

# Linezolid Susceptibility Surveillance in Europe for 2013: ZAAPS Program (5,076 strains)

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## ABSTRACT

**Objectives:** To report the most recent full-year of linezolid (LZD) resistance surveillance (ZAAPS Program) monitoring European (EU) medical centers for 2013. A total of 5,076 Gram-positive organisms were consecutively collected (prevalence design) from 42 hospitals in 20 countries. Results represent the thirteenth yearly EU sample for this central laboratory-based study.

**Methods:** All susceptibility (S) tests were performed applying reference CLSI broth microdilution methods, using validated panels (TREK Diagnostics). The number of strains tested were: *S. aureus* (SA; 2,542, 23.4% MRSA), coagulase-negative staphylococcal species (CoNS; 499, 79.4% methicillin-resistant [R]), *Enterococcus* spp. (ENT; 722 with 420 *E. faecalis* [EF] and 291 *E. faecium* [EFM]), *S. pneumoniae* (SPN, 693), viridans gr. (VGS, 189) and beta-haemolytic (BHS, 431). Staphylococcal strains having elevated MICs to LZD (MIC,  $\geq$ 4 mg/L) were tested by molecular methods (PCR/sequencing, PFGE) to determine R mechanisms (23S target, L3 or L4 mutations and *cf*r).

**Results:** LZD potency for indicated species in the ZAAPS Program was consistent across species (MIC<sub>90</sub> at 1 mg/L), see Table. Nearly all MIC values for LZD were 0.5, 1 or 2 mg/L. One LZD-R isolate was detected among SA which contained the *cf*r gene. LZD-R strains were also detected in the CoNS group (2 *S. epidermidis*) both with G2576T mutations (1 strain with the L3 mutation M156R and 1 strain with L3 mutation M156T). These countries had LZD-R strains: Italy (Genova; 2 *S. epidermidis* strains) and Spain (Madrid; *S. aureus*) with LZD-R MIC values at 8-32 mg/L. Agents other than LZD with  $>90\%$  S rates versus SA were: clindamycin (90.3%), gentamicin (95.6%), tetracycline (93.0%), teicoplanin (100.0%), trimethoprim/sulfamethoxazole (99.5%) and vancomycin (100.0%). MRSA rates varied greatly among countries. Six countries exhibited rates greater than 40%, four countries rates between 20-30%, eight countries between 10-20% and two countries (Sweden and Slovenia) less than 10%. Sweden had the lowest rate (0.6%, 1/177) and Portugal the highest (78.1%, 57/73). Erythromycin-R and clindamycin-R rates among MRSA were 72.1% and 33.7%, respectively. The vancomycin-non-susceptible rate for Enterococci was 12.2%. LZD was active against all streptococci with an MIC<sub>90</sub> of 1 mg/L. Penicillin-R rate for SPN was 12.0% and ceftriaxone-non-S for SPN was 4.7% (CLSI criteria) or 14.0% (EUCAST criteria).

**Conclusions:** LZD-R rate for 2013 EU ZAAPS Program was only 0.06% and showed no escalation of R compared to previous reports from the ZAAPS programs. LZD-R occurrences were among CoNS and SA, which included one acquired *cf*r gene among the 3,041 staphylococci screened in EU.

### Abstract Table

Organism group (no. tested)	LZD MIC (mg/L):			% Susceptible (EUCAST Criteria)
	50%	90%	Range	
<i>S. aureus</i>				
MSSA (1,948)	1	1	$\leq 0.12$ -2	100.0
MRSA (594)	1	1	$\leq 0.12$ -8	99.83 <sup>a</sup>
CoNS (499)	0.5	1	0.25->8	99.80 <sup>b</sup>
ENT (722)	1	1	0.25-2	100.0
SPN (693)	1	1	$\leq 0.12$ -2	100.0
VGS (189)	0.5	1	$\leq 0.12$ -1	100.0
BHS (431)	1	1	0.25-1	100.0

a. 1 strain was R (Spain; *cf*r positive)  
b. 2 strains were R (Italy; G2576T mutations [2]), both *S. epidermidis*.

## INTRODUCTION

Linezolid was the first oxazolidinone agent to be approved for clinical use by various national or regional regulatory agencies, and has become an important therapeutic agent for infections caused by commonly occurring antimicrobial-resistant Gram-positive pathogens. Oxazolidinone resistance has been detected, mainly among Enterococcus species and the coagulase-negative staphylococci (CoNS; *S. epidermidis* and some other species), but the occurrence rates remain rare among indicated *Staphylococcus aureus* and streptococci. These documented resistance occurrences were associated with recognized risk factors such as prolonged therapeutic exposure, but oxazolidinone-resistant strains have also emerged in patients without prior linezolid exposure, each attributable to clonal dissemination from other patients in the same hospital environment. Also, new mechanisms of resistance (L3 and/or L4 protein alterations) have been detected in CoNS, but in the more recent reports, the mobile *cf*r gene has been observed in *S. aureus* and CoNS human infections on several continents.

Linezolid is used globally and the need for potency and resistance surveillance remains critical to the continued success of the oxazolidinone class. Comprehensive in vitro susceptibility testing studies of linezolid before regulatory approval, showed an excellent spectrum against nearly all Gram-positive pathogens isolated worldwide, including those in Europe. This report for 2013 ZAAPS Program, presents data from laboratories in Europe that forwarded cultures for the central laboratory-based surveillance study (20 nations). The total number of ZAAPS Program strains reported from 2006-2012 was 43,997 (6,285/year average) strains. All tests were performed in a GLP-compliant and CLIA-certified reference laboratory (JMI Laboratories, North Liberty, Iowa, USA) using CLSI broth microdilution methods and published CLSI interpretive criteria [M07-A9, 2012; M100-S24, 2014], as well as EUCAST interpretive breakpoints (2014). All surveillance results for these surveillance programs through 2012 have been reported in peer-reviewed publications.

## MATERIALS AND METHODS

A total of 5,076 Gram-positive strains were collected from 42 medical centers in Europe (20 countries; Table 1). Each site submitted 250-500 isolates to reach a targeted  $\geq 200$  Gram-positive organisms per country. JMI Laboratories performed confirmatory organism identification tests and reference broth microdilution susceptibility testing by CLSI methods (M07-A9, 2012; M100-S24, 2014). Susceptibility testing was performed using validated, microdilution panels with cation-adjusted Mueller-Hinton broth (2.5-5% lysed horse blood added for testing streptococci). For 2013 in Europe, all results were interpreted for the susceptibility category by both CLSI (M100-S24, 2014) and EUCAST (2014) criteria/breakpoints, where available.

Isolates with elevated MIC values to linezolid (MIC,  $\geq 4$  mg/L) upon initial testing were confirmed by repeat dry-and frozen-form broth microdilution testing, Etest and disk diffusion methods (CLSI M02-A10, 2012). Molecular testing was performed to identify the target site mutation (23S rRNA, L3 and/or L4 proteins) or gene (*cf*r)-based mechanisms, and possible epidemic clonality using pulsed field gel electrophoresis (PFGE) and/or various other PCR-based tests (Table 4).

## RESULTS

- A total of 18 European countries plus Israel and Turkey contributed isolates to the 2013 ZAAPS Program (Table 1).
- Six major Gram-positive pathogen groups were monitored with sampled organisms ranging from 2,542 strains of *S. aureus* to a sample of only 189 viridans group streptococci (Table 2). Three (0.06%) organisms had linezolid MIC values of  $>2$  mg/L (possible non-susceptibility).
- Linezolid potency at a MIC<sub>50</sub>/MIC<sub>90</sub> of 0.5/1 mg/L was consistent across all sampled species; and was consistent with ZAAPS results published since 2004. Over 95.0% of tested strains had linezolid MIC values of either 0.5 or 1 mg/L (Table 2).
- The MRSA rates among countries ranged from 0.56% (Sweden) to 78.08% (Portugal). Only one linezolid-resistant MRSA isolate was detected in Spain (MIC, 8 mg/L; *cf*r- positive from Madrid, see Table 4). This occurrence appears to be a continuation of a local epidemic.
- Among the 499 CoNS strains, two *S. epidermidis* were linezolid-resistant (MIC values, 16 and 32 mg/L). Both organisms were methicillin-resistant and contained a variety of target site mutations in 23S rRNA and/or L3 protein (Table 4).
- One in eight enterococci were vancomycin-resistant, usually a VAN-A phenotype (Table 3); and 27.6% of pneumococci were penicillin-non-susceptible by EUCAST criteria (Table 3). *S. pneumoniae* were also less susceptible to ceftriaxone (86.0%), erythromycin (74.9%), clindamycin (84.0%), tetracyclines (78.3%) and TMP/SMX (79.7%).
- The most complete activity against all sampled Gram-positive pathogens was noted for linezolid (99.94% susceptible) > vancomycin (100.0% except for enterococci [87.8% susceptible]); see Tables 2 and 3.

**Table 1.** Countries participating in the 2013 ZAAPS linezolid resistance surveillance program when monitoring Europe, Israel and Turkey.

Belgium	Germany	Poland	Spain
Bulgaria	Greece	Portugal	Sweden
Croatia	Hungary	Romania	United Kingdom
Czech Republic	Ireland	Slovakia	Israel
France	Italy	Slovenia	Turkey

**Table 2.** Linezolid MIC population distributions for six organism groups for Gram-positive pathogens in Europe, 2013 (5,076 strains).

Organism (no. tested)	No. occurrences (cum. %) inhibited by linezolid at MIC in mg/L:								MIC <sub>50</sub>	MIC <sub>90</sub>
	$\leq 0.12$	0.25	0.5	1	2	4	8	$>8$		
<i>S. aureus</i> (2542)	2 (0.1)	5 (0.3)	382 (15.3)	2,064 (96.5)	88 (100.0)	--	1 (100.0)	--	1	1
MSSA (1948)	1 (0.1)	3 (0.2)	269 (14.0)	1,605 (96.4)	70 (100.0)	--	--	--	1	1
MRSA (594)	1 (0.2)	2 (0.5)	113 (19.5)	459 (96.8)	18 (99.8)	0 (99.8)	1 (100.0)	--	1	1
CoNS (499)	0 (0.0)	67 (13.4)	349 (83.4)	80 (99.4)	1 (99.6)	0 (99.6)	0 (99.6)	2 (100.0)	0.5	1
MSCoNS (103)	0 (0.0)	13 (12.6)	74 (84.5)	16 (100.0)	--	--	--	--	0.5	1
MRCoNS (396)	0 (0.0)	54 (13.6)	275 (83.1)	64 (99.2)	1 (99.5)	0 (99.5)	0 (99.5)	2 (100.0)	0.5	1
<i>Enterococcus</i> spp. (722)	0 (0.0)	5 (0.7)	111 (16.1)	560 (93.6)	46 (100.0)	--	--	--	1	1
vanc.-susc. (634)	0 (0.0)	5 (0.8)	86 (14.4)	498 (92.9)	45 (100.0)	--	--	--	1	1
vanc.-non-susc. (88)	0 (0.0)	0 (0.0)	25 (28.4)	62 (98.9)	1 (100.0)	--	--	--	1	1
<i>S. pneumoniae</i> (693)	1 (0.1)	6 (1.0)	209 (31.2)	472 (99.3)	5 (100.0)	--	--	--	1	1
VGS (189)	2 (1.1)	10 (6.3)	94 (56.1)	83 (100.0)	--	--	--	--	0.5	1
BHS (431)	0 (0.0)	1 (0.2)	159 (37.1)	271 (100.0)	--	--	--	--	1	1
All Organisms (5076)	5 (0.1)	94 (2.0)	1,304 (27.6)	3,530 (97.2)	140 (99.9)	0 (99.9)	1 (99.9)	2 (100.0)	1	1

**Table 3.** Comparative activity of linezolid tested against 5,076 Gram-positive pathogens in the European ZAAPS Program (2013).

Organism (no. tested)/ antimicrobial agent	MIC (mg/L):			CLSI <sup>a</sup>		EUCAST <sup>a</sup>	
	50%	90%	Range	%S / %I / %R			
<i>S. aureus</i> (2,542)							
Linezolid	1	1	$\leq 0.12$ -8	$>99.9 / 0.0 / <0.1$	$>99.9 / 0.0 / <0.1$	$>99.9 / 0.0 / <0.1$	$>99.9 / 0.0 / <0.1$
Oxacillin	0.5	$\geq 2$	$\leq 0.25$ ->2	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4
Ceftriaxone	4	$>8$	0.5->8	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4	76.6 / 0.0 / 23.4
Erythromycin	0.25	$>16$	$\leq 0.12$ ->16	71.4 / 1.8 / 26.8	71.4 / 1.8 / 26.8	71.5 / 0.6 / 27.9	71.5 / 0.6 / 27.9
Clindamycin	$\leq 0.25$	$\leq 0.25$	$\leq 0.25$ ->2	90.3 / 0.1 / 9.6	90.3 / 0.1 / 9.6	90.0 / 0.3 / 9.7	90.0 / 0.3 / 9.7
Gentamicin	$\leq 1$	$\leq 1$	$\leq 1$ ->8	95.6 / 0.1 / 4.3	95.6 / 0.1 / 4.3	95.1 / 0.0 / 4.9	95.1 / 0.0 / 4.9
Levofloxacin	0.25	$\geq 4$	$\leq 0.12$ ->4	77.8 / 0.4 / 21.8	77.8 / 0.4 / 21.8	77.8 / 0.4 / 21.8	77.8 / 0.4 / 21.8
Tetracycline	0.25	0.25	0.06->32	93.0 / 0.5 / 6.5	92.5 / 0.3 / 7.2	92.5 / 0.3 / 7.2	92.5 / 0.3 / 7.2
TMP/SMX	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$ ->4	99.5 / 0.0 / 0.5	99.5 / 0.0 / 0.5	99.5 / 0.1 / 0.4	99.5 / 0.1 / 0.4
Teicoplanin	$\leq 2$	$\leq 2$	$\leq 2$ -4	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	99.8 / 0.0 / 0.2	99.8 / 0.0 / 0.2
Vancomycin	1	1	$\leq 0.12$ -2	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
CoNS (499) <sup>b</sup>							
Linezolid	0.5	1	0.25->8	99.6 / 0.0 / 0.4	99.6 / 0.0 / 0.4	99.6 / 0.0 / 0.4	99.6 / 0.0 / 0.4
Oxacillin	$\geq 2$	$\geq 2$	$\leq 0.25$ ->2	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4
Ceftriaxone	$>8$	$>8$	0.5->8	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4	20.6 / 0.0 / 79.4
Erythromycin	$>16$	$>16$	$\leq 0.12$ ->16	32.9 / 0.8 / 66.3	32.9 / 0.8 / 66.3	32.9 / 0.6 / 66.5	32.9 / 0.6 / 66.5
Clindamycin	$\leq 0.25$	$\geq 2$	$\leq 0.25$ ->2	66.3 / 1.0 / 32.7	66.3 / 1.0 / 32.7	65.5 / 0.8 / 33.7	65.5 / 0.8 / 33.7
Gentamicin	$\leq 1$	$>8$	$\leq 1$ ->8	54.5 / 8.6 / 36.9	54.5 / 8.6 / 36.9	51.3 / 0.0 / 48.7	51.3 / 0.0 / 48.7
Levofloxacin	4	$\geq 4$	$\leq 0.12$ ->4	44.9 / 3.6 / 51.5	44.9 / 3.6 / 51.5	44.9 / 3.6 / 51.5	44.9 / 3.6 / 51.5
Tetracycline	0.5	32	0.06->32	81.5 / 1.8 / 16.7	81.5 / 1.8 / 16.7	76.7 / 2.6 / 20.7	76.7 / 2.6 / 20.7
TMP/SMX	$\leq 0.5$	$\geq 4$	$\leq 0.5$ ->4	64.7 / 0.0 / 35.3	64.7 / 0.0 / 35.3	64.7 / 16.9 / 18.4	64.7 / 16.9 / 18.4
Teicoplanin	$\leq 2$	8	$\leq 2$ -16	98.2 / 1.8 / 0.0	98.2 / 1.8 / 0.0	84.6 / 0.0 / 15.4	84.6 / 0.0 / 15.4
Vancomycin	1	2	$\leq 0.12$ -2	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Enterococci (722) <sup>c</sup>							
Linezolid	1	1	0.25->2	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Ampicillin	2	$>8$	0.5->8	61.8 / 0.0 / 38.2	61.8 / 0.0 / 38.2	61.4 / 0.4 / 38.2	61.4 / 0.4 / 38.2
Piperacillin/tazobactam	8	$\geq 64$	1->64	61.4 / - / -	61.4 / - / -	- / - / -	- / - / -
Erythromycin	$>16$	$>16$	$\leq 0.12$ ->16	6.6 / 28.6 / 64.8	6.6 / 28.6 / 64.8	- / - / -	- / - / -
Levofloxacin	$\geq 4$	$\geq 4$	0.25->4	44.9 / 1.6 / 53.5	44.9 / 1.6 / 53.5	- / - / -	- / - / -
Teicoplanin	$\leq 2$	16	$\leq 2$ ->16	89.8 / 1.2 / 9.0	89.8 / 1.2 / 9.0	89.6 / 0.0 / 10.4	89.6 / 0.0 / 10.4
Vancomycin	1	$>16$	0.5->16	87.8 / 0.4 / 11.8	87.8 / 0.4 / 11.8	87.8 / 0.0 / 12.2	87.8 / 0.0 / 12.2
<i>S. pneumoniae</i> (693)							
Linezolid	1	1	$\leq 0.12$ -2	100.0 / - / -	100.0 / - / -	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Penicillin	$\leq 0.06$	2	$\leq 0.06$ -4	72.4 / 15.6 / 12.0	72.4 / 15.6 / 12.0	72.4 / 23.7 / 3.9	72.4 / 23.7 / 3.9
Amoxicillin/clavulanate	$\leq 1$	2	$\leq 1$ ->8	93.9 / 2.3 / 3.8	93.9 / 2.3 / 3.8	- / - / -	- / - / -
Ceftriaxone	$\leq 0.06$	1	$\leq 0.06$ -2	95.3 / 4.7 / 0.0	95.3 / 4.7 / 0.0	86.0 / 14.0 / 0.0	86.0 / 14.0 / 0.0
Erythromycin	$\leq 0.12$	$>16$	$\leq 0.12$ ->16	74.9 / 0.7 / 24.4	74.9 / 0.7 / 24.4	74.9 / 0.7 / 24.4	74.9 / 0.7 / 24.4
Clindamycin	$\leq 0.25$	$>2$	$\leq 0.25$ ->2	84.0 / 0.0 / 16.0	84.0 / 0.0 / 16.0	84.0 / 0.0 / 16.0	84.0 / 0.0 / 16.0
Levofloxacin	1	1	0.25->4	99.0 / 0.1 / 0.9	99.0 / 0.1 / 0.9	99.0 / 0.0 / 1.0	99.0 / 0.0 / 1.0
Tetracycline	0.25	32	0.06->32	78.3 / 0.4 / 21.3	78.3 / 0.4 / 21.3	78.3 / 0.4 / 21.3	78.3 / 0.4 / 21.3
TMP/SMX	$\leq 0.5$	4	$\leq 0.5$ ->4	73.0 / 10.6 / 16.4	73.0 / 10.6 / 16.4	79.7 / 3.9 / 16.4	79.7 / 3.9 / 16.4
Vancomycin	0.25	0.5	$\leq 0.12$ -0.5	100.0 / - / -	100.0 / - / -	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Viridans gr. streptococci (189) <sup>d</sup>							
Linezolid	0.5	1	$\leq 0.12$ -1	100.0 / - / -	100.0 / - / -	- / - / -	- / - / -
Penicillin	$\leq 0.06$	1	$\leq 0.06$ -8	74.6 / 21.7 / 3.7	74.6 / 21.7 / 3.7	83.6 / 12.7 / 3.7	83.6 / 12.7 / 3.7
Ceftriaxone	0.25	0.5	$\leq 0.06$ ->8	93.7 / 2.6 / 3.7	93.7 / 2.6		