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In Vitro Activity of the Oxazolidinone RWJ-416457 against Linezolid-Resistant and -Susceptible Staphylococci and Enterococci[▽]

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RWJ-416457, a novel oxazolidinone, had modal MICs of 0.5 to 1 μg/ml for linezolid-susceptible staphylococci and enterococci, versus linezolid MICs for these organisms of 1 or 2 μg/ml. RWJ-416457 MICs for mutants with 23S rRNA mutations were 2 to 32 μg/ml, versus linezolid MICs of 8 to 64 μg/ml; actual values reflected the proportion of gene copies mutated.

Oxazolidinones are among the very few genuinely new antimicrobial classes developed in the past 30 years, with linezolid the sole analogue currently marketed. At launch, linezolid had nearly universal activity against gram-positive bacteria, with MICs for staphylococci, enterococci, and streptococci tightly clustered from 0.5 to 4 μg/ml (3). Licensing trials showed equivalence to standard therapies in pneumonia infections and in skin and skin structure infections (10), while pooled subgroup analysis suggested superiority over vancomycin in methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia (11), and a recent prospective trial indicated superiority over vancomycin in MRSA skin and skin structure infections (9).

Linezolid resistance is sometimes selected in vivo, particularly if treatment is prolonged or if the drug dosage is reduced (3). Most of the clinical mutants have G2576T mutations in domain V of their 23S rRNA genes, giving G2576U in the corresponding rRNA product, although other 23S rRNA mutations are occasionally found. More than one 23S rRNA gene copy must be altered to confer resistance, meaning that internal recombination events must follow modification of the first gene copy (4). This complexity doubtless explains the continued rarity of resistance, which remains essentially undetectable in large-scale epidemiological surveys.

Numerous further oxazolidinone analogues have been synthesized in the search for improvements over linezolid, but none has yet progressed beyond phase 1 testing. RWJ-416457 is a novel investigational analogue (1) now being developed by Ortho McNeil (Raritan, NJ). We evaluated its activity against linezolid-susceptible and -resistant staphylococci and enterococci. The MICs of RWJ-416457, linezolid, and other comparators (quinupristin-dalfopristin, ampicillin, oxacillin, gentamicin, vancomycin, and teicoplanin) were determined by CLSI agar dilution methodology (5) for clinical isolates (ca. 100 each) of linezolid-susceptible *Staphylococcus aureus*, coagulase-negative staphylococci, and enterococci, all collected in the United Kingdom. These collections were designed to include equal proportions of MRSA and methicillin-susceptible *S. aureus* and of *Enterococcus faecium* and *Enterococcus faecalis*. The enterococcal collections were deliberately loaded with vancomycin-resistant organisms, since oxazolidinones are particularly used in infections due to these organisms. Both the staphylococci and enterococci were selected for epidemiological diversity, being sourced from a wide geographic spread of hospitals. Nevertheless, the MRSA collections were dominated by the nationally prevalent epidemic MRSA-15 and -16 lineages, which account for ca. 95% of all invasive MRSA isolates from infections in the United Kingdom (2). We also tested 23 linezolid-resistant clinical isolates of enterococci and *S. aureus* and 7 linezolid-selected *S. aureus* laboratory mutants. Most of the resistant clinical isolates were from patients in the United Kingdom, but a few were from patients in Brazil and Austria (see below). The resistant laboratory mutants were variously selected from a clinical isolate, ST/02/2121, and from strain RN4220 and its hypermutable derivative RN4220Δ*mutS* (6) by successive passages in broth with arithmetically increasing linezolid concentrations from 0.5 to 10 μg/ml, followed by repeated subculture on agar with 10 μg/ml linezolid. The mutations present in the 23S rRNA genes of the linezolid-resistant organisms were characterized by PCR restriction fragment length polymorphisms or sequencing; the proportions of mutated gene copies were estimated by pyrosequencing (8).

MIC distributions of RWJ-416457 for linezolid-susceptible organisms were tightly clustered within species, as with linezolid, spanning only two to four doubling dilutions (Table 1); modal values were either 0.5 or 1 μg/ml, according to the species group, compared with 1 to 2 μg/ml for linezolid. Seven of 49 (14%) *E. faecalis* and 43 of 49 (86%) *E. faecium* isolates were vancomycin resistant, compared with national rates among bacteraemia isolates of ca. 3 and 20%, respectively (http://www.bsacsurv.org) (7). Four of 107 coagulase-negative staphylococci were teicoplanin resistant and 8 were intermediate, as was 1 MRSA (MIC, 16 μg/ml); all the staphylococci

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TABLE 1. MIC distributions of oxazolidinones for linezolid-susceptible isolates

Organism (no. of isolates)	Antimicrobial	No. of isolates with MIC (μg/ml) of:[a]					
		0.12	0.25	0.5	1	2	4
MRSA (47)	RWJ-416457	1	4	17	**25**		
	Linezolid			1	10	**22**	14
MSSA (56)	RWJ-416457		1	21	**34**		
	Linezolid			1	0	**35**	20
Methicillin-resistant coagulase-negative staphylococci (51)	RWJ-416457	1	17	**35**	1		
	Linezolid		1	2	22	**26**	
Methicillin-susceptible coagulase-negative staphylococci (56)	RWJ-416457		17	**39**			
	Linezolid			1	**34**	21	
E. faecalis (49)	RWJ-416457			**31**	18		
	Linezolid					**48**	1
E. faecium (49)	RWJ-416457			19	**30**		
	Linezolid					**44**	5

[a] Modal values are shown in bold font.

were susceptible to vancomycin and only 1, also methicillin-resistant, was resistant to quinupristin-dalfopristin (MIC, 16 μg/ml). Gentamicin resistance was present in 42% and 2% of the MRSA and methicillin-susceptible *S. aureus* isolates, respectively, and in 68% and 5.5% of the methicillin-resistant and -susceptible coagulase-negative staphylococci, respectively. High-level gentamicin resistance was present in 46% and 34% of *E. faecalis* and *E. faecium* isolates, respectively. There was no evidence of cross-resistance between RWJ-416457 or linezolid and these various nonoxazolidinone drugs (not shown).

Oxazolidinone MICs for the linezolid-resistant clinical isolates are shown in Table 2. All these organisms, except *E. faecium* H0 4136-0043, had G2576U mutations, but they varied in the proportion of gene copies altered; the mechanism of resistance in *E. faecium* H0 4136-0043 remains under investigation, as this strain did not harbor any previously recognized 23S rRNA mutation. The MICs of RWJ 416457 were increased for all the linezolid-resistant isolates, but remained two- to fourfold below those of linezolid, and for 14 of 23 linezolid-resistant organisms, the MICs of RWJ-416457 were only 2 to 8 μg/ml. These organisms, which included all the linezolid-resistant MRSA isolates, were heterozygous for G2576U. Higher RWJ-416457 MICs, up to 32 μg/ml, were seen for those enterococci that were homozygous for G2576U. The MICs of both linezolid and RWJ-416457 were increased also for *S. aureus* laboratory mutants with a range of different 23S rRNA mutations, including G2447U, T2504C, and G2576U (Table 3); in all cases, the MIC of RWJ-416457 remained below that of linezolid.

In summary, we found that RWJ-416457 was two- to fourfold more active than linezolid against susceptible staphylococci and enterococci and that its MICs, like those of linezolid, were tightly clustered. Its activity was reduced or lost against isolates with G2576U or other linezolid-compromising rRNA

TABLE 2. MICs for linezolid-resistant clinical isolates of *S. aureus* and enterococci

Species and strain no.	Heterozygous (Rr) or homozygous (RR) for G2576U	Source	MIC (μg/ml) of:	
			RWJ-416457	Linezolid
E. faecalis				
H0 4450-0446	Rr	London, United Kingdom	2	8
1 (L2)	Rr	Austria	16	32
25	Rr	Southeast United Kingdom	16	32
37	Rr	Midlands, United Kingdom	8	32
45	Rr	Southeast United Kingdom	8	32
9	Rr	Austria	16	64
E. faecium				
H0 5114-0276	Rr	East Anglia, United Kingdom	2	8
28	Rr	London	8	16
44	Rr	Scotland	4	16
H0 4418-0062	Rr	London, United Kingdom	16	32
18	Rr	Southeast United Kingdom	8	32
31	Rr	London	8	32
2 (L1)	RR	Austria	32	64
3	Rr	Austria	16	64
16	RR	United States	16	64
34	RR	Dublin, Ireland	32	64
H0 4136-0043	rr[a]	Dublin, Ireland	16	64
MRSA				
H0 4536-0367	Rr	Southeast United Kingdom	2	8
H0 4280-0256	Rr	Midlands, United Kingdom	4	16
H0 4536-0368	Rr	Southeast United Kingdom	8	32
H0 4280-0257	Rr	Midlands, United Kingdom	8	32
H0 4280-0258	Rr	Midlands, United Kingdom	8	32
B2 (posttherapy)	Rr	Brazil	8	32

[a] This strain has a novel mechanism of resistance.

TABLE 3. MICs and mutations of linezolid-selected laboratory mutants of *S. aureus*

Strain or mutant	23S rRNA mutation	MIC (μg/ml) for:	
		RWJ-416457	Linezolid
RN4220 (parent)		0.5	2
RN4220	G2447T	8	16
RN4220	G2576U	4	16
RN4220 Δ*mutS* (parent)		0.5	2
RN4220 Δ*mutS*	G2576U	8	16
RN4220 Δ*mutS* (revertant)	G2576U	8	16
RN4220 Δ*mutS*	G2576U, A2503G	8	32
ST/02/2121 (parent)		1	2
ST/02/2121	T2504C	8	16
ST/02/2121	G2447T	4	16

mutations, though its MICs remained lower than those for linezolid.

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