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Report of Linezolid Resistance from the 2007 Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program (Europe, Latin America, Asia-Pacific)

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ABSTRACT

Objectives: To monitor the in vitro activity of linezolid, the ZAAPS Program (year 6; 2007) was initiated in various geographic areas of the world. LZD, the first oxazolidinone agent clinically applied, has become an important therapeutic addition for infections caused by antimicrobial-R Gram-positive (GP) pathogens. LZD-R has been observed particularly in enterococci (ENT) and recently among coagulase-negative staphylococci (CoNS), but occurrence rates are extremely low for indicated *S. aureus* (SA) and streptococci.
Methods: 5,591 GP strains were collected from 64 sites in 23 countries in 2007. Strains were received from the following organism groups: SA (3000), CoNS (716), ENT (906), *S. pneumoniae* (SPN; 452), viridans group (VGS; 155) and Beta-haemolytic streptococci (BHS; 362). At least 200 isolates from each country (expect China [800] and United Kingdom [400]) were requested to be sent to a reference laboratory for CLSI broth microdilution susceptibility (S) testing.
Results: The ZAAPS reports from 2006 and 2007 cited LZD-R *S. epidermidis* from the same hospital in Rome (Table). Further examination of other *S. epidermidis* isolates from the same hospital showed five other strains with LZD MIC values of 4 or 8 mg/L. MRSA rates ranged from 1.7% (Sweden) to 68.0% (Japan). Among vancomycin resistant enterococci, Korea had the highest rate of 38.6%. SPN had overall penicillin and erythromicin resistance rates of 20.1% and 33.6%, respectively. All streptococci had LZD MIC values of ≤2 mg/L. Overall LZD-R was 0.07% (0.12% in 2006). LZD-R rates among organism groups were: SA (0.03%), CoNS (0.28%), and ENT (0.11%).

Table: Linezolid-R isolates found in the 2007 ZAAPS Program.

Species	City/Country	LZD MIC (mg/L)	R- mechanism (23S mutation)
S. aureus	Dublin/Ireland	8	G2576T
S. epidermidis[a]	Rome/Italy	8	G2576T
S. epidermidis[a]	Rome/Italy	8	G2576T
E. faecalis	Brasilia/Brazil	>8	G2576T

a. Clonal.

Conclusions: LZD remained highly active against contemporary pathogens from indicated organism groups with an overall S rate of 99.83%. As LZD use continues to evolve, the need for continued monitoring of the LZD in vitro activity versus Gram-positive pathogens and for the emergence of R is apparent.

INTRODUCTION

Glycopeptide antimicrobial agents (vancomycin or teicoplanin) have become widely used for the treatment of serious infections caused by Gram-positive organism especially methicillin (oxacillin)- resistant *Staphylococcus aureus* (MRSA). Linezolid, a member of the antimicrobial class, oxazolidinones, offers an alternative therapy for the treatment of infections due to multidrug-resistant (MDR) Gram-positive species as well as uncommonly isolated pathogens that may infect compromised hosts. Favorable clinical trial outcomes have been demonstrated in subjects with complicated skin and skin structure infections and pneumonia due to MDR pathogens.

Linezolid resistance, though uncommon, has been well described with the majority of linezolid resistance due to single-nucleotide changes in varying numbers of encoding gene copies (dosing) for 23S rRNA. Rare cases of oxazolidinone resistance due to acquisition of the *cfr* gene encoding a methyltransferase that methylates 23S rRNA at position A2503 has been reported. The latter mechanism of oxazolidinone resistance also confers co-resistances to phenicols (chloramphenicol), lincosamides (clindamycin), pleuromutilins (retapamulin) and streptogramin A agents.

Current surveillance studies are designed to monitor the in-vitro activities of linezolid and comparator antimicrobials and detect the development of resistance. The most notable oxazolidinone in-vitro surveillance networks have been the LEADER Program for the United States (USA) and ZAPS and ZAAPS Programs for the rest of the world. This presentation summarizes the surveillance results for the 2007 Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program, with organisms monitored from 64 sites in 23 countries outside of the USA.

MATERIALS AND METHODS

The number of processed strains (5,591; 103.5% compliance to protocol) were all from Gram-positive species collected from 64 medical centers in 23 countries (Table 1). Each participating country with the exception of the United Kingdom and China which submitted more isolates, forwarded a target total of 200 consecutive, non-duplicate patient isolates from infections of the bloodstream, respiratory tract, urinary tract, or wound/skin and skin structure.

All isolates were identified by the submitting laboratory and confirmed by the central facility using standardized and commercial methods (VITEK 2 system; bioMerieux, Hazelwood, Missouri, USA). Isolates were grouped for analysis as follows: *S. aureus* (3,000 strains), CoNS (716 strains), β-haemolytic streptococci (362 strains), viridans group streptococci (155 strains), *Streptococcus pneumoniae* (452 strains), and enterococci (906 strains).

Antimicrobial susceptibility testing (linezolid and comparators) was performed using validated microdilution panels with cation-adjusted Mueller-Hinton broth (2-5% lysed horse blood supplement for testing fastidious streptococci) produced by TREK Diagnostics (Cleveland, Ohio, USA). The categorical interpretations of MIC results were those published by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) in M100-S18 [2008] and quality control (QC) organism (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619) results were within the acceptable published ranges. Isolates having linezolid MIC values in the non-susceptible range were repeated by the CLSI M7-A7 method and further subjected to alternative tests using disk diffusion and Etest (AB BIODISK, Solna, Sweden) methods.

Molecular testing was performed to identify the 23S rRNA target site mutation and possible epidemic clonality using pulsed-field gel electrophoresis (PFGE), automated ribotyping and various PCR tests as previously described. Furthermore, molecular tests to identify the *cfr* gene encoding resistances to oxazolidinones were performed as described by Mendes et al, 2008. Other potential target site modifications associated with increased linezolid MICs were also examined.

RESULTS

- A total of 3,000 *S. aureus* isolates were tested (1,416/2,276 tested in 2005/2006) from 23 nations. MRSA rates were as follows: Canada (55.7%; compliant to local protocol asking for >50% MRSA isolates to be submitted), for Latin America (average 50.1%, range 29.0% [Brazil] to 64.1% [Mexico]), for Europe (average 28.2%, range 1.7% [Sweden] to 56.2% [Ireland]) and the APAC region (average 44.2%; range 25.3% [Australia] to 68.0% [Japan]) (Tables 1 and 2).
- For *S. aureus* tested against linezolid, the MIC_{50} and MIC_{90} was 2 mg/L and the MIC distribution illustrates a broad mode at 1 and 2 mg/L (data not shown). One MRSA strain from Ireland was detected with a non-susceptible linezolid value at 8 mg/L (Table 3).
- Among the 716 CoNS tested, oxacillin resistance rates ranged from 57.1% (Thailand) to 95.8% (Korea) and 13 of the 23 nations had rates greater than 80%. Linezolid was consistently more potent by one doubling dilution step against CoNS when compared to the *S. aureus* isolates (Table 2).
- Two *S. epidermidis* isolates were detected with linezolid MIC results at 8 mg/L (non-susceptible). One of these CoNS strains was isolated in a cancer treatment medical center in Rome, the same location where similar strains were detected in 2006. The other non-susceptible CoNS was found in Genoa, Italy (Table 3).
- Among 906 summarized enterococcal isolates (Table 2), the species distribution was *E. faecalis* (545), and *E. faecium* (336) that included 97.2% of isolates tested. Vancomycin-resistant isolates (VRE) were tabulated at 8.2% overall (includes intermediate category; 8.3% in 2006), however the rates of VRE varied widely among nations.
- Linezolid resistances were rare in enterococci (0.1%) with only one resistant *E. faecalis* from Brasilia, Brazil having the G2576T ribosomal target mutation (Table 3).
- Linezolid demonstrated excellent in vitro activity vs. streptococci (Table 2). The MIC_{50} and MIC_{90} was 1 mg/L for each of the three streptococcal groups tested.
- Overall, linezolid remains stable and without significant occurrence of isolates with MIC values of ≥8 mg/L (four isolates or 0.07% of all organisms tested).

Table 1. Distribution of organism identifications for the 2006 and 2007 ZAAPS samples indexed by nation of origin (4,216/5,591 strains).

	No. of strains						Totals	
Nation (no. medical centers)	*S. aureus*	CoNS	Enterococci	*S. pneumoniae*	vir. gr. streptococci	β-streptococci	2006	2007
Canada (2)	106	32	20	20	10	12	991	200
Argentina (2)	100	50	20	20	6	14	211	210
Brazil (4)	100	44	20	21	2	18	258	205
Chile (2)	120	25	20	30	0	19	234	214
Mexico (2)	128	29	20	17	0	27	199	221
Belgium (1)	88	50	32	33	5	21	NS[a]	229
France (5)	100	47	30	20	10	12	209	219
Germany (4)	106	40	20	10	14	34	210	224
Ireland (2)	130	11	20	21	6	20	NS	208
Italy (3)	98	53	20	20	0	18	207	209
Poland (1)	62	2	36	42	0	1	NS	143
Spain (2)	124	20	20	30	2	15	211	211
Sweden (2)	120	21	20	20	10	10	218	201
Turkey (2)	86	73	25	39	6	1	NS	230
United Kingdom (2)	223	57	40	40	21	20	208	401
Australia (5)	486	27	39	32	8	46	269	638
China (10)	403	29	365	0	3	2	343	802
Hong Kong (1)	50	1	6	0	7	12	101	76
Indonesia (5)	18	11	7	0	12	2	NS	50
Japan (2)	100	41	20	19	10	8	NS	198
Korea (2)	155	24	57	5	4	27	220	272
Taiwan (2)	73	22	28	9	14	15	127	161
Thailand (1)	24	7	21	4	5	8	NS	69
TOTAL (64)	3,000	716	906	452	155	362	4,216	5,591

a. NS = not sampled.

Table 2. Comparative activity of linezolid tested against 5,591 Gram-positive cocci from 23 nations in the ZAAPS Program (2007).

Organism (no. tested)/ antimicrobial agent	MIC (mg/L) 50%	MIC (mg/L) 90%	% by category[a] Susceptible/ Resistant
S. aureus			
All strains (3,000)			
Linezolid	2	2	>99.9 / -
Ceftriaxone[a]	4	>32	61.8 / 38.2
Ciprofloxacin	0.5	>4	64.2 / 34.9
Clindamycin	≤0.25	>2	74.3 / 25.6
Erythromycin	0.5	>2	57.6 / 42.0
Gentamicin	≤2	>8	79.7 / 19.8
Levofloxacin	≤0.5	>4	65.2 / 34.6
Oxacillin[a]	0.5	>2	61.8 / 38.2
Penicillin	8	>32	11.6 / 88.4
Q/D[b]	≤0.25	0.5	>99.9 / <0.1
Teicoplanin	≤2	≤2	100.0 / 0.0
Tetracycline	≤2	>8	80.2 / 19.6
TMP/SMX[b]	≤0.5	≤0.5	93.1 / 6.9
Vancomycin	1	1	100.0 / 0.0
MRSA (1,147)			
Linezolid	1	2	99.9 / -
MSSA (1,853)			
Linezolid	2	2	100.0 / -
Coagulase-negative staphylococci (716)[c]			
Linezolid	1	1	99.7 / -
Ceftriaxone[a]	16	>32	20.4 / 79.6
Ciprofloxacin	4	>4	43.3 / 54.5
Clindamycin	≤0.25	>2	69.0 / 30.2
Erythromycin	>2	>2	35.8 / 64.0
Gentamicin	≤2	>8	59.7 / 31.6
Levofloxacin	4	>4	43.7 / 52.4
Oxacillin[a]	>2	>2	20.4 / 79.6
Penicillin	4	32	13.7 / 86.3
Q/D[b]	≤0.25	0.5	98.9 / 0.6
Teicoplanin	≤2	8	97.5 / 0.3
Tetracycline	≤2	>8	82.7 / 16.6
TMP/SMX[b]	≤0.5	>2	60.5 / 39.5
Vancomycin	1	2	99.9 / 0.0

Organism (no. tested)/ antimicrobial agent	MIC (mg/L) 50%	MIC (mg/L) 90%	% by category[a] Susceptible/ Resistant
Enterococci			
All strains (906)[d]			
Linezolid	2	2	99.3 / 0.1
Ampicillin[a]	2	>16	64.0 / 36.0
Ciprofloxacin	>4	>4	39.3 / 55.2
Erythromycin	>2	>2	4.4 / 78.6
Gentamicin (HL)	1000	>1000	49.7 / 50.3
Levofloxacin	>4	>4	45.0 / 52.5
Penicillin[a]	4	>32	61.7 / 38.3
P/T[ab]	8	>64	64.0 / -
Q/D[b]	>2	>2	26.6 / 63.0
Streptomycin (HL)	≤1000	>2000	59.9 / 40.1
Teicoplanin	≤2	≤2	94.2 / 5.1
Vancomycin	1	4	91.8 / 7.1
VRE (74)			
Linezolid	1	2	97.3 / 0.0
VSE (832)			
Linezolid	2	2	99.5 / 0.1
S. pneumoniae			
All strains (452)			
Linezolid	1	1	100.0 / -
A/C[b]	≤1	2	92.0 / 4.2
Ceftriaxone	≤0.25	1	92.3 / 1.8
Ciprofloxacin	1	2	(2.2)[e]
Clindamycin	≤0.25	>2	81.9 / 17.9
Erythromycin	≤0.25	>2	65.9 / 33.6
Levofloxacin	1	1	98.9 / 0.7
Penicillin	≤0.03	2	66.2 (92.7) / 20.1 (1.1)[f]
Q/D[b]	0.5	1	99.2 / 0.0
Tetracycline	≤2	>8	73.0 / 25.9
TMP/SMX[b]	≤0.5	>2	61.9 / 26.8
Vancomycin	≤1	≤1	100.0 / -
MDR-3[g] (38)			
Linezolid	1	1	100.0 / -
MDR-4[h] (35)			
Linezolid	1	1	100.0 / -
MDR-5[i] (23)			
Linezolid	0.5	1	100.0 / -

Organism (no. tested)/ antimicrobial agent	MIC (mg/L) 50%	MIC (mg/L) 90%	% by category[a] Susceptible/ Resistant
Viridans group streptococci (155)[j]			
Linezolid	1	1	100.0 / -
A/C[ab]	≤1	2	72.9 / -
Ceftriaxone	≤0.25	1	93.5 / 4.5
Ciprofloxacin	1	4	(21.9)[e]
Clindamycin	≤0.25	0.5	89.0 / 9.7
Erythromycin	≤0.25	>2	63.9 / 34.2
Levofloxacin	1	2	94.8 / 2.6
Penicillin[a]	0.06	1	72.9 / 4.5
Q/D[b]	0.5	1	94.8 / 1.3
Tetracycline	≤2	>8	53.5 / 44.5
Vancomycin	0.5	1	100.0 / -
β-haemolytic streptococci (362)[k]			
Linezolid	1	1	100.0 / -
A/C[ab]	≤1	≤1	100.0 / -
Ceftriaxone	≤0.25	≤0.25	100.0 / -
Ciprofloxacin	0.5	1	(1.4)[e]
Clindamycin	≤0.25	≤0.25	92.5 / 7.2
Erythromycin	≤0.25	>2	84.5 / 14.4
Levofloxacin	≤0.5	1	98.6 / 1.4
Penicillin[a]	≤0.015	0.06	100.0 / -
Q/D[b]	≤0.25	0.5	100.0 / 0.0
Tetracycline	≤2	>8	53.0 / 45.6
Vancomycin	0.5	0.5	100.0 / -

a. Criteria as published by the CLSI [2008]. beta-lactam susceptibility should be directed by the oxacillin test results for staphylococci and by ampicillin or penicillin for Enterococcus or streptococci.
b. Q/D=quinupristin/dalfopristin, TMP/SMX=trimethoprim/sulfamethozazole, P/T=piperacillin/tazobactam, and A/C=amoxicillin/clavulanic acid.
c. Includes: 16 species (514 strains) and unspecated coagulase-negative staphylococci (202 strains).
d. Includes: *Enterococcus faecalis* (545 strains), *Enterococcus faecium* (336 strains), and 25 other enterococci.
e. Percentage of pneumococci or other streptococci with ciprofloxacin MICs at ≥4 mg/L, indicating possible QRDR mutations.
f. CLSI 2008 susceptibility breakpoints for parenteral penicillin (nonmeningits).
g. MDR-3 = resistant to three agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, and clindamycin ≥1 mg/L.
h. MDR-4 = resistant to four agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, clindamycin ≥1 mg/L, and tetracycline ≥8 mg/L.
i. MDR-5 = resistant to five agents eg. penicillin ≥2 mg/L, erythromycin ≥1 mg/L, clindamycin ≥1 mg/L, tetracycline ≥8 mg/L, and trimethoprim-sulfamethoxazole ≥4 mg/L.
j. Includes: 12 species (109 strains), *Streptococcus bovis* group (3 strains), unspecated viridians group streptococci (43 strains).
k. Includes: Group A (183 strains), Group B (127 strains), Group C (8 strains), Group F (2 strains), Group G (28 strains), unspecated beta-haemolytic streptococci (2 strains), and two other species (12 strains).

Table 3. Comparisons of the 2006 and 2007 ZAAPS Program results for linezolid MIC distributions by cumulative percentages of staphylococcal and enterococcal isolates inhibited.

Organism group	Study Year (no.tested)	Cum. % inhibited at linezolid MIC (mg/L) ≤0.12	0.25	0.5	1	2	4	≥8	% Susc.
S. aureus	2006 (2,276)	0.0	0.2	1.7	43.0	>99.9	100.0	-	100.00
	2007 (3,000)	0.0	0.1	1.5	49.0	99.9	99.9	100.0[a]	99.97
CoNS	2006 (615)	0.5	2.0	33.3	97.7	99.5	99.5	99.7[b]	99.51
	2007 (716)	0.1	2.0	44.8	98.2	99.6	99.8	100.0[c]	99.77
Enterococci	2006 (423)	0.0	0.0	2.6	60.8	99.1	99.5[d]	100.0[e]	99.05
	2007 (906)	0.0	0.0	2.0	49.0	99.3	99.9[f]	100.0[f]	99.34

a. One strain from Ireland (G2576T mutation).
b. Three strains from two medical centers (Brazil and Italy).
c. Two strains from two different medical centers in Italy.
d. Two strains.
e. Two strains (China and Germany).
f. Five strains from China at 4 mg/L (Intermediate) and one strain from Brazil at >8 mg/L.

CONCLUSIONS

- Linezolid, monitored in 23 countries, maintained high activity and a wide Gram-positive spectrum with rare occurrences of resistance (0.07%) and no evidence of "MIC creep" compared to results from prior years of the ZAAPS Program.
- Linezolid resistance occurred in only four strains, one isolate each from Brazil and Ireland with two strains from Italy.
- Other Gram-positive spectrum agents tested (vancomycin, teicoplanin, and quinupristin/dalfopristin) exhibited activity that was less than perfect (100% susceptible rates), each having a small number of resistant isolates (see Table 2).

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