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Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program 2008 (Europe, Latin America, Canada, Asia Pacific): Linezolid Global In Vitro Susceptibility Analyses

J ROSS[1], U UTSUKI[2], I FUMIAKI[3], I KOBAYASHI[3], RN JONES[1], P HOGAN[4]

[1]JMI Laboratories, North Liberty, Iowa, USA; [2]Toho University Sakura Medical Center, Chiba, Japan; [3]Mitsubishi Kagaku Bio Clinical Akita Laboratory, Tokyo, Japan; [4]Pfizer Inc., New York, New York, USA

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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT

Background: The seventh year of the ZAAPS Program continues to monitor the in vitro activities of linezolid (LZD) and comparator agents tested against Gram positive (GP) pathogens in Latin America (LA), Europe (EU), Canada and Asia Pacific (APAC). LZD is an oxazolidinone approved for the treatment of complicated skin and soft tissue infections and nosocomial pneumonia due to MRSA.

Methods: Isolates were submitted from 64 medical centers (24 countries) for a total of 6,121 strains. Each country was requested to send 200 isolates in 6 targeted GP categories to a central processing laboratory, except UK [400], Japan [400], and China [800] where more strains were sampled. CLSI broth microdilution susceptibility (S) testing methods were used to susceptibility (S) test the following organism groups: *S. aureus* (SA; 3240), coag-neg staphylococci (CoNS; 748), enterococci (ENT; 864), *S. pneumoniae* (655), viridans group (297), and ß-haemolytic streptococci (317).

Results: Five LZD resistant (R) isolates were detected in four countries (Italy [2], France, China, Brazil) in ENT (2; *E. faecalis* and *E. faecium*) and CoNS (3; *S. epidermidis*). One LZD-R ENT (Brazil) contained the 23S G2576T mutation, the other ENT (China) R mechanism was undeterminable. One CoNS (Italy) contained the mobile *cfr* gene, one 23S G2576T mutation (France) and the other CoNS (Italy) mechanism was unknown. Vancomycin-R enterococci ranged from 0.0% in several countries to 59.4% in Taiwan. All streptococci were LZD-S.

Table: *S. aureus* resistant rates by region found in the 2008 ZAAPS Program.

Region	Antimicrobial	Resistance rates (%) Lowest	Highest
Canada	LZD	0.0	-
	Oxacillin	7.2	-
	Clindamycin	6.2	-
Latin America	LZD	0.0	0.0
	Oxacillin	27.3 (Mexico)	60.8 (Chile)
	Clindamycin	20.0 (Argentina)	60.0 (Chile)
Europe	LZD	0.0	0.0
	Oxacillin	1.5 (Sweden)	50.5 (Belgium)
	Clindamycin	0.9 (Turkey)	24.7 (Poland)
Asia Pacific	LZD	0.0	0.0
	Oxacillin	9.0 (New Zealand)	78.2 (Singapore)
	Clindamycin	1.0 (New Zealand)	57.3 (China)

Conclusions: The activity of LZD appears uniform across the regions studied. Documented LZD-R remains rare (only 0.1%) among the 24 countries sampled within 6 GP pathogen groups. Rates of clindamycin-R and the frequency of MRSA varies by geographic region and requires continued surveillance.

INTRODUCTION

Linezolid, the first oxazolidinone agent to be clinically released by regulatory agencies, has become an important therapeutic addition for infections caused by commonly occurring antimicrobial-resistant Gram-positive pathogens. Resistances to linezolid, however have been observed, particularly among enterococci (*E. faecium* or *E. faecalis*) and the coagulase-negative staphylococci (CoNS), but the occurrence rates remain rare for indicated *Staphylococcus aureus* and streptococci; and these occurrences usually were associated with recognized risk factors such as prolonged therapeutic exposure and/or infected indwelling devices. Some oxazolidinone-resistant strains have emerged in patients without prior drug exposure, each event likely attributable to clonal, epidemic spread from other patients in the same hospital environment. Also, some mechanisms of resistance (*cfr* gene) have been detected in CoNS of animal origins, but in the more recent ZAAPS and LEADER (USA surveillance) reports these genes were noted in *S. aureus* and CoNS isolates from human infections.

The ZAAPS Program has collected over 4,000 Gram-positive isolates each of the last seven years worldwide to monitor for any emerging linezolid resistance. The most recent surveillance data is presented here for 2008.

MATERIALS AND METHODS

Organism collection

A total of 6,121 isolates were sent to the central monitoring site (JMI Laboratories, North Liberty, Iowa, USA, or Women's and Children's Hospital, North Adelaide, Australia) from 24 different nations in Europe, Asia Pacific, North America, and Latin America. Each participating medical center (64 total) forwarded clinically significant Gram-positive isolates in the following organism groups: *S. aureus* (3,240 strains), CoNS (748 strains), enterococci (864 strains), ß-haemolytic streptococci (317 strains), viridans group streptococci (297 strains), and *S. pneumoniae* (655 strains). All processed isolates were identified by the submitting laboratory and confirmed by the central facility using the Vitek System (bioMerieux, Hazelwood, MO) or conventional manual methods.

Susceptibility testing

Antimicrobial susceptibility testing was performed using validated microdilution panels with cation-adjusted Mueller-Hinton broth (2-5% lysed horse blood added for testing streptococci) prepared by TREK Diagnostics (Cleveland, Ohio, USA). The categorical interpretations of MIC results followed Clinical and Laboratory Standards Institute (CLSI) document M100-S19. Quality control organism (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619) results were within the acceptable ranges as published by CLSI (M100-S19, 2009).

All isolates were tested against antimicrobial agents active against Gram-positive organisms including: linezolid, ciprofloxacin, erythromycin, levofloxacin, penicillin, quinupristin/dalfopristin, rifampin, teicoplanin, and vancomycin. Other drugs tested against selected pathogen subgroups were: ampicillin, ceftriaxone, clindamycin, doxycycline, gentamicin (high-level resistance screen), piperacillin/tazobactam, streptomycin (high-level resistance screen), tetracycline, and trimethoprim/sulfamethoxazole (TMP/SMX).

All linezolid-resistant isolates (MIC, ≥8 µg/ml), if detected, were confirmed by Etest (AB BIODISK, Solna, Sweden) and disk diffusion methods. The determination of the domain V 23S ribosomal target mutation(s) was performed by polymerase chain reaction (PCR) amplification and sequence analysis. Any potential clonality within or between medical centers was identified using pulsed field gel electrophoresis (PFGE) and/or automated ribotyping. Staphylococci that were negative for the common G2576T or U mutation were also screened for the phenicol resistance mediating gene, *cfr*.

RESULTS

- A total of 3,240 *S. aureus* isolates were tested (1,416/2,276/3,000 tested in 2005/2006/2007) from 24 nations. MRSA rates were as follows: Canada (7.2%; initial year of prevalence design); for Latin America (average 42.0%, range 27.3 [Mexico] to 60.8% [Chile]); for Europe and Turkey (average 25.9%, range 1.5 [Sweden] to 50.5% [Belgium]) and the APAC region (average 41.9%; range 9.0 [New Zealand] to 78.2% [Singapore]; Tables 1 and 2).
- No evidence of linezolid resistance was noted during 2008 and the $MIC_{50/90}$ result for linezolid was 2 µg/ml against *S. aureus* (Tables 2 and 3). Linezolid activity was not affected by methicillin resistance in *S. aureus* as the $MIC_{50/90}$ was 2 µg/ml for both MRSA and MSSA.
- Among the 748 CoNS tested, oxacillin resistance rates ranged from 60.5 (Japan) to 100.0% (Singapore) and 17 of the 24 monitored nations had rates greater than 70%. Linezolid was consistently more potent by one doubling dilution step against CoNS when compared to *S. aureus* (Table 2).
- Three *S. epidermidis* isolates were detected with linezolid MIC results at >8 µg/ml (non-susceptible). One of these CoNS strains (isolated in Rome, Italy) had a detected *cfr*. The other non-susceptible CoNS were found in Genoa, Italy (no mutation or resistance gene found) and Metz, France (G2576T mutation).

Table 1. Distribution of organism identifications for the 2008 ZAAPS sample indexed by nation of origin (6,121 strains).

Nation (no. medical centers)	No. of strains *S. aureus*	CoNS	ENT	SPN	VGS	ß-strep	Total
Canada (2)	97	42	23	21	8	11	202
Argentina (2)	100	49	20	29	11	20	229
Brazil (4)	100	40	20	21	3	17	201
Chile (2)	120	41	20	20	2	19	222
Mexico (2)	150	15	20	14	3	14	216
Belgium (1)	91	51	13	20	7	3	185
France (5)	100	40	20	20	45	10	235
Germany (3)	99	41	20	20	11	10	201
Ireland (2)	150	4	20	20	6	15	215
Italy (3)	59	69	22	54	1	10	215
Poland (1)	66	20	51	32	9	49	227
Spain (2)	140	19	20	20	2	11	212
Sweden (2)	134	24	20	20	16	10	224
Turkey (2)	110	50	64	15	2	0	241
United Kingdom (2)	265	22	20	81	6	34	428
Australia (7)	273	44	20	76	28	33	474
China (10)	415	5	329	0	0	5	754
Hong Kong (1)	66	0	5	0	5	16	92
Japan (2)	193	86	20	39	18	20	376
Korea (3)	153	35	20	45	12	33	298
New Zealand (3)	199	16	20	71	12	33	351
Singapore (1)	110	2	13	7	1	3	136
Taiwan (1)	20	10	32	9	3	9	83
Thailand (1)	30	23	32	1	5	13	104
TOTAL (64)	3,240	748	864	655	216	398	6,121

Abbreviations: CoNS = Coagulase negative staphylococci, ENT = Enterococci, SPN = *S. pneumoniae*, VGS = viridans group streptococci, ß-strep = ß-streprococci.

- Among 864 summarized enterococcal isolates (Table 2), the species distribution was *E. faecalis* (515), and *E. faecium* (329) that included 97.7% of isolates tested. Vancomycin-resistant isolates (VRE) were tabulated at 10.0% overall (includes intermediate category; 8.2% in 2007), however the rates of VRE varied widely among nations.
- Linezolid resistances were rare in enterococci (0.6%) from five different countries. Three of the five isolates (2 *E. faecium*, 1 *E. faecalis*) had the G2576T ribosomal target mutation. Only one strain was found at the intermediate MIC of 4 µg/ml for linezolid.
- Penicillin resistance among 655 strains of *S. pneumoniae* averaged 19.2% with a range of the lowest rates in Canada, Sweden, United Kingdom and Mexico (0.0%) to the highest rates in Taiwan (88.9%), Korea (64.4%) and Turkey (53.3%).
- Linezolid demonstrated excellent in vitro activity against streptococci (Table 2). The MIC_{50} and MIC_{90} was 1 µg/ml for each of the three streptococcal groups tested.
- Overall, linezolid remains stable and without significant occurrence of isolates with MIC values of ≥8 µg/ml (eight isolates or 0.13% of all organisms tested).

Table 2. Comparative activity of linezolid tested against 6,121 Gram-positive cocci from 24 nations in the ZAAPS Program (2008).

Organism (no. tested)/ antimicrobial agent	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible/Resistant
S. aureus				
All strains (3,240)				
Linezolid	2	2	0.5-4	100.0 / -
Ceftriaxone[a]	4	>32	1->4	66.3 / 33.7
Ciprofloxacin	0.5	>4	≤0.03->4	66.4 / 32.1
Clindamycin	≤0.25	>2	≤0.25->2	77.3 / 22.6
Daptomycin	0.25	0.5	≤0.06-2	99.9 / -
Erythromycin	0.5	>2	≤0.25->2	60.6 / 38.4
Gentamicin	≤2	>8	≤2->8	79.4 / 20.1
Levofloxacin	≤0.5	>4	≤0.5->4	68.2 / 31.4
Oxacillin[b]	0.5	>2	≤0.25->2	66.3 / 33.7
Penicillin[c]	8	>32	≤0.015->32	12.2 / 87.8
Q/D[b]	0.5	0.5	≤0.25->2	99.9 / <0.1
Teicoplanin	≤2	≤2	≤2->16	>99.9 / <0.1
Tetracycline	≤2	>8	≤2->8	79.9 / 19.8
TMP/SMX[c]	≤0.5	≤0.5	≤0.5->2	92.8 / 7.2
Vancomycin	1	1	0.25-4	99.9 / 0.0
MRSA (1,092)				
Linezolid	2	2	0.5-4	100.0 / -
MSSA (2,148)				
Linezolid	2	2	0.5-4	100.0 / -
Coagulase-negative staphylococci (748)[c]				
Linezolid	1	1	0.25->8	99.6 / -
Ceftriaxone[a]	16	>32	≤0.25->32	24.2 / 75.8
Ciprofloxacin	4	>4	≤0.03->4	45.7 / 51.9
Clindamycin	≤0.25	>2	≤0.25->2	65.5 / 33.3
Daptomycin	0.25	0.5	≤0.06-1	100.0 / -
Erythromycin	>2	>2	≤0.25->2	37.4 / 62.2
Gentamicin	≤2	>8	≤2->8	54.8 / 37.7
Levofloxacin	4	>4	≤0.5->4	47.2 / 50.8
Oxacillin[a]	>2	>2	≤0.25->2	24.2 / 75.8
Penicillin	4	>32	≤0.015->32	15.9 / 84.1
Q/D[b]	≤0.25	0.5	≤0.25->2	98.7 / 0.9
Teicoplanin	≤2	8	≤2->16	97.2 / 0.4
Tetracycline	≤2	>8	≤2->8	83.0 / 15.9
TMP/SMX[c]	≤0.5	>2	≤0.5->2	64.8 / 35.2
Vancomycin	1	2	≤0.12-4	100.0 / 0.0
Enterococci				
All strains (864)[d]				
Linezolid	2	2	≤0.06->8	99.3 / 0.6
Ampicillin[a]	2	>16	≤1->16	63.8 / 36.2
Ciprofloxacin	>4	>4	≤0.03->4	33.9 / 56.5
Daptomycin	1	4	0.12->8	99.5 / -
Erythromycin	>2	>2	≤0.25->2	5.8 / 76.0
Gentamiain (HL)	≤500	>1000	≤500->1000	51.0 / 49.0
Levofloxacin	>4	>4	≤0.5->4	43.9 / 53.6
Penicillin[a]	4	>32	0.25->32	59.7 / 40.3
P/T[e]	8	>64	≤0.5->64	63.8 / -
Q/D[b]	>2	>2	≤0.25->2	27.9 / 63.9
Streptomycin (HL)	≤1000	>2000	≤1000->2000	54.4 / 45.6
Teicoplanin	≤2	≤2	≤2->16	93.1 / 6.8
Vancomycin	1	4	0.25->16	90.0 / 8.9
VRE (86)				
Linezolid	1	2	0.5->8	97.7 / 2.3
VSE (778)				
Linezolid	2	2	≤0.06->8	99.5 / 0.4

Organism (no. tested)/ antimicrobial agent	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible/Resistant
S. pneumoniae				
Linezolid	1	1	≤0.12-2	100.0 / -
A/C[b]	≤1	2	≤1->16	91.9 / 5.2
Ceftriaxone	≤0.25	2	≤0.25->32	89.1 / 2.4
Ciprofloxacin	1	2	0.25->4	(6.9)[f]
Clindamycin	≤0.25	>2	≤0.25->2	76.3 / 23.4
Erythromycin	≤0.25	>2	≤0.25->2	65.5 / 34.5
Levofloxacin	1	1	≤0.5->4	97.3 / 2.4
Penicillin	≤0.03	2	≤0.03-16	70.5(92.4) / 19.2(1.2)[f]
Q/D[b]	0.5	1	≤0.25-2	99.7 / 0.0
Tetracycline	≤2	>8	≤2->8	69.0 / 30.2
TMP/SMX[c]	≤0.5	>2	≤0.5->2	66.1 / 23.1
Vancomycin	≤1	≤1	≤1	100.0 / -
Viridans group streptococci (216)[g]				
Linezolid	1	1	≤0.06-2	100.0 / -
A/C[eb]	≤1	2	≤1->16	74.5 / -
Ceftriaxone	≤0.25	1	≤0.25->32	90.3 / 4.6
Ciprofloxacin	1	4	≤0.03->4	(14.4)[f]
Clindamycin	≤0.25	>2	≤0.25->2	87.5 / 12.5
Erythromycin	≤0.25	>2	≤0.25->2	64.4 / 32.9
Levofloxacin	1	2	≤0.5->4	95.8 / 3.2
Penicillin[a]	0.06	1	≤0.015->32	74.5 / 6.9
Q/D[b]	0.5	1	≤0.25-2	98.6 / 0.0
Tetracycline	≤2	>8	≤2->8	69.9 / 29.2
Vancomycin	0.5	1	≤0.12-1	100.0 / -
ß-haemolytic streptococci (398)[h]				
Linezolid	1	1	0.25-2	100.0 / -
A/C[eb]	≤1	≤1	≤1	100.0 / -
Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0 / -
Ciprofloxacin	0.5	1	≤0.03->4	(2.0)[f]
Clindamycin	≤0.25	≤0.25	≤0.25->2	92.0 / 7.5
Erythromycin	≤0.25	>2	≤0.25->2	84.4 / 15.1
Levofloxacin	≤0.5	1	≤0.5->4	98.7 / 1.3
Penicillin[a]	≤0.015	0.06	≤0.015-0.12	100.0 / -
Q/D[b]	≤0.25	0.5	≤0.25-1	100.0 / 0.0
Tetracycline	≤2	>8	≤2->8	53.8 / 44.0
Vancomycin	0.5	0.5	0.25-1	100.0 / -

a. Criteria as published by the CLSI (2009), 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/sulfamethoxazole, P/T=piperacillin/tazobactam, and A/C=amoxicillin/clavulanic acid.
c. Includes: 15 species (487 strains) and unspeciated coagulase-negative staphylococci (261 strains).
d. Includes: *Enterococcus faecalis* (515 strains), *Enterococcus faecium* (329 strains), and 20 other enterococci.
e. Percentage of pneumococci or other streptococci with ciprofloxacin MICs at ≥4 µg/ml, indicating possible QRDR mutations.
f. CLSI 2008 susceptibility breakpoints for parenteral penicillin (nonmeningitis).
g. Includes: 12 species (162 strains), *Streptococcus bovis* group (3 strains), unspeciated viridians group streptococci (51 strains).
h. Includes: Group A (164 strains), Group B (178 strains), Group C (14 strains), Group G (36 strains), and *Streptococcus dysgalactiae* (6 strains).

Table 3. Cumulative % inhibited results at each linezolid MIC when testing six different groups of Gram-positive cocci isolated on four continents (ZAAPS, 2008)[a].

Organism group (no. tested)	Cum. % inhibited at linezolid MIC (µg/ml) ≤0.12	0.25	0.5	1	2	4	≥8
ß-haemolytic streptococci (398)	0.0	0.3	13.3	98.0	100.0	-	-
S. pneumoniae (655)	0.5	3.4	31.0	97.4	100.0	-	-
Viridans group streptococci (216)	1.9	5.6	42.6	96.8	100.0	-	-
S. aureus (3,240)	0.0	0.0	1.0	36.0	99.9	100.0	-
CoNS (748)	0.0	0.1	31.3	96.5	99.6	99.6	100.0
Enterococci (864)	0.1	0.5	2.4	39.7	99.3	99.4	100.0

a. Organism groups were ranked in decreasing order of susceptibility to the oxazolidinone.

CONCLUSIONS

- Linezolid remained highly active against contemporary pathogens from indicated organism groups including *S. aureus* (MIC_{90}, 2 µg/ml), CoNS (MIC_{90}, 1 µg/ml), various enterococcal species (MIC_{90}, 2 µg/ml) and three groups of streptococci (MIC_{90}, 1 µg/ml).
- Overall, linezolid remained active against 99.87% of tested strains that were processed in the 2008 ZAAPS Program.
- As other commonly used antimicrobials continue to be compromised by evolving resistances, linezolid refractory strains appear to be unusual, yet most likely to occur among the enterococci and CoNS species exposed to long courses of therapy or to endemic clones/outbreaks.

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