Eight-year (2002-2009) Summary of the Zyvox Annual Appraisal of Potency and Spectrum Program in European Countries

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AMENDED ABSTRACT

Objectives: To document the rates of susceptibility (S) for the oxazolidinone, linezolid (LZD), when tested against a longitudinal resistance (R) surveillance sample of European (EU) medical center isolates (ZAAPS; 2002-2009). Samples from 12-24 sites annually in 10 countries were monitored by a central laboratory design using reference methods (CLSI) and regional interpretations (EUCAST).

Methods: A total of 13,965 Gram-positive pathogens were tested from 6 pathogen groups: *S. aureus* (SA; 6,096), coagulase-negative staphylococci (CoNS; 2,073), enterococci (2,054), *S. pneumoniae* (2,267), beta-haemolytic (BHS; 947) and viridans gr. (VGS; 528) streptococci. CLSI (M07-A8, 2009) methods and interpretations (M100-S20, 2010) were used, supplemented by EUCAST (2010) breakpoints. At least 15 comparator agents were tested. LZD-R strains (MIC, ≥8 mg/L) were confirmed by a second method (disk, Etest) and then by molecular tests to determine R-mechanisms (*cfr*, target mutations) and clonality by PFGE and/or automated riboprints for perceived clusters.

Results: LZD generally remained without documented R from 2002-2005, but beginning in 2006 LZD-R strains emerged at very low rates ≤1.1% among SA (G2576T mutant in Ireland, 2007), CoNS (usually *S. epidermidis*; France and Italy, 2006-2008) and enterococci (*E. faecium* in Germany [2008, 2009], *E. faecalis* in Sweden and UK [2008]), each strain having a target mutation. A mobile *cfr* was detected in an Italian CoNS strain (2008), and clonal spread was noted for LZD-R strains at that site (PFGE results). Overall the LZD-S rates were >99.9, 99.7 and 99.8% for SA, CoNS and enterococci, respectively. All LZD MIC_{90} results ranged from 1 to 2 mg/L. All streptococci were LZD-S (≤2 mg/L), but penicillin-R was 27.7% in pneumococci and fluoroquinolone-R was >1.1% in pneumococci, BHS and VGS. Other resistances noted were: MRSA and MRCoNS ranging from 20.0-30.1% and 37.5-83.8%, respectively, without trends toward greater R. VRE rates increased from 6.9% (2002) to 16.0% (2009), with 83.8% having VanA phenotype in 2009.

Abstract Table

	MIC_{90} (mg/L)/% susceptible by pathogen group				
Year	*S. aureus*	CoNS	Enterococci	Streptococci[b]	Sample no.
2002	2/100.0	2/100.0	2/99.4	1/100.0	1323
2003	2/100.0	1/100.0	2/100.0	1/100.0	1283
2004	2/100.0	1/100.0	2/100.0	1/100.0	1198
2005	2/100.0	1/100.0	2/100.0	1/100.0	1238
2006	2/100.0	1/99.5[a]	2/99.2	1/100.0	1263
2007	2/>99.9	1/99.5	2/100.0	1/100.0	2276
2008	2/100.0	1/99.1[b]	2/98.9	1/100.0	2383
2009	2/100.0	1/100.0	2/99.7	1/100.0	3001
All years	2/>99.9	1/99.7	2/99.8	1/100.0	13965

a. Underline indicates documented resistances by molecular tests.
b. *cfr* discovered in Italy.

Conclusions: ZAAPS surveillance for LZD-S rates confirmed high levels (≥99.7% S) for staphylococci and enterococci from 2002-2009 and without R among streptococci. No trends toward LZD MIC creep or escalating R rates were detected in this multi-year post-marketing surveillance program for the EU (see Abstract Table).

INTRODUCTION

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program has eight years of resistance surveillance information for linezolid, the first oxazolidinone class agent to be licensed for clinical use. Linezolid has been used primarily to treat multidrug-resistant (MDR) Gram-positive pathogens found in complicated skin and soft tissue infections (cSSTI) and nosocomial pneumonias, after its United States Food and Drug Administration (US-FDA) approval in early 2000. Linezolid has emerged as a valuable treatment alternative for infections caused by Gram-positive organisms that are MDR to conventional drugs, such as methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae* 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 G2447T target site mutations have been the typical mechanism, however, mobile resistance elements (*cfr*) have recently been described in staphylococci.

The 2002 and 2003 ZAAPS Program monitored countries around the world including the United States (USA). The 2004 and onward ZAAPS programs surveyed only the "rest of the world" (not USA) while the USA was separated in 2004 (LEADER Program) and expanded to more than 50 monitored sites in an effort to concentrate on emerging resistance and different drug usage patterns. The 2009 program monitored 23 medical centers in 10 European countries for the emergence of linezolid resistance; results are presented here and compared to the earlier years of the ZAAPS Program surveillance initiative in Europe.

MATERIALS AND METHODS

Organism collection: A total of 13,965 isolates were forwarded to the central monitoring site (JMI Laboratories, North Liberty, Iowa, USA) from 11 different nations between 2002 and 2009 for the ZAAPS Program. Each participating site (12 to 24 total) or country forwarded a target total of 200 clinically significant Gram-positive isolates (Table 1) in a prevalence style sampling.

Isolates were grouped for analysis as follows: *S. aureus* (6,096 strains), coagulase-negative staphylococci (CoNS; 2,073 strains), β-haemolytic streptococci (947 strains), viridans group streptococci (528 strains), *S. pneumoniae* (2,267 strains) and enterococci (2,054 strains). All processed isolates were identified by the submitting laboratory and confirmed by the central facility using the Vitek standard system (bioMerieux, Hazelwood, Missouri, 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, Ohio, USA). The categorical interpretations of MIC results followed Clinical and Laboratory Standards Institute (CLSI) document M100-S20. Quality control (QC) organism (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619) results were within the acceptable MIC QC ranges as published by CLSI (2010).

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 mg/L), if detected, were confirmed by Etest (bioMerieux, 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.

Furthermore, molecular tests to identify the *cfr* gene encoding resistances to oxazolidinones in staphylococci were performed as described by Mendes et al, [2008]. Other potential target site modifications associated with increased linezolid MIC results were also examined.

RESULTS

- A total of 13,965 Gram-positive cocci were sampled across Europe by the ZAAPS linezolid resistance surveillance program (2002-2009). All tests were performed in a reference laboratory by standardized CLSI methods (Tables 1-3 and Figure 1).
- Resistance to methicillin did not adversely affect the linezolid resistance (Table 2).
- No linezolid resistance was detected from samples taken in 2002-2005. However, organisms with linezolid MIC values at ≥8 mg/L were found among *S. aureus* (one isolate; Ireland in 2007), *S. epidermidis* (six isolates; Italy and France in 2006-2008) and enterococci (five isolates; Germany, Sweden and UK in 2006-2009), see Table 3.
- Linezolid resistance rates remain very low: *S. aureus* (0.00 to 0.10%; 0.02% over 8 years); *S. epidermidis* (0.00 to 0.90%; 0.29% over all years); and *E. faecalis/E. faecium* (0.00 to 1.10%; 0.24% across all years). No oxazolidinone non-susceptible strains were identified among 3,742 sampled streptococci (Table 1).
- The most common linezolid resistance mechanisms (Table 3) were: G2576T mutations (3); G2447T mutation (1); L3 + L4 mutations (3); *cfr* + L3 + L4 mutations (3); and no recognized mechanism (1). Epidemic clones of linezolid-resistant *S. epidermidis* (Italy) and *E. faecium* (Germany) have persisted in some monitored medical centers for more than four study years (Table 3).
- The overall linezolid resistance rate was only 0.09% (Figure 1) with nearly all MIC values occurring at 0.5, 1 or 2 mg/L. Only three isolates had a 4 mg/L MIC result and the clear modal MIC was 1 mg/L.

Table 1. Linezolid activity as measured by the ZAAPS Program in Europe (2002-2009) for 13,965 Gram-positive pathogens, and compared to selected other antimicrobials (8-11) by reference (CLSI) methods.

Pathogen/ Antimicrobial agent	Year (no. tested)	MIC (mg/L): 50%	90%	Range	% Susceptible[a] CLSI/EUCAST
S. aureus					
Linezolid	2002 (502)	2	2	0.25-2	100.0 / 100.0
	2003 (373)	2	2	1-2	100.0 / 100.0
	2004 (419)	2	2	0.25-2	100.0 / 100.0
	2005 (405)	1	2	0.5-2	100.0 / 100.0
	2006 (657)	2	2	0.5-2	100.0 / 100.0
	2007 (1,138)	1	2	0.5-8	99.9 / 99.9
	2008 (1,214)	2	2	0.5-4	100.0 / 100.0
	2009 (1,328)	2	2	0.5-2	100.0 / 100.0
	All (6,096)	2	2	0.25-8	99.98[b]
Oxacillin	2009 (1,328)	0.5	>2	≤0.25->2	77.0 / 77.0
Erythromycin	2009 (1,328)	0.5	>2	≤0.25->2	71.1 / 72.1
Clindamycin	2009 (1,328)	≤0.25	≤0.25	≤0.25->2	91.1 / 90.4
Daptomycin	2009 (1,328)	0.25	0.5	≤0.06-1	100.0 / 100.0
Gentamicin	2009 (1,328)	≤1	≤1	≤1-28	96.1 / 95.3
Levofloxacin	2009 (1,328)	≤0.5	>4	≤0.5->4	75.2 / 75.2
QD[c]	2009 (1,328)	0.5	0.5	0.5->2	99.7 / 99.7
Tetracycline	2009 (1,328)	≤1	≤1	≤1->8	95.0/94.7
TMP/SMX[c]	2009 (1,328)	≤0.5	≤0.5	≤0.5->2	99.0 / 99.0
Teicoplanin	2009 (1,328)	≤2	≤2	≤2-8	100.0 / 99.3
Vancomycin	2009 (1,328)	1	1	≤0.12-2	100.0 / 100.0
CoNS[c]					
Linezolid	2002 (178)	1	1	0.25-2	100.0 / 100.0
	2003 (261)	1	1	0.25-2	100.0 / 100.0
	2004 (186)	1	1	0.25-2	100.0 / 100.0
	2005 (140)	1	1	0.5-2	100.0 / 100.0
	2006 (200)	1	1	≤0.06-8	99.5 / 99.5
	2007 (373)	0.5	1	0.25-8	99.5 / 99.5
	2008 (340)	1	1	0.25->8	99.1 / 99.1
	2009 (394)	1	1	0.25-4	100.0 / 100.0
	All (2,072)	1	1	≤0.06->8	99.71[b]
Oxacillin	2009 (394)	>2	>2	≤0.25->2	16.2 / 31.5
Erythromycin	2009 (394)	>2	>2	≤0.25->2	35.8 / 36.6
Clindamycin	2009 (394)	≤0.25	>2	≤0.25->2	68.5 / 66.0
Daptomycin	2009 (394)	0.25	0.5	≤0.06-1	100.0 / 100.0
Gentamicin	2009 (394)	≤1	4	≤1->8	53.3 / 49.0
Levofloxacin	2009 (394)	4	>4	≤0.5->4	42.9 / 42.9
QD[c]	2009 (394)	≤0.25	0.5	≤0.25->2	97.5 / 97.5
Tetracycline	2009 (394)	≤1	>8	≤1->8	85.0 / 78.7
TMP/SMX[c]	2009 (394)	≤0.5	>2	≤0.5->2	61.8 / 61.8
Teicoplanin	2009 (394)	≤2	8	≤2->16	98.0 / 66.2
Vancomycin	2009 (394)	1	2	0.25-4	100.0 / 98.7
Enterococci					
Linezolid	2002 (173)	2	2	0.5-4	99.4 / 99.4
	2003 (234)	2	2	0.5-2	100.0 / /100.0
	2004 (187)	2	2	0.5-2	100.0 / 100.0
	2005 (203)	2	2	0.5-2	100.0 / 100.0
	2006 (120)	1	2	0.5-8	99.2 / 99.2
	2007 (263)	1	2	0.5-2	100.0 / 100.0
	2008 (270)	1	2	0.25->8	98.9 / 98.9
	2009 (574)	2	2	0.25-2	100.0 / 100.0
	All (2,054)	2	2	0.25->8	99.76[b]
Ampicillin	2009 (574)	2	>16	≤1->16	61.2 / 60.8
Ciprofloxacin	2009 (574)	>4	>4	≤0.5->4	33.6 / -[d]
Daptomycin	2009 (574)	1	2	0.12-4	100.0 / -
Levofloxacin	2009 (574)	>4	>4	≤0.5->4	45.5 / -
QD[c,e]	2009 (574)	>2	>2	≤0.25->2	36.2 / -
Tetracycline	2009 (574)	>8	>8	≤2->8	42.7 / -
Teicoplanin	2009 (574)	≤2	≤2	≤2->16	86.6 / 85.2
Vancomycin	2009 (574)	1	>16	0.5->16	84.0 / 84.0

Pathogen/ Antimicrobial agent	Year (no. tested)	MIC (mg/L): 50%	90%	Range	% Susceptible[a] CLSI/EUCAST
S. pneumoniae					
Linezolid	2002 (376)	1	1	≤0.06-2	100.0 / 100.0
	2003 (246)	1	1	0.12-2	100.0 / 100.0
	2004 (237)	1	1	≤0.12-2	100.0 / 100.0
	2005 (274)	1	1	≤0.12-2	100.0 / 100.0
	2006 (120)	1	1	0.25-2	100.0 / 100.0
	2007 (275)	1	1	≤0.12-2	100.0 / 100.0
	2008 (302)	1	1	≤0.12-2	100.0 / 100.0
	2009 (437)	1	1	≤0.12-2	100.0 / 100.0
	All (2,267)	1	1	≤0.12-2	100.00[b]
Penicillin[f]	2009 (437)	≤0.03	2	≤0.03-8	72.3 / 72.3
Amoxyclav	2009 (437)	≤1	2	≤1>16	941 / 72.3
Ceftriaxone	2009 (437)	≤0.25	1	≤0.25-4	92.5 / 81.7
Erythromycin	2009 (437)	≤0.25	>8	≤0.25->8	74.1 / 74.1
Clindamycin	2009 (437)	≤0.25	>2	≤0.25->2	81.0 / 81.7
Levofloxacin	2009 (437)	1	1	≤0.5->4	98.9 / 98.9
Tetracycline	2009 (437)	≤2	>8	≤2->8	77.6 / 77.6
TMP/SMX[c]	2009 (437)	≤0.5	>2	≤0.5->2	78.2 / 84.2
Teicoplanin	2009 (437)	≤2	≤2	≤2	- / 100.0
Vancomycin	2009 (437)	1	1	≤1	100.0 / 100.0
β-haemolytic streptococci					
Linezolid	2002 (47)	1	1	0.5-2	10.0 / 100.0
	2003 (78)	1	1	0.5-1	100.0 / 100.0
	2004 (117)	1	1	0.25-2	100.0 / 100.0
	2005 (116)	1	1	0.25-2	100.0 / 100.0
	2006 (107)	1	1	0.5-1	100.0 / /100.0
	2007 (152)	1	1	0.5-1	100.0 / 100.0
	2008 (152)	1	1	0.5-1	100.0 / 100.0
	2009 (178)	1	1	0.5-2	100.0 / 100.0
	All (947)	1	1	0.25-2	100.00[b]
Penicillin	2009 (178)	0.03	0.06	≤0.015-0.12	100.0 / 100.0
Ceftriaxone	2009 (178)	≤0.25	≤0.25	≤0.25-0.5	100.0 / 100.0
Erythromycin	2009 (178)	≤0.25	>2	≤0.25->2	81.5 / 81.5
Clindamycin	2009 (178)	≤0.25	0.5	≤0.25->2	89.8 / 91.0
Levofloxacin	2009 (178)	≤0.5	1	≤0.5-2	100.0 / 92.1
Daptomycin	2009 (178)	0.12	0.25	≤0.06-0.5	100.0 / 100.0
Teicoplanin	2009 (178)	≤2	≤2	≤2	- / 100.0
Vancomycin	2009 (178)	0.5	0.5	0.25-1	100.0 / 100.0

a. Interpretive breakpoint criteria of the CLSI and EUCAST (2010).
b. All-year linezolid susceptibility rate; range, 98.9-100.0%.
c. QD = quinupristin/dalfopristin; TMP/SMX = trimethoprim/sulfamethoxazole; CoNS = coagulase-negative staphylococci.
d. - = no criteria published.
e. Active only against *E. faecium*.
f. Criteria at ≤0.06 mg/L for both organizations.

Figure 1. Linezolid MIC distribution for all isolates from 2002-2009 ZAAPS Program in European nations (13,965 strains).

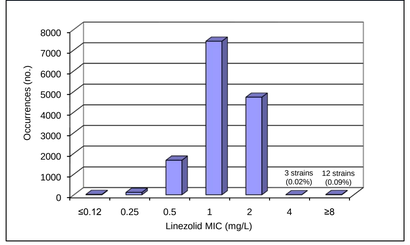

Table 2. Distributions of linezolid MIC results for *S. aureus* isolates from Europe (2009) indexed by methicillin (oxacillin) susceptibility.

Oxacillin pattern (no. tested)	No. (%) by MIC (mg/L) 0.25	0.5	1	2	4
Susceptible (1,023)	0 (0.0)	3 (0.3)	282 (27.6)	738 (72.1)	0 (0.0)
Resistant (305)	0 (0.0)	3 (1.0)	79 (25.9)	223 (73.1)	0 (0.0)

Table 3. Listing of various linezolid non-susceptible Gram-positive cocci detected in European medical centers during the 2002-2009 ZAAPS resistance surveillance program.

Year	Organism	Country	Linezolid MIC	Mechanism
2002-05	-	-	-	-
2006	*E. faecium*	Germany	8	G2576T
	S. epidermidis	Italy	8	L3 (L101V, F147L, A157R), L4 (N158S)
2007	*S. aureus*	Ireland	8	G2576T
	S. epidermidis	Italy	8	L3 (L101V, F147L, A157R), L4 (N158S)
	S. epidermidis	Italy	8	L3 (L101V, F147L, A157R), L4 (N158S, K68R)
2008	*E. faecalis*	Sweden	8	Unknown[a]
	E. faecalis	UK	>8	G2576T
	E. faecium	Germany	>8	G2576T
	S. epidermidis	France	>8	G2576T
	S. epidermidis	Italy	>8	G2447T
	S. epidermidis	Italy	>8	*cfr*, L3 (L101V, F147L, A157R), L4 (N158S)
2009	*E. faecium*	Germany	8	G2576T

a. Mechanisms remain under study via L3, L4, and L22 protein analysis.

CONCLUSIONS

- Linezolid resistance in Europe remains unusual (<0.1%), but focused among staphylococci (CoNS > *S. aureus*) and enterococci.
- Diverse geographic locations of the linezolid-resistant strains were noted in six countries (France, Germany, Ireland, Italy, Sweden and UK) with clonal dissemination in several medical centers.
- Resistance mechanisms were also diverse (Table 3) but included only one *cfr* mobile gene found in 2008 (Italy).
- Monitoring should be continued to assure the recognition of linezolid-refractory strains and the potential for wider dissemination geographically in Europe.

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