Susceptibility of *Enterococcus* species including Vancomycin-resistant phenotypes: A European perspective 2004-2009

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Revised Abstract

Background: *Enterococcus faecium* and *E .faecalis* are significant clinical pathogens in hospital patients causing a variety of infections including those of the urinary tract, skin and soft tissue, and blood stream. The increasing incidence of vancomycin-resistant *E. faecium* and *E. faecalis* worldwide has been documented by the continued monitoring of these phenotypes globally. The Tigecycline European Surveillance Trial (TEST) evaluated the activity of linezolid, tigecycline, and comparators against 5,420 *Enterococcus* species in 27 European countries from 2004-2009. **Methods:** 591 cumulative sites in 27 European countries collected 5,420 clinically significant *Enterococcus* species from 2004-2009. MICs were performed as specified by CLSI and manufacturer guidelines at each site using custom supplied microbroth panels and interpreted according to EUCAST guidelines (2009). **Results**: The % susceptible and MIC_{90} (mcg/ml) of linezolid, tigecycline and comparators vs. *E. faecalis and E. faecium* including vancomycin-susceptible (VS) and –resistant (VR) phenotypes are shown in the following table.

	E. faecalis VS		*E. faecium* VS		*E. faecalis* VR		*E. faecium* VR	
Drug	% S	MIC_{90}	% S	MIC_{90}	% S	MIC_{90}	% S	MIC_{90}
Tigecycline	99.9	0.25	100	0.25	100	0.25	98.1	0.25
Ampicillin	99.4	2	17.3	>16	91.1	4	4.3	>16
Levofloxacin	69.7	>32	17.7	>32	13.3	>32	3.8	>32
Linezolid	99.9	2	99.9	2	100	2	99.1	2
Penicillin	99.5	4	17.6	>8	91.1	8	4.3	>8
Vancomycin	100	2	100	1	0	>32	0	>32
N	3,688		1,335		45		210	

Conclusions: In European TEST isolates 13.6 % and 1.2 % of *E. faecium* and *E. faecalis* respectively were vancomycin-resistant. Linezolid and tigecycline demonstrated potent *in vitro* activity against both vancomycin-susceptible and -resistant isolates.

Introduction

Enterococcus species are significant opportunistic pathogens in hospital patients. The most common species, *Enterococcus faecium* and *Enterococcus faecalis*, cause a variety of infections including those of the urinary tract, skin and soft tissue, and blood stream [1]. Enterococci display intrinsic resistance to a number of antimicrobial agents and have a propensity for acquiring resistance to antimicrobials, seriously limiting therapeutic options when infections occur. Vancomycin-resistant enterococci (VRE) are a major cause of nosocomial infections in healthcare facilities. The increasing incidence of vancomycin-resistant *E. faecium* and *E. faecalis* worldwide has been documented globally [2].

Tigecycline and linezolid are synthetic antibiotics developed for the treatment of serious infections caused by different types bacteria, including multi-drug resistant strains [3].

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 [3-6]. In addition, tigecycline has shown potent activity in animal models infected with selected strains of multi-drug resistant *E. faecium* and *E. faecalis* with diverse genotypes van-A, -B and -C [7].

Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by gram-positive bacteria that are resistant to several other antibiotics [8]. 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 Surveillance Trial (T.E.S.T.), a global surveillance study, has monitored the *in vitro* activity of linezolid, tigecycline, and comparators against *Enterococcus* species in 27 European countries from 2004-2009.

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 January 2004 and December 2009 from 591 cumulative sites in 27 European countries. Isolates were identified to the species level and tested 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. All sites identified each study isolate utilizing local laboratory site criteria.
- Antimicrobial resistance was interpreted according to European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints [9].

Antimicrobial Susceptibility Testing

- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [10]. 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 [9].
- 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 CLSI (2010) guidelines [11].

References

1. Hidron, A. I., J. R. Edwards, J. Patel, T. C. Horan, D. M. Sievert, D. A. Pollock, and S. K. Fridkin. 2008. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect. Control Hosp. Epidemiol. 29:996-1011.
2. Deshpande, L. M., T. R. Fritsche, G. J. Moet, D. J. Biedenbach, and R. N. Jones. 2007. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. Diagn. Microbiol. Infect. Dis. **58:**163-170.
3. Hoban DJ, Bouchillon SK, Johnson BM, et al. In vitro activity of tigecycline against 6792 Gram-negative and Gram-positive clinical isolates from the global Tigecycline Evaluation and Surveillance Trial (TEST Program, 2004). Diagn Microbiol Infect Dis 2005;52:215-27
4. Abbanat, D., M. Macielag, and K. Bush, Novel antibacterial agents for the treatment of serious Gram-positive infections. Expert Opin Investig Drugs, 2003. 12(3): p. 379-99.
5. Mercier, R.C., C. Kennedy, and C. Meadows, Antimicrobial activity tigecycline (GAR-936) against *Enterococcus faecium* and *Staphylococcus aureus* used alone and in combination. Pharmacotherapy, 2002. 22(12): p. 1517-23.
6. Patel, R., et al., In vitro activity of GAR-936 against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant Streptococcus pneumoniae. Diagn Microbiol Infect Dis, 2000. 38(3): p. 177-9.
7. Murphy, T.M., et al., Therapeutic efficacy of GAR-936, a novel glycylcycline, in a rat model of experimental endocarditis. Antimicrob Agents Chemother, 2000. 44(11): p. 3022-7.
8. Livermore DM. Linezolid *in vitro*: mechanism and antibacterial spectrum. J Antimicrob Chemother (2003) 51(Suppl S2):ii9–16.
9. European Committee on Antimicrobial Susceptibility Testing (EUCAST) website, http://www.eucast.org, 2009-12-22 (version 1.0).
10. CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition*, in *Document M7-A8*. 2009: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
11. CLSI, *Performance Standards for Antimicrobial Susceptibility Testing*, in *Document M100-S20*. 2010: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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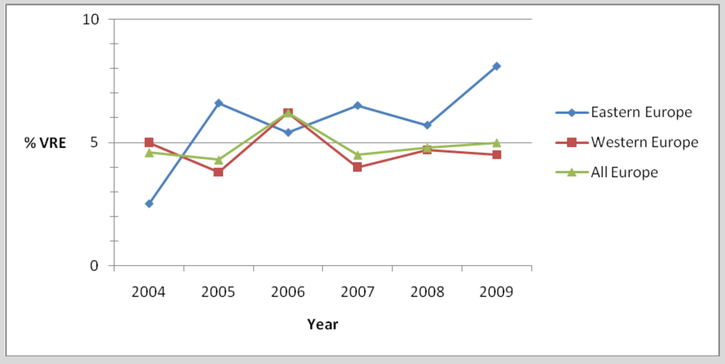
Results

Table 1. *In vitro* activity of tigecycline, linezolid and comparative agents against 5,420 European *Enterococcus* spp. isolates.

					MIC (mcg/mL)	
Organism	**Drug**	**%Sus**	**%Int**	**%Res**	**MIC_{50}**	**MIC_{90}**
E. faecalis (n=3733)	Tigecycline	99.8	0.1	0.1	0.12	0.25
	Ampicillin	98.9	0.5	0.6	1	2
	Levofloxacin	68.7	1.5	29.9	1	>32
	Linezolid	100	na	0	2	2
	Minocycline	30.8	28.5	40.7	8	>8
	Penicillin	98.8	na	1.2	2	4
	Vancomycin	98.8	na	1.2	1	2
E. faecalis, vancomycin resistant (n=45)	Tigecycline	100	0	0	0.12	0.25
	Ampicillin	91.1	0	8.9	2	4
	Levofloxacin	13.3	6.7	80.0	32	>32
	Linezolid	100	na	0	1	2
	Minocycline	26.7	33.3	40.0	8	>8
	Penicillin	91.1	na	8.9	4	8
	Vancomycin	0	Na	100	>32	>32
E. faecium (n=1545)	Tigecycline	99.6	0.3	0.1	0.06	0.25
	Ampicillin	15.6	1.1	83.3	>16	>16
	Levofloxacin	15.5	5.2	79.3	>32	>32
	Linezolid	99.7	na	0.3	2	2
	Minocycline	73.5	9.5	17.0	≤0.25	>8
	Penicillin	15.8	na	84.2	>8	>8
	Vancomycin	86.4	na	13.6	1	>32
E. faecium, vancomycin resistant (n=210)	Tigecycline	98.1	1.0	0.9	0.06	0.25
	Ampicillin	4.3	1.4	94.3	>16	>16
	Levofloxacin	3.8	1.0	95.2	>32	>32
	Linezolid	99.1	na	0.9	2	2
	Minocycline	75.7	9.5	14.8	≤0.25	>8
	Penicillin	4.3	na	95.7	>8	>8
	Vancomycin	0	na	100	>32	>32
Other *Enterococcus* spp.* (n=142)	Tigecycline	100	0	0	0.06	0.25
	Ampicillin	61.3	2.1	36.6	1	>16
	Levofloxacin	58.5	7.0	34.5	2	>32
	Linezolid	99.3	na	0.7	2	2
	Minocycline	71.1	14.1	14.8	≤0.25	>8
	Penicillin	62.0	na	38.0	2	>8
	Vancomycin	92.3	na	7.8	1	4

* *Enterococcus* spp. (n) = *E. avium* (37), *E. casseliflavus* (30), *E. durans* (36), *E. gallinarum* (20), *Enterococcus* Group D (2), *E. hirae* (5), *E. raffinosus* (7), *Enterococcus*, non-speciated (5)

Figure 1. Percent of VRE in Europe from 2004 to 2009.



Conclusions

- Tigecycline and linezolid show excellent *in vitro* activity against all *Enterococcus* species, including VRE, with ≥98% susceptible.
- Tigecycline MIC50/90 values of 0.06/0.25 mcg/ml for *E. faecium* and 0.12/0.25 mcg/ml for *E. faecalis* were the lowest of all compounds tested.
- Vancomycin resistance in *Enterococcus* spp. in Europe has remained stable over the past six years, with a slight, but statistically non-significant increase ($p>0.05$) in Eastern European countries in 2009.
- The *in vitro* activity of tigecycline in this study suggests that tigecycline is highly active against vancomycin-resistant *Enterococcus* species and may be an effective treatment option for these frequently difficult to treat phenotypes.