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Antibiotic and Disinfectant Susceptibility Profiles of Vancomycin-Resistant *Enterococcus faecium* (VRE) Isolated from Community Wastewater in Texas

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Abstract Vancomycin-resistant *Enterococcus faecium* (VRE) from human wastewater effluents in a nonclinical semiclosed agri-food system in Texas were characterized for susceptibility to antibiotics and disinfectants. The 50 VRE were resistant to eight fluoroquinolones and 10 of 17 antimicrobials typically active against Gram-positive organisms. The VRE were susceptible to quinupristin/dalfopristin and linezolid. Lack of the insertion element IS*1251* correlated with VRE susceptibility to streptomycin and gentamicin at $p < 0.0001$ and $p = 0.033$, respectively. An association was observed between pulsed-field gel electrophoresis genotypes Ic and II and susceptibility to streptomycin at $p = 0.0006$. VRE susceptibility for nine disinfectants and five disinfectant components is shown. Ninety-two percent of the isolates had a minimum inhibitory concentration (MIC) for triclosan ≥2 ppm. Triclosan MICs for many of the VRE were well over expected product application levels. No association was observed between antibiotic resistance and disinfectant susceptibility in these VRE. Enterococci multiply-resistant to vancomycin and aminoglycosides were found in a non-hospital environment where one would not expect to find them.

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Disinfectants are widely used in medicine, agriculture, food processing plants, manufacturing, and household settings for sanitation and infectious disease control. These chemicals inevitably become distributed into the general aquatic environment from medical wastewater, sewer systems, and agricultural runoff. For example, about 80 million pounds of quaternary ammonium compounds were sewered in 1979 (Boethling 1984), and their fate in the environment is concerning because they are toxic at low concentrations (Núñez et al. 2004). Triclosan has been found in urban streams and domestic and municipal water wells in Colorado (Sprague and Battaglin 2005), where it may degrade into highly toxic dioxins (Prada et al. 2004). And, chlorhexidine was present in wastewater from a medical wastewater treatment plant up to 1.94 mg/L (Matsushiman and Sakurai 1984). Although disinfectants are widely present in the environment there has been little research into correlation between disinfectant resistance and antibiotic resistance. We recently demonstrated an association between chlorhexidine resistance among beta-hemolytic *Escherichia coli* from neonatal swine and resistance to the antibiotics gentamicin and streptomycin (Beier et al. 2005).

Vancomycin-resistant enterococci (VRE) cause significant human infections and are widely disseminated in institutions (e.g., hospitals) (Bonten et al. 2001), where both disinfectants and antibiotics are extensively used. Infections due to VRE are associated with higher treatment costs, prolonged morbidity, and greater mortality rates (Calfee et al. 2003). The widespread use of the antimicrobial avoparcin (similar to vancomycin) in the agricultural industry of

Europe resulted in endemic VRE colonization of farm animals and healthy people (Bonten et al. 2001). However, a community reservoir such as seen in Europe seems to be absent in the U.S. (Bonten et al. 2001).

Researchers from our laboratory screened seven community wastewater locations of a semiclosed agri-food system in the state of Texas for VanA or VanB VRE and found 49 VRE possessing *vanA*, and one possessing the *vanB* gene cluster (Poole et al. 2005). VanA and VanB phenotypes demonstrate a high level of resistance to vancomycin and this trait is acquired from mobile DNA elements (Cetinkaya et al. 2000). This work demonstrated for the first time the presence of high level resistant VRE in the U.S. in non-hospital community wastewater. A large number of swine fecal samples were also screened, all of which were susceptible to vancomycin (Poole et al. 2005); therefore, it is unlikely that agriculture was the source of these VRE.

There is concern over the development of cross-resistance between antibiotics and disinfectants, and research directed at surveillance and cross-resistance patterns is needed (Beier et al. 2004). Nothing is known of the disinfectant resistance profiles of enterococci in non-hospital settings where there is widespread use of many disinfectants, particularly with respect to the resistance patterns of organisms present in specialized niches, such as in sewer effluent. The aim of this study was to describe the minimum inhibitory concentration (MIC) distribution of VRE isolated from wastewater in respect to antibiotics and disinfectants and determine whether the isolates demonstrate a link between antibiotic resistance and disinfectant susceptibility.

Materials and Methods

Fifty vancomycin-resistant *Enterococcus faecium* isolates (49 VanA and one VanB) with 7, 14, 3, 13, 5, 5, and 3 VRE isolated from locations TX1, TX2, and TX3 (situated within 20 miles of Huntsville, TX, USA), TX4 and TX5 (located south of Houston, TX, USA), and TX6 and TX7 (located within Huntsville, TX, USA), respectively (Poole et al. 2005). These VRE were from human wastewater effluents at a non-clinical semiclosed agri-food system with restricted access and a long-term permanent worker population in Texas (Scott et al. 2005). TX6 has a hospital unit and TX7 is where new workers are processed and assigned to a swine farm (TX1-TX5). Bacterial isolates were stored and reconstituted as previously described (Beier et al. 2005).

MICs were determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) standards for antimicrobial susceptibility testing (CLSI 2002, 2005). MICs were determined as the lowest concentration of compound that inhibited the visible growth of the microorganisms (Andrews 2001). Antibiotic MICs were obtained using National Antimicrobial Resistance Monitoring System (NARMS) susceptibility plates (No's CMV1AGPF and CMV1DW), demineralized water, and cation-adjusted Mueller-Hinton broth with TES (Trek Diagnostic systems Inc., Cleveland, OH, USA). The following organisms were used as controls for all susceptibility testing: *Enterococcus faecalis* 29212, *E. faecalis* 51299, and *Pseudomonas aeruginosa* 27853. *E. faecium* CF3 1.3 (*E. faecium* Y) isolated from the ceca of an adult broiler chicken is susceptible to vancomycin (Corrier et al. 1995), and was compared with the VRE in this study.

The disinfectants chlorhexidine diacetate (Nolvasan® solution), DC&R®, Enforcer®, and Tek-Trol® were purchased at Producers Cooperative Association (Bryan, TX, USA). The disinfectant P-128 was obtained from Burns Veterinary Supply, Inc. (Farmers Branch, TX, USA). Betadine® solution, 10% providone-iodine (P-I), was obtained from The Pharmacy Shop (Bryan, TX, USA). J.T. Baker 37% formaldehyde solution was obtained from VWR International, Inc. (Marietta, GA, USA). The disinfectants benzalkonium chloride (BKC) and triclosan, and the disinfectant components benzyldimethylhexadecylammonium chloride (C16AC), benzyldimethyltetradecylammonium chloride (C14AC), and tris(hydroxylmethyl)nitromethane (THN) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). The disinfectant component didecyldimethylammonium chloride (C10AC) was obtained from Lonza Inc. (Fairlawn, NJ, USA), and benzyldimethyldodecylammonium chloride (C12AC) was obtained from Chem Service (West Chester, PA, USA). Sterile dimethyl sulfoxide (DMSO), and Hybri-Max were obtained from Sigma-Aldrich (St. Louis, MO, USA). Reverse osmosis water (H_2O) was produced on site by a reverse osmosis system obtained from Millipore Corp. (Bedford, MA, USA). The following disinfectants exist as mixtures of multiple components: DC&R® has the following active ingredients: THN 19.2%; (C12AC 67%, C14AC 25%, C16AC 7%, and (C8, C10, C18 1%)benzyldimethylammonium chlorides) 3.08%; and formaldehyde 2.28%. Active ingredients of Enforcer® are the following: (C14AC 60%, C16AC 30%, C12AC 5%, (C18 5%)benzyldimethylammonium chlorides) 0.105%; and (C12AC 68%, C14AC 32%) 0.105%. Active ingredients of Tek-Trol® are the following: *o*-Phenylphenol 12%; *o*-benzyl-*p*-chlorophenol 10%; and *p*-tert-amylphenol 4%; and P-128 has the following active ingredients: C10AC 4.61%; and (C14AC 50%, C12AC 40%, C16AC 10%) 3.07%.

The disinfectants and disinfectant components were diluted with H_2O to make standard solutions and then filtered using a 0.2 μm × 25 mm syringe filter (No. 431224, Corning Inc., Corning, NY, USA). DMSO was added to triclosan, C14AC, C16AC, and THN to aid chemical solubility, and the final Mueller Hinton broth (DIFCO brand Mueller Hinton Broth, No. 275730, Fisher Scientific, Houston, TX, USA)

solution with the highest concentration of these chemicals contained 5% DMSO. The method of disinfectant susceptibility determination was similar to that used for susceptibility testing of chlorhexidine in *E. coli* (Beier et al. 2005), and resulted in the following concentrations of disinfectants or disinfectant components used: DC&R®, 1024–1 μg/mL; Tek-Trol®, 512–0.5 μg/mL; chlorhexidine, 64–0.06 μg/mL; triclosan, 128–0.12 μg/mL; Enforcer®, 64–0.06 μg/mL; P-128, 64–0.06 μg/mL; BKC, 256–0.25 μg/mL; P-I, 16384–16 μg/mL; formaldehyde, 2048–2 μg/mL; THN, 4096–4 μg/mL; C10AC, 64–0.06 μg/mL; C12AC, 512–0.5 μg/mL; C14AC, 128–0.12 μg/mL; and C16AC, 64–0.06 μg/mL.

MICs of the 17 antimicrobials on the NARMS 2005 panel (CMV1AGPF) [bacitracin (BAC), chloramphenicol (CHL), ciprofloxacin (CIP), daptomycin (DAP), erythromycin (ERY), flavomycin (FLA), gentamicin (GEN), kanamycin (KAN), lincomycin (LIN), linezolid (LZD), nitrofurantoin (NIT), penicillin (PEN), quinupristin/dalfopristin (QDA), streptomycin (STR), tetracycline (TET), tylosin (TYL), and vancomycin (VAN)], and the 8 fluoroquinolones on the CMV1DW panel [Ciprofloxacin (CIP), danofloxacin (DANO), difloxacin (DIF), enrofloxacin (ENRO), gatifloxacin (GAT), levofloxacin (LEVO), marbofloxacin (MARB), and orbifloxacin (ORB)] were determined by broth microdilution according to methods described by CLSI (2002, 2005). MICs were determined using the Sensititre automated antimicrobial susceptibility system according to the manufacturer's instructions (Trek Diagnostic Systems Inc., Cleveland, OH, USA).

Statistical analysis was conducted in JMP 6.0©, SAS Institute Inc. (Cary, NC). Data was analyzed using multivariate hierarchical clustering techniques and Ward's minimum variance method to produce dendrograms that helped demonstrate relationships between MICs and sample location, sample type (workers, non-workers, or mixed workers and non-workers), PFGE genotypes, and occurrence of IS*1251*. Associations among isolates were evaluated through correlation analysis. Kendall's tau nonparametric correlation coefficient was used since the variables were not continuous. Significant correlations were assessed at a 0.05 probability of Type I error.

Results and Discussion

Vancomycin-resistant *E. faecium* resistance profiles for the NARMS 2005 Gram-positive antibiotic panel are shown in Table 1 with a comparison to the MICs of a vancomycin susceptible *E. faecium* CF3 1.3. All VRE were resistant to the antibiotics CIP, ERY, FLA, KAN, and VAN, and most were resistant to the antibiotics GEN, LIN, PEN, STR, and TYL at a percentage of resistance of 76%, 94%, 98%, 86%, and 94%, respectively. Thirty-six percent of the VRE were resistant to TET and 54% had intermediate resistance, while they demonstrated a low percentage of resistance at 20% and 2% for BAC and CHL, respectively, but had a high intermediate percentage of resistance of 72% and 46%, respectively. The 50 VRE were highly susceptible to LZD and QDA with only 1 isolate being resistant to LZD and 2 isolates showing intermediate resistance to QDA. The LZD, and QDA MIC_{50} and MIC_{90} at 2 and 2 μg/mL, and $\leq$1 and $\leq$1 μg/mL, respectively, were low. Strain CF3 1.3 had lower MIC values for most antibiotics than the VRE, and it clustered with the antibiotic susceptible isolates from the hospital unit and new workers processing unit, TX6 and TX7. CF3 1.3 was resistant to only FLA and had intermediate resistance to BAC, KAN, LIN, NIT and TET.

TET resistant VRE were only found at TX2, TX4, and TX6 locations and comprised 38.5, 61.5, and 83.3% of the isolates at these locations, respectively. TX2 and TX4 are swine farms and TX6 has a hospital. VRE resistant to BAC were not found at one farm location, TX3, and at location TX7 where workers were processed into the system and assigned living quarters. VRE susceptible to STR were only found at the TX4, TX6, and TX7 locations, and TX1, TX2, TX3, and TX5 only had STR resistant VRE. GEN susceptible VRE were found at the TX2, TX4, TX5, TX6, and TX7 locations.

The fluoroquinolone MICs and resistance profiles for the VRE compared with strain CF3 1.3 are shown in Table 2. The 50 VRE were resistant to CIP, GAT, and LEVO. No published *Enterococcus* breakpoints were available for DANO, DIF, ENRO, MARB, and ORB. However, both *Enterococcus* and *Pseudomonas* have identical breakpoints for CIP, GAT, and LEVO, and the observed high level of MIC_{50}s for DANO, DIF, ENRO, MARB, and ORB would suggest that the 50 VRE are highly resistant to these fluoroquinolones as well. Strain CF3 1.3 demonstrated some resistance to DIF and ENRO with MICs of 4 and 2 μg/mL, respectively. Overall, the CF3 1.3 MICs were much lower for all the fluoroquinolone antibiotics than were the VRE MICs.

Susceptibility profiles for disinfectants and their disinfectant-components are shown in Table 3. Some of the disinfectants DC&R®, Tek-Trol®, Enforcer®, and P-128 are mixtures of several disinfectant components and, therefore, the MICs shown for these disinfectants were determined on the composite mixtures. Vancomycin resistant enterococci are more resistant to the antimicrobial action of DC&R®, Tek-Trol®, and PI than they are to chlorhexidine, triclosan, Enforcer®, P-128, and benzalkonium chloride, or to several of the individual components, C10AC, C14AC, and C16AC.

DC&R® is made up of THN (19.2%), ammonium chlorides (primarily C12AC, C14AC, and C16AC) (3.08%), and formaldehyde (2.28%). An interesting relationship can be

Table 1 NARMS 2005 antibiotic MICs and resistance profiles among VRE[a]

Antibiotic	No. (%) Resistant[b]	No. (%) Intermediate[b]	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	CF3 1.3 MIC (μg/mL)
Bacitracin (BAC)	10 (20)	36 (72)	64	128	64
Chloramphenicol (CHL)	1 (2)	23 (46)	8	16	8
Ciprofloxacin (CIP)	50 (100)	0	>4	>4	1
Daptomycin (DAP)	NA[c]	NA	4	8	2
Erythromycin (ERY)	50 (100)	0	>8	>8	≤0.5
Flavomycin (FLA)	50 (100)	0	>32	>32	>32
Gentamicin (GEN)	38 (76)	NA	>1024	>1024	≤128
Kanamycin (KAN)	50 (100)	0	>1024	>1024	256
Lincomycin (LIN)	47 (94)	0	>32	>32	16
Linezolid (LZD)	1 (2)	0	2	2	2
Nitrofurantoin (NIT)	NA	14 (28)	>64	>64	>64
Penicillin (PEN)	49 (98)	NA	>16	>16	2
Quinupristin/ Dalfopristin (QDA)	0	2 (4)	≤1	≤1	≤1
Streptomycin (STR)	43 (86)	NA	>2048	>2048	≤512
Tetracycline (TET)	18 (36)	27 (54)	8	16	8
Tylosin (TYL)	47 (94)	0	>32	>32	4
Vancomycin (VAN)	50 (100)	0	>32	>32	≤0.5

[a] MIC profiles of 50 high-level vancomycin-resistant *E. faecium* isolated from community waste water in Texas (Poole et al. 2005) compared to *E. faecium* CF3 1.3 susceptible to vancomycin (Corrier et al. 1995)

[b] Resistant and intermediate indicates the level of antibiotic resistance

[c] NA, not applicable; due to the lack of interpretive criteria for enterococci

Table 2 Fluoroquinolone MICs and resistance profiles among VRE[a]

Antibiotic	No. (%) Resistant	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	CF3 1.3 MIC (μg/mL)
Ciprofloxacin (CIP)	50 (100)	>32	>32	1
Danofloxacin (DANO)	NA[b]	32	>32	1
Difloxacin (DIF)	NA	>32	>32	4
Enrofloxacin (ENRO)	NA	32	>32	2
Gatifloxacin (GAT)	50 (100)	>16	>16	0.5
Levofloxacin (LEVO)	50 (100)	>32	>32	1
Marbofloxacin (MARB)	NA	>32	>32	2
Orbifloxacin (ORB)	NA	>32	>32	4

[a] MIC profiles of 50 high-level vancomycin-resistant *E. faecium* isolated from community waste water in Texas (Poole et al. 2005) compared to *E. faecium* CF3 1.3 susceptible to vancomycin (Corrier et al. 1995)

[b] NA, not applicable; due to the lack of interpretive criteria for enterococci

seen when the concentrations of the individual components are examined and related to the DC&R® MIC. To evaluate an individual component concentration, the DC&R® MIC was multiplied by the component of interest percentage and divided by the sum of all the components percentages in DC&R®. For example, to calculate the level of ammonium chloride components of DC&R® MIC = 8 μg/mL, ammonium chloride component level = 8 × 3.08/24.56 = 1 μg/mL. Similarly, the THN portion of DC&R® results in a distribution of 6.25, 12.5, and 25 μg/mL THN for DC&R® MICs of 8, 16, and 32 μg/mL, and the formaldehyde portion results in a distribution of 0.74, 1.49, and 2.97 μg/mL. The levels of both components are well below the VRE MICs for THN and formaldehyde, and these levels are too low to contribute to disinfection of VRE (Table 3). However the third component, comprised of ammonium chlorides (primarily C12AC, C14AC, and C16AC), results in a distribution of 1.0, 2.0, and 4.01 μg/mL. This ammonium chloride concentration would have accomplished the entire antimicrobial activity of DC&R® against the VRE. The distribution is the same as seen for MICs of C14AC and C16AC (Table 3). If THN is a weak disinfectant against other bacteria too, its use is questionable. However, while DC&R® MICs are higher than those of other disinfectants

Table 3 Disinfectant and disinfectant component susceptibility profiles among VRE[a]

Disinfectant[b]	MIC (μg/mL)													MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	CF3 1.3 MIC (μg/mL)
	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048			
DC&R®					4[c]	26	20							16	32	16
Tek-Trol®						4	34	12						32	64	64
Chlorhexidine	12	13	25											1	2	1
Triclosan	1	3	13	16	17									4	8	8
Enforcer®	5	41	4											1	1	1
P-128	14	22	14											1	2	1
BKC			7	37	6									4	8	4
P-I												1	49	2048	2048	2048
C10AC[d]	17	30	3											1	1	0.5
C12AC[d]			7	18	23	2								4	8	4
C14AC[d]		6	39	5										2	2	1
C16AC[d]		8	41	1										2	2	1
THN[d]										7	28	10	5	512	1024	1024
Formaldehyde[d]								21	29					128	128	128

[a] Susceptibility profiles of 50 high-level vancomycin-resistant *E. faecium* isolated from community waste water in Texas (Poole et al. 2005) compared to *E. faecium* CF3 1.3 susceptible to vancomycin (Corrier et al. 1995)

[b] Disinfectant and disinfectant component abbreviations: benzalkonium chloride (BKC), providone-iodine (P-I), didecyldimethylammonium chloride (C10AC), benzyldimethyldodecylammonium chloride (C12AC), benzyldimethyltetradecylammonium chloride (C14AC), benzyldimethylhexadecylammonium chloride (C16AC), and tris(hydroxylmethyl)nitromethane (THN)

[c] Indicates the total number of isolates out of 50 VRE that exhibited the specified MIC

[d] These entries are disinfectant components

shown in Table 3, its intended application rate of 1,919 μg/mL is well over the level required for killing VRE (32 μg/mL).

Enforcer® contains both ethylbenzyl- and benzyldimethylammonium chlorides and the VRE MICs for Enforcer® are similar to or less than those of the components C14AC and C16AC (Table 3). The disinfectant P-128 contains C10AC (60% of its active ingredients). The MICs for P-128 follow quite closely the MICs for the component C10AC. C10AC is the most active single ammonium chloride component against the *Enterococcus*. We have also observed C10AC to be the most active ammonium chloride component against beta-hemolytic *E. coli* (unpublished).

A high concentration of P-I is needed to prevent the growth of VRE (Table 3). Forty-nine of 50 isolates required a P-I of 2048 μg/mL for growth inhibition. One isolate was slightly more sensitive than the rest with an MIC of 1024 μg/mL. These were the highest MICs observed among the disinfectants except for the component THN. However, the application of P-I is for minor cuts and scrapes, and a 100,000-μg/mL solution of P-I is suggested to be used directly without dilution. This is about a 49-fold more concentrated solution than is required for disinfecting *Enterococcus*.

The MIC results for triclosan span across a broad range of disinfectant concentrations (Table 3). Forty-six VRE isolates (92%) were inhibited at triclosan concentrations of 2 through 8 μg/mL. The strains with the highest MICs were more resistant than other published VRE strains (Suller and Russell 1999), and also exceed values for methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Bamber and Neal 1999). The use of triclosan in commercial products with respect to the VRE MICs was explored. In a clinical situation, patients may be asked to bathe in water containing 1 sachet of Aquasept solution (28.5 mL, 2% triclosan). If one assumes 140 L of bath water, the final concentration of triclosan would be 4.6 μg/mL (Bamber and Neal 1999). In a home situation, dish soap commonly contains 0.1% triclosan. If one assumes 18 mL of dish soap for 8.5 L water, the final concentration of triclosan would be 2.1 μg/mL. In both applications the expected level of triclosan was well below the observed triclosan MICs for some of the VRE; 34% and 66% of the isolates in the clinical and home situation, respectively.

The MICs for BKC show a distribution of 2, 4, and 8 μg/mL, which are similar to the triclosan MICs for the major number of isolates. Thirty-four percent of the isolates have a MIC for BKC of 8 μg/mL, which is a higher MIC for BKC than is demonstrated by four other VRE (Suller and Russell 1999). According to Sidhu et al. (2002), the VRE here are sensitive to BKC.

It is interesting that the *E. faecium* CF3 1.3 from an adult broiler chicken has among the higher MICs for Tek-Trol®, triclosan, THN and formaldehyde. Tek-Trol® is a phenolic

disinfectant used in farm situations, and THN and formaldehyde are components of DC&R®, which is also used on the farm. But, strain CF3 1.3 is related to lower VRE resistance for other disinfectants.

PCR analysis of the intergenic regions of Tn*1546* of the 49 *vanA* isolates demonstrated that 39 VRE carried the insertion sequence, IS*1251* (Poole et al. 2005). The mobile DNA element (transposon) Tn*1546* contains the *vanA* gene cluster, which confers high-level resistance to vancomycin and teicoplanin (Arthur et al. 1995). The IS*1251* insertion sequence is found in the *vanS-vanH* intergenic region within Tn*1546* (Handwerger et al. 1995). VRE not containing the IS*1251* element were only isolated at locations TX4, TX6, and TX7. Ninety percent of the VRE susceptible to STR and GEN correlated with the absence of IS*1251*. We observed a correlation of 0.30 at $p = 0.033$ for GEN and 0.66 at $p < 0.0001$ for STR susceptibility with the absence of IS*1251*. In general, there was a strong correlation between VRE without IS*1251* of 0.93 at $p = 0.0037$ between PFGE genotype and isolation location. Prior to the first study with these VRE, IS*1251* had only been identified in VRE isolated from hospitals in the United States (Poole et al. 2005). Ninety-three percent of the VRE containing the IS*1251* genomic element were from locations TX1-TX5 and TX7, which did not have a hospital unit.

PFGE analysis of these VRE resulted in 5 unique genotypes Ia, Ib, Ic, II, and III (Poole et al. 2005). An association was observed between genotypes Ic and II (10 of 16 isolates having the absence of IS*1251*) and susceptibility to STR ($p = 0.0006$). Genotypes II and III were associated with 58.3% and 25% of the GEN-susceptible VRE, respectively. Approximately 88% of genotype II and 100% of genotype III were susceptible to GEN.

Forty-two percent of the VRE clustered together because they had higher MICs for most disinfectants. This group of VRE comprised 62% of TX4 isolates, 100% of TX6 isolates, and 100% of TX7 isolates. Location is a good predictor of isolate clustering based on MICs. It was predominately at locations TX6 (with a hospital) and TX7 (with a holding facility for new workers and non-workers) where disinfectant MICs were the highest. The isolates from these three locations (TX4, TX6, and TX7) were also the only isolates susceptible to STR. Further, 86% of the isolates (18 of 21) with higher disinfectant MICs were predominately made up of the PFGE genotypes Ic, II, and III at 100%, 87.5%, and 100%, respectively. However, genotype Ic also was associated with lower MICs for Tek-Trol®, triclosan, and THN, and in some cases formaldehyde. Three of the 21 isolates in this higher disinfectant MIC group have genotypes Ia (2 isolates) and Ib (1 isolate). The two genotype Ia isolates had lower MICs to Tek-Trol® and THN. All VRE without the genomic insertion element IS*1251* had increased MICs to the disinfectants; however, 27.5% of the isolates that possessed IS*1251* were also found in the group with increased MICs to the disinfectants.

Finally, enterococci from non-hospital environments were multiple resistant to vancomycin and both the aminoglycosides, gentamicin and streptomycin. A non-hospital environment is a place where one would not expect to find these types of isolates. No correlation was found between antibiotic resistance and disinfectant susceptibility patterns (Data not shown), and this differed from our earlier results with beta-hemolytic *E. coli* (Beier et al. 2005).

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