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Longitudinal Evaluation of Linezolid Activity: Results from the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Surveillance Program (2002-2005)

JE ROSS, TR FRITSCHE, HS SADER, RN JONES
JMI Laboratories, North Liberty, IA

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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 global ZAAPS Program has evaluated over 14,000 Gram-positive isolates monitoring for emergence of linezolid resistance (R) during 2002-2005. Here we present a longitudinal analysis of results from 16 countries on 5 continents (excluding the USA).

Methods: A total of 14,750 isolates were forwarded to the central monitor from 16 nations. Each participating site (39 to 41) or country submitted 200 consecutive, non-duplicate patient isolates yearly. Major organism groups tested included: *S. aureus* (5,151, see Table), coagulase-negative staphylococci (CoNS; 2,376), enterococci (2,547), *S. pneumoniae* (2,987), viridans group streptococci (704), and ß-hemolytic streptococci (985). Susceptibility testing (broth microdilution) was performed adhering to CLSI methods and interpretive criteria (M100-S16; 2006).

Results: The linezolid MIC_{90} for all streptococci and CoNS tested was 1 µg/ml; that for enterococci and *S. aureus* was 2 µg/ml. The modal linezolid MIC (1 µg/ml) for *S. aureus* decreased slightly (one log_2 dilution) in 2005 when compared to 2004. Linezolid $MIC_{50/90}$ results for *S. aureus,* however, did not change over the study interval and the percentage of strains with MIC values >2 µg/ml has remained low (<0.9% for 2002-2005).

		Cumulative % of *S. aureus* strains inhibited at linezolid MIC (µg/ml)				
Year	No. tested	≤0.25	0.5	1	2	4
2002	1,209	0.2	0.7	30.7	99.1	100.0
2003	1,104	0.1	0.5	31.6	99.5	100.0
2004	1,422	0.3	1.4	26.6	99.9	100.0
2005	1,416	0.1	1.3	55.9	100.0	-

Conclusions: No linezolid-R strains have been detected to date in the ZAAPS Program (non-USA). Generally, linezolid MIC distributions covered only 2 or 3 log_2 dilution steps across all years and were not influenced by methicillin or vancomycin R patterns. No trend toward greater linezolid R (MIC creep) was noted; in fact, for *S. aureus* in 2005 the modal MIC (1 µg/ml) was actually lower compared to 2002–2004 results (not significant).

INTRODUCTION

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program has four years of resistance surveillance information for linezolid, the first oxazolidinone class agent to be licensed for use in clinical practice. Linezolid has been used primarily to treat multidrug-resistant Gram-positive pathogens in complicated skin and soft tissue infections (SSTI) and nosocomial pneumonias, after its United States Food and Drug Administration approval in 2000. Linezolid has emerged as a viable alternative for infections caused by Gram-positive organisms that are resistant to conventional drugs, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* (DRSP) and vancomycin-resistant enterococci (VRE). Therefore, it is prudent to monitor the potency and potential emerging resistance to linezolid as the use of this agent increases, in volume and geographic distributions.

The linezolid mechanism of action has been described as selective binding to the 50S ribosomal subunit of the 23S rRNA molecule with resultant inhibition of protein synthesis. Among the rare cases of linezolid resistance reported to date among staphylococci and enterococci, G2576U or T2500A target site mutations have been the typical mechanism.

The 2002 and 2003 ZAAPS Program monitored countries around the world including the United States (USA). The 2004 and 2005 ZAAPS program surveyed only the "rest of the world" (not USA) while the USA was separated in 2004 (LEADER Program) and expanded to 50 monitored sites in an effort to concentrate on emerging resistance and different drug usage patterns. The ZAAPS Program has studied for surveillance purposes a large volume collection (>14,000) of Gram-positive isolates over its four years of existence. The 2005 program monitored 39 medical centers for the emergence of linezolid resistance; results are presented here and compared to the earlier years of the ZAAPS surveillance initiative.

MATERIALS AND METHODS

Organism collection

A total of 14,750 isolates were forwarded to the central monitoring sites from 16 different nations between 2002 and 2005 for the ZAAPS Program. Each participating site (39 to 41 total) or country forwarded a target total of 200 clinically significant Gram-positive isolates (Table 1).

Isolates were grouped for analysis as follows: *S. aureus* (5,151 strains), coagulase-negative staphylococci (CoNS; 2,376 strains), ß-hemolytic streptococci (985 strains), viridans group streptococci (704 strains), *S. pneumoniae* (2,987 strains) and enterococci (2,547 strains). All processed isolates were identified by the submitting laboratory and confirmed by the central facility using the Vitek standard system (bioMerieux, Hazelwood, MO, USA).

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, OH, USA). The categorical interpretations of MIC results followed Clinical and Laboratory Standards Institute (CLSI) document M100-S16. 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.

All isolates were tested against antimicrobial agents active against Gram-positive organisms including: linezolid, chloramphenicol, 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.

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.

RESULTS

- Over 50% of the organisms submitted (2002-2005) were staphylococci (Table 1). The 2005 MRSA rates varied greatly between countries with the highest rates in Taiwan (78.8%) and Mexico (61.1%), and the lowest rates in Sweden (3.3%). Although the total MRSA rate decreased compared to the previous year (2004), nine nations displayed an increase while seven countries showed a rate decrease (data not shown). Even the country with the lowest MRSA rate over the last four years, Sweden, has demonstrated an increase from no MRSA in 2002 to 3.3% in 2005.
- The linezolid MIC_{50} for 2002–2005 was 2 µg/ml, however, the MIC_{50} decreased in 2005 to 1 µg/ml (Table 2 and Figure 1a). Greater than 99% of all *S. aureus* had MIC values at 2 µg/ml or below for all four years. Nearly all MIC results ranged from 0.5 to 2 µg/ml.
- Linezolid MIC values were generally two-fold lower for CoNS (≥11 species tabulated) when compared to *S. aureus* MIC results (Table 2 and Figure 1b). The linezolid $MIC_{50/90}$ in 2005 were both 1 µg/ml (range of MICs, 0.25-2 µg/ml), unchanged from prior years. In 2005, teicoplanin (96.4% susceptible), vancomycin (100.0%) and quinupristin/dalfopristin (99.2%) exhibited high activity against these staphylococci (data not shown).
- Linezolid-resistant enterococci remain undetected in the "rest of world" ZAAPS Program with over 90% of MIC values at 1 and 2 µg/ml (Table 2 and Figure 1c). As linezolid and the glycopeptides (teicoplanin and vancomycin) show the most potent activity against enterococci, it is important to note increasing rates of VRE (5.7% in 2005, an increase of 0.4% over 2004) and the likelihood of increased linezolid usage. The highest VRE rates in 2005 were in the UK (43%), Korea (22%) and Germany (16%).
- Linezolid MIC trends in streptococci demonstrated little variation in MIC values, although linezolid MICs decreased markedly among viridans group *Streptococcus* spp. (Figures 1d, 1e, 1f). In contrast, the erythromycin resistance rate among *S. pneumoniae* increased from 22.6% in 2004 to 24.0% in 2005. The MIC_{90} for all three groups of streptococci across all years remained at 1 µg/ml (Table 2).

Table 1. Distribution of organism identifications for ZAAPS (2002-2005) indexed by nation of origin (14,750 strains).[a]

	No of strains:						
Region (no. nations)	*S. aureus*	CoNS	Enterococci	*S. pneumoniae*	viridans gr. streptococci	ß-streptococci	Total
North America (1)	1,138	631	753	765	214	297	3,798
Latin America (4)	1,240	608	465	333	48	109	2,803
Europe (6)	1,664	716	803	1128	181	345	4,837
Asia-Pacific (5)	1,109	421	526	761	261	234	3,312
TOTAL (16)	5,151	2,376	2,547	2,987	704	985	14,750

a. Not all nations or sites participated in each year.

Table 2. Cumulative percentage of tested strains inhibited at each linezolid MIC when testing six different groups of Gram-positive cocci (14,750) isolated on five continents (ZAAPS Program, 2002-2005).

		Cumulative % of strains inhibited at linezolid MIC (µg/ml)				
Organism group (no. tested)	Years	≤0.25	0.5	1	2	4
ß-hemolytic streptococci (985)	2002-2005	0.8	16.0	98.7	100.0	-
S. pneumoniae (2,987)	2002-2005	0.4	32.9	96.5	99.9	100.0
viridans group streptococci (704)	2002-2005	0.4	36.1	97.5	99.9	100.0
Enterococci (2,547)	2002-2005	0.1	2.2	47.8	99.5	100.0
Staphylococci						
CoNS (2,376)	2002-2005	0.7	16.9	92.8	99.9	100.0
S. aureus (1,209)	2002	0.2	0.7	30.7	99.1	100.0
S. aureus (1,104)	2003	0.1	0.5	31.6	99.5	100.0
S. aureus (1,422)	2004	0.3	1.4	26.6	99.9	100.0
S. aureus (1,416)	2005	0.1	1.3	55.9	100.0	-

Figure 1. Linezolid MIC distributions in the ZAAPS Program (2002-2005) for *S. aureus* (Figure 1a), coagulase-negative staphylococci (Figure 1b), enterococci (Figure 1c), beta-hemolytic streptococci (Figure 1d), *Streptococcus pneumoniae* (Figure 1e), and viridans group streptococci (Figure 1f).

Figure 1a. *S. aureus* (5,151strains)

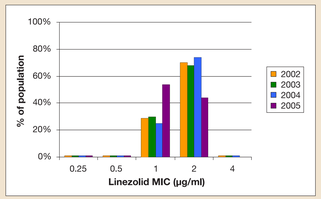


Figure 1b. Coagulase-negative staphylococci (2,376 strains)

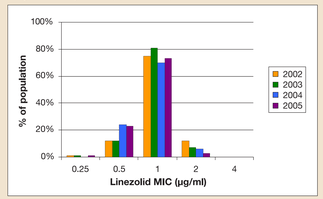


Figure 1c. Enterococci (2,547 strains)

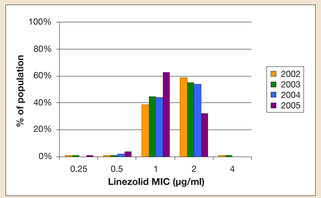


Figure 1d. Beta-hemolytic streptococci (985 strains)

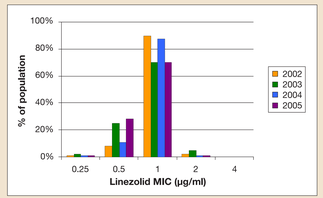


Figure 1e. *Streptococcus pneumoniae* (2,987 strains)

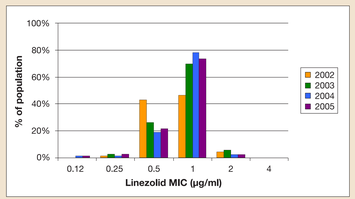
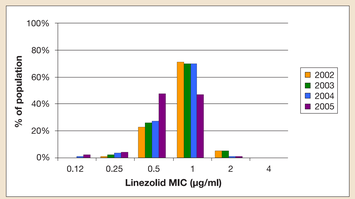


Figure 1f. Viridans group streptococci (704 strains)



- Generally, MIC distributions for all displayed Gram-positive species, were observed to be two to three log_2 dilution steps across all years (2002-2005). No trend toward greater resistance was consistently seen.

CONCLUSIONS

- No linezolid resistant strains were detected in the protocol isolates from the non-USA sample through 2005.
- Linezolid continues to show excellent activity with a narrow MIC range among all six Gram-positive subsets including VRE, MRSA and DRSP.
- No trend toward greater linezolid resistance (MIC creep) was observed throughout the ZAAPS Program.
- As linezolid-resistant isolates emerge in areas of increased exposure (USA), continued surveillance appears prudent and should also include baseline sampling from nations having limited linezolid use (ZAAPS Program).
- After submission of this abstract, one linezolid-resistant CoNS (MIC, 8 µg/ml) was detected in Rome, Italy; year 2006 ZAAPS sample.

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