ECCMID 2021 | Poster #051

# Epidemiology and Molecular Characterization of Linezolid Non-Susceptible *Enterococcus* spp. Clinical Isolates from the SENTRY Antimicrobial Surveillance Program (2017–2019)

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## Introduction

- The clinically available oxazolidinones, linezolid and tedizolid, are considered drugs of last resort for the treatment of Gram-positive severe infections.
- Oxazolidinones are mainly used to treat multidrug-resistant Gram-positive strains such as methicillin-resistant staphylococci, vancomycin-resistant enterococci, and isolates displaying elevated minimal inhibitory concentration (MIC) values to daptomycin.
- Although the vast majority of *Enterococcus* spp. remain susceptible to oxazolidinones, resistant isolates have been reported worldwide.
- Ribosomal mutations, including alterations in the oxazolidinone binding sites (23S rRNA and L3 and L4 ribosomal proteins), are the most common mechanisms of oxazolidinone resistance.
- However, plasmid-borne resistance genes, such as *cfr*, *cfr*(B), *cfr*(C), *poxtA* and *optrA*, have been detected as newer mechanisms responsible for a decreased susceptibility to either linezolid and/or tedizolid.
- We evaluated the epidemiology and resistance mechanisms to oxazolidinones in a worldwide collection of linezolid non-susceptible (NS) *Enterococcus faecalis* and *E. faecium* from the SENTRY Antimicrobial Surveillance Program.

## Materials and Methods

**Bacterial isolates**

- A total of 30 linezolid-nonsusceptible (MIC, ≥4 mg/L; CLSI breakpoint) *Enterococcus* spp. clinical isolates, including 18 *E. faecalis* and 12 *E. faecium*, were included (Table 1).
- These isolates collected from 20 medical centres spread across Asia-Pacific (10 isolates; 6 medical centres; 6 countries), Europe (10 isolates; 6 medical centres; 5 countries), North America (9 isolates; 7 medical centres; 1 country), and Latin America (1 isolate; 1 medical centre; 1 country) during 2017–2019 (Figure 1).
- These isolates were recovered from patients with bloodstream infections (BSIs; 10 isolates), skin and skin structure infections (SSSIs; 9 isolates), urinary tract infections (UTIs; 8 isolates), intra-abdominal infections (IAIs; 2 isolates), and respiratory tract infections (RTIs, 1 isolate) (Figure 2).
- Only isolates determined to be clinically significant by local criteria as the reported probable cause of infection were included in the program.
- All isolates were identified by standard microbiology methods and/or MALDI-TOF.

**Susceptibility testing and molecular characterization**

- The broth microdilution method was conducted according to CLSI guidelines using 96-well panels manufactured by JMI Laboratories (North Liberty, Iowa, USA).
- Susceptibility was based on EUCAST (2021) and CLSI (2021) breakpoint criteria.
- The mechanisms of resistance to oxazolidinone were evaluated by whole genome sequencing on a MiSeq sequencer following the manufacturer's instructions (Illumina, San Diego, CA).
  - Assembled genomes were subjected to a proprietary software (JMI Laboratories) to screen for the presence of *cfr*, *cfr*(B), *cfr*(C), *optr*A, and *poxt*A genes.
  - DNA sequences associated with the 23S rRNA and ribosomal proteins (L3, L4, and L22) were analysed for the presence of mutations, as previously described.
- These isolates were also subjected to multilocus sequence typing (MLST).

## Results

- Among 18 linezolid-nonsusceptible *E. faecalis*, the vast majority of isolates (83.3%; 15/18) had a linezolid MIC of 4 mg/L (intermediate susceptibility per CLSI), whereas other 3 isolates had MIC of 8 mg/L or >8 mg/L (resistant per CLSI; Table 2).
  - All isolates with a linezolid MIC of 4 mg/L (83.3%) were considered as susceptible by the EUCAST breakpoint (i.e., ≤4 mg/L; Table 1).
  - A total of 13 (72.2%) isolates showed tedizolid MIC values of 0.5 mg/L and were categorized as susceptible based on the CLSI breakpoint (i.e. ≤0.5 mg/L) (Tables 1 and 2).
  - All but 1 *E. faecalis* carried *optr*A gene (94.4%), and these isolates originated mainly from Asia-Pacific (9 isolates; 50%) and Europe (6 isolates; 33.3%) (Table 2).
  - One *E. faecalis* isolate from Italy had G2576T substitutions in the 23S rRNA (Table 2).
- Ampicillin, vancomycin, and daptomycin inhibited 100.0% of *E. faecalis* isolates at their respective breakpoints (Table 1).
- Twelve clonal complexes (CC) were noted among *optr*A-carrying *E. faecalis* (Table 2).
- A total of 12 linezolid-nonsusceptible *E. faecium* were observed per CLSI criteria.
  - A total of 8 isolates (66.7%) had a linezolid MIC of 4 mg/L (intermediate susceptibility per CLSI), whereas 4 isolates had MIC of 8 mg/L or >8 mg/L (resistant per CLSI; Table 2).
  - A total of 58.3% (7/12) isolates had tedizolid MIC of 0.5 mg/L and considered as susceptible based on the CLSI breakpoint (i.e, ≤0.5 mg/L; Tables 1 and 2).
- All *E. faecium* isolates were susceptible to daptomycin (MIC, ≤2 mg/L) when the CLSI dose-dependent breakpoint was applied.
- G2576T substitutions were observed in 8 *E. faecium* isolates (66.7%), followed by *poxt*A (33.3%; 4 isolates), *optr*A genes (16.7%; 2 isolates), and *cfr*(B) (8.3%; 1 isolate).
  - Four (50%) *E. faecium* isolates displaying G2576T amino acid substitutions had linezolid MIC of 4 mg/L and were considered as susceptible when the linezolid EUCAST breakpoint was applied.
- *E. faecium* isolates were mainly recovered from North America (7; 58.3%) and Europe (4; 33.3%) and belonged to CC17.

**Figure 1. Distribution of linezolid-nonsusceptible *Enterococcus* spp. isolates from the SENTRY Surveillance Program (2017–2019) by geographic region and country of origin**

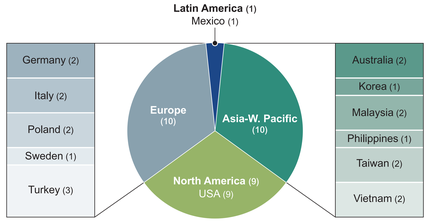


**Figure 2. Linezolid-nonsusceptible *Enterococcus* spp. isolates recovered from SENTRY Surveillance Program during 2017–2019 by infection type**

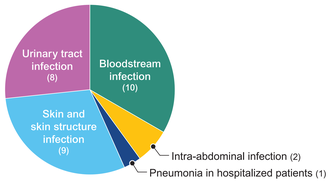


**Table 1. Activity of antimicrobial agents tested against linezolid-nonsusceptible *E. faecalis* and *E. faecium* isolates from SENTRY Surveillance Program (2017–2019)**

| Antimicrobial agent / No. of isolates | MIC (mg/L) | | EUCAST[a] | |
|---|---|---|---|---|
| | $MIC_{50}$ | $MIC_{90}$ | %S | %R |
| *E. faecalis* (18) | | | | |
| Ampicillin | 1 | 1 | 100.0 | 0.0 |
| Daptomycin | 0.5 | 2 | 100.0 [b] | 0.0 |
| Levofloxacin | 2 | >4 | 50.0 [c] | 50.0 |
| Linezolid | 4 | 8 | 83.3 | 16.7 |
| Streptomycin (high-level) | ≤512 | >1024 | 61.1 | 38.9 |
| Tedizolid | 0.5 | 1 | 72.2 [b] | |
| Teicoplanin | 0.25 | 0.5 | 100.0 | 0.0 |
| Tigecycline | 0.12 | 0.12 | 100.0 | 0.0 |
| Vancomycin | 1 | 2 | 100.0 | 0.0 |
| *E. faecium* (12) | | | | |
| Ampicillin | >16 | >16 | 0.0 | 100.0 |
| Daptomycin | 1 | 2 | 100.0 [d] | 0.0 |
| Levofloxacin | >4 | >4 | 16.7 [c] | 83.3 |
| Linezolid | 4 | >8 | 66.7 | 33.3 |
| Streptomycin (high-level) | ≤512 | >1024 | 58.3 | 41.7 |
| Tedizolid | 0.5 | 1 | 58.3 [b] | |
| Teicoplanin | 0.5 | >16 | 58.3 | 41.7 |
| Tigecycline | 0.06 | 0.12 | 91.7 | 8.3 |
| Vancomycin | 2 | >16 | 58.3 | 41.7 |

[a] Criteria as published by EUCAST (2021).
[b] Using the CLSI (2021) breakpoint.
[c] Uncomplicated UTI only.
[d] Based on a dosage regimen of 8-12 mg/kg (CLSI 2021).

**Table 2. Characterization of oxazolidinone resistance mechanisms in linezolid-nonsusceptible *E. faecalis* and *E. faecium* isolates (2017–2019)**

| Organism | Year | Region | MIC (mg/L) | | ST (CC) | Resistance mechanism | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Linezolid | Tedizolid | | 23S rRNA gene/ L3-L4-L22 amino acid alterations | methyltransferase *cfr* | ribosomal protection *poxtA* | ribosomal protection *optrA* |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 0.5 | 16 (CC16) | - | | - | + |
| *E. faecalis* | 2018 | Europe | >8 | >1 | 28 (CC28) | G2576T | | - | - |
| *E. faecalis* | 2017 | Latin America | 4 | 0.5 | 55 (C55) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 1 | 69 (CC69) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 0.5 | 116 (CC116) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 1 | 116 (CC116) | - | | - | + |
| *E. faecalis* | 2019 | Asia-W. Pacific | 4 | 0.5 | 179 (CC16) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 0.5 | 207 (CC207) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 0.5 | 444 (CC444) | - | | - | + |
| *E. faecalis* | 2019 | Europe | 4 | 0.5 | 476 (CC116) | - | | - | + |
| *E. faecalis* | 2019 | Europe | 4 | 0.5 | 476 (CC116) | - | | - | + |
| *E. faecalis* | 2019 | Europe | 4 | 0.5 | 476 (CC116) | - | | - | + |
| *E. faecalis* | 2018 | North America | 4 | 0.5 | 476 (CC116) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 4 | 0.5 | 480 (CC480) | - | | - | + |
| *E. faecalis* | 2019 | Europe | 4 | 0.5 | 480 (CC480) | - | | - | + |
| *E. faecalis* | 2017 | Europe | 4 | 0.5 | 585 (CC585) | - | | - | + |
| *E. faecalis* | 2018 | Asia-W. Pacific | 8 | 1 | 958 (CC16) | - | | - | + |
| *E. faecalis* | 2019 | North America | 8 | 1 | 960 (CC960) | - | | - | + |
| *E. faecium* | 2017 | Europe | 4 | 1 | 117 (CC17) | G2576T | | - | - |
| *E. faecium* | 2018 | Europe | >8 | 1 | 80 (CC17) | G2576T | | - | - |
| *E. faecium* | 2019 | Asia-W. Pacific | 8 | 0.5 | 817 (CC17) | G2576T | | - | - |
| *E. faecium* | 2019 | Europe | 4 | 0.5 | 18 (CC17) | - | | + | + |
| *E. faecium* | 2017 | Europe | 4 | 0.5 | 18 (CC17) | - | | + | + |
| *E. faecium* | 2018 | North America | 4 | 0.5 | 794 (CC17) | G2576T | *cfr*(B) | - | - |
| *E. faecium* | 2019 | North America | 4 | 0.5 | 18 (CC17) | G2576T | | - | - |
| *E. faecium* | 2017 | North America | >8 | 1 | 736 (CC17) | G2576T | | - | - |
| *E. faecium* | 2017 | North America | 4 | 0.5 | 117 (CC17) | G2576T | | - | - |
| *E. faecium* | 2018 | North America | 8 | 1 | 1523 (CC17) | G2576T | | - | - |
| *E. faecium* | 2018 | North America | 4 | 1 | 1641 (CC17) | - | | + | - |
| *E. faecium* | 2018 | North America | 4 | 0.5 | 1641 (CC17) | - | | + | - |

## Conclusions

- The resistance mechanisms for oxazolidinone differed between *E. faecalis* and *E. faecium* isolates.
- G2576T substitutions were the main mechanism for resistance in *E. faecium* isolates, while *optr*A appeared to be almost ubiquitous among the linezolid-nonsusceptible *E. faecalis* isolates.
- The EUCAST breakpoint for linezolid and CLSI/EUCAST breakpoints for tedizolid may fail to detect *Enterococcus* isolates carrying the resistance mechanisms presented here.
- In addition, the *E. faecium* isolates included in this study may represent a continuous selection and expansion of linezolid-nonsusceptible isolates of a well-established nosocomial population (CC17), whereas the clonal diversity among *E. faecalis* may indicate the dissemination capability of *optr*A-carrying plasmids in this species.

## Acknowledgements

This study and poster presentation were supported by JMI Laboratories. The authors appreciate the assistance from Amy Chen and Judy Oberholser at JMI Laboratories.

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