

Revised Abstract

Background: *Enterococcus faecium* and *E. faecalis* are significant pathogens, primarily in hospital patients, causing infections of the urinary tract, skin/skin structure and blood stream. Increasingly, these pathogens are also found in community infections. The increasing prevalence of vancomycin-resistant *Enterococcus* spp. worldwide dictates the continued monitoring of these phenotypes in all countries. The Tigecycline Evaluation and Surveillance Trial (TEST) evaluated the activity of linezolid, tigecycline and comparators to 796 *E. faecium/faecalis* isolates in 9 Asian countries between 2004-2010. **Methods:** In Asia, 93 cumulative sites in 8 countries collected 796 significant *Enterococcus* species during 2004-2010. MICs were performed at each site using prepared broth microdilution panels and interpreted according to CLSI guidelines. **Results:** The % susceptible and MIC₉₀ (mcg/ml) of tigecycline, linezolid and comparators to *Enterococcus* spp. including vancomycin-susceptible and -resistant phenotypes are shown in the following table.

| | <i>E. faecalis</i> VS ¹ | | <i>E. faecium</i> VS ¹ | | <i>E. faecalis</i> VR ² | | <i>E. faecium</i> VR ² | |
|--------------|------------------------------------|-------------------|-----------------------------------|-------------------|------------------------------------|-------------------|-----------------------------------|-------------------|
| Drug | % S | MIC ₉₀ | % S | MIC ₉₀ | % S | MIC ₉₀ | % S | MIC ₉₀ |
| Ampicillin | 99.8 | 2 | 11.9 | > 16 | 1/1 | -- | 0 | > 16 |
| Levofloxacin | 59.8 | > 32 | 9.3 | > 32 | 0/1 | -- | 0 | > 32 |
| Linezolid | 100 | 2 | 100 | 2 | 1/1 | -- | 100 | 2 |
| Penicillin | 98.5 | 8 | 14.0 | > 8 | 1/1 | -- | 2.6 | > 8 |
| Tigecycline | 100 | 0.25 | 100 | 0.25 | 1/1 | -- | 100 | 0.25 |
| Vancomycin | 100 | 2 | 100 | 1 | 0/1 | -- | 0 | > 32 |
| N | 477 | | 239 | | 1 | | 76 | |

¹ Vancomycin susceptible ; ² Vancomycin resistant. For n<10, %susceptible and MIC₉₀ are not reported; results are shown as susceptible n/total n.

Conclusions: In Asia 24.4% and 0.2% of *E. faecium* and *E. faecalis*, respectively, were vancomycin-resistant during the 2004-2010 study periods. In comparison to other global regions, vancomycin-resistant *E. faecalis* were distinctly uncommon. Linezolid demonstrated potent *in vitro* activity against both vancomycin-susceptible and -resistant isolates with percent susceptible of 100% for all phenotypes and species and MIC₉₀ of 2 mcg/ml irrespective of phenotype. Tigecycline also demonstrated percent susceptible of 100% for all phenotypes and species and MIC₉₀ of 0.25 mcg/ml regardless of phenotype. *In vitro* tigecycline and linezolid demonstrated continued activity against all phenotypes of *Enterococcus* spp. in Asia.

Introduction

Enterococcus species are significant opportunistic pathogens in hospitalized patients and have become endemic in many hospitals throughout the world. *E. faecium* and *E. 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, fluoroquinolones and vancomycin. Vancomycin-resistant enterococci (VRE) are a major cause of nosocomial infections in healthcare facilities with vancomycin-resistant *E. faecium* increasingly common in haematology/oncology and bone marrow transplantation units. 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. 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 including those carrying the van-A, B and C genes.

Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by gram-positive bacteria that are resistant to several other antibiotic 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 9 Asian countries from 2004-2010.

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 2004 and 2010 from 93 cumulative sites in 8 Asian 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.
- 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, Cleveland, OH). 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 CLSI or FDA guidelines [2, 3].
- 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 (2011) guidelines [2].
- Fisher's exact test was used to compare each country's proportion of vancomycin-resistant *E. faecium* to the overall Asian average; 95% confidence intervals were calculated using the adjusted Wald method.

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
- Clinical and Laboratory Standards Institute. 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI Document. Wayne, PA.
- Tygacil®, 2010. Tigecycline FDA, prescribing information. Pfizer, Inc., Collegeville, PA, USA.

Acknowledgements

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Results

Table 1. *In vitro* activity of linezolid and comparative agents against 796 Asian *Enterococcus* spp. isolates.

| Organism | Drug | %Sus* | %Int | %Res | MIC (mcg/mL) | |
|--|--------------|-------|------|------|-------------------|-------------------|
| | | | | | MIC ₅₀ | MIC ₉₀ |
| <i>E. faecalis</i> (n=479) | Ampicillin | 99.8 | -- | 0.2 | 1 | 2 |
| | Levofloxacin | 59.9 | 1.7 | 38.4 | 1 | >32 |
| | Linezolid | 100 | 0 | 0 | 2 | 2 |
| | Penicillin | 98.3 | -- | 1.7 | 2 | 8 |
| | Tigecycline | 100 | -- | -- | 0.12 | 0.25 |
| | Vancomycin | 99.6 | 0.2 | 0.2 | 1 | 2 |
| <i>E. faecalis</i> , vancomycin resistant (n=1) | Ampicillin | 1/1 | -- | 0/1 | -- | -- |
| | Levofloxacin | 0/1 | 0/1 | 1/1 | -- | -- |
| | Linezolid | 1/1 | 0/1 | 0/1 | -- | -- |
| | Penicillin | 1/1 | -- | 0/1 | -- | -- |
| | Tigecycline | 1/1 | -- | -- | -- | -- |
| | Vancomycin | 0/1 | 0/1 | 1/1 | -- | -- |
| <i>E. faecalis</i> vancomycin sensitive (n=477) | Ampicillin | 99.8 | -- | 0.2 | 1 | 2 |
| | Levofloxacin | 60.0 | 1.7 | 38.4 | 1 | >32 |
| | Linezolid | 100 | 0 | 0 | 2 | 2 |
| | Penicillin | 98.3 | -- | 1.7 | 2 | 8 |
| | Tigecycline | 100 | -- | -- | 0.12 | 0.25 |
| | Vancomycin | 100 | -- | -- | 1 | 2 |
| <i>E. faecium</i> , (n=317) | Ampicillin | 9.2 | -- | 90.9 | >16 | >16 |
| | Levofloxacin | 7.3 | 1.9 | 90.6 | >32 | >32 |
| | Linezolid | 100 | -- | 0 | 2 | 2 |
| | Penicillin | 11.4 | -- | 88.6 | >8 | >8 |
| | Tigecycline | 100 | -- | -- | 0.06 | 0.25 |
| | Vancomycin | 75.4 | 0.6 | 24.0 | 1 | >32 |
| <i>E. faecium</i> vancomycin resistant (n=76) | Ampicillin | 0 | -- | 100 | >16 | >16 |
| | Levofloxacin | 0 | 0 | 100 | >32 | >32 |
| | Linezolid | 100 | 0 | 0 | 2 | 2 |
| | Penicillin | 2.6 | -- | 97.4 | >8 | >8 |
| | Tigecycline | 100 | -- | -- | 0.06 | 0.25 |
| | Vancomycin | 0 | 0 | 100 | >32 | >32 |
| <i>E. faecium</i> vancomycin sensitive (n=239) | Ampicillin | 11.7 | -- | 88.3 | >16 | >16 |
| | Levofloxacin | 9.2 | 2.5 | 88.3 | >32 | >32 |
| | Linezolid | 100 | 0 | 0 | 2 | 2 |
| | Penicillin | 13.8 | -- | 86.2 | >8 | >8 |
| | Tigecycline | 100 | -- | -- | 0.06 | 0.25 |
| | Vancomycin | 100 | 0 | 0 | 0.5 | 1 |

* Susceptibility defined by CLSI document M100-S21 (2011), where available. Tigecycline FDA indications and breakpoints are for vancomycin-susceptible *E. faecalis*; breakpoints are generalized to all enterococci for comparative purposes only. For n<10, %susceptible and MIC₉₀ are not reported; results are shown as susceptible n/total n.

Figure 1. Number of ALL *E. faecalis* and *E. faecium* isolates by country in Asia 2004-2010.

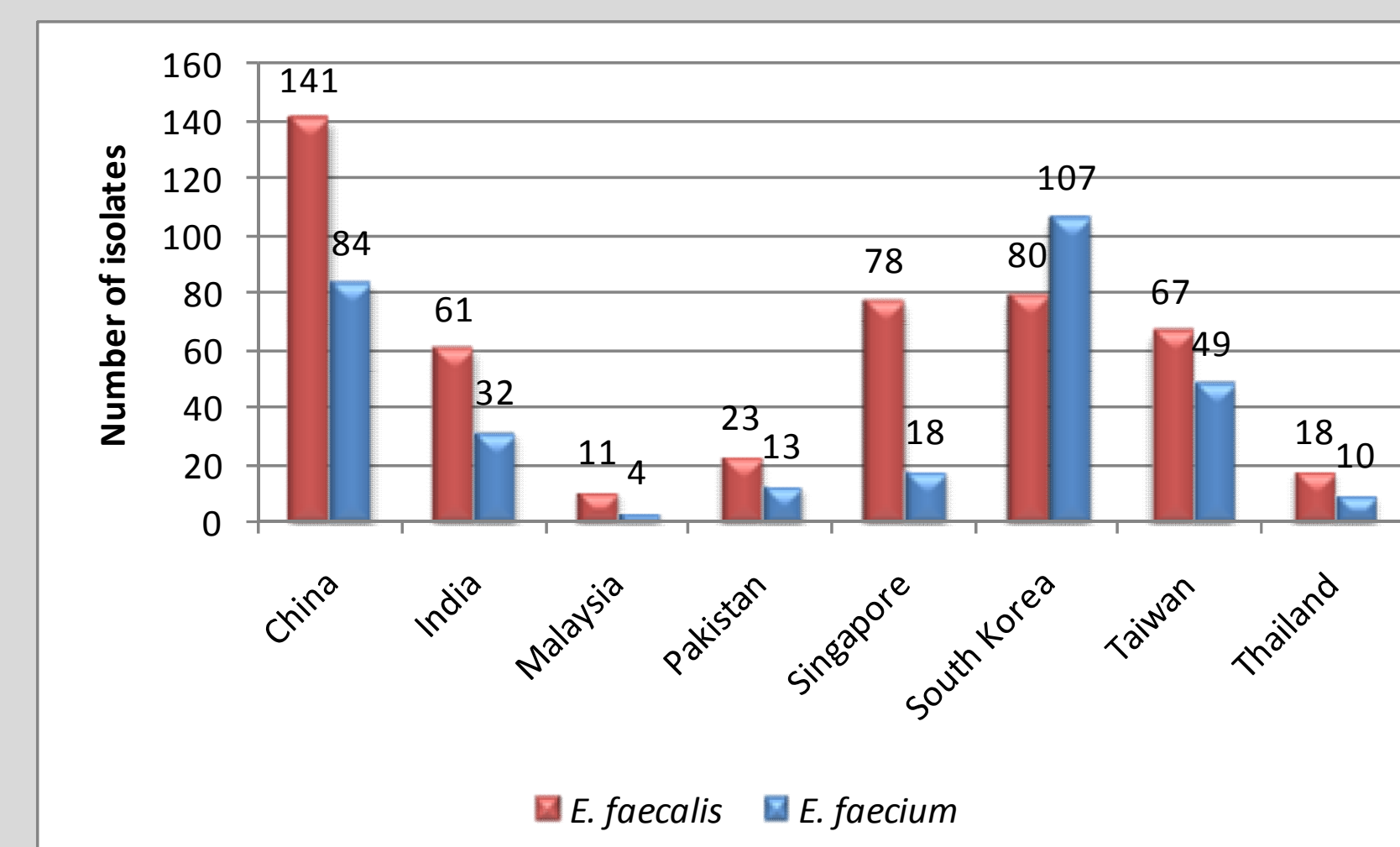
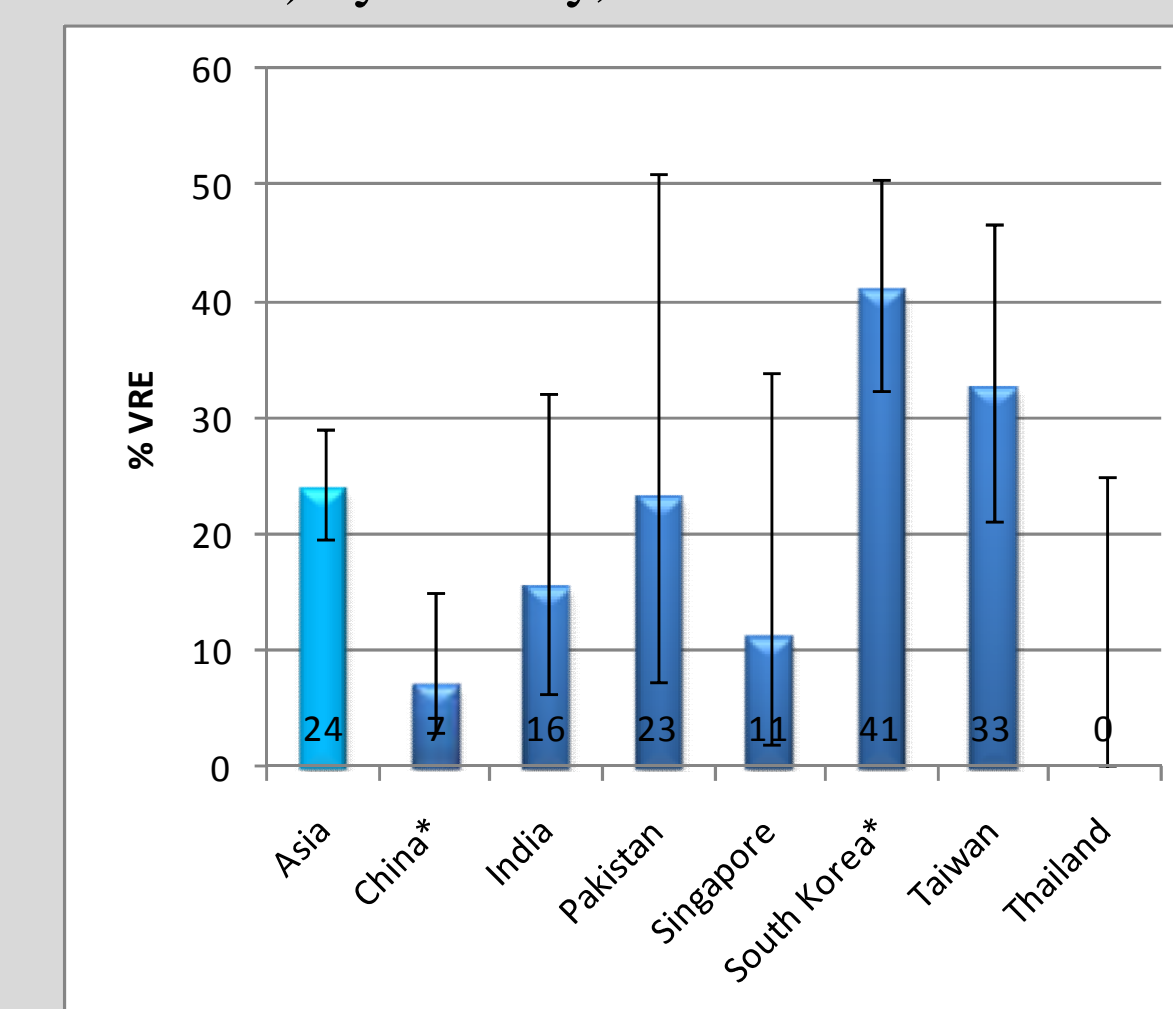


Figure 2. Proportion of *E. faecium* that are vancomycin-resistant (with 95% confidence intervals) by country, 2004-10.



* % VRE differs significantly from the Asian average (p<0.05). Note: Only countries with *E. faecium* n≥10 are included in this figure.

Conclusions

- Tigecycline and linezolid showed excellent *in vitro* activity against all *Enterococcus* species, including VRE, with 100% susceptible.
- Tigecycline MIC_{50/90} 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 Asia varied by country, with China demonstrating a significantly lower proportion of VRE than the Asian average, while South Korea's rate was significantly higher (p<0.05).
- The *in vitro* activity of tigecycline in this study suggests that tigecycline is highly active against vancomycin-resistant *Enterococcus* species.