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Susceptibility to quinupristin/dalfopristin and other antibiotics of vancomycin-resistant enterococci from the UK, 1997 to mid-1999

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Susceptibility to quinupristin/dalfopristin and other antibiotics was studied for clinical isolates of vancomycin-resistant enterococci (VRE) referred by UK hospitals between January 1997 and June 1999. Single isolates of VRE from 858 patients in 136 hospitals were received, of which 76% were *Enterococcus faecium* and 21% were *Enterococcus faecalis*, the remainder comprising minor species. Most isolates were multi-resistant. After allowing for the effect of blood, which raised the MICs of quinupristin/dalfopristin four-fold, 98.3% of *E. faecalis* isolates and all the *Enterococcus avium*, *Enterococcus casseliflavus* and *Enterococcus gallinarum* appeared resistant to quinupristin/dalfopristin, whereas 98.8% of the *E. faecium* isolates and the single *Enterococcus raffinosus* isolate were susceptible.

Introduction

Enterococci are important opportunistic nosocomial pathogens and have intrinsic and acquired resistance to many antibiotics. During the past decade, a major interest has been the emergence and spread of enterococci resistant to vancomycin. Following their initial recognition in a south London hospital in 1987, vancomycin-resistant enterococci (VRE) had been isolated in at least 71 UK hospitals by 1995.[1] They are an even greater problem in the USA, and have been isolated in many other countries.[2] Although 85–90% of clinical isolates of enterococci are *Enterococcus faecalis*, most VRE are *Enterococcus faecium*.[2]

The streptogramin quinupristin/dalfopristin (Synercid) was recently licensed in the UK and the USA, with treatment of infections caused by glycopeptide-resistant *E. faecium* as an indication. The PHLS Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) regularly receives isolates of VRE from many hospitals throughout the UK. We report on the activity of quinupristin/dalfopristin and other agents against clinical isolates of VRE referred between January 1997 and June 1999, immediately before the drug's launch.

Materials and methods

Susceptibility testing

For the past decade, Gram-positive bacteria (comprising predominantly pneumococci, enterococci, viridans streptococci and staphylococci) submitted to the ARMRL have been tested for resistance to ampicillin, penicillin, erythromycin, gentamicin, vancomycin, teicoplanin, rifampicin, tetracycline, ciprofloxacin and chloramphenicol. Quinupristin/dalfopristin was added to this panel in January 1997. MICs were determined on Diagnostic Sensitivity Test (DST) agar (Oxoid, Basingstoke, UK) containing 5% lysed horse blood (TCS Microbiology, Buckingham, UK) to promote the growth of fastidious species. Inocula comprise 10^4–10^5 cfu/spot, and plates are incubated for 18 h at 37°C in air. Originally, the MICs were recorded by eye, but since May 1997 they have been read with a Domino image analyser (Perceptive Instruments, Haverhill, UK). Before analysis, records for isolates from non-human sources (e.g. those from food, animals or the environment) were deleted, as were those for repeat isolates from individual patients. Isolates were categorized as susceptible or resistant using breakpoints recommended by the British Society for Antimicrobial Chemotherapy (BSAC)[3,4] as follows:

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ampicillin, 8 mg/L; chloramphenicol, 8 mg/L; ciprofloxacin, 1 mg/L; erythromycin, 0.5 mg/L; gentamicin (high-level), 500 mg/L; rifampicin, 1 mg/L; teicoplanin, 4 mg/L; tetracycline, 1 mg/L; vancomycin, 4 mg/L.

Results

Referred isolates of VRE

Over the 2.5 year study period, isolates of VRE from 858 different patients in 136 hospitals were referred to the ARMRL. Speciation of the VRE by PCR,[5] undertaken in the Epidemiological Typing Unit of the Laboratory of Hospital Infection at Colindale, showed that 650 (76%) were *E. faecium*, 179 (21%) *E. faecalis*, and the remainder comprised *Enterococcus avium* (n = 3), *Enterococcus casseliflavus* (n = 7), *Enterococcus gallinarum* (n = 18) and *Enterococcus raffinosus* (n = 1). The number of hospitals referring isolates of vancomycin-resistant *E. faecium* increased from 52 in 1997 to 76 in 1998, with 43 referring isolates in the first 6 months of 1999. Corresponding figures for *E. faecalis* were 28, 41 and 15. Many of the referred *E. faecium* and *E. faecalis* isolates were resistant to multiple antibiotics (Table I). Approximately 83% of vancomycin-resistant isolates of both these species were resistant to teicoplanin, over 96% to erythromycin, 52% to high levels of gentamicin and 99% to ciprofloxacin. Approximately 99% of *E. faecalis* isolates, but only 37.5% of *E. faecium*, were resistant to tetracycline. All *E. faecalis* isolates, but none of *E. faecium*, were susceptible to ampicillin. All 29 isolates of the four minor species were sensitive to ampicillin (MICs 0.5–8 mg/L), with 32% also sensitive to erythromycin; four (14%) showed high-level resistance to gentamicin. The MICs of vancomycin for *E. casseliflavus* and *E. gallinarum* isolates were 8–16 mg/L, but these species were sensitive to teicoplanin (MICs 0.5–2 mg/L), as is typical of the VanC phenotype.[2] The three isolates of *E. avium* and the one *E. raffinosus* were resistant to both vancomycin and teicoplanin (MICs > 32 mg/L).

Activity of quinupristin/dalfopristin

MIC distributions of quinupristin/dalfopristin on DST agar containing 5% lysed horse blood are shown in Table II. Of the 650 *E. faecium* isolates, 77.8% were susceptible to quinupristin/dalfopristin at ⩽2 mg/L, and the MIC_{90} was 4 mg/L. In contrast, MICs for 98.3% of the isolates of *E. faecalis* were ⩾8 mg/L, as were those for 14 of 18 *E. gallinarum* isolates. The MICs for the *E. faecium* isolates appeared higher than those reported elsewhere[6,7] and, as several groups have shown that quinupristin/dalfopristin MICs are influenced by blood,[7–9] 50 selected *E. faecium* isolates were re-tested with quinupristin/dalfopristin on DST with and without lysed blood. As shown in Table II, MICs without blood were about four-fold lower than those obtained with 5% blood.

Table I. Antimicrobial susceptibility of isolates of vancomycin-resistant *E. faecium* and *E. faecalis* referred to Antibiotic Resistance Monitoring and Reference Laboratory January 1997 to June 1999

		MIC (mg/L)			
Species (n)	Antibiotic	range	MIC_{50}	MIC_{90}	Susceptible (%)
Enterococcus faecium (650)	ampicillin	>8	>8	>8	0
	erythromycin	⩽0.25–>16	>16	>16	1.7
	gentamicin	4–>2000	>2000	>2000	48.3
	vancomycin	>32	>32	>32	0
	teicoplanin	⩽0.5–>32	16	>32	16.6
	rifampicin	⩽0.25–>1	>1	>1	30.5
	tetracycline	⩽0.5–>8	1	>8	62.5
	ciprofloxacin	2–>8	>8	>8	0
	chloramphenicol	4–>8	8	>8	62.2
Enterococcus faecalis (179)	ampicillin	1–8	2	4	100
	erythromycin	0.25–>16	>16	>16	4
	gentamicin	4–>2000	>2000	>2000	46.9
	vancomycin	8–>32	>32	>32	0
	teicoplanin	⩽0.5–>32	>32	>32	16.8
	rifampicin	⩽0.25–>1	>1	>1	39.6
	tetracycline	1–>8	>8	>8	1.1
	ciprofloxacin	1–>8	>8	>8	0.6
	chloramphenicol	4–>8	>8	>8	45.5

Table II. Activity of quinupristin/dalfopristin against vancomycin-resistant enterococci

Species	No. of isolates	Medium	No. of isolates with indicated MIC (mg/L)						
			0.25	0.5	1	2	4	8	⩾16
Enterococcus faecium	650	DST + 5% blood	–	3	137	366	100	37	7
Enterococcus faecalis	179	DST + 5% blood	–	–	–	3	–	7	169
Enterococcus avium	3	DST + 5% blood	–	–	–	–	–	–	3
Enterococcus casseliflavus	7	DST + 5% blood	–	–	–	–	2	2	3
Enterococcus gallinarum	18	DST + 5% blood	–	–	–	–	3	14	1
Enterococcus raffinosus	1	DST + 5% blood	–	–	–	1	–	–	–
Medium comparison									
E. faecium	50	DST + 5% blood	–	1	0	22	11	14	2
E. faecium	50	DST-No blood	2	19	11	9	8	0	1

Discussion

Quinupristin/dalfopristin was recently licensed in the UK, *inter alia*, for treatment of infections due to vancomycin-resistant *E. faecium*. We outline here its activity against a large collection of clinical VRE isolates (predominantly *E. faecium*, many multiply resistant), referred to the ARMRL from a sizeable number of UK hospitals, in the 2.5 years immediately before its launch.

As noted by others,[6,7] quinupristin/dalfopristin was more active against *E. faecium* than against *E. faecalis*. However, determination of the proportion of isolates resistant to quinupristin/dalfopristin posed some difficulty. When this study was initiated, there was no defined breakpoint for quinupristin/dalfopristin. In 1998, the BSAC advocated a breakpoint of 2 mg/L for enterococci,[4] and on this basis, only 77.8% of the referred isolates of *E. faecium* would be regarded as susceptible. However, the BSAC guidelines note that the *in vitro* activity of quinupristin/dalfopristin is reduced by blood, as reported also by others.[7–9] This effect was confirmed here, the MICs of quinupristin/dalfopristin on blood-free DST agar being about four-fold lower than on DST agar with 5% lysed blood. The BSAC guidelines advocate that the disc zone diameter breakpoint of ⩾20 mm for quinupristin/dalfopristin should be reduced to ⩾15 mm if the medium contains blood. If the same broad principle is applied here, and the breakpoint raised four-fold to 8 mg/L to compensate for the effect of the blood, about 98.8% of the vancomycin-resistant *E. faecium* isolates would be regarded as sensitive. On this basis we confirm that quinupristin/dalfopristin is highly active against most vancomycin-resistant *E. faecium* from UK hospitals. Nevertheless, the occurrence of a few resistant *E. faecium* isolates gives some cause for concern. Resistance has already been noted in clinical *E. faecium* isolates and those from foodstuffs,[10] with the latter perhaps reflecting the use of a related streptogramin (virginiamycin) as a growth promoter. Resistance may become more prevalent under the selective pressure of increasing quinupristin/dalfopristin usage; monitoring of this situation will require the inclusion of quinupristin/dalfopristin in resistance surveillance programmes.

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