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Five-Year Longitudinal Assessment of Linezolid Activity in Europe: Results from the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS)

JE ROSS, RN JONES, HS SADER, TR FRITSCHE, P HOGAN, D SHEEHAN
JMI Laboratories, North Liberty, Iowa, USA;
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 worldwide ZAAPS program has accumulated 5 years of LZD surveillance for *S. aureus* (SA) including MRSA and other resistant(R) organisms (vancomycin-R enterococci [VRE], and multidrug-resistant [MDR] *S. pneumoniae* [SPN]) from serious infections. Here we prospectively monitored linezolid (LZD) in vitro activity among Gram-positive pathogens isolated in European (EU) medical centers (14; 6 countries) using a central monitor.

Methods: From 2002 through 2006, a total of 4,366 Gram-positive strains were collected in EU as a component of ZAAPS. Sites within each nation contributed isolates toward a total of 200 isolates/nation for reference identification and broth microdilution MIC susceptibility (S) testing. Major organism groups tested were: SA(2,424), coagulase-negative staphylococci(CoNS; 956), enterococci(946), SPN(1,316), viridans group streptococci(259) and beta-haemolytic streptococci(BHS; 465).

Results: One CoNS strain from Rome, Italy, was confirmed as LZD-R(<0.1% overall rate). LZD remains highly active against all SA (see Table). The overall MRSA rate (24.5%) remained constant across EU for the 5 year interval, although dramatic differences between countries were apparent. The overall VRE rate (increasing over time) for EU was 4.8% with a range of 3.2% (2004) to a high of 6.7% (2006). The LZD MIC_{50} for enterococci, however decreased from 2 µg/ml in 2004 to 1 µg/ml in 2005 and 2006.

Nations (no. tested)	% MRSA	LZD $MIC_{50/90}$ µg/ml (%S)
France (354)	29	2/2 (100)
Germany (364)	14	2/2 (100)
Italy (370)	33	2/2 (100)
Spain (418)	4	2/2 (100)
Sweden (369)	2	2/2 (100)
Switzerland (99)	16	2/2 (100)
UK (450)	46	2/2 (100)

Conclusions: LZD continues to be highly active against Gram-positive strains from Europe, including MRSA. Emerging LZD-R CoNS and VRE appear to be rare but greater in USA (data not shown). As rates of MRSA, VRE, and MDR increase, continued surveillance of the in vitro activity of LZD and comparator Gram-positive-active agents versus these pathogens is needed.

INTRODUCTION

Linezolid, the first oxazolidinone class agent to be FDA approved, has become a welcome addition for the treatment of infections caused by commonly occurring antimicrobial-resistant Gram-positive pathogens. Linezolid is indicated for the treatment of complicated skin and soft tissue infections and nosocomial pneumonia. Resistance to linezolid has been infrequently observed among enterococci (*E. faecium* > *E. faecalis*) and more recently among coagulase-negative staphylococci. For *S. aureus* and streptococci, the occurrence of linezolid resistance has been classified as rare to very rare. Linezolid resistance is associated with prolonged therapy (weeks or months) and in the treatment of undrained abscesses and unremoved prosthetic devices. Some oxazolidinone-resistant strains have emerged in patients without prior drug exposure, each event attributed to clonal, epidemic spread from other patients in the same hospital environment.

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program has collected over 4,000 Gram-positive isolates in the last five years from Europe to monitor for any emerging linezolid resistance.

MATERIALS AND METHODS

Organisms studied:
A total of 4,366 isolates were sent to the central monitoring site from 6 different nations in Europe between 2002 and 2006 (ZAAPS Program). Each participating medical center (14 total) forwarded a target total of 200 clinically significant Gram-positive isolates.

Isolates were grouped for analysis as follows: *S. aureus* (2,424 strains), coagulase-negative staphylococci (CoNS; 956 strains), enterococci (946 strains), ß-hemolytic streptococci (465 strains), viridans group streptococci (259 strains), and *S. pneumoniae* (1,316 strains). All processed isolates were identified by the submitting laboratory and results were confirmed by the central facility using the Vitek System (bioMerieux, Hazelwood, MO).

Susceptibility test:
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). The categorical interpretations of MIC results followed Clinical and Laboratory Standards Institute (CLSI) document M100-S17. 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-S17, 2007).

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. Any potential clonality within a medical center was identified using pulse field gel electrophoresis (PFGE) and automated ribotyping.

RESULTS

- The most active antimicrobials against *S. aureus* were linezolid (100.0% susceptible), vancomycin (100.0%), teicoplanin (99.9%), and quinupristin/dalfopristin (Q/D, 99.6%; see Table 1).
- MRSA rates ranged from 2.2% in Sweden to 45.6% in the UK (Figure 1) with an overall rate of 24.5% in the seven European countries which has remained consistent throughout the ZAAPS program (2002-2006).
- CoNS MIC values for linezolid were generally two-fold lower than those of *S. aureus* (Tables 1 and 2).
- One CoNS strain (*S. epidermidis*) from Italy was non-susceptible to linezolid (8 µg/ml). Four other CoNS isolates from the same medical center (not included in the ZAAPS program) were found to be clonal with this strain having MIC values varying from 4 to 8 µg/ml. The G2576T target mutation was not detected in these five strains.
- Although VRE rates increased in Europe from 3.2% (2004) to 6.7% (2006), the linezolid $MIC_{50/90}$ (1/2 µg/ml, respectively) remained unchanged or decreased in 2006 compared to previous years. The highest linezolid MIC value for *Enterococcus* spp. was 4 µg/ml (1 strain; intermediate). Teicoplanin and vancomycin remain the most active agents against enterococci ranked after linezolid (Table 1).
- Linezolid showed excellent activity against all streptococci with all MIC results at ≤2 µg/ml. Over 80% (376 strains) of ß-haemolytic streptococci had a linezolid MIC value of 1 µg/ml. In *S. pneumoniae*, penicillin resistance varied from only 1.9% in Sweden to 32.8% in France (Figure 1).
- Linezolid $MIC_{50/90}$ results among all Gram-positive organism groups have remained stable during the course of the ZAAPS Program indicating the lack of MIC creep.

Figure 1. Resistance rates by country (seven) for VRE (vancomycin MIC, ≥8 µg/ml), MRSA (oxacillin MIC, ≥4 µg/ml) and penicillin-resistant *S. pneumoniae* (PRSP; penicillin MIC, ≥2 µg/ml) for ZAAPS Europe (2002-2006).

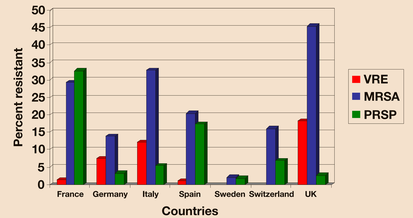

Table 1. Comparative activity of linezolid tested against Gram-positive cocci from six European nations in the ZAAPS Program (2002-2006).

Organism (no. tested)/antimicrobial agent	MIC (µg/ml) 50%	MIC (µg/ml) 90%	MIC (µg/ml) Range	% by category[a] Susceptible/Resistant
S. aureus (2,424)				
Linezolid	2	2	0.12-2	100.0/-
Ciprofloxacin	0.5	>4	0.06->4	69.8/29.3
Clindamycin	≤0.25	>2	≤0.25->2	85.1/14.7
Erythromycin	≤0.25	>8	≤0.25->8	69.7/29.3
Gentamicin	≤2	4	≤2->8	90.0/9.6
Levofloxacin	≤0.5	>4	≤0.5->4	70.9/27.8
Oxacillin[b]	0.5	>2	≤0.25->2	75.6/24.4
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25->2	99.6/0.3
Teicoplanin	≤2	≤2	≤2-16	>99.9/0.0
Tetracycline	≤4	≤4	≤4->8	94.8/4.8
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	98.3/1.7
Vancomycin	1	1	≤0.12-2	100.0/0.0
CoNS[b] (956)				
Linezolid	1	1	≤0.25-8	99.9/-
Ciprofloxacin	4	>4	≤0.03->4	44.7/51.5
Clindamycin	≤0.25	>8	≤0.25->8	69.8/29.8
Erythromycin	>8	>8	≤0.25->8	39.1/60.5
Gentamicin	≤2	>8	≤2->8	57.4/35.2
Levofloxacin	2	>4	≤0.5->4	46.2/45.7
Oxacillin[b]	>2	>2	≤0.25->2	26.5/73.5
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25->2	99.6/0.3
Teicoplanin	≤2	4	≤2->16	97.3/0.5
Tetracycline	≤4	>8	≤4->8	85.1/14.3
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	62.9/37.1
Vancomycin	1	2	≤0.12-4	100.0/0.0
Enterococcus spp. (946)				
Linezolid	2	2	0.5-4	99.9/0.0
Ampicillin	≤2	>16	≤2->16	77.6/22.4
Ciprofloxacin	2	>4	0.06->4	47.8/46.0
Doxycycline	8	>8	≤1->8	46.9/21.3
Erythromycin	>8	>8	≤0.25->8	9.2/59.4
Gentamicin (HL)a	≤500	>1000	≤500->1000	66.1/43.9
Levofloxacin	2	>4	≤0.5->4	54.1/43.3
Penicillin	4	>32	≤0.015->32	76.0/24.0
Piperacillin/tazobactam	8	>64	1->64	-/-
Quinupristin/dalfopristin	>2	>2	≤0.25->2	17.7/72.9
Rifampin	2	>2	≤0.5->2	25.2/48.6
Streptomycin (HL)[a]	≤1000	>2000	≤1000->2000	56.6/43.4
Tetracycline	>8	>8	≤2->8	35.3/64.1
Teicoplanin	≤2	≤2	≤2->16	96.1/3.5
Vancomycin	1	2	0.25->16	93.7/4.8
S. pneumoniae (1,316)				
Linezolid	1	1	≤0.25-2	100.0/-
Amoxicillin/clavulanic acid	≤2	≤2	≤2->16	97.9/1.4
Ceftriaxone	≤0.25	0.5	≤0.25-8	91.1/0.9
Clindamycin	≤0.25	>2	≤0.25->2	80.4/18.5
Erythromycin	≤0.25	>2	≤0.25->2	73.3/25.8
Levofloxacin	1	1	≤0.5->4	98.9/1.0
Penicillin	≤0.03	1	≤0.03->4	79.7/9.4
Tetracycline	≤2	>8	≤2->8	71.6/22.3
Trimethoprim/sulfamethoxazole	≤0.5	4	≤0.5->4	75.7/11.6
Vancomycin	0.25	0.5	≤0.12-1	100.0 /-
Viridans group streptococci (259)				
Linezolid	1	1	≤0.06-2	100.0/-
Amoxicillin/clavulanic acid	≤1	≤1	≤1-16	-/-
Ceftriaxone	≤0.25	0.5	≤0.25-8	95.4/2.7
Clindamycin	≤0.25	1	≤0.25->8	88.0/10.5
Erythromycin	≤0.25	4	≤0.25->8	66.8/30.1
Levofloxacin	1	1	≤0.5->4	98.5/1.5
Penicillin	0.06	0.5	≤0.015-8	81.5/2.7
Tetracycline	≤2	>8	≤2->8	50.2/34.0
Vancomycin	0.5	1	≤0.12-1	100.0/-
ß-haemolytic streptococci (465)				
Linezolid	1	1	≤0.06-2	100.0/-
Amoxicillin/clavulanic acid	≤2	≤2	≤2	-/-
Ceftriaxone	≤0.25	≤0.25	≤0.25-1	99.8/-
Clindamycin	≤0.25	≤0.25	≤0.25->2	93.1/6.7
Erythromycin	≤0.25	>2	≤0.25->2	81.5/17.8
Levofloxacin	≤0.5	1	≤0.5->4	99.1/0.6
Penicillin	≤0.015	0.06	≤0.015-0.12	100.0/-
Tetracycline	≤2	>8	≤2->8	51.6/41.9
Vancomycin	0.25	0.5	≤0.12-1	100.0/-

a. Criteria as published by the CLSI ß-lactam susceptibility for staphylococci should be directed by the oxacillin test results; "-" = no interpretive criteria have been published by the CLSI; HL= high-level resistance screen.
b. CoNS = coagulase-negative staphylococci.

Table 2. Linezolid $MIC_{50/90}$ (µg/ml) results for six Gram-positive cocci groups in Europe.

Organism	$MIC_{50/90}$ results by year: 2002	2003	2004	2005	2006
S. aureus	2/2	2/2	2/2	1/2	2/2
Coagulase-negative staphylococci	1/2	1/1	1/1	1/1	1/1
Enterococcus spp.	2/2	2/2	2/2	1/2	1/2
S. pneumoniae	1/1	1/1	1/1	1/1	1/1
Viridans group streptococci	1/1	1/1	1/1	1/1	1/1
ß-haemolytic streptococci	1/1	1/1	1/1	1/1	1/1

CONCLUSIONS

- Linezolid remains highly active against Gram-positive organism groups including MRSA and VRE.
- Among >4,000 isolates tested during the last five years, only one linezolid-resistant organism from Europe has been identified. This represents the initial linezolid-resistant CoNS in the European ZAAPS sample.
- A clonal outbreak of linezolid-resistant CoNS was identified in Italy, underscoring the need for continued monitoring as well as epidemiologic typing to guide local infection control investigations.

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