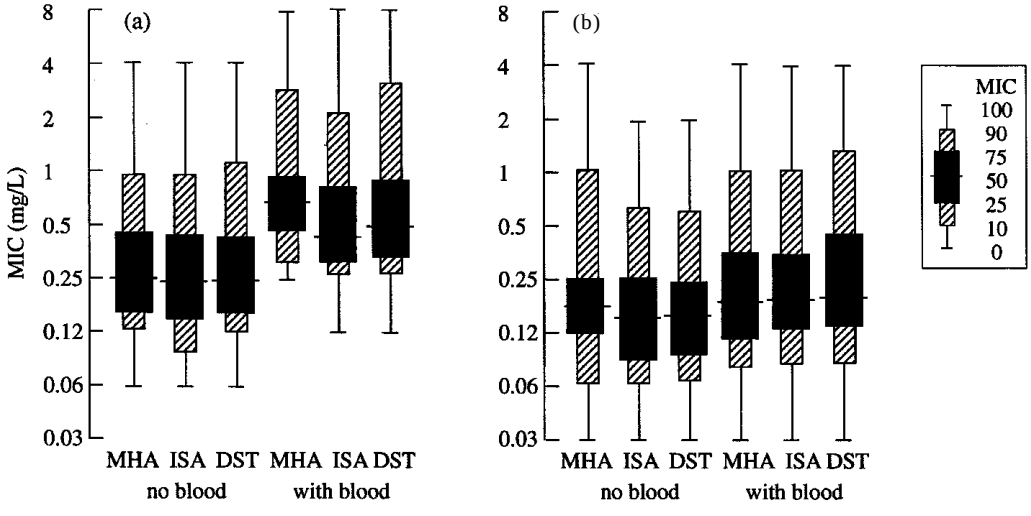

Figure 4. MICs of (a) quinupristin/dalfopristin and (b) RPR 106972 for 45 enterococci and 23 staphylococci tested with and without added blood on MHA, ISA and DST.

large zones in relation to MICs for *M. catarrhalis* and some *Lactobacillus* spp., this being most obvious with ISA (Figure 3).

For the 68 isolates tested on all three media with and without blood, the addition of lysed horse blood increased quinupristin/dalfopristin MICs 2- to 4-fold, with MHA showing the greatest increase (Figure 4a). The effect was also seen with RPR 106972 but to a much smaller degree (Figure 4b). This resulted in enterococci being recategorized from sensitive to resistant to quinupristin/dalfopristin for one isolate on ISA, three on MHA and three on DST, and to RPR 106972 for two isolates on DST. There were also minor changes of categorization from sensitive to intermediate and from intermediate to resistant on all three media for both agents.

Zone diameters were also affected by the addition of blood, with a decrease in diameter of about 3–4 mm, the biggest effect being on ISA (Figure 5). This again resulted in two isolates of *E. faecalis* and two of *E. faecium*, all with quinupristin/dalfopristin MICs of 2–4 mg/L and all of which grew poorly without blood, being recategorized from sensitive to resistant.

Discussion

Our results on the activity of quinupristin/dalfopristin against Gram-positive cocci, *M. catarrhalis* and *H. influenzae* confirm those of other workers.[10,16] We concentrated mainly on multi-resistant isolates, particularly Gram-positive cocci, and found no evidence of cross-resistance or associated resistance with any of the antimicrobials tested, which confirms and extends existing

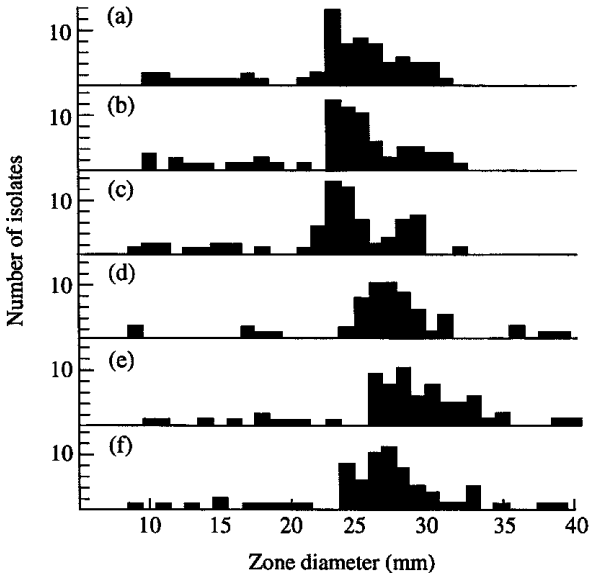

Figure 5. Zone diameters of quinupristin/dalfopristin for 45 enterococci and 23 staphylococci tested with (a, b, c) and without (d, e, f) added blood on three media: MHA (a, d), ISA (b, e), and DST (c, f).

knowledge,[10,17] especially for vancomycin-resistant isolates. We have demonstrated that quinupristin/dalfopristin was more active than RPR 106972 against *S. aureus*, particularly methicillin-resistant isolates, but for other species the oral streptogramin RPR 106972 was 1–2-fold more active *in vitro* than quinupristin/dalfopristin. However, the two drugs have different pharmacokinetics and it is probable that blood levels of RPR 106972 are lower than those of the injectable agent, which may result in lowering of the breakpoints. Furthermore, when isolates were sensitive to RPR 106972 and resistant to quinupristin/dalfopristin the results for both were close to the breakpoints. Both drugs were active against most aerobic Gram-positive cocci, with the exception of *E. faecalis.* They were also active against *M. catarrhalis* but less so against *H. influenzae.*

There is current anxiety that technical and methodological differences might contribute to different perceptions of activity of antibiotics, therefore we tested these streptogramins on three commonly used media. The quality of growth on the media, often not noted in descriptions of susceptibility testing methods, does influence the results but only to a minor extent. In general, organisms grew better on DST than on ISA or MHA and zone sizes tended to be slightly smaller on DST, but there was very little difference in MICs. Our results, determined on Iso-Sensitest agar, compare very closely to those of Barry *et al.*[13] who used NCCLS methodology, and thus Mueller–Hinton agar. The MIC_{50} and MIC_{90} were either the same (for staphylococci and *H. influenzae*) or within one dilution (for *E. faecalis*, *S. pyogenes*, *S. agalactiae* and *M. catarrhalis*) for most organisms. However, one of Barry *et al.*'s isolates of *E. faecium* and two of *E. faecalis*, with quinupristin/dalfopristin MICs of 16 mg/L, were clearly more resistant than any of ours. In contrast, we found *S. pneumoniae*, both penicillin-sensitive and penicillin-resistant, to be less sensitive (MIC_{50} 1 mg/L versus 0.25 mg/L, and MIC_{90} 1 mg/L versus 0.5 mg/L). Results for α-haemolytic streptococci were difficult to compare, since we examined more isolates and the proportion of each species differed. However, some isolates grow poorly or not at all from a standard inoculum on one or other of the media and this made a small number of resistant isolates appear sensitive. This error is virtually impossible to detect when only one medium is used.

The addition of blood to the medium had a noticeable but small effect on both zone sizes and MICs. However, it did result in changes of categorization of a few organisms from sensitive to resistant. Our results differ from those of Barry *et al.*,[13] who concluded that addition of blood made no difference.

With a tentative breakpoint of $\leqslant$1 mg/L for isolates sensitive to quinupristin/dalfopristin, we recommend a zone diameter breakpoint of $\geqslant$19 mm. Since there were no highly resistant organisms in our study, the appropriateness of this recommendation requires confirmation. However, it must be remembered that streptogramin MICs for *E. faecalis* isolates are around the breakpoint (1–4 mg/L) and that all have small zones and are clearly resistant. Conversely, *H. influenzae* have larger zones than expected for the corresponding MIC and the setting of appropriate breakpoints must await clinical experience.

Acknowledgements

This study was supported by grants from Rhône-Poulenc Rorer and from the Special Trustees of St Thomas's Hospital (project no. 804).

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Received 12 January 1998; returned 29 January 1998; revised 17 February 1998; accepted 30 June 1998