Multicenter Assessment of the Linezolid Spectrum and Activity Using the Disk Diffusion and Etest Methods: Report of the Zyvox® Antimicrobial Potency Study in Latin America (LA-ZAPS)

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Linezolid was the first clinically applied member of the new antimicrobial class called the "oxazolidinones". These agents have a powerful spectrum of activity focussed against Gram-positive organisms including strains with documented resistances to other antimicrobial classes. We conducted a multicenter surveillance (Zyvox Antimicrobial Potency Study; ZAPS) trial of qualifying Gram-positive isolates from 24 medical centers in eight countries in Latin America. The activity and spectrum of linezolid was compared to numerous agents including glycopeptides, quinupristin/dalfopristin, β-lactams and fluoroquinolones when testing 2,640 strains by the standardized disk diffusion method or Etest (AB BIODISK, Solna, Sweden). The linezolid spectrum was complete against staphylococci (median zone diameter, 29 - 32 mm), as was the spectrum of vancomycin and quinupristin/dalfopristin. Among the enterococci, no linezolid resistance was detected, and the susceptibility rate was 93.1 - 96.4%. Only the vancomycin-susceptible *Enterococcus faecium* strains remained susceptible (92.8%) to quinupristin/dalfopristin. Marked differences in the glycopeptide resistance patterns (*van A* versus *van B*) were noted for the 22 isolates of VRE, thus requiring local susceptibility testing to direct therapy. *Streptococcus pneumoniae* and other species were very susceptible (100.0%) to linezolid, MIC_{90} at 0.75 μg/ml. Penicillin non-susceptible rate was 27.7% and erythromycin resistance was at 17.4%. Other streptococci were also completely susceptible to linezolid (MIC_{90}, 1 μg/ml). These results provide the initial benchmark of potency and spectrum for linezolid in Latin American medical centers. Future comparisons should recognize that the oxazolidinones possess essentially a complete spectrum coverage of the monitored staphylococci, enterococci and streptococcal isolates in 2000-2001. This positions linezolid as the widest spectrum empiric choice against multi-resistant Gram-positive cocci, a spectrum of activity greater than available glycopeptides and the streptogramin combination.

Key Words: Linezolid, oxazolidinones, antimicrobial surveillance, resistant Gram-positive cocci, ZAPS.

The global emergence of resistances among Gram-positive species isolates has necessitated the rapid discovery and development of alternative agents to the penicillins, macrolides and glycopeptides [1-6]. Examples of newer classes have been the streptogramins [7], everninomicins [8] and the fluoroquinolones with expanded Gram-positive spectra, such as trovafloxacin. However, each of these cited classes do not possess an overall spectrum greater than that of glycopeptides such as vancomycin [7, 8], and emergence of resistance while on chemotherapy has been reported [9]. In fact, quinupristin/dalfopristin only inhibits *Enterococcus faecium* among the enterococci leaving some endemic or epidemic problems of vancomycin-resistant *E. faecalis* in Latin America with

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limited therapeutic options [6, 7, 10]. As the new agents are introduced into practice in Brazil and other Latin American nations, baseline susceptibility information becomes essential to monitor for subsequent emerging resistance to these novel classes [11].

Linezolid has become the first widely used agent in the oxazolidinone class to be used for problematic vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) or -coagulase-negative staphylococci (MR-CoNS), and drug-resistant *Streptococcus pneumoniae* (DRSP) [12-17]. Earlier agents in the class, such as DuP105 and DuP721 [18] exhibited promising potency, but toxicities prevented advanced human developmental trials. Scientists at Pharmacia & Upjohn described modifications of the basic oxazolidinone structure producing the safe agents, linezolid (PNU- or U-100766) and eperezolid (PNU- or U-100692), linezolid being advanced to clinical development [13]. Premarketing and post-marketing surveillance study results in Europe [19, 20] and in North America [21] have documented essentially total inhibition by linezolid of all tested native isolates of staphylococci, enterococci, streptococci and many other Gram-positive genera. Also excellent post-antibiotic effects [15], and a well defined mechanism of action have been reported [16]. One series of these multicenter surveillance investigations, the Zyvox Antimicrobial Potency Study (ZAPS), had a component that monitored the linezolid comparative activity and spectrum in 29 recruited Latin American medical centers found in nine nations [21]. The results from this controlled study using standardized, reference-quality methods [22, 23] represents the largest, geographicaly diverse study in the region for this novel oxazolidinone.

Materials and Methods

Participants. A total of 29 Latin American recruited investigators from nine countries contributed results from isolates to this study including medical centers in Argentina, Brazil, Chile, Colombia, Costa Rica, Guatemala, Mexico, Peru, and Venezuela. Each site was requested to test 150 local isolates divided among defined numbers of staphylococci, enterococci, and streptococci. The protocol design yielded a large number of species for each genus group and the comparisons of resistant phenotypes resulted in a significant number of evaluable strains. A total of 330 isolates meeting defined screening resistance criteria [24] were requested by the regional coordinator (Sao Paulo, Brazil) based on zone diameter values of linezolid (≤ 20 mm), vancomycin (≤ 14 mm), quinupristin/dalfopristin (≤ 18 mm) and teicoplanin (≤ 10 mm). Streptococcal MIC referral criteria were designated as followers for linezolid (> 4 μg/ml), trovafloxacin (> 1 μg/ml) and quinupristin/dalfopristin (> 1 μg/ml). Among the isolates meeting these criteria, only 202 viable strains were received by the regional coordinator and 67 were confirmed. Only isolates with glycopeptide or streptogramin resistance were confirmed, and these were then forwarded to the international microbiology coordinator (Iowa, USA) for further characterization.

Bacterial isolate collection. The activity of spectrum for linezolid and comparative Gram-positive and broad-spectrum antimicrobial agents was evaluated against a total of 2,640 qualifying bacterial isolates. These strains were clinically significant isolates from a wide variety of patient infections and only one strain per patient was to be included during the study period (1999-2000) according to protocol guidelines. Three genus groups (150 total strains/site) were to be tested including *S. aureus* (50 strains), CoNS (35 strains), enterococci (40 strains) and streptococci (25 strains). The collected data was sent to the regional coordinator and the international monitors in the United States (Iowa and New York, USA), the latter of which entered and processed all data sets. Identification to species level using Vitek (bioMerieux, St. Louis, MO, USA) and/or biochemical tests was performed if an unusual susceptibility pattern was of concern.

Among the tested organisms, a total of 1,582 isolates of *Staphylococcus* spp. were included in the study of which 586 and 262 strains were oxacillin-susceptible among *S. aureus* and CoNS, respectively. Oxacillin-

resistant species were also represented and included 378 strains of *S. aureus* and 356 strains of CoNS. A large collection of *Enterococcus* spp. (599 strains) were dominated by *E. faecalis* (496 strains) followed by *E. faecium* and unspeciated enterococci tested with only 22 confirmed vancomycin-resistant strains. The remaining 437 isolates were streptococcal species which included *S. pneumoniae* (339 strains), viridans group and β-haemolytic streptococci. The non-pneumococcal strains were combined for analyses purposes as the susceptibility among these two groups did not significantly vary among the tested compounds. Quality assurance using appropriate ATCC disk diffusion and MIC quality control (QC) strains included *S. aureus* ATCC 25923 and 29213 and *E. faecalis* ATCC 29212. The overall testing of these QC strains yielded 212 results for each tested antimicrobial depending upon method (Etest or disk diffusion). Data from the sites (five) with numerous aberrant QC results were eliminated from the study or if only a single drug's result were noted to be consistently problematic, all data for that agent was not included in the final analysis. Final analyses used susceptibility test data from 24 participants in eight nations.

Susceptibility testing methods. All participants used the standardized disk diffusion method [22] for non-fastidious Gram-positive pathogens or the Etest (MIC) methodology (AB BIODISK, Solna, Sweden) when testing streptococcal species. Thirteen antimicrobial agents were evaluated against the non-streptococcal isolates using the disk diffusion method on Mueller-Hinton agar plastes and included Gram-positive spectrum compounds, as well as broader spectrum agents. These tested drugs included: linezolid, MLS_B compounds, β-lactams, glycopeptides, trovafloxacin, chloramphenicol, doxycycline and gentamicin (high-level; *Enterococcus* spp. only). A total of six compounds (linezolid, quinupristin/dalfopristin, penicillin, erythromycin, ceftriaxone and trovafloxacin) were tested against the streptococcal isolates on Mueller-Hinton agar supplemented with 5% sheep blood. All methods and interpretive criteria complied with NCCLS [22-24] and/or manufacturer's recommendations. The disk diffusion interpretive criteria for linezolid were [24-26]: for *Staphylococcus* spp., *S. pneumoniae*, and *Streptococcus* other than *S. pneumoniae*, susceptible at ≥ 21 mm; and for *Enterococcus* spp., susceptible at ≥ 23 mm and resistant at ≤ 20 mm. When using MIC methods (Etest) for linezolid susceptibllity was defined at ≤ 2 μg/ml for the streptococci [24].

Results

Linezolid activity against staphylococci. Table 1 shows the comparative activity and spectrum of linezolid tested by the standardized disk diffusion method [] against 1,582 staphylococci. Linezolid, quinupristin/dalfopristin and vancomycin exhibited complete activity (100% susceptibility rates) against all tested staphylococci. Teicoplanin was almost as effective *in vitro*, but rare (0.7 - 6.7%) strains showed zones in the non-susceptible range. The median zone diameters for the four most effective agents varied only to a minor degree, most evident was the reduced potency of quinupristin/dalfopristin against *S. aureus* strains (25 or 26 mm) compared to the CoNS isolates (29-30 mm). This variation was approximately equal to a one $\log_2$ dilution step reduction in potency for *S. aureus* isolates.

The activity of the other seven comparison agents was lower among oxacillin-resistant strains. For example, the susceptibility rates of oxacillin-resistant *S. aureus* (ORSA) was reduced by 99.5% for cefazolin, 94.2% for ceftriaxone, 78.6% for clindamycin, 69.1% for erythromycin, 43.2% for chloramphenicol, 19.5% for doxycycline and 10.4% for trovafloxacin. The newer fluoroquinolone, trovafloxacin, remained active against 89.1% of ORSA and 87.7% of OR-CoNS.

Linezolid activity against vancomycin-susceptible enterococci. The activity of linezolid by the disk diffusion test against 599 strains of vancomycin-susceptible *E. faecalis* and *E. faecium* plus 27 other enterococcal species are summarized in Table 2. Only linezolid, vancomycin and teicoplanin demonstrated

Table 1. Antimicrobial activity and spectrum of linezolid compared to 10 other agents tested against 1,582 strains of staphylococci isolated in Latin America medical centers

Organism (no. tested)	Antimicrobial agent	Zone diameter (mm)		% by category:[a]	
		Median	Range	Susceptible	Resistant
S. aureus					
oxacillin-susceptible (586)	Linezolid	29	21-44	100.0	-[b]
	Quinupristin/Dalfopristin	25	19-42	100.0	0.0
	Vancomycin	18	15-34	100.0	-[b]
	Teicoplanin	17	6-30	99.2	0.2
	Erythromycin	25	6-40	76.5	14.7
	Clindamycin	26	6-36	95.4	2.4
	Chloramphenicol	24	6-35	96.4	3.0
	Doxycycline	28	10-45	95.2	1.3
	Trovafloxacin	32	6-49	99.5	0.3
	Cefazolin	28	6-50	99.5	0.3
	Ceftriaxone	26	6-38	94.2	1.0
oxacillin-resistant (378)	Linezolid	30	22-44	100.0	-[b]
	Quinupristin/Dalfopristin	26	19-39	100.0	0.0
	Vancomycin	19	15-30	100.0	-[b]
	Teicoplanin	16	11-25	98.0	0.0
	Erythromycin	6	6-33	7.4	91.6
	Clindamycin	6	6-33	16.8	81.9
	Chloramphenicol	19	6-35	53.2	45.5
	Doxycycline	19	6-37	75.7	4.9
	Trovafloxacin	21	6-37	89.1	3.5
	Cefazolin	6	6-33	0.0	100.0
	Ceftriaxone	6	6-30	0.0	100.0
Coagulase-negative					
oxacillin-susceptible (262)	Linezolid	32	24-44	100.0	-[b]
	Quinupristin/Dalfopristin	29	20-40	100.0	0.0
	Vancomycin	20	16-30	100.0	-[b]
	Teicoplanin	18	13-32	99.3	0.0
	Erythromycin	28	6-46	72.7	25.2
	Clindamycin	28	6-44	92.8	6.1
	Chloramphenicol	25	6-44	92.3	6.9
	Doxycycline	29	6-44	90.0	6.9
	Trovafloxacin	33	16-48	99.3	0.0
	Cefazolin	34	12-48	99.6	0.4
	Ceftriaxone	30	6-43	98.1	0.4
oxacillin-resistant (356)	Linezolid	30	24-50	100.0	-[b]
	Quinupristin/Dalfopristin	30	19-40	100.0	0.0
	Vancomycin	19	15-30	100.0	-[b]
	Teicoplanin	17	6-27	93.3	0.3
	Erythromycin	6	6-42	24.7	74.0
	Clindamycin	6	6-41	44.2	54.5
	Chloramphenicol	21	6-38	55.8	41.7
	Doxycycline	25	6-42	82.1	13.6
	Trovafloxacin	25	6-43	87.7	6.2
	Cefazolin	24	6-42	0.0	100.0
	Ceftriaxone	15	6-36	0.0	100.0

a. Susceptibility categories defined by the NCCLS [24].
b. - = no category of resistance published in current standards [24].

Table 2. Antimicrobial activity and spectrum of linezolid compared to 11 other agents tested against 599 strains of enterococci isolated in Latin America medical centers

Organism (no. tested)	Antimicrobial agent	Zone diameter (mm)		% by category:[a]	
		Median	Range	Susceptible	Resistant
E. faecalis					
vancomycin-susceptible (496)	Linezolid	26	21-36	93.1	0.0
	Quinupristin/Dalfopristin	12	6-34	13.4	75.5
	Vancomycin	19	17-28	100.0	0.0
	Teicoplanin	18	14-25	99.8	0.0
	Ampicillin	25	6-39	96.5	3.5
	Erythromycin	6	6-36	35.7	39.3
	Clindamycin	6	6-30	1.8	97.8
	Chloramphenicol	20	6-30	64.2	27.6
	Doxycycline	15	6-40	44.6	26.7
	Trovafloxacin	22	6-35	73.4	22.4
	Cefazolin	14	6-38	15.1	53.4
	Ceftriaxone	6	6-35	6.3	85.1
E. faecium					
vancomycin-susceptible (76)	Linezolid	27	21-37	94.7	0.0
	Quinupristin/Dalfopristin	22	12-32	92.8	2.4
	Vancomycin	22	17-28	100.0	0.0
	Teicoplanin	18	12-25	98.8	0.0
	Ampicillin	15	6-30	42.2	57.8
	Erythromycin	11	6-34	9.6	61.4
	Clindamycin	6	6-30	20.5	77.1
	Chloramphenicol	22	6-31	78.3	3.6
	Doxycycline	18	6-36	59.0	30.1
	Trovafloxacin	19	6-31	66.3	20.5
	Cefazolin	6	6-34	8.4	84.3
	Ceftriaxone	6	6-34	9.6	85.5
Enterococcus spp.[b] (27)	Linezolid	28	22-40	96.4	0.0
	Quinupristin/Dalfopristin	19	12-38	57.1	35.7
	Vancomycin	19	16-27	96.4	0.0
	Teicoplanin	18	15-26	100.0	0.0
	Ampicillin	27	6-32	92.9	7.1
	Erythromycin	20	6-29	35.7	39.3
	Clindamycin	11	6-27	28.6	64.3
	Chloramphenicol	23	12-29	92.9	7.1
	Doxycycline	28	13-34	85.7	0.0
	Trovafloxacin	23	11-30	71.4	28.6
	Cefazolin	13	6-22	35.7	50.0
	Ceftriaxone	15	6-36	28.6	35.7

a. Susceptibility categories defined by the NCCLS [24].
b. Includes unspeciated enterococci and other non-faecalis, non-faecium isolates such as *E. avium, E. durans, E. gallinarum* and *E. casseliflavus.*

Table 3. Zone diameter distribution for vancomycin-resistant enterococci (22 strains)[a] tested against linezolid, quinupristin/dalfopristin (Q/D), ampicillin, chloramphenicol and doxycycline. Results from Latin American medical centers

	No. of occurrences for:				
Zone diameter in mm	**Linezolid**	**Q/D**	**Ampicillin**	**Chloramphenicol**	**Doxycycline**
6-11		7	13	8	
12				0[b]	3[b]
13					2
14		1			2
15		1[b]			
16			0[b]		
17			0[c]		0[c]
18		1			
19		0[c]	1	1[c]	
20	0[b]	1		1	
21				6	
22		1	2		2
23	0[c]	6	2	1	
24	1	1	2	1	2
≥25	21	3	2	4	11
% susceptible	100.0	54.5	40.9	63.6	63.6
% resistant	0.0	36.4	59.1	36.4	13.6

a. Includes *E. faecalis* (seven strains), *E. faecium* (14 strains) and *Enterococcus* spp., NOS (one strain).
b. Indicates the resistant breakpoint zone diameter [24].
c. Indicates the susceptible breakpoint zone diameter [24].

Table 4. Linezolid antimicrobial activity compared to five other agents tested by Etest (AB BIODISK, Solna, Sweden) against 339 strains of *S. pneumoniae* isolated from Latin American medical centers.

		Penicillin susceptibility pattern (no. tested)[a]			
Antimicrobial agent	**Parameter**	**Susceptible (225)**	**Intermediate (74)**	**Resistant (20)**	**All strains (339)**
Linezolid	% S/R[a]	100.0/-[b]	100.0/-	100.0/-	100.0/-
	$MIC_{50/90}$[c]	0.5/0.75	0.5/0.75	0.5/0.75	0.5/0.75
Quinupristin/Dalfopristin	% S/R	100.0/0.0	100.0/0.0	100.0/0.0	100.0/0.0
	$MIC_{50/90}$	0.5/1	0.5/1	0.5/1	0.5/1
Erythromycin	% S/R	89.8/9.8	64.9/33.8	65.0/35.0	82.6/16.8
	$MIC_{50/90}$	0.064/0.19	0.125/6	0.094/>256	0.064/3
Trovafloxacin	% S/R	100.0/0.0	100.0/0.0	100.0/0.0	100.0/0.0
	$MIC_{50/90}$	0.094/0.19	0.094/0.125	0.064/0.19	0.094/0.19
Ceftriaxone	% S/R	100.0/0.0	100.0/0.0	90.0/0.0	99.4/0.0
	$MIC_{50/90}$	0.012/0.047	0.19/0.5	0.75/1	0.016/0.38
Penicillin	% S/R	100.0/0.0	0.0/0.0	0.0/100.0	69.3/5.8
	$MIC_{50/90}$	0.016/0.047	0.25/1	2/4	0.023/0.75

a. Susceptibility (S) and resistant (R) criteria of the NCCLS [24].
b. - = no criteria published for intermediate or resistant MICs.
c. MIC in µg/ml.

Table 5. MIC distributions for linezolid and five comparison agents tested by Etest (AB BIODISK, Solna, Sweden) against 98 non-pneumococcal *Streptococcus* spp. isolates from Latin America

	Cum. % inhibited at MIC (μg/ml) of:											
Antimicrobial agent	**0.004**	**0.008**	**0.016**	**0.032**	**0.064**	**0.125**	**0.25**	**0.5**	**1**	**2**	**≥4**	**% susc.[a]**
Linezolid	0.0	0.0	0.0	0.0	0.0	3.1	14.3	52.0	91.8	100.0[b,c]	-	100.0/100.0
Quinupristin/Dalfopristin	0.0	0.0	0.0	0.0	1.9	4.9	41.7	83.5	99.0[b,c]	100.0	-	99.0/99.0
Erythromycin	0.0	1.0	5.1	27.6	53.1	69.4	72.4[b,c]	78.6	79.6	81.6	100.0	72.4/72.4
Trovafloxacin	0.0	1.0	2.0	14.3	38.8	76.5	95.9	98.0	100.0[b,c]	-	-	100.0/100.0
Ceftriaxone	2.0	7.1	37.8	63.3	76.5	88.8	94.9	96.9[b]	98.0[c]	98.0	100.0	96.9/98.0
Penicillin	4.1	28.6	54.1	63.3	83.7	86.7[b,c]	92.9	94.9	96.9	98.0	100.0	86.7/86.7

a. Susceptibility rates using the NCCLS [24] criteria for β-haemolytic species/viridans group strains.
b. Susceptibility breakpoint used for β-haemolytic streptococci [24].
c. Susceptibility breakpoint used for viridans group streptococci [24].

high *in vitro* spectra against these strains (93.1 to 100.0% susceptibility with no resistant isolates). Quinupristin/dalfopristin was active against 92.8% of vancomycin-susceptible *E. faecium*, but only 13.4% of *E. faecalis* and 57.1% of other enterococci. Ampicillin remained a treatment option against *E. faecalis* (96.5% susceptible) and the other *Enterococcus* spp. (92.9%), but not for *E. faecium* (42.2%). Cephalosporins, macrolides and clindamycin were generally not effective *in vitro*. Widest spectrums among the other tested agents were found for chloramphenicol (64.0% susceptible) and trovafloxacin (73.4%) against *E. faecalis*, and the same two drugs plus doxycycline against *E. faecium* (59.0 - 78.3%).

Linezolid activity against vancomycin-resistant enterococci. Vancomycin resistance among the enterococci (VRE) isolated in this multicenter investigation was relatively uncommon and only 22 strains (3.5%) were detected. These strains were distributed by species as follows (Table 3): *E. faecalis* (seven strains), *E. faecium* (14 strains) and *Enterococcus* spp., NOS (one strain). Only two countries (Argentina and Brazil) contributed VRE strains. In Argentina, 14 VRE isolates were identified of which one strain had a *van C* phenotype and 13 *E. faecium* strains had the *van A* phenotype. In contrast, Brazil had only one VRE *E. faecium* (*van A*) and the seven remaining isolates were *van A* phenotypes found in *E. faecalis* [10].

Table 3 shows the distribution of reported zone diameter around five selected antimicrobial disks (linezolid, quinupristin/dalfopristin, ampicillin, chloramphenicol, doxycycline) when testing the 22 VRE in Latin America. All linezolid zone diameters were in the susceptible range (≥ 24 mm), in contrast to the alternative agents displayed in Table 3. The susceptibility rates for the other agents ranged from 40.9% (ampicillin) to 63.6% (chloramphenicol and doxycycline). Quinupristin/dalfopristin was active against 84.6% of the VRE *E. faecium* isolates from Argentina; the Brazil strain had an intermediate susceptibility pattern. None of the Brazilian VRE (*E. faecalis*) were susceptible to the newer streptogramin combination.

Linezolid activity against *S. pneumoniae*. The pneumococcal strains in this *in vitro* trial were tested by Etest (AB BIODISK) and precise MIC results were generated over a 15 $\log_2$ dilution scale (Table 4). Linezolid, quinupristin/dalfopristin and trovafloxacin were active against all 339 tested pneumococci at MIC values at or below NCCLS breakpoints [24]. Each of these potent drugs did not have their activity adversely influenced by resistances to penicillin (Table 4) or macrolides (data not shown). The MIC_{50} (0.5 μg/ml) and MIC_{90} (0.75 μg/ml) for linezolid did not vary for *S. pneumoniae* strains that were susceptible, intermediate or resistant to penicillin (Table 4). In contrast, erythromycin and ceftriaxone potency and/

or spectrums diminished as the resistance to penicillin increased. Although ceftriaxone was at least 60-fold less active against penicillin-resistant *S. pneumoniae* strains, this "third-generation" cephalosporin was judged to be effective against 90.0% of strains (MIC, ≤ 1 μg/ml) [24]. For the macrolides, resistance rates increased from 9.8 to 35.0% among penicillin-resistant pneumococci.

Linezolid activity against other streptococci. The MIC results for the remaining β-haemolytic and viridans group streptococci (98 strains) were combined and presented as a cumulative percentage inhibited chart (Table 5). All linezolid MIC values were ≤ 2 μg/ml, and the MIC_{50} and MIC_{90} results were 0.5 and 1 μg/ml, respectively. Trovafloxacin also inhibited all strains at concentrations at or below its breakpoint, and only one isolate was resistant to quinupristin/dalfopristin. The least effective agent *in vitro* was erythromycin at 72.5% susceptible, and the susceptibility rate for penicillin was only 86.7%. All penicillin-resistant strains were among the viridans group streptococcal isolates. The ceftriaxone susceptibility rate ranged from 96.9 to 98.0%.

Discussion

The development of the oxazolidinones [12] has successfully met the needs for a class of antimicrobials that can effectively treat problematic, resistant Gram-positive cocci [13]. The results of this study establishes the universal activity of linezolid against these Gram-positive species isolated from patients in Latin American nations. Previously published reports by individual investigations have established the extent of linezolid potency against the enterococci, staphylococci and streptococci with the activity ranging across a narrow range of MIC values (0.5 - 4 μg/ml) [14, 15, 17]. These cited results have been substantiated by various national level [20, 21, 27] and regional surveillance studies [19] of linezolid spectrum generally using acceptable reference-quality methods [22-24].

The unique mode of action directed against protein synthesis where the oxazolidinone inhibits the initiation complex by binding to the 50S ribosomal subunit minimizes the probability of prior selected mutations, and no cross-resistance has been observed with other agents [13-16]. However, linezolid-resistant strains have emerged on extended chemotherapy during the clinical trials especially among patients with infected indwelling devices [28]. Since the clinical release of linezolid, two reports have also been published describing linezolid refractory *Enterococcus* spp. [29] and one patient having a *S. aureus* with a linezolid MIC of > 32 μg/ml [30]. The mechanisms of resistance have been studied and these organisms contain 23S rRNA gene mutations at G2447U and G2528U for laboratory-derived mutants [13] and at G2576U among various clinical cases [28-30]. These mutational events are rare and patients receiving prolonged courses of linezolid should be closely monitored for evolving resistant strains; in fact the linezolid MIC of the mutant strains may only elevate to 8 μg/ml (one $\log_2$ dilution above the susceptible breakpoint) requiring the use of quality susceptibility testing procedures and reagents. Mutational events to resistance have been observed earlier with Gram-positive pathogens from patients receiving glycopeptides, streptogramin combinations [9], rifampin, macrolides and other classes [9, 13]. Furthermore, evidence has emerged that environmental contamination by linezolid- or quinupristin/dalfopristin-resistant enterococci can compromise patients via nosocomial infections, where no evidence of prior oxazolidinone patient exposure could be determined [31].

Currently in Latin America, linezolid activity and spectrum was the most complete among monitored agents, and the oxazolidinones appear applicable to a wide range of infection therapies for multi-resistant Gram-positive cocci. This baseline evaluation should serve as a reference to all subsequent investigations [11] of newer agents directed against enterococci, staphylococci and streptococci (streptogramins, oxazolidinones, later-generation fluoroquinolones, novel glycopeptides). Regardless of the favorable spectrum of linezolid, susceptibility testing should be performed to guide therapy, with strains having non-susceptible range test results forwarded to reference

laboratories for confirmation and genetic characterization. This prudent practice [11] appears to be a necessity to prevent false-resistant information from negatively impacting the use of newer agents such as that described for quinupristin/dalfopristin [7, 32].

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