Linezolid Susceptibility in Europe for 2010: ZAAPS Program (5,532 strains)

1367

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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 2010. A total of 5,532 Gram-positive organisms were consecutively collected (prevalence design) from 39 hospitals in 17 countries. Results represent the ninth 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). Sampled strains came from bacteremias (52.4%), respiratory tract (25.5%) and skin infections (22.1%) sources; one organism per patient episode. The number of strains tested were: *S. aureus* (SA; 2,452, 27.0% MRSA), coagulase-negative staphylococcal species (CoNS; 593, 71.7% methicillin-resistant [R]), *Enterococcus* spp. (ENT; 864 with 542 *E. faecalis* [EF] and 288 *E. faecium* [EFM]), *S. pneumoniae* (SPN, 747), viridans gr. (VGS, 250) and beta-haemolytic (BHS, 626). Streptococcal strains R to LZD (MIC, ≥4 mg/L) were tested by molecular methods (PCR/sequencing, PFGE) to determine R mechanism (23S target, L3, L4 mutations and *cfr*).

Results: LZD potency for indicated species in the ZAAPS Program remained stable (MIC_{90} for all species at 1 mg/L), see Table. Nearly all MIC values for LZD were 0.5, 1 or 2 mg/L. No LZD-R isolates were detected among SA and the three streptococcal pathogens monitored. LZD-R strains were EFM (1 strain with G2576T mutation) and 2 *S. epidermidis* strains (both with G2576T mutations) and no other mechanisms were detected. Three countries had LZD-R strains: Germany (Frankfurt), Italy (Genoa) and Spain (Madrid) with LZD-R MIC values at 8-64 mg/L. Other agents with >90% S rates versus SA were: fusidic acid (92.9%), daptomycin (99.9%), doxycycline (94.0%), gentamicin (91.4%), mupirocin (98.1%), quinupristin/dalfopristin (99.8%), tigecycline (100.0%), and vancomycin (100.0%).

Organism group (no. tested)	LZD MIC (mg/L): 50%	90%	Range	% Susceptible (EUCAST Criteria)
S. aureus				
MSSA (1,791)	1	1	0.25-2	100.00
MRSA (661)	1	1	0.25-2	100.00
CoNS (593)	0.5	1	≤0.12->8	99.66[a]
ENT (864)	1	1	0.25-8	99.88[b]
SPN (747)	1	1	≤0.12-2	100.00
VGS (250)	1	1	≤0.12-2	100.00
BHS (626)	1	1	0.5-1	100.00

a. 2 strains were R (Italy and Spain; G2576T mutations [2]), both *S. epidermidis*.
b. 1 strains was R (Germany; G2576T mutation), an EFM.

Conclusions: LZD-R rate for the worldwide 2009 ZAAPS Program was only 0.14% and these 2010 EU ZAAPS results showed no escalation of R (0.05%; 3/5,532) with no evidence of clonality. CoNS and ENT species have the highest LZD-R occurrences and no mobile *cfr* genes were identified among 3,045 staphylococci screened in EU.

INTRODUCTION

There is a limited number of therapeutic options for the treatment of serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Linezolid is an oxazolidinone agent with a unique mechanism of action that inhibits the formation of the initiation complex necessary for protein synthesis. Approved by the United States (USA) Food and Drug Administration (FDA) in the year 2000, linezolid remains the only commercially available oxazolidinone. Since its launch in the USA, many European countries have subsequently approved the use of linezolid. This agent is recognized as an important therapeutic alternative for infections caused by commonly occurring multidrug-resistant (MDR) Gram-positive pathogens, and its use continues on a global level.

There are limited side effects associated with linezolid therapy including nausea, vomiting, thrombocytopenia, and myelosuppression among the more commonly documented adverse events from published clinical studies. In the USA, linezolid is approved for the treatment of infections caused by vancomycin-resistant (VRE) *Enterococcus faecium*, with or without concurrent bacteremia, nosocomial pneumonia caused by *S. aureus* (methicillin-susceptible [MSSA] and MRSA strains), or *Streptococcus pneumoniae* including MDR strains. Other indications include complicated skin and skin structure infections (cSSSI) caused by *S. aureus*, *S. pyogenes*, or *S. agalactiae*, including diabetic foot infections without concomitant osteomyelitis.

Confirmed resistance to linezolid has been detected in several countries and among certain clinically important species, such as *E. faecium*, *E. faecalis* and coagulase-negative staphylococci (CoNS) including *S. epidermidis*. The occurrence of linezolid resistance remains rare (<0.1%) for *S. aureus* and streptococci. Documented resistance has been associated with risk factors such as prolonged therapeutic exposure, intermittent use or the presence of undrained abscesses. In contrast, some oxazolidinone-resistant strains have been reported in patients who have not been exposed to linezolid therapy and have been attributed to clonal or epidemic spread of linezolid-resistant strains in the hospital environment.

The most common mechanism of resistance to linezolid is target site mutations in 23S rRNA and/or ribosomal proteins L3 and L4. More recently, resistance mediated by the *cfr* gene has been described in CoNS, enterococcus and *S. aureus,* including European strains. Cfr resistance is transmissible and is associated with cross resistance to other antimicrobial classes.

Continued monitoring of the *in vitro* activity of linezolid versus Gram-positive pathogens as well as for the detection of emerging resistance are necessary to ensure optimial use of this unique agent and to preserve its clinical efficacy.

The history of linezolid surveillance programs began before product launch with a series of susceptibility testing studies including ZAPS international studies followed by this investigation (ZAAPS; Zyvox® Annual Appraisal of Potency and Spectrum). The study results summarized here, analyze year 2010 expanded sampling of Gram-positive species collected from European medical centers.

MATERIALS AND METHODS

Bacterial strains: The year 2010 ZAAPS organism collection for European nations was expanded from 10 to 17 countries (39 sites) and from 2,200 to over 5,000 sampled Gram-positive pathogens. These organisms were grouped for analyses as follows: *S. aureus* (2,452 strains, 27.0% MRSA), CoNS (593 strains; 72.7% methicillin-resistant), *Enterococcus* species (864 strains; 62.7% *E. faecalis*), *S. pneumoniae* (747 strains), viridans group streptococci (VGS; 250 strains) and β-haemolytic streptococci (626 strains). The organisms were derived from various infection sites including bacteremias (52.4%), lower respiratory tract (25.5%), and skin and skin structure (22.1%), collected in a prevalence study design.

The countries participating were: Belgium, Czech Republic, France, Germany, Greece, Ireland, Italy, Poland Portugal, Russia, Slovenia, Spain, Sweden, United Kingdom (UK) and the Ukraine. Medical centers in Israel and Turkey also contributed strains.

Susceptibility testing: The ZAAPS Program processes all submitted strains in a central, reference laboratory design using reference Clinical and Laboratory Standards Institute (CLSI) methods (M07-A8 [2009] and M100-S21 [2011]). Forwarded organism identifications were confirmed and the strains were tested against linezolid. Breakpoints published by the CLSI (M100-S21, 2011) and EUCAST (2011) were used to interpret MIC results. Concurrent quality control (QC) was performed with at least three QC strains (ATCC 29213, 29212, 49619), with all results within published limits.

Organisms determined to have higher linezolid MIC values (≥4 mg/L) were subjected to a series of molecular tests to detect 23S rRNA target mutations and/or L3, L4 or L22 protein alterations, and/or the presence of *cfr* (see Table 1) as described in earlier ZAAPS Program publications (Biedenbach et al., 2010).

RESULTS

- ZAAPS Program sampling of European medical centers expanded by more than two-fold from 2,441 strains in 2009 to 5,532 strains in 2010 (Table 1).
- Modal linezolid MIC values ranged from 0.5 mg/L (CoNS) to 1 mg/L (all other monitored species), and results were consistent with all prior ZAAPS surveillance years (2002-2009), see Table 2.
- Linezolid MIC values at 4 mg/L were not observed (very rare in earlier reports) and only three (3) oxazolidinone-resistant strains were detected; one *E. faecium* from Germany, and two *S. epidermidis* strains from Italy and Spain.
- Molecular studies for detection resistance mechanisms revealed a 23S mutation (G2576T) in each strain. Neither the *cfr* gene, nor alterations in ribosomal L proteins were observed (Table 1).
- Linezolid resistance rates (Table 2) remain stable across all studied Gram-positive species and were highest among MR-CoNS (0.5%) and *E. faecium* (0.3%).

Table 1. Distribution of sampled Gram-positive organisms by country and location/type of oxazolidinone resistance.

Country (no. tested)	No. (%) Linezolid-resistant	Species resistant/mechanism
Belgium (169)	-	-
Czech Republic (75)	-	-
France (1,100)	-	-
Germany (717)	1 (0.14%)	*E. faecium* / 23S (G2576T)
Greece (148)	-	-
Ireland (382)	-	-
Italy (316)	1 (0.32%)	*S. epidermidis* / 23S (G2576T)
Poland (70)	-	-
Portugal (264)	-	-
Russia (293)	-	-
Slovenia (96)	-	-
Spain (498)	1 (0.20%)	*S. epidermidis* / 23S (G2576T)
Sweden (472)	-	-
UK (505)	-	-
Ukraine (80)	-	-
Israel (113)	-	-
Turkey (234)	-	-
All (5,532)	3 (0.05%)	-

- Non-susceptibility rates were also noted for several comparison agents (EUCAST breakpoint criteria):
  - Daptomycin versus *S. aureus* and CoNS, <0.1 and 0.2% respectively.
  - Teicoplanin versus CoNS and enterococci, 6.7 and 7.1%, respectively.
  - Vancomycin versus CoNS and enterococci, 0.3 and 8.7%, respectively (See Table 3).

Table 3. Comparative activity of linezolid and selected agents tested against species most commonly associated with oxazolidinone resistance mechanisms (3,909 strains from Europe in 2010).

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L) 50%	90%	% susceptible[b] CLSI	EUCAST
S. aureus (2,452)				
Linezolid	1	1	100.0	100.0
Ciprofloxacin	0.25	>4	69.7	69.7
Clindamycin	≤0.25	1	89.9	**89.5**
Daptomycin	0.25	0.5	>99.9	>99.9
Doxycycline	0.12	0.25	98.1	**94.0**
Erythromycin	≤0.25	>4	71.0	71.0
Gentamicin	≤1	≤1	92.4	**91.4**
Oxacillin	0.5	>2	73.0	73.0
Teicoplanin	≤1	≤1	100.0	100.0
TMP/SMX[c]	≤0.5	≤0.5	99.2	99.2
Vancomycin	1	1	100.0	100.0
CoNS (593)				
Linezolid	0.5	1	99.7	99.7
Ciprofloxacin	4	>4	43.7	43.7
Clindamycin	≤0.25	>2	71.0	**68.6**
Daptomycin	0.25	0.5	99.8	99.8
Doxycycline	0.25	2	94.4	**89.5**
Erythromycin	>4	>4	36.3	36.3
Gentamicin	≤1	>8	64.4	**56.1**
Oxacillin	>2	>2	28.3	28.3
Teicoplanin	2	4	98.7	**92.9**
TMP/SMX[c]	≤0.5	>4	62.2	62.2
Vancomycin	1	2	100.0	**99.7**
Enterococcus spp. (864)[d]				
Linezolid	1	1	99.9	99.9
Ampicillin	≤1	>8	67.5	**66.8**
Ciprofloxacin	>4	>4	41.3	-
Daptomycin	1	2	100.0	-
Doxycycline	8	>8	48.0	-
Q/D[c]	>4	>4	27.7	27.7
Teicoplanin	≤1	≤1	93.5	**93.3**
Vancomycin	1	2	92.7	**91.3**

a. CoNS = coagulase-negative staphylococci.
b. Breakpoint criteria of the CLSI (2011) and EUCAST (2011), where published. - = no interpretive criteria available. BOLDED percentages highlight differences between CLSI and EUCAST interpretive results.
c. TMP/SMX = trimethoprim/sulfamethoxazole (1:19 ratio, TMP concentration listed). Q/D = quinupristin/dalfopristin.
d. Includes: *E. faecalis* (542 strains), *E. faecium* (288 strains), *E. avium* (nine strains), *E. casseliflavus* (seven strains), *E. durans* (six strains), and five strains each of *E. gallinarum* and *E. raffinosus*. Two strains were not speciated.

Table 2. Linezolid MIC distributions for six major genus/species groups as well as resistance subsets in the European ZAAPS Program sample for 2010 (5,532 strains).

Organism (no. tested)[a]	Occurrences (cum%) at MIC in mg/L: ≤0.12	0.25	0.5	1	2	4	8[b]	MIC 50%	90%	% susceptible[c] CLSI	EUCAST
S. aureus											
All (2,452)	-	4 (0.2)	76 (3.3)	2,192 (92.7)	180 (100.0)	-	-	1	1	100.0	100.0
MSSA (1,791)	-	1 (0.1)	40 (2.3)	1,619 (92.7)	131 (100.0)	-	-	1	1	100.0	100.0
MRSA (661)	-	3 (0.5)	36 (5.9)	573 (92.6)	49 (100.0)	-	-	1	1	100.0	100.0
CoNS											
All (593)	3 (0.5)	28 (5.2)	405 (73.5)	155 (99.7)	0 (99.7)	0 (99.7)	0 (99.7)	0.5	1	99.7	99.7
MS (168)	-	9 (5.4)	110 (70.8)	49 (100.0)	-	-	-	0.5	1	100.0	100.0
MR (425)	3 (0.7)	19 (5.2)	295 (74.6)	106 (99.5)	0 (99.5)	0 (99.5)	0 (99.5)	0.5	1	99.5	99.5
Enterococci											
All (864)	-	1 (0.1)	45 (5.3)	757 (92.9)	60 (99.9)	0 (99.9)	1 (100.0)	1	1	99.9	99.9
E. faecalis (542)	-	1 (0.2)	27 (5.2)	469 (91.7)	45 (100.0)	-	-	1	1	100.0	100.0
E. faecium (288)	-	-	12 (4.2)	263 (95.5)	12 (99.7)	0 (99.7)	1 (100.0)	1	1	99.7	99.7
S. pneumoniae (747)	2 (0.3)	11 (1.7)	136 (20.0)	591 (99.1)	7 (100.0)	-	-	1	1	100.0	100.0
VGS (250)	3 (1.2)	10 (5.2)	89 (40.8)	143 (98.0)	5 (100.0)	-	-	1	1	100.0	-
BHS (626)	-	-	82 (13.1)	544 (100.0)	-	-	-	1	1	100.0	100.0

a. MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus*, MS = methicillin-susceptible, MR = methicillin-resistant, VGS = viridans group streptococci, and BHS = β-haemolytic streptococcoci (Group A, 241 strains; and Group B, 261 strains).
b. Highest tested linezolid concentration. Two *S. epidermidis* strains had MIC values at >8 mg/L.
c. Breakpoint interpretive criteria of the CLSI (2011) and EUCAST (2011).

CONCLUSIONS

- After 10 years of clinical use, linezolid remains very active versus >99% of Gram-positive isolates collected from European medical centers.
- The overall linezolid resistance rate was only 0.05% (3/5,532), but was as high as 0.5% for MR-CoNS. No plasmidic oxazolidinone resistance elements (*cfr*) were detected.
- *In vitro* surveillance programs (ZAAPS, LEADER, SENTRY, etc.) have evolved into valuable sources of epidemiologic and molecular-based resistance data on linezolid and other agents.
- CLSI and EUCAST breakpoints for linezolid have become harmonized and provide identical susceptibility results (% susceptible).

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REFERENCES

1. Anderegg TR, Sader HS, Fritsche TR, Ross JE, Jones RN (2005). Trends in linezolid susceptibility patterns: Report from the 2002-2003 worldwide Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program. *Int J Antimicrob Agents* 26: 13-21.
2. Beibei L, Yun C, Mengli C, Nan B, Xuhong Y, Rui W (2010). Linezolid versus vancomycin for the treatment of gram-positive bacterial infections: meta-analysis of randomised controlled trials. *Int J Antimicrob Agents* 35: 3-12.
3. Biedenbach DJ, Farrell DJ, Mendes RE, Ross JE, Jones RN (2010). Stability of linezolid activity in an era of mobile oxazolidinone resistance determinants: Results from the 2009 Zyvox(R) Annual Appraisal of Potency and Spectrum program. *Diagn Microbiol Infect Dis* 68: 459-467.
4. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2011). *M100-S21. Performance standards for antimicrobial susceptibility testing: 21st informational supplement.* Wayne, PA: CLSI.
6. EUCAST (2011). Breakpoint tables for interpretation of MICs and zone diameters. Version 1.3, January 2011. Available at: http://www.eucast.org/clinical_breakpoints/, March 18, 2011.
7. Jones RN, Ross JE, Bell JM, Utsuki U, Fumiaki I, Kobayashi I, Turnidge JD (2009). Zyvox Annual Appraisal of Potency and Spectrum program: Linezolid surveillance program results for 2008. *Diagn Microbiol Infect Dis* 65: 404-413.
8. Kunkel M, Chastre JE, Kollef M, Niederman M, Shorr AF, Wunderink RG, McGee W, Olvey S, Reisman A, Baruch A (2010). Linezolid vs vancomycin in the treatment of nosocomial pneumonia proven due to methicillin-resistant *Staphylococcus aureus*. Abstr. LB-49. *48th IDSA, October 21-24, 2010,* Vancouver, B.C. Canada.
9. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B (2006). The *cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* 50: 2500-2505.
10. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN (2008). First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob Agents Chemother* 52: 2244-2246.
11. Morales G, Picazo JJ, Baos E, Candel FJ, Arribi A, Pelaez B, Andrade R, de la Torre MA, Fereres J, Sanchez-Garcia M (2010). Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. *Clin Infect Dis* 50: 821-825.