# European Trends in Linezolid Susceptibility Patterns in 2003: Report from the Worldwide ZAAPS Program

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## ABSTRACT

**Objective**: To compare and contrast susceptibility (S) testing results for linezolid (LZD) in European isolates of Gram-positive cocci (GPC) with those from the "rest of the world" (ROW; North and South America, Asia). Results from the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program surveillance study of LZD activity initiated in 2002 worldwide (7,921 strains) were compared to program results continued in 2003.
**Methods**: The ZAAPS Program monitors for oxazolidinone (LZD) resistance (R) in >60 medical centers worldwide, each country or site sending 200 isolates to a central laboratory for reference NCCLS testing. In 2003, a total of 8,089 strains were tested, 1,349 strains from Europe (7 countries; 18 sites). Interpretive criteria of the NCCLS M100-S15 (2005) were applied (LZD-R or non-S at ≥ 8 mg/L) for staphylococci and enterococci; and ≥ 4 mg/L for streptococci. LZD-R strains were studied to determine the mechanism via target site sequencing, and patient demographics were also sought.
**Results**: Six groups of GPC were monitored: *S. aureus* (SA), coagulase-negative staphylococci (CoNS), *Enterococcus* spp. (ENT), *S. pneumoniae* (SPN), viridans group streptococci (VgS) and beta-haemolytic streptococci (BST). The 1,349 European strains were processed and results compared to ROW data, see table:

| | LZD MIC (mg/L) | | | % S | |
|---|---|---|---|---|---|
| Organisms (no. tested) | Range | 50% | 90% | Europe | ROW |
| SA (375) | 0.12-2 | 2 | 2 | 100.0 | 100.0 |
| CoNS (263) | 0.25-2 | 1 | 1 | 100.0 | 100.0 |
| ENT (264) | 0.5-2 | 2 | 2 | 100.0 | 99.2 |
| SPN (307) | 0.12-2 | 1 | 1 | 100.0 | 100.0 |
| VgS (62) | 0.25-2 | 1 | 1 | 100.0 | 100.0 |
| BST (78) | ≤0.06-1 | 1 | 1 | 100.0 | 100.0 |

Characteristics of the European collection included: 1) 26.7% MRSA; 2) 76.4% MR-CoNS; 3) 5.3% VRE, 58% *vanA*; 4) SPN penicillin-R and macrolide-non-S at 17.2% and 25.0%, respectively; and 5) macrolide-R BST at 29.0%. Seven LZD-R ENT had G2576U ribosomal target mutations (6 from USA, 1 from Greece; separately submitted, not in ZAAPS monitored nations). No significant changes in LZD potency by organism group or in rates of LZD-R strains were detected between 2002 and 2003 samples.
**Conclusions**: LZD, as monitored by the ZAAPS Program, remained highly active against European isolates of GPC, having a potency identical to that observed in other geographic regions and in various pre-marketing trials (ZAPS). LZD-R strains (ENT) appeared in patient infections in the USA, each with established ribosomal target alternatives. Continued monitoring seems prudent, but LZD-R rates remain very low at 0.07%.

## INTRODUCTION

Antimicrobial resistance rates are increasing in serious Gram-positive infections. Physicians need to have alternative treatments available as the commonly used drugs become rapidly ineffective. Linezolid is a newer oxazolidinone for the treatment of Gram-positive pathogens, including vancomycin-resistant enterococci (VRE). Resistance has been rare, and has been reported dominantly in enterococci in the United States. The resistance mechanism most commonly identified has been a G2576U mutation in the domain V region of the 23S rRNA.

The Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program was established to monitor and report linezolid-resistant organisms isolated from patients throughout the world. In 2002, the ZAAPS Program tested 7,971 Gram-positive isolates in Europe: no resistant isolates were detected during that year. Given the rare, but sporadic appearance of linezolid-resistant strains discovered to date in staphylococci, streptococci and enterococci, continued monitoring for the emergence of resistance remains a prudent measure.

## MATERIALS AND METHODS

A total of 8,089 bacterial isolates were tested in 2003 from the ZAAPS Program. A total of 1,349 of these isolates were collected from Europe (seven countries; 18 sites). The central laboratory selected 200 Gram-positive strains from documented infections from each participating country for reference quality susceptibility testing.

Isolates were tested against a Gram-positive panel that included 22 antimicrobial agents (linezolid, amoxicillin/clavulanate, ampicillin, cefepime ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, doxycycline, erythromycin, gentamicin, levofloxacin, nitrofurantoin, oxacillin, penicillin, quinupristin/dalfopristin, rifampin, streptomycin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin).

Isolate species and groups included *Staphylococcus aureus* (375 strains), coagulase-negative staphylococci (CoNS; 263 strains), enterococci (264 strains), *Streptococcus pneumoniae* (309 strains), viridans group streptococci (62 strains) and ß-haemolytic streptococci (78 strains). Susceptibility testing was performed on validated commercial dry-form reference broth microdilution panels supplied by TREK Diagnostics (Cleveland, OH) using methods recommended by the Clinical Laboratory Standards Institute (CLSI; formerly NCCLS). An initial bacterial suspension equal to a 0.5 McFarland Standard was prepared for each isolate, diluted 1/200, then inoculated with a Sensititre autoinoculator into the test panels. Isolates were incubated in an ambient air environment at 35ºC for 20 - 24 hours. The NCCLS M100-S15 (2005) interpretive criteria were used to determine linezolid resistance or non-susceptibility at ≥ 8 mg/L for staphylococci and enterococci and ≥ 4 mg/L for streptococci. Concurrent susceptibility quality control strains (*Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619) were processed along with PCR and gene sequencing methodologies to determine the presumed target site mutation. Patient demographics and drug-use data were also sought.

**Table 1.** Antimicrobial activity of linezolid tested against Gram-positive isolates in 2003 from infected patients in Europe compared to North America, Latin America and Asia.

| | | MIC (mg/L) | | | |
|---|---|---|---|---|---|
| Organism/antimicrobial | Continent (no. tested) | Range | 50% | 90% | % susceptible[a] |
| *Staphylococcus aureus* | | | | | |
| Oxacillin | Europe (375) | ≤0.25->8 | 0.5 | >8 | 73.3 |
| Erythromycin | Europe (375) | ≤0.06->8 | 0.5 | >8 | 38.7 |
| Clindamycin | Europe (375) | ≤0.06->8 | 0.12 | >8 | 81.3 |
| Ciprofloxacin | Europe (375) | 0.06->4 | 0.5 | >4 | 66.4 |
| Quinupristin/Dalfopristin | Europe (375) | ≤0.25-1 | 0.5 | 1 | 100.0 |
| Vancomycin | Europe (375) | ≤0.12-4 | 1 | 1 | 100.0 |
| Teicoplanin | Europe (374) | ≤2->16 | ≤2 | ≤2 | 99.7 |
| Linezolid | Europe (375)[b] | 0.12-2 | 2 | 2 | 100.0 |
| | North America (1,681) | 0.5-4 | 2 | 2 | 100.0 |
| | Latin America (242) | 0.25-4 | 2 | 2 | 100.0 |
| | Asia (332) | 1-4 | 2 | 2 | 100.0 |
| Coag-neg staphylococci | | | | | |
| Oxacillin | Europe (263) | ≤0.25->8 | 4 | >8 | 23.6 |
| Erythromycin | Europe (263) | ≤0.06->8 | >8 | >8 | 37.3 |
| Clindamycin | Europe (263) | ≤0.06->8 | 0.12 | >8 | 66.9 |
| Ciprofloxacin | Europe (263) | 0.06->4 | 4 | >4 | 42.6 |
| Quinupristin/Dalfopristin | Europe (263) | ≤0.25->2 | ≤0.25 | 0.5 | 99.2 |
| Teicoplanin | Europe (262) | ≤2->16 | ≤2 | 4 | 96.6 |
| Vancomycin | Europe (263) | 0.5-4 | 2 | 2 | 100.0 |
| Linezolid | Europe (263) | 0.25-2 | 1 | 1 | 100.0 |
| | North America (540) | 0.12-2 | 1 | 1 | 100.0 |
| | Latin America (139) | 0.12-2 | 1 | 2 | 100.0 |
| | Asia (159) | 0.12-2 | 1 | 2 | 100.0 |
| *Enterococcus* spp. | | | | | |
| Ampicillin | Europe (264) | ≤1->16 | 2 | >16 | 76.3 |
| Chloramphenicol | Europe (264) | ≤2->8 | 8 | >16 | 76.5 |
| Ciprofloxacin | Europe (264) | ≤0.03->4 | 2 | >4 | 43.0 |
| Gentamicin | Europe (264) | ≤500->1000 | ≤500 | >1000 | 65.9 |
| Quinupristin/Dalfopristin | Europe (264) | ≤0.25->2 | >2 | >2 | 18.9 |
| Teicoplanin | Europe (262) | ≤2->16 | ≤2 | ≤2 | 96.9 |
| Vancomycin | Europe (264) | 0.5->16 | 1 | 2 | 94.7 |
| Linezolid | Europe (264) | 0.5-2 | 2 | 2 | 100.0 |
| | North America (1,512) | ≤0.06->8[c] | 2 | 2 | 99.1 |
| | Latin America (120) | 1-4 | 2 | 2 | 98.3 |
| | Asia (141) | 0.5-2 | 2 | 2 | 100.0 |
| *Streptococcus pneumoniae* | | | | | |
| Penicillin | Europe (309) | ≤0.03-4 | ≤0.03 | 1 | 82.8 |
| Ceftriaxone | Europe (309) | ≤0.008-2 | 0.016 | 0.5 | 99.7 |
| Erythromycin | Europe (308) | ≤0.25->32 | ≤0.25 | >32 | 75.0 |
| Clindamycin | Europe (309) | ≤0.06->2 | 0.25 | >2 | 82.8 |
| Levofloxacin | Europe (309) | 0.25->4 | 1 | 1 | 99.4 |
| Quinupristin/Dalfopristin | Europe (308) | ≤0.5-1 | ≤0.5 | ≤0.5 | 100.0 |
| Vancomycin | Europe (309) | ≤0.12-1 | 0.25 | 0.5 | 100.0 |
| Linezolid | Europe (307) | 0.12-2 | 1 | 1 | 100.0 |
| | North America (1,225) | ≤0.06-4 | 1 | 1 | 100.0 |
| | Latin America (80) | 0.25-2 | 1 | 1 | 100.0 |
| | Asia (87) | 0.5-2 | 1 | 1 | 100.0 |
| viridans group streptococci | | | | | |
| Penicillin | Europe (62) | ≤0.016-2 | 0.06 | 0.25 | 83.9 |
| Ceftriaxone | Europe (62) | ≤0.25->32 | ≤0.25 | 0.5 | 95.2 |
| Erythromycin | Europe (62) | ≤0.06->8 | ≤0.06 | >8 | 71.0 |
| Clindamycin | Europe (62) | ≤0.06->8 | ≤0.06 | 8 | 88.7 |
| Levofloxacin | Europe (62) | 0.12->4 | 1 | 1 | 98.4 |
| Quinupristin/Dalfopristin | Europe (62) | ≤0.25-1 | 0.5 | 1 | 100.0 |
| Vancomycin | Europe (62) | 0.25-2 | 0.5 | 1 | 98.4 |
| Linezolid | Europe (62) | 0.25-2 | 1 | 1 | 100.0 |
| | North America (84) | ≤0.06-2 | 1 | 1 | 100.0 |
| | Latin America (16) | 0.5-1 | 1 | 1 | 100.0 |
| | Asia (74) | 0.25-2 | 1 | 1 | 100.0 |
| ß-streptococci | | | | | |
| Penicillin | Europe (78) | ≤0.016-0.12 | ≤0.016 | 0.06 | 100.0 |
| Ceftriaxone | Europe (78) | ≤0.25-1 | ≤0.25 | ≤0.25 | 98.7 |
| Erythromycin | Europe (78) | ≤0.06->8 | ≤0.06 | 8 | 87.2 |
| Clindamycin | Europe (78) | ≤0.06->8 | ≤0.06 | ≤0.06 | 93.6 |
| Levofloxacin | Europe (78) | 0.25-4 | 0.5 | 1 | 98.7 |
| Quinupristin/Dalfopristin | Europe (78) | ≤0.25-0.5 | ≤0.25 | 0.5 | 100.0 |
| Vancomycin | Europe (78) | ≤0.12-0.5 | 0.25 | 0.5 | 100.0 |
| Linezolid | Europe (78) | ≤0.06-1 | 1 | 1 | 100.0 |
| | North America (252) | ≤0.06-2 | 1 | 1 | 100.0 |
| | Latin America (28) | 0.5-2 | 1 | 1 | 100.0 |
| | Asia (25) | 1-2 | 1 | 1 | 100.0 |

a. Susceptibility as defined by NCCLS M100-S15 (2005).
b. Results from 18 medical centers in Europe.
c. Linezolid-resistant strains were only discovered among ZAAPS Program isolates in North America (USA). One separately submitted *E. faecium* strain from Greece was noted to be resistant, not from one of the monitored nations.

## RESULTS

- Results from a total of 1,349 European strains are listed in Tables 1 and 2. No MIC values higher than 2 mg/L for linezolid were observed among isolates collected by the ZAAPS Program in Europe.
- Only vancomycin and linezolid demonstrated complete (100.0%) susceptibility for staphylococci (Table 1). Methicillin resistance, as measured by oxacillin MICs, was 26.7% for *S. aureus* and 76.4% for CoNS.
- Linezolid ($MIC_{90}$, 2 mg/L; 100.0% susceptible in Europe) was the most active antimicrobial agent tested against enterococci, followed by teicoplanin ($MIC_{90}$, ≤ 2 mg/L; 96.9% susceptible) and vancomycin ($MIC_{90}$, 2 mg/L; 94.7% susceptible). Among VRE, 58.0% of isolates were *vanA* phenotypes.
- The linezolid $MIC_{90}$ and modal MIC was 1 mg/L for all streptococci (Table 1). The penicillin resistance rate for *S. pneumoniae* was 17.2% in Europe and 16.2% worldwide; the macrolide non-susceptible rate was 25.0% in Europe and 27.2% worldwide.
- Quinupristin/dalfopristin, penicillin, vancomycin and linezolid all demonstrated 100.0% activity against ß-haemolytic streptococci (Table 1). Macrolide resistance for European ß-haemolytic streptococci was elevated, 29.0%.
- The 2003 ZAAPS Program found six linezolid-resistant enterococci in the United States, all with the G2576U ribosomal target mutation. One *S. aureus* isolate from Greece was also detected as linezolid-resistant in 2003, but was not recovered in a ZAAPS monitored country (Table 3).
- Between 2002 and 2003, there was no significant change in linezolid potency or percent increase in resistance.

**Table 2.** MIC population distributions of linezolid for clinical isolates of staphylococci, enterococci and selected streptococcal species from Europe compared to the "rest of the world (ROW)".

| | | Cum. % inhibited at MIC (mg/L): | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism | Continent (no. tested) | ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | >8 |
| *S. aureus* | Europe (375) | 0.0 | 0.3 | 0.3 | 0.5 | 25.3 | 100.0 | - | - | - |
| | ROW (2,255) | 0.0 | 0.0 | 0.1 | 0.5 | 32.6 | 99.4 | 100.0 | - | - |
| CoNS[a] | Europe (263) | 0.0 | 0.0 | 0.4 | 9.1 | 94.7 | 100.0 | - | - | - |
| | ROW (838) | 0.0 | 0.4 | 0.7 | 12.8 | 92.6 | 100.0 | - | - | - |
| Enterococci | Europe (264) | 0.0 | 0.0 | 0.0 | 2.7 | 37.9 | 100.0 | - | - | - |
| | ROW (1,773) | 0.1 | 0.1 | 0.2 | 1.5 | 46.5 | 99.1 | 99.7 | 99.8[b] | 100.0[b] |
| *S. pneumoniae* | Europe (307) | 0.0 | 0.7 | 2.3 | 30.0 | 96.7 | 100.0 | - | - | - |
| | ROW (1,392) | 0.3 | 0.8 | 3.7 | 26.5 | 95.0 | 99.9 | 100.0 | - | - |
| viridans group streptococci | Europe (62) | 0.0 | 0.0 | 3.2 | 43.5 | 96.8 | 100.0 | - | - | - |
| | ROW (174) | 1.1 | 1.7 | 4.0 | 33.3 | 96.6 | 100.0 | - | - | - |
| ß-haemolytic streptococci | Europe (78) | 1.3 | 1.3 | 1.3 | 21.8 | 100.0 | - | - | - | - |
| | ROW (305) | 1.0 | 1.0 | 1.0 | 11.1 | 98.4 | 100.0 | - | - | - |

a. CoNS = coagulase-negative staphylococci.
b. Resistant enterococci had documented G2576U ribosomal target mutations.

**Table 3.** Characteristics of only European isolate found to be linezolid-resistant (not a ZAAPS Programe monitored country).

| | | Antimicrobial agent | MIC (mg/L) |
|---|---|---|---|
| Species | S. aureus | Linezolid | 8 |
| Country | Greece | Ampicillin | >16 |
| | | Cefepime | >16 |
| Sex | Male | Chloramphenicol | 16 |
| | | Clindamycin | 0.25 |
| Age | 48 years | Erythromycin | 0.25 |
| | | Gentamicin | 8 |
| Date isolated | October 9, 2003 | Levofloxacin | >4 |
| | | Oxacillin | 2 |
| Other | Dialysis patient with prior history of linezolid exposure | Quinupristin/Dalfopristin | 0.5 |
| | | Teicoplanin | ≤2 |
| | | Trimethoprim/Sulfamethoxazole | 2 |
| | | Vancomycin | 1 |

## CONCLUSIONS

- Linezolid remains a highly potent oxazolidinone for Gram-positive organisms recovered from patients in European countries.
- Linezolid demonstrated 100.0% susceptibility in all six Gram-positive groups monitored in the European ZAAPS countries.
- Linezolid resistance remains extremely low among Gram-positive cocci (0.07%) and when it occurs is mediated by a common, G2574U ribosomal target mutation.

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