

Revised Abstract

Background: *Enterococcus faecium* and *E. faecalis* are significant pathogens both in community and hospital patients causing infections of the urinary tract, skin/skin structure and blood stream. The increasing prevalence of vancomycin-resistant *Enterococcus* spp. worldwide dictates the continued monitoring of these phenotypes in all countries. The Tigecycline European Surveillance Trial (TEST) evaluated the activity of linezolid, tigecycline and comparators to over 4,100 *Enterococcus* isolates in 25 European countries 2007-2010. **Methods:** In Europe 489 cumulative sites in 25 countries collected 4,245 significant *Enterococcus* species during 2007 - 2010. MICs were performed at each site using prepared broth microdilution panels and interpreted according to EUCAST guidelines. **Results:** The % susceptible and MIC₉₀ (mg/L) of tigecycline, linezolid and comparators to *Enterococcus* spp. including vancomycin-susceptible and -resistant phenotypes are shown in the following table.

Drug	<i>E. faecalis</i> VS ¹		<i>E. faecium</i> VS ¹		<i>E. faecalis</i> VR ²		<i>E. faecium</i> VR ²	
	% S	MIC ₉₀	% S	MIC ₉₀	% S	MIC ₉₀	% S	MIC ₉₀
Ampicillin	99.5	2	15.8	>16	96.8	4	4.0	>16
Amox-clav acid	99.5	1	16.2	>8	90.3	4	2.9	>8
Linezolid	99.9	2	99.7	2	100	2	98.9	2
Tigecycline	99.8	0.25	99.8	0.25	100	0.25	97.7	0.25
Vancomycin	100	2	100	1	0	>32	0	>32
N	2,897		1,143		31		174	

Conclusions: In Europe 13.3% and 1.0% of *E. faecium* and *E. faecalis*, respectively, were vancomycin resistant during 2007-2010. Linezolid demonstrated potent *in vitro* activity against both vancomycin-susceptible and -resistant isolates with percent susceptible ranging from 98.9-100% and MIC₉₀ of 2 mg/L regardless of phenotype. Tigecycline demonstrated percents susceptible ranging from 97.7-100% and MIC₉₀ of 0.25 mg/L regardless of phenotype. Linezolid and tigecycline continue to demonstrate *in vitro* activity against all phenotypes of *Enterococcus* spp.

Introduction

Enterococcus species are significant opportunistic pathogens in hospitalized patients and have become endemic in many hospitals throughout the world. *Enterococcus faecium* and *Enterococcus faecalis* remain the most common enterococci and cause a variety of infections including those of the urinary tract, skin and soft tissue, and blood stream. Enterococci display intrinsic resistance to a number of antimicrobial agents and have acquired resistance to several antimicrobials including ampicillin/penicillin and vancomycin. The increasing incidence of vancomycin-resistant *E. faecium* and *E. faecalis* worldwide warrants continued monitoring.

Tigecycline and linezolid are synthetic antibiotics developed for the treatment of serious infections caused by different genera of bacteria, including multi-drug resistant strains.

Tigecycline was developed to provide activity against tetracycline- and multi-drug-resistant gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms.

Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by gram-positive bacteria that are resistant to several other antibiotics classes. A member of the oxazolidinone class of drugs, linezolid is active against most gram-positive bacteria that cause disease, including streptococci, VRE, and methicillin-resistant *Staphylococcus aureus* (MRSA). The main indications of linezolid are infections of the skin and soft tissues and pneumonia (particularly hospital-acquired pneumonia).

The Tigecycline Evaluation and Surveillance Trial (TEST), a global surveillance study, has monitored the *in vitro* activity of linezolid, tigecycline and comparators against *Enterococcus* species in Western and Eastern Europe from 2007-2010.

Materials & Methods

> All isolates were derived from blood, respiratory tract, urine, skin, wound, body fluids and other defined sources. Only one isolate per patient was accepted into the study. Clinical isolates were collected and tested between 2007 and 2010 from 489 cumulative sites in 25 European countries. Isolates were identified to the species level and MICs determined at each site by the participating laboratory.

> Organism collection, transport, confirmation of organism identification, as well as, development and management of a centralized database was coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.

> All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age or gender.

> Fisher's exact test was used to compare the proportion of vancomycin resistant enterococcal isolates between the Western and Eastern European samples.

> Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [1]. Custom broth microdilution panels were supplied by MicroScan (Siemens, West Sacramento, CA, USA) and TREK (TREK Diagnostic Systems, West Sussex, England). The following antimicrobial agents were included on the panels with their dilution ranges (expressed in mcg/ml): amoxicillin/clavulanic acid (0.12/0.06-32/16); ampicillin (0.06-16); ceftriaxone (0.06-64); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.008-8); meropenem (0.12-16); minocycline (0.5-16); tigecycline (0.008-16); penicillin (0.06-8); piperacillin/tazobactam (0.06/4-128/4) and vancomycin (0.12-32). MIC interpretive criteria followed EUCAST guidelines [2].

> Quality controls (QC) were performed by each testing site on each day of testing using ATCC control strains *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to EUCAST guidelines [2].

References

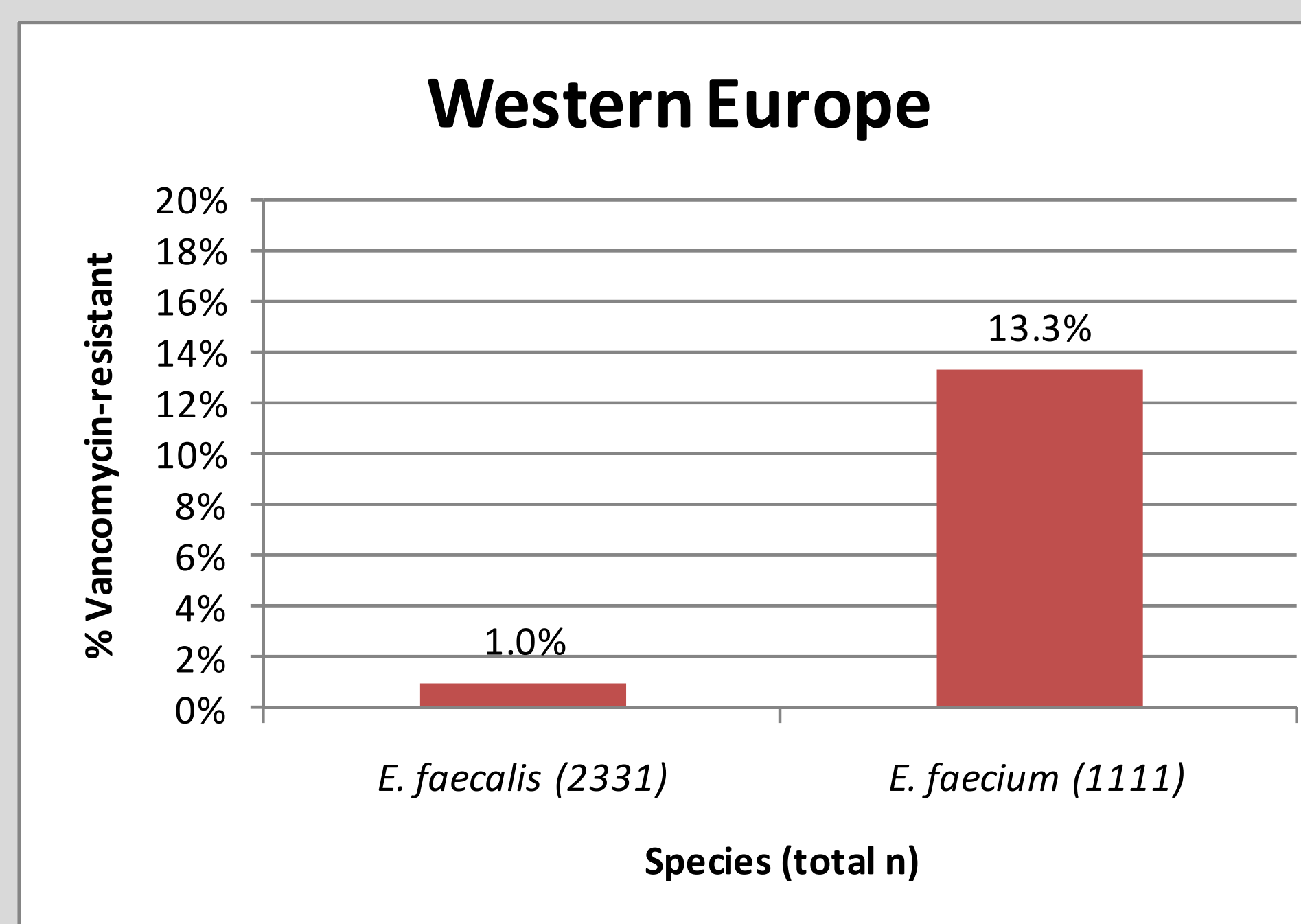
- Clinical Laboratory Standards Institute. 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards -- Eighth Edition. CLSI document M07-A8. Wayne, PA.
- European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2011. Breakpoint tables for interpretation of MICs and zone diameters, version 1.3, <http://www.eucastr.org>.

Acknowledgements

We gratefully acknowledge the contributions of the investigators, laboratory personnel, and all members of the Tigecycline European Surveillance Trial program group. This study was sponsored by Pfizer Inc.

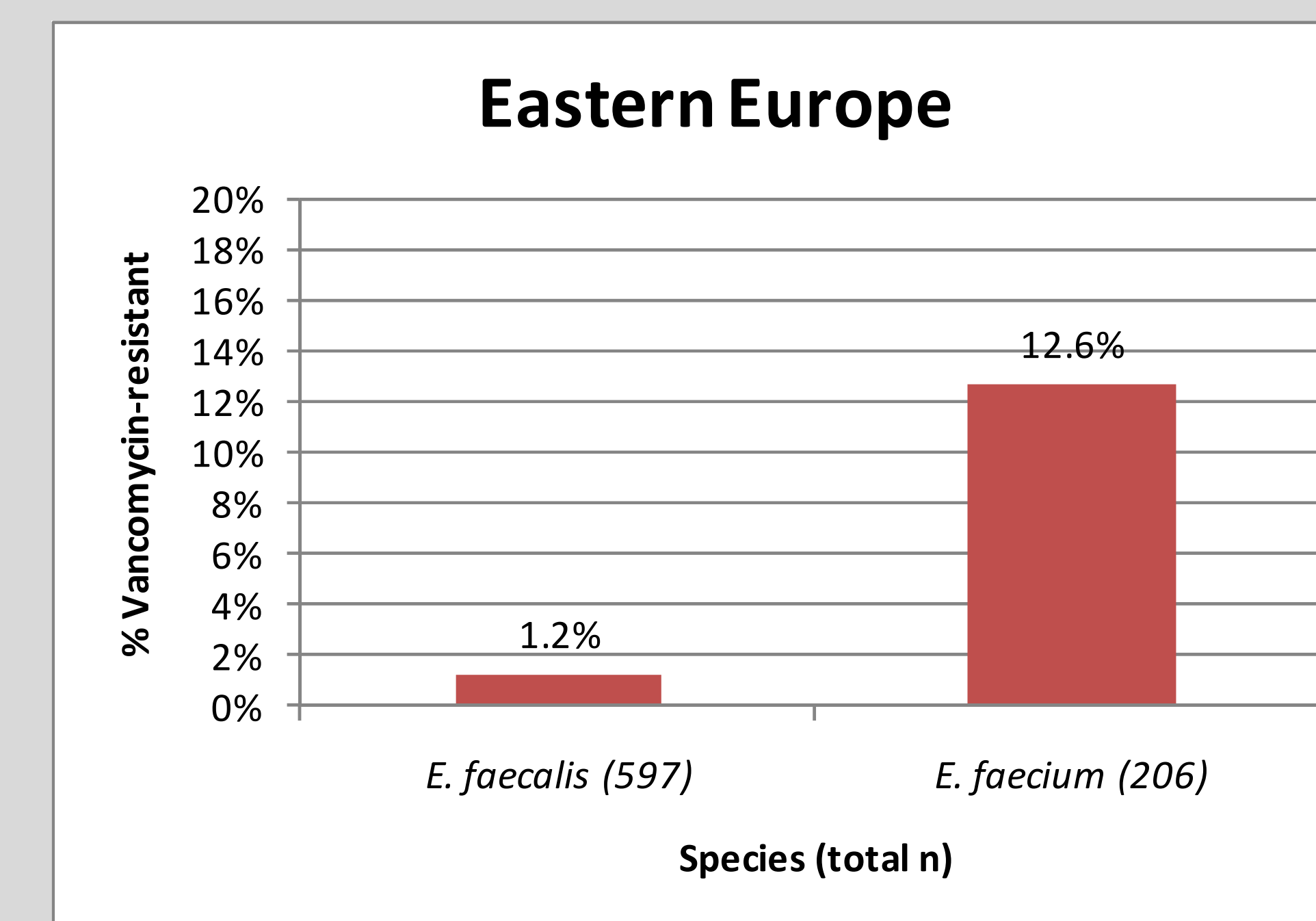
Results

Figure 1. Percent of vancomycin-resistant enterococci in Western Europe.*



*There was no statistically significant difference between Western and Eastern Europe in the vancomycin resistance rate (p>0.05).

Figure 2. Percent of vancomycin-resistant enterococci in Eastern Europe.*



*There was no statistically significant difference between Western and Eastern Europe in the vancomycin resistance rate (p>0.05).

Table 1. *In vitro* activity of linezolid and comparative agents against 3,442 *Enterococcus* isolates from Western Europe.

Organism	Drug	MIC (mg/L)		%S*	%I	%R
		MIC ₅₀	MIC ₉₀			
<i>Enterococcus faecalis</i> (n=2,331)	Amox-clav acid	1	1	99.4	0.2	0.4
	Ampicillin	1	2	99.4	0.4	0.2
	Linezolid	2	2	99.9	0	0.1
	Tigecycline	0.12	0.25	99.8	0.2	0.0
	Vancomycin	1	2	99.0	0	1.0
<i>Enterococcus faecalis</i> , vancomycin-resistant (n=24)	Amox-clav acid	1	8	87.5	8.3	4.2
	Ampicillin	2	4	95.8	0	4.2
	Linezolid	2	2	100	0	0
	Tigecycline	0.12	0.25	100	0	0
	Vancomycin	>32	>32	0	0	100
<i>Enterococcus faecium</i> (n=1,111)	Amox-clav acid	>8	>8	14.6	4.6	80.8
	Ampicillin	>16	>16	14.3	0.9	84.8
	Linezolid	2	2	99.6	0	0.4
	Tigecycline	0.06	0.25	99.5	0.4	0.1
	Vancomycin	1	>32	86.7	0	13.3
<i>Enterococcus faecium</i> , vancomycin-resistant (n=148)	Amox-clav acid	>8	>8	3.4	2.0	94.6
	Ampicillin	>16	>16	4.1	1.4	94.5
	Linezolid	2	2	99.3	0	0.7
	Tigecycline	0.12	0.25	97.3	1.4	1.3
	Vancomycin	>32	>32	0	0	100

*Susceptibility defined by EUCAST breakpoints.

Table 2. *In vitro* activity of Linezolid and comparative agents against 803 *Enterococcus* isolates from Eastern Europe.

Organism	Drug	MIC (mg/L)		%S*	%I	%R
		MIC ₅₀	MIC ₉₀			
<i>Enterococcus faecalis</i> (n=597)	Amox-clav acid	1	1	99.0	0.5	0.5
	Ampicillin	1	2	99.5	0.5	0
	Linezolid	2	2	100	0	0
	Tigecycline	0.12	0.25	100	0	0
	Vancomycin	1	2	98.8	0	1.2
<i>Enterococcus faecalis</i> , vancomycin-resistant** (n=7)	Amox-clav acid	--	--	7/7	0/7	0/7
	Ampicillin	--	--	7/7	0/7	0/7
	Linezolid	--	--	7/7	0/7	0/7
	Tigecycline	--	--	7/7	0/7	0/7
	Vancomycin	--	--	0/7	0/7	7/7
<i>Enterococcus faecium</i> (n=206)	Amox-clav acid	>8	>8	13.6	3.4	83.0
	Ampicillin	>16	>16	14.1	1.5	84.4
	Linezolid	2	2	99.5	0	0.5
	Tigecycline	0.06	0.25	100	0	0
	Vancomycin	1	>32	87.4	0	12.6
<i>Enterococcus faecium</i> , vancomycin-resistant (n=26)	Amox-clav acid	>8	>8	0	7.7	92.3
	Ampicillin	>16	>16	3.9	0	96.1
	Linezolid	2	2	96.2	0	3.8
	Tigecycline	0.06	0.25	100	0	0
	Vancomycin	>32	>32	0	0	100

*Susceptibility defined by EUCAST breakpoints. ** For n=10, MIC₅₀, MIC₉₀, and %S are not reported; susceptibility results are shown as n/S n total.

Conclusions

- > Linezolid and tigecycline both show excellent *in vitro* activity against all *Enterococcus* species from both Eastern and Western Europe, including vancomycin-resistant isolates, with >96.2% susceptible.
- > The linezolid MIC₉₀ of 2 mg/L was consistent for all enterococcal isolates, regardless of vancomycin resistance or region.
- > Tigecycline exhibited the lowest MIC₉₀ at ≤0.25 mg/L of all antimicrobials tested and regardless of region.
- > The results for linezolid and tigecycline suggest that both antimicrobials are highly active *in vitro* against vancomycin-resistant *Enterococcus* species.