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Activity of Tigecycline Tested against Vancomycin-Resistant *Enterococcus faecium* Isolated in North America (NA) and Europe (EU), Including Clonal Complex-17 (CC-17) Strains

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ABSTRACT

Background:
CC-17 characterizes a lineage of *E. faecium* (EFM) with resistance (R) to ampicillin (AMP) and ciprofloxacin (CIP) with or without R to vancomycin (VANC), a pathogenicity island and an association with hospital outbreaks, which have spread globally. We evaluated the activity of tigecycline (TIG) against clinical strains of VANC-R EFM collected in NA and EU, including strains characterized as being CC-17.

Methods:
EFM strains were submitted from 41 medical centers in NA and 33 in EU during 2000-2005. Strains were susceptibility (S) tested against TIG and >20 antimicrobials using CLSI broth microdilution methods and US-FDA interpretative criteria (TIG S at ≤0.25 µg/ml). VanA VREFM strains R to AMP and CIP were characterized as a CC-17. CC-17 strains showing rifampin-R and high-level R to gentamicin or streptomycin (multidrug R [MDR] phenotype) collected in 2003 via a focused SENTRY Program surveillance objective were further characterized by PFGE and PCR for *esp* gene.

Results:
1,832 EFM (50.2% VANC-R) strains were collected and 678 (37.0%) showed a CC-17 phenotype. The *esp* gene was detected in 23 CC-17 MDR strains from 7 medical centers; grouped in 14 PFGE patterns and 6 epidemic clusters. Activity of TIG is shown in the table.

Organism	Cumulative % inhibited at TIG MIC (µg/ml) of:					
(no. tested)	≤0.03	0.06	0.12	0.25	0.5	1
All EFM (1,832)	27.5	59.0	81.6	97.9	99.7	100.0
VANC-R EFM (920)	27.2	59.5	82.9	99.5	100.0	-
CC-17 VANC-R phenotype (673)	27.9	62.1	84.8	99.6	100.0	-
esp(+) VANC-R strains (23)	17.4	82.6	95.7	100.0	-	-

TIG was very active against VANC-R EFM (MIC_{90}, 0.25 µg/ml; 99.5% S) and all MDR *esp*(+) strains were TIG-S (MIC_{90}, 0.12 µg/ml). TIG was also highly active against the collection of EFM (MIC_{90}, 0.25 µg/ml; 97.9% S).

Conclusions:
TIG represents an important therapeutic option for infections caused by VANC-R EFM, including the epidemic CC-17 strains. Various R mechanisms do not appear to adversely affect TIG activity against EFM.

INTRODUCTION

Enterococcus appears fourth in North America (10.2%), and fifth in Europe (7.2%) in rank order of pathogens causing bacteremia as monitored by the SENTRY Antimicrobial Surveillance Program; a much lower incidence is found in Latin America (3.3%). Isolation of vancomycin-resistant enterococci (VRE) has consistently increased in the United States (USA) as well as in some parts of Europe over the past decade. An observation from the early years of the SENTRY Program (1997-1999) illustrated that USA isolates were considerably more resistant to vancomycin (17% in 1999), than those from patients in the rest of the world.

Increased use of vancomycin for treatment of infections caused by oxacillin-resistant *Staphylococcus* spp. has been a leading factor for selection of these resistant Enterococcus phenotypes among other factors such as clonal spread and various environmental gene pools, such as animal healthcare in the EU. Gastrointestinal colonization with VRE typically precedes infection. Furthermore, VRE infections occur most commonly among "at-risk" patients like those in ICUs, on hemodialysis, in nursing homes, the immunocompromised or those being treated with multiple antimicrobial agents. Greater morbidity and mortality related to VRE infections compared to those by susceptible enterococci appears secondary to limited therapeutic options and potentially greater pathogenicity owing to acquisition of virulence genes.

In the year 2003, a SENTRY Program objective was designed to study the genotypic and phenotypic expression of resistance among VRE isolates from medical centers in North America (NA), Europe (EU), Israel and Turkey. The participant medical centers contributed up to 50 consecutive, clinically significant VRE (vancomycin MIC, >4 µg/ml) isolates, regardless of the resistance pattern (VanA, B, etc.) or site of infection. Epidemiologic typing was performed on isolates showing similar susceptibility patterns. Microbiologic and clinical characteristics of the geographic collections were compared and contrasted. Virulence pathogenicity island (PAI) genes (*esp*) were determined on a subset of *E. faecium* strains and for those isolates phenotypically and genotypically consistent with Clonal Complex-17 (CC17; Willems et al., 2005). These strains and 1,832 other *E. faecium* isolates from NA and EU (2000-2005 collection) were specifically studied for susceptibility to tigecycline and other therapeutic options by reference broth microdilution tests (CLSI, 2006).

Tigecycline, the first glycylcycline used in clinical practice, has broad-spectrum coverage of Gram-positive pathogens, Enterobacteriaceae, anaerobic bacteria and some non-fermentative Gram-negative bacilli (example: *Acinetobacter baumannii*). United States Food and Drug Administration (US-FDA)-approved indications include skin and soft tissue and intra-abdominal infections. In both types of infection, enterococci can complicate therapy associated with other defined pathogens.

MATERIALS AND METHODS

Bacterial isolates. All *E. faecium* strains were collected by the SENTRY Program (2000-2005) and were placed into four categories for analysis:

1. All *E. faecium* isolates from NA and EU (1,832 strains);
2. Vancomycin-resistant *E. faecium* (920 strains);
3. CC-17 resistance phenotypes (673 strains); and
4. CC-17 with a defined PAI (23 strains).

These enterococci were submitted for testing from 41 medical centers, across more than a dozen nations.

Antimicrobial Susceptibility Testing: All 1,832 strains were susceptibility tested against tigecycline and comparator antimicrobials using validated, dry-form broth microdilution panels with cation-adjusted Mueller-Hinton medium (TREK Diagnostics, Cleveland, OH) according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines. The results were interpreted as specified by the M100-S16 CLSI document (2006). For tigecycline, breakpoints approved by the US-FDA were applied, i.e. ≤0.25 µg/ml for susceptibility with no resistant breakpoint.

PFGE. A subset of 158 isolates based on similar multidrug-resistant (MDR) profiles, and temporal and spatial proximity of isolation were selected for analysis using PFGE. Briefly, DNA was digested with *Sma*I. The restriction fragments were separated by electrophoresis on the CHEF DR II (Bio-Rad, Hercules, CA) with the following conditions: 1% agarose, 0.5X TBE, 200V at 5-30 sec switch time interval over 23 hours. Ethidium bromide stained gels were examined visually. Isolates showing < three bands difference were considered identical/clonally related.

Detection of *esp* from *E. faecium* isolates. A total of 101 unique VR *E. faecium* isolates based on PFGE patterns (one isolate per cluster or unique VR *E. faecium* isolates) were tested for presence of *esp* using PCR primers and protocol described by Leavis et al. (2003). These strains were consistent with the CC-17 phenotype.

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RESULTS

- The rank order of susceptibility rates for tested agents versus *E. faecium* from North America and Europe (2000-2005) was: linezolid (99.1%) > tigecycline (97.9%) > chloramphenicol (90.3%) > quinupristin/dalfopristin (84.1%). All other agents had susceptibility rates of ≤59.0%.

- Aminoglycoside co-drug synergy testing showed potential synergy for 67.8 and 41.7% of *E. faecium* strains with gentamicin and streptomycin, respectively.

Table 1. Comparative antibiograms of four subsets of *E. faecium* strains isolated from North America and Europe (2000-2005) showing tigecycline activity.

Organism group (no. tested)	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible	Resistant
All Strains (1,832)					
Tigecycline	0.06	0.25	≤0.03-1	97.9	-[b]
Ampicillin	>16	>16	≤2->16	10.6	89.4
Vancomycin	8	>16	≤0.12->16	49.4	49.8
Teicoplanin	≤2	>16	≤2->16	54.6	38.0
Q-D[c]	1	2	≤0.25->2	84.1	6.4
Chloramphenicol	8	8	≤2->16	90.3	3.4
Ciprofloxacin	>4	>4	≤0.25->4	5.4	89.2
Linezolid	1	2	≤0.25->8	99.1	0.7
Nitrofurantoin	>32	>32	≤16->32	31.3	68.7
Tetracycline	≤2	>8	≤2->8	59.0	40.3
Gentamicin[d]	≤500	>1000	≤500->1000	67.8	-
Streptomycin[d]	2000	>2000	≤1000->2000	41.7	-
Vancomycin-resistant (920)					
Tigecycline	0.06	0.25	≤0.03-0.5	99.5	-
Ampicillin	>16	>16	≤2->16	1.4	98.6
Teicoplanin	>16	>16	≤2->16	9.8	75.5
Q-D	1	1	≤0.25->2	93.9	3.0
Chloramphenicol	8	8	≤2->16	95.1	2.1
Ciprofloxacin	>4	>4	0.5-4	0.8	98.2
Linezolid	1	2	0.5->8	98.4	1.2
Nitrofurantoin	>32	>32	≤16->32	32.6	67.4
Tetracycline	>8	>8	≤2->8	50.8	48.3
Gentamicin[d]	≤500	>1000	≤500->1000	60.2	-
Streptomycin[d]	>2000	>2000	≤1000->2000	32.4	-
CC-17 (678)					
Tigecycline	0.06	0.25	≤0.03-0.5	99.6	-
Q-D	1	1	≤0.25->2	95.6	1.5
Chloramphenicol	8	8	≤2->16	95.9	1.1
Linezolid	1	2	0.5->8	98.2	1.6
Nitrofurantoin	>32	>32	≤16->32	28.9	71.1
Tetracycline	>8	>80	≤2->8	48.7	50.1
Gentamicin[d]	≤500	>1000	≤500->1000	59.0	-
Streptomycin[d]	>2000	>2000	≤1000->2000	30.4	-
esp-positive (23)					
Tigecycline	0.06	0.12	≤0.03-0.25	100.0	-
Q-D	1	1	0.5-2	95.7	0.0
Chloramphenicol	8	>16	4->16	82.6	17.4
Linezolid	2	2	1-2	100.0	0.0
Nitrofurantoin	>32	>32	32->32	17.4	82.6
Tetracycline	>8	>8	≤2->8	39.1	60.9
Gentamicin[d]	>1000	>1000	≤500->1000	30.4	-
Streptomycin[d]	>2000	>2000	≤1000->2000	17.4	-

a. Susceptibility criteria of the CLSI M100-S16 (2006) or the US-FDA for tigecycline.
b. - = no interpretive criteria established for that category.
c. Q-D = quinupristin/dalfopristin.
d. Testing for high-level aminoglycoside resistance. Susceptibility implies a high probability of enhanced killing combined with a cell-wall active agent.

- Tigecycline was uniformly active against all 4 groups of *E. faecium* tested.
  - All strains (MIC_{90}, 0.25 µg/ml; 97.9% susceptible)
  - Vancomycin-resistant strains (MIC_{90}, 0.25 µg/ml; 99.5% susceptible)
  - CC-17 phenotypes (MIC_{90}, 0.25 µg/ml; 99.6% susceptible)
  - *esp*-positive isolates (MIC_{90}, 0.12 µg/ml; 100.0% susceptible)
  - "MDR-*E. faecium* clones tended to be more tigecycline-susceptible."

Table 2. Occurrence of *E. faecium* clonal dissemination among sampled North American and European medical centers (2003 only).

Continent	Site #	No. isolates tested for clonality	No. clusters (No. isolates)	*esp*[c]	Resistances
North America (USA only)	001	5	1(4)	+	MDR[a]
	002	10	3(10)	+/-	MDR; Q/D-R
	004	5	1(5)	+	MDR
	012	5	1(3)	+	MDR
	013	8	1(6)	+	MDR
	014	8	3(8)	+	MDR; Q/D-R
	015	6	1(2)	+	MDR
	017	6	1(3)	-	MDR
	019	8	2(7)	+	MDR; VanB
	021	6	2(6)	+/-	MDR
	024	9	1(5)	+	MDR; CAT+ (nonclonal)[b]
	025	6	1(6)	+	MDR
	029	6	2(6)	+/-	MDR
	030	5	1(3)	+	MDR
	051	9	2(5)	+	MDR
	082	6	2(6)	+/-	MDR
	106	3	1(3)	+	MDR
	107	6	1(5)	+	MDR
	109	3	1(3)	+	MDR
	110	6	2(4)	+/-	MDR
Europe	062	2	0	+	Q/D-R
	075	3	0	-	MDR
	095	7	2(5)	+/-	Q/D-R; VanB
	096	3	1(3)	+	MDR

a. Isolates with MDR resistance phenotypes were resistant to vancomycin (VanA or B), ampicillin, ciprofloxacin, aminoglycosides, rifampin and in some cases doxycycline.
b. CAT+ = positive chloramphenicol acetyl transferase test.
c. +/- indicates that *esp*-positive and –negative clones were detected.

Table 3. Summary of tigecycline activity versus the *E. faecium* collection (1,832 strains).

Organism group (no. tested)	Cumulative % inhibited at MIC (µg/ml) ≤0.03	0.06	0.12	0.25	0.5	1
All strains (1,832)	27.5	59.0	81.6	97.9[a]	99.7	100.0
Vancomycin-susceptible (912)	27.9	58.4	80.3	96.3	99.5	100.0
Vancomycin-resistant (920)	27.2	59.5	82.9	99.5	100.0	-
CC-17 (678)	27.9	62.1	84.8	99.6	100.0	-
esp-positive (23)	17.4	82.6	95.7	100.0	-	-

a. Underlined value indicates percentage susceptibility by the US-FDA breakpoint criteria.

- Clonality was greater in the USA compared to Canada and Europe (Table 2), and a total of 33 epidemic clusters were detected in 2003 alone, 30 from the USA (20 medical centers) and 3 from Europe (2 sites). The vast majority of the clusters were MDR *vanA* isolates consistent with the CC-17 phenotype.

- *esp* was detected in 76.0% of strains from PFGE-proven epidemic clusters.

- Overall (Table 3), tigecycline had a clear modal MIC of 0.06 µg/ml for these *E. faecium* strains. Only 2.1% of tigecycline MIC results were at 0.5 or 1 µg/ml with only five strains having a MIC at 1 µg/ml over the 6-year period.

CONCLUSIONS

- Epidemic MDR-*E. faecium* isolates were detected across North America and Europe, many consistent with CC-17.

- Tigecycline has potent activity against these problematic *E. faecium* isolates with a modal MIC at only 0.06 µg/ml (MIC_{90}, 0.25 µg/ml; >99% susceptible against MDR and CC-17 isolates).

- Along with traditional or newer agents (chloramphenicol, some tetracyclines, linezolid and quinupristin/dalfopristin), tigecycline has emerged as a valuable treatment option for MDR-*E. faecium*.