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Trends in Linezolid Susceptibility Patterns in 2002 and 2003: Report from the Worldwide ZAAPS Program

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ABSTRACT

Background: Oxazolidinones have become reliable agents to be utilized for multi-resistant (R) Gram-positive cocci (GPC), especially VRE and MRSA. However, mutational R in the ribosomal target has been described for several GPC species. Longitudinal surveillance remains necessary to monitor for evolving linezolid (LZD)-R.
Methods: A survey of LZD and comparison GPC-focused agents (13 - 15) was initiated in 2002 (7,971 strains) and continued in 2003 (8,089; 99.8% compliance by sites). Sites in the USA, Canada, Europe (EU; 7 nations), South America (3) and the Asia-Pacific (3) forwarded 200 GPC strains for central reference MIC processing and confirmation of organism identity. LZD-R (MIC, ≥ 8 µg/ml) was established by alternative susceptibility testing methods and target characterization. Concurrent drug-use demographics were collected.
Results: LZD activity against the six major organism groups did not vary between years or geographic areas. In contrast, penicillin R increased 2% in SPN; macrolide R was stable in BHS (19 - 21%), but increased in SPN (+ 2%); MRSA rates increased 4% and MR-CoNS 3%; and VRE was present at 39.6% in the 2003 sample. Non-clonal LZD-R strains were detected (4/7) in 2002/2003, each having a G2576U ribosomal target mutation. Also in 2003 the first LZD-R, non-USA strain (SA) was isolated in Greece, unrelated to LZD use (48 ypm on dialysis).
Conclusions: LZD via ZAAPS Program results retained near 100% activity versus contemporary GPC isolates on 4 monitored continents and in centers utilizing oxazolidinones. Rare LZD-R strains were identified, usually ENT, in Greece and USA. Poor correlations of LZD-R to drug use continues and indicates the important role of hospital hygiene practice in dissemination.

INTRODUCTION

Linezolid is the initial oxazolidinone (oral and parenteral) to be approved by the FDA and represents the first new antimicrobial agent class to be introduced in two decades. Uniformly active against most clinically-significant Gram-positive cocci, this agent is indicated for treatment of vancomycin-resistant *Enterococcus faecium* and methicillin- or oxacillin-resistant *Staphylococcus aureus* (MRSA) infection. Oxazolidinone resistance is exceedingly rare, but does occur during therapy in wild-type enterococci, staphylococci and streptococci with mutations occurring in domain V of the 23S rRNA (primarily G2576U mutation).

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program was developed to monitor for emergence of linezolid resistance in over 50 medical centers located in North America, South America, Europe and the Asia-Pacific region. Recent reports of decreasing vancomycin potency among MRSA, including the recovery of three vancomycin-resistant isolates, underscores the need for continued monitoring of agents commonly used against multidrug-resistant (MDR) Gram-positive organisms.

This international surveillance study presents results of the analysis for 8,089 contemporary (2003) isolates of staphylococci, streptococci and enterococci tested against linezolid and selected comparator antimicrobial agents used for empiric and directed therapy of infections caused by Gram-positive organisms.

MATERIALS AND METHODS

Specimen collection: A total of 8,089 bacterial isolates were collected as part of the ZAAPS Program for 2003 from sites in South America (three nations/eight sites), Europe (seven nations/18 sites), Asia-Pacific (three nations/six sites) and North America (two nations/30 sites; **Table 1**). Each participant site forwarded a total of 200 consecutive, non-duplicate patient specimens to the central monitor (JMI Laboratories, Iowa, USA) originating from patients with bloodstream infections, respiratory tract infections, wounds or skin and soft tissue infections and urinary tract infections.

The collection included *S. aureus* (2,630 strains), *Streptococcus pneumoniae* (1,701 strains), coagulase-negative staphylococci (1,101 strains), enterococci (2,038 strains), ß-haemolytic streptococci (383 strains) and viridans group streptococci (236 strains). Isolates were identified by the submitting laboratory and confirmed by the monitoring facility using standard and commercial methods. These results were compared to similar findings for the year 2002 ZAAPS Program (7,071 strains); total of 16,060 Gram-positive cocci were tested.

Susceptibility testing: All strains were tested by the reference broth microdilution method [NCCLS, 2003] in cation-adjusted Mueller-Hinton broth (with 2 - 5% lysed horse blood added for testing of streptococci) against a variety of antimicrobial agents representing the most common classes and examples of drugs used in the empiric or directed treatment of the indicated pathogen. Dry-form microdilution panels and broth reagents were purchased from TREK Diagnostics (Cleveland, Ohio, USA).

Interpretation of quantitative MIC results was in accordance with NCCLS [2004] criteria. Quality control strains utilized included *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619.

RESULTS

- The distribution of the processed Gram-positive pathogens was: *S. aureus* (2,630 strains) > enterococci (2,038 strains) > *S. pneumoniae* (1,701 strains) > CoNS (1,101 strains) > other streptococci (619 strains; Table 1). The largest national sample was 4,433 strains from the USA (54.8%) followed by 1,352 strains from Europe.

- The MRSA rates varied among nations in the monitored areas: North America, 7.1% (Canada) to 45.0% (USA); South America, 26.0% (Brazil) to 38.2% (Mexico); Europe, 0.0% (Sweden) to 56.2% (UK); and Asia, 56.0% (Korea) to 78.7% (Taiwan). The MRSA rates within the USA varied between census zones (33.1 to 70.0%). The rates by zone (60 - 232 strains/zone) were: 1. Pacific (41.8%), 2. Mountain (42.0%), 3. West north central (33.1%; lowest), 4. West south central (49.7%), 5. East north central (45.7%), 6. East south central (49.4%), 7. New England (70.0%; highest), 8. Mid-atlantic (50.3%), and 9. South atlantic (40.5%).

- Linezolid retains potent activity (> 99.9% susceptible) against the vast majority of staphylococci, enterococci and streptococci with MIC_{50} and MIC_{90} values of 1 or 2 µg/ml (Table 2).

- *S. pneumoniae* remained fully susceptible to linezolid despite resistance to penicillin (16.2%), macrolides (27.2%), clindamycin (13.5%), tetracycline (18.7%) and trimethoprim/sulfamethoxazole (30.2%). Linezolid was also very active against ß-haemolytic streptococci (MIC_{90}, 1 µg/ml) and viridans group streptococci (MIC_{90}, 1 µg/ml; Table 2).

- Resistance to linezolid was only detected in the USA in six (0.07% of isolates overall) isolates of *Enterococcus* spp. (two *E. faecalis* and four *E. faecium*); each strain had the well-characterized ribosomal target mutation at G2576U (Table 3).

- Given that linezolid resistance is the result of mutations in the 23S rRNA, the likelihood of transferable resistance would be remote, however, nosocomial transmission of resistant clones has been proven to occur.

Table 1. Distribution of organism identifications for the 2003 ZAAPS sample indexed by nation of origin (8,089 strains).

Nation (no. medical centers)	No. of strains: ß-streptococci	vir. gr. streptococci	*S. pneumoniae*	Enterococci	*S. aureus*	CoNS	Total
Canada (5)	54	26	257	183	197	144	861
United States (25)	198	58	968	1,329	1,484	396	4,433
Argentina (2)	9	13	46	22	90	45	225
Brazil (4)	14	3	33	49	50	51	200
Mexico (2)	5	0	1	49	102	43	200
France (5)	10	10	37	43	50	50	200
Germany (3)	6	16	31	40	49	58	200
Italy (3)	5	2	52	41	50	50	200
Spain (3)	9	7	39	50	50	45	200
Sweden (1)	20	5	53	41	61	20	200
Switzerland (1)	8	14	38	19	42	31	152
United Kingdom (2)	20	8	59	31	73	9	200
Japan (3)	16	33	16	65	207	119	456
Korea (1)	3	18	47	50	50	32	200
Taiwan (2)	6	23	24	26	75	8	162
TOTAL (62)	383	236	1,701	2,038	2,630	1,101	8,089

Table 2: Comparisons of linezolid and other antimicrobials tested as part of the ZAAPS surveillance protocol in all nations (2003) against Gram-positive pathogens (8,089 strains).

Organism(no. tested)/ antimicrobial agent	MIC (µg/ml): 50%	90%	Range	% by category(2002 %)[a]: Susceptible	Resistant
S. aureus (2,630)					
Linezolid	2	2	0.12-4	100.0(>99.9)	-
Amoxicillin/Clavulanate[b]	≤2	>16	≤2->16	58.5	41.5
Ceftriaxone[b]	4	>32	0.5->32	58.5	41.5
Chloramphenicol	8	8	≤2->16	93.1	2.2
Ciprofloxacin	0.5	>2	0.06->2	55.5	43.5
Clindamycin	0.12	>8	≤0.06->8	64.8	34.9
Erythromycin	>8	>8	≤0.06->8	45.4	53.8
Gentamicin	≤2	>8	≤2->8	85.4	13.6
Imipenem[c]	≤0.5	>8	≤0.5->8	58.5	41.5
Oxacillin[c]	1	>8	≤0.25->8	58.5	41.5
Piperacillin/Tazobactam[b]	2	>64	≤0.5->64	58.5	41.5
Quinupristin/Dalfopristin	0.5	0.5	≤0.25-2	99.9	0.0
Rifampin	≤0.25	≤0.25	≤0.25->2	96.3	2.9
Teicoplanin	≤2	≤2	≤2-16	99.9	0.0
Tetracycline	≤4	>8	≤4->8	87.4	12.1
Trimethoprim/Sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	94.0	6.0
Vancomycin	1	1	≤0.12-4	100.0	0.0
CoNS (1,101)					
Linezolid	1	1	0.12-2	100.0(>99.9)	-
Amoxicillin/Clavulanate[b]	≤2	16	≤2->16	19.2	80.8
Ceftriaxone[b]	16	>32	≤0.25->32	19.2	80.8
Chloramphenicol	4	8	≤2->16	91.1	8.3
Ciprofloxacin	>2	>2	0.06->2	43.9	53.3
Clindamycin	0.12	>8	≤0.06->8	60.2	39.4
Erythromycin	>8	>8	≤0.06->8	33.7	65.9
Gentamicin	4	>8	≤2->8	57.3	31.7
Imipenem[c]	≤0.5	>8	≤0.5->8	19.2	80.8
Oxacillin[c]	>2	>2	≤0.25->2	19.2	80.8
Piperacillin/Tazobactam[b]	2	32	≤0.5->64	19.2	80.8
Quinupristin/Dalfopristin	0.25	0.5	≤0.25->2	99.5	0.2
Rifampin	≤0.25	2	≤0.25->2	88.7	9.9
Teicoplanin	≤2	8	≤2->16	95.7	1.0
Tetracycline	≤4	>8	≤4->8	83.3	15.7
Trimethoprim/Sulfamethoxazole	≤0.5	>1	≤0.5->1	59.5	40.5
Vancomycin	1	2	≤0.12-4	100.0	0.0
Enterococci (2,038)					
Linezolid	2	2	≤0.06->8	99.2(>99.9)	0.3(0.01)[d]
Ampicillin	≤2	>16	≤2->16	55.7	44.3
Chloramphenicol	8	>16	≤2->16	87.3	10.4
Ciprofloxacin	2	>2	≤0.03->2	29.1	66.8
Erythromycin	>8	>8	≤0.06->8	5.8	72.3
Gentamicin[e]	≤500	>1000	≤500->1000	63.7	-
Nitrofurantoin	≤32	>32	≤32->32	67.9	32.1
Quinupristin/Dalfopristin	>2	>2	≤0.25->2	42.1	52.1
Streptomycin[c]	≤1000	>2000	≤1000->2000	51.0	-
Teicoplanin	≤2	>16	≤2->16	67.1	29.8
Tetracycline	>8	>8	≤4->8	36.3	62.8
Vancomycin	2	>16	0.25->16	60.4	38.2
S. pneumoniae (1,701)					
Linezolid	1	1	≤0.06-4	>99.9(100.0)[e]	-
Amoxicillin/Clavulanate	≤2	≤2	≤2->8	94.5	3.4
Penicillin	≤0.03	2	≤0.03->4	69.8	16.2
Cefdinir	0.06	4	≤0.03->4	74.5	18.5
Cefuroxime	≤0.06	4	≤0.06->8	78.8	16.2
Cefepime	≤0.12	1	≤0.12->8	96.2	0.6
Ceftriaxone	≤0.25	1	≤0.25->16	97.6	0.9
Erythromycin	≤0.25	>8	≤0.25->8	72.1	27.2
Clindamycin	≤0.25	>2	≤0.25->2	86.3	13.5
Quinupristin/Dalfopristin	≤0.5	≤0.5	≤0.5->4	99.7	0.1
Levofloxacin	1	1	0.06->4	99.2	0.6
Rifampin	≤0.5	≤0.5	≤0.5->2	99.3	0.4
Tetracycline	≤4	>8	≤4->8	79.6	18.7
Trimethoprim/Sulfamethoxazole	≤0.5	4	≤0.5->4	69.8	30.2
Vancomycin	0.25	0.5	≤0.12-2	>99.9[f]	-
ß-haemolytic streptococci (383)					
Linezolid	1	1	≤0.06-2	100.0(100.0)	-[g]
Amoxicillin/Clavulanate	≤2	≤2	≤2->16	100.0	-
Cefepime	≤0.12	≤0.12	≤0.12-0.5	100.0	-
Ceftriaxone	≤0.25	≤0.25	≤0.25-1	99.7	-
Chloramphenicol	≤2	4	≤2->16	99.7	0.3
Clindamycin	≤0.06	≤0.06	≤0.06->8	92.4	7.3
Erythromycin	≤0.06	4	≤0.06->8	80.9	18.8
Levofloxacin	0.5	1	0.06->4	99.0	0.8
Penicillin	≤0.016	0.06	≤0.016-0.5	99.7	-
Quinupristin/Dalfopristin	≤0.25	0.5	≤0.25-1	100.0	0.0
Rifampin	≤0.25	≤0.25	≤0.25->2	-	-
Tetracycline	8	>8	≤4->8	44.6	50.1
Vancomycin	0.5	0.5	≤0.12-1	100.0	-
viridans group streptococci (236)					
Linezolid	1	1	≤0.06-2	100.0(99.5)	-[h]
Amoxicillin/Clavulanate	≤2	≤2	≤2->16	-	-
Cefepime	≤0.12	1	≤0.12->16	91.9	3.0
Ceftriaxone	≤0.25	0.5	≤0.25->32	93.2	3.8
Chloramphenicol	≤2	4	≤2-8	99.2	0.0
Clindamycin	≤0.06	8	≤0.06->8	88.6	11.0
Erythromycin	≤0.06	>8	≤0.06->8	66.1	30.9
Levofloxacin	1	2	≤0.03->4	98.3	0.4
Penicillin	0.06	1	≤0.016-16	77.1	5.1
Quinupristin/Dalfopristin	0.5	1	≤0.25-2	99.6	0.0
Rifampin	≤0.25	≤0.25	≤0.25->2	-	-
Tetracycline	≤4	>8	≤4->8	63.6	36.4
Vancomycin	0.5	1	≤0.12-2	98.7[i]	-

a. Susceptibility interpretive criteria of the NCCLS [2004].
b. Oxacillin susceptibility predicted by the oxacillin test results. Percentage of susceptibility will not exceed that of oxacillin when test results are correctly reported by NCCLS guidelines.
c. High-level resistance screen. Susceptible result predicts synergistic killing when combined with a cell-wall active agent.
d. Six (6) linezolid-resistant strains (MIC, ≥ 8 µg/ml) were detected in the USA.
e. One strain had a MIC at 4 µg/ml.
f. One strain had a reproducible MIC at 2 µg/ml.
g. Three strains had a reproducible MIC values of 2 µg/ml e.g. one log$_2$ dilution greater than the NCCLS breakpoint.

- Although oxazolidinone resistance detected during the ZAAPS Program was only found in the USA, a separately referred *S. aureus* isolate from Athens, Greece was observed to have a linezolid MIC of > 16 µg/ml. A G2576U ribosomal target alteration was identified.

- The most common patient demographics associated with linezolid-resistant enterococci were: female sex, average age at 53.3 years (range, 40 - 66 years) and hospitalization in an eastern USA university medical center. Susceptibility to 1 to 6 alternative agents was observed (Table 3).

- Whereas sporadic linezolid resistance was previously reported among non-enterococcal isolates of *S. aureus*, coagulase-negative staphylococci and viridans group streptococci in ZAAPS 2002, none was apparent in these groups in the 2003 sample (Table 4).

Table 3. Oxazolidinone-resistant strains discovered during the ZAAPS protocol (2003).

Parameter	Organism number: (15-699V)	(4-4V)	(17-982A)	(107-9175A)	(108-12402A)	(21-2870E)
Species	*E. faecium*	*E. faecalis*	*E. faecium*	*E. faecalis*	*E. faecium*	*E. faecium*
Age	58	66	52	43	40	61
Sex	F	F	M	F	F	F
State	New York	Ohio	New York	Kentucky	Tennessee	Washington
MIC (µg/ml)						
Linezolid	8	>8	>8	>8	8	8
Vancomycin	>16	>16	0.5	1	>16	1
Teicoplanin	>16	>16	≤2	≤2	>16	≤2
Quinupristin/Dalfopristin	1	>2	1	>2	1	1
Erythromycin	>8	>8	2	>8	>8	>8
Clindamycin	>8	>8	0.25	>8	>8	>8
Levofloxacin	>4	>4	>4	>4	>4	>4
Gentamicin	≤500	>1000	≤500	>1000	≤500	≤500
Ampicillin	>16	≤1	>16	≤1	>16	>16
Chloramphenicol	16	16	8	>16	8	16
Tetracycline	≤2	>8	>8	>8	>8	≤2
Trim/Sulfa[a]	>2	>2	>2	>2	>2	>2
Mutation	G2576U	G2576U	G2576U	G2576U	G2576U	G2576U

a. Trimethoprim/Sulfamethoxazole

Table 4. Linezolid activity when tested against the six major organism groups.

Organism (no. tested 2002/2003)	Linezolid MIC$_{50}$/highest MIC/% S by year: 2002	2003
S. aureus (3,687/2,630)	2/16/>99.9	2/4/100.0[a]
CoNS (870/1,101)	2/8/>99.9	1/2/100.0
Enterococci (1,070/2,038)	2/>8/>99.9	2/>8/99.2
ß-streptococci (387/383)	1/2/100.0	1/2/100.0
viridans group streptococci (187/236)	1/8/99.5	1/2/100.0
S. pneumoniae (1,770/1,701)	1/2/100.0	1/4/>99.9

a. One linezolid-resistant strain was processed from Greece, outside of the ZAAPS Program (2003).

CONCLUSIONS

- Results from the 2003 ZAAPS Program demonstrate that linezolid retains near complete activity in vitro against contemporary isolates of staphylococci, enterococci and streptococci, including *S. pneumoniae* from the four monitored continents (15 countries).

- Rare linezolid-resistant strains of *E. faecalis* and *E. faecium* were identified, recovered only in the USA; all resistant strains contained a G2576U ribosomal target mutation.
  - 0.07% resistance overall
  - 0.14% resistance in USA strains
  - 0.45% resistance among USA enterococci

- Published studies correlating linezolid resistance with drug utilization indicates the greater importance of hospital hygiene practices in dissemination and chart reviews, where available, noted linezolid resistance developing with and without drug exposure.

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