Antimicrobial Resistance and Molecular Epidemiology of VRE Isolates from North America and Europe: A Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: The occurrence of VRE has consistently increased in the USA and EU in the past 5 years. A SENTRY Program (2003) objective monitored VRE isolates with respect to antimicrobial susceptibility (S) trends, clonal dissemination and geographic resistance (R) variability.
Methods: In 2003, VRE isolates from North America (NA; 839 ; 26 sites) and Europe (EU; 56; 10) were S tested against > 30 antimicrobials using NCCLS methods. Based on R patterns, 158 isolates were selected with similar MDR profiles and close occurrences in time/place to be tested by PFGE for possible clonal spread. Molecular methods were applied for determination of CAT-mediated chloramphenicol (CHL)-R, VAN-R genotype and *vat*E or D for quinupristin/dalfopristin (Q/D)-R.
Results: The vast majority of isolates were *E. faecium* (EFM; 91.0%) followed by *E. faecalis* (EF; 7.8%). The table shows % R for EF and EFM in EU and NA.

	% R	
	Europe	NA
Antimicrobials	EF (n=14)/EFM (40)	EF (n=56)/EFM (776)
Ampicillin	0.0/97.5	0.0/98.7
CHL	7.1/15.0	28.6/0.5
Ciprofloxacin	85.7/87.5	100.0/99.5
Linezolid	0.0/0.0	1.8/0.3
Q/D	100.0/10.0	98.2/0.6
Teicoplanin	85.7/67.5	48.2/78.5
Gentamicin (HL)	85.7/42.5	76.8/37.0
Rifampin	21.4/67.5	5.4/65.9

The VanA R phenotype was more prevalent in NA (76%) than EU (40%) in this R enhanced data set of isolates. 35 MDR epidemic clusters were identified by PFGE in 21 NA medical centers and 3 EU hospitals including: VanA (20 sites; 27 clonal occurrences) and VanB (1 site; 2 clonal occurrences), elevated Q/D MIC results (not *vat*D/E; 3 sites) and CHL-R (CAT-positive; 3 sites).
Conclusions: VRE isolates had very high R rates to alternative therapeutic antimicrobial classes. R profiles to key antimicrobials showed variation between continents including: 1) CHL-R among EF and EFM (clonal), 2) non-S to ciprofloxacin in NA, 3) rare occurrences of linezolid-R in NA (0.3 - 1.8%), 4) higher Q/D-R in EU EFM strains, and 5) higher rifampin-R in EU EF. Generally, high S rates were observed for linezolid and Q/D. Clonal spread appears to be a dominating cause of dissemination of MDR-VRE strains on both continents.

INTRODUCTION

There is a worldwide trend for the increasing occurrence of enterococci and the emerging pattern of antimicrobial resistance among such isolates. Isolation of vancomycin-resistant enterococci (VRE) has consistently increased in the United States (USA) and in some parts of Europe over the past five years. An observation from the early years of the SENTRY Antimicrobial Surveillance 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 the leading factor speculated for selection of this resistant enterococcus phenotype, among other factors such as clonal spread and various gene pools in certain environments.

In the year 2003, a SENTRY Program objective ("V" for VRE) was designed to study the genotypic and phenotypic expression of resistance among VRE isolates from medical centers in North America and Europe, 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. The medical centers also provided data on the prevalence of VRE in the year prior to the study (2002). The isolates were tested for susceptibility to more than 30 antimicrobials; genotypes were determined and compared with respect to glycopeptide and streptogramin resistances. Epidemiologic typing was performed on isolates showing similar susceptibility patterns. Microbiologic and clinical characteristics of the geographic collections (North America and Europe) were compared and contrasted.

MATERIALS AND METHODS

Bacterial isolates. One of the SENTRY Program protocols monitored occurrence of VRE in the year 2003. A total of 839 isolates were collected from 26 North American sites (average, 32.3 strains per center per protocol), while 10 European sites contributed only 56 isolates (5.6 strains per center per protocol). All the strains were unique patient isolates causing significant infections.

Susceptibility testing. The isolates were tested for susceptibility against more than 30 antimicrobials on reference broth microdilution trays made by TREK Diagnostics (Cleveland, OH). Susceptibility test results were interpreted per NCCLS criteria (M100-S14). ATCC quality control strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were included in the testing series for quality assurance.

Where necessary for an expanded antimicrobial dilution range, quinupristin/dalfopristin Etests (AB BIODISK, Solna, Sweden) were applied.

PFGE. A subset of 158 isolates based on similar multi-drug resistant (MDR) profiles, and temporal and spatial proximity of isolation were selected for analysis using PFGE. Briefly, bacterial cells grown overnight were embedded in agarose, lysed and deproteinated to isolate near intact genomic DNA. The 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, 0.5 X TBE, 200V at 5 - 30 sec switch interval over 23 hours. Ethidium bromide stained gels were examined visually. Isolates showing < three bands difference were considered identical/clonally related.

Chloramphenicol acetyl transferase assay (CAT). Based on resistance to chloramphenicol, 29 isolates were subjected to CAT assay. The CAT assay was performed as described in the American Society for Microbiology Manual of Clinical Microbiology (1992). The bacterial cultures were induced by culture in the presence of chloramphenicol before determining CAT activity.

RESULTS

- VRE occurrence was more than five-fold greater in the USA compared to Europe.
- *E. faecium* constituted the largest number of isolates (91.0%) in the 2003 VRE protocol of the SENTRY Program. *E. faecalis* isolates accounted for only 7.8% of the total VRE population.
- Resistance profiles of *E. faecalis* and *E. faecium* isolates from Europe and North America showed significant differences (Table 1).
- vanA phenotype was more common among *E. faecalis* from Europe (85.7%), while vanB phenotype was more prevalent among *E. faecalis* from North America, mainly the USA (51.8%).
- Higher rates of chloramphenicol resistance were observed among *E. faecalis* from North America, whereas quinupristin/dalfopristin-resistant *E. faecium* were more prevalent in Europe (Table 1).
- Two linezolid-resistant *E. faecium* and one *E. faecalis* were isolated only from US sites.
- Table 2 presents the analysis of PFGE results of the VRE isolates. A total of 35 epidemic clusters were identified by PFGE, 32 from North America (20 sites) and three from Europe (two sites).
- The majority of the MDR clusters exhibited the vanA VRE phenotype.
- Quinupristin/dalfopristin MIC values for isolates with elevated MICs (> 2 µg/ml) were repeated using Etest. None of the isolates showed MIC results (> 8 µg/ml) consistent with *vat*D/E.
- Four vanB clusters were detected, two of which occurred in New York City sites with closely related/identical PFGE patterns (Figure 1).

Table 1. Comparative resistance profiles of VRE isolates from Europe and North America to selected, therapeutically important antimicrobials (SENTRY Program, 2003).

	Europe			North America		
Organism/antimicrobial agent	MIC_{50}	MIC_{90}	% resistant[a]	MIC_{50}	MIC_{90}	% resistant[a]
E. faecalis (no. tested)		(14)			(56)	
Ampicillin	2	4	0.0	2	2	0.0
Chloramphenicol	8	8	7.1	8	>16	28.6
Ciprofloxacin	>4	>4	85.7	>4	>4	100.0
Linezolid	1	2	0.0	1	2	1.8
Quinupristin/Dalfopristin	>2	>2	100.0	>2	>2	98.2
Teicoplanin	>16	>16	85.7	4	>16	48.2
Gentamicin (HL)	>1000	>1000	85.7	1000	>1000	62.5
Rifampin	2	>2	21.4	1	2	5.4
E. faecium (no. tested)		(40)			(776)	
Ampicillin	>16	>16	97.5	>16	>16	98.7
Chloramphenicol	8	>16	15.0	8	8	0.5
Ciprofloxacin	>4	>4	87.5	>4	>4	99.5
Linezolid	1	2	0.0	2	2	0.3
Quinupristin/Dalfopristin	0.5	1	10.0	1	1	0.6
Teicoplanin	>16	>16	67.5	>16	>16	78.5
Gentamicin (HL)	≤500	>1000	42.5	≤500	>1000	37.0
Rifampin	>2	>2	67.5	>2	>2	65.9

a. Resistance breakpoints per NCCLS criteria.

- All the chloramphenicol-resistant *E. faecalis* isolates showed a positive CAT test, whereas only three of 10 *E. faecium* were positive for CAT. CAT-positive *E. faecalis* from New York City sites (015 and 082) were clonal, which also had the vanB resistance phenotype (Figure 1).
- Two more CAT-positive *E. faecium* were detected from another site in North America, but were not clonally related.

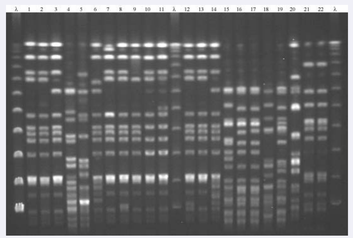
Table 2. Occurrence of clonal dissemination for enterococci among North American and European SENTRY Program sites.

Continent	Site #	No. isolates tested for clonality	No. clusters (# isolates)	Species	Resistances
North America	001	5	1(4)	EFM	MDR[b]
	002	10	3(10)	EFM	MDR;Q/D-R
	004	5	1(5)	EFM	MDR
	012	5	1(3)	EFM	MDR
	013	8	1(6)	EFM	MDR
	014	8	3(8)	EFM	Q/D-R;MDR
	015	3	1(3)	EF	CAT+;vanB
		6	1(2)	EFM	MDR
	017	6	1(3)	EFM	MDR
	019	8	2(7)	EFM	vanB;MDR
	021	6	2(6)	EFM	MDR
	024	9	1(5)	EFM	MDR;CAT+ (nonclonal)
	025	6	1(6)	EFM	MDR
	029	6	2(6)	EFM	MDR
	030	5	1(3)	EFM	MDR
	038	2	0	EFM	MDR
	051	9	2(5)	EFM	MDR
	082	9	1(9)	EF	vanA;vanB;CAT+
		6	2(6)	EFM	MDR
	106	3	1(3)	EFM	MDR
	107	6	1(5)	EFM	MDR
	109	3	1(3)	EFM	MDR
	110	6	2(4)	EFM	MDR
Europe	062	2	0	EFM	Q/D-R
	075	3	0	EFM	MDR
	095	7	2(5)	EFM	Q/D-R;vanB
	096	3	1(3)	EFM	MDR

a. Isolates with MDR resistance phenotype were resistant to vancomycin (van A or B), ampicillin, ciprofloxacin, aminoglycosides, rifampin and in some cases doxycycline.
b. CAT+ = positive chloramphenicol acetyl transferase test.

Figure 1. PFGE profiles of VRE strains.

FIGURE LEGEND:
Lanes 1 - 3 represent CAT +/vanB isolates from site 015, lanes 6 - 14 represent CAT +/vanB isolates from site 082. Both the medical centers are in New York City.



CONCLUSIONS

- Vancomycin-resistant enterococci (VRE) showed high rates of co-resistances to other therapeutic antimicrobial classes.
- Clonal spread was common among VRE isolates in North America and Europe.
- Resistance to chloramphenicol in North America was over-represented due to clonal spread among two centers in New York City, which harbored a common MDR, vanB *E. faecalis* cluster.
- Linezolid resistance was present and rare among USA isolates and non-existent in European VRE.
- *vat*D- or *vat*E-mediated quinupristin/dalfopristin resistance was not observed in the large number of VRE isolates studied, even among European strains (MICs at 2 - 8 µg/ml).
- VRE continues to be an expanding clinical problem in North America and Europe, exacerbated by common clonal dissemination within and between monitored medical centers.

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