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Identification and Characterization of Multiple Species of Vancomycin-Resistant Enterococci, Including an Evaluation of Vitek Software Version 7.1

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A total of 374 clinical isolates of *Enterococcus* spp. were characterized to determine the species distribution and vancomycin resistance. The ability of the Vitek system (bioMerieux Inc, Hazelwood, St. Louis, Mo.) to identify enterococci to the species level and to recognize vancomycin resistance by using computer software version 7.1 was evaluated. Conventional methods were used for identification and agar dilution was used for susceptibility testing, the results of which were as follows (presented as number of vancomycin-resistant isolates/number of members of that species identified): 219/234 *E. faecium*, 9/112 *E. faecalis*, 2/3 *E. mundtii*, 0/1 *E. durans*, 0/1 *E. hirae*, 0/1 *E. raffinosis*, and 0/1 *E. avium*. Ten enterococci were in the vancomycin-intermediate category (six *E. gallinarum*, two *E. casseliflavus*, and one each of *E. faecium* and *E. faecalis*). The Vitek GPI card correctly identified 98% of *E. faecium* isolates, 99% of *E. faecalis* isolates, and only two isolates of other enterococcal species. The GPS-TA card was 98% sensitive and 95% specific for the detection of vancomycin resistance, generating a total of four very major (1.6%) and five minor errors (1.3%).

The resistance of enterococci to vancomycin has been reported with increasing frequency in both Europe and North America (7, 10, 11, 15, 19). A diversity of resistance phenotypes has been described in *E. faecium* and *E. faecalis* (1, 9, 10, 20–23), and resistance has also been detected in isolates of *E. gallinarum*, *E. casseliflavus* (6, 14, 25), *E. avium*, and *E. durans* (5, 6, 10). We identified clinical enterococcal isolates to the species level and determined their resistance phenotypes. We used these isolates to evaluate the Vitek system (bioMerieux Inc., Hazelwood, St Louis, Mo.) by using software version 7.1 to detect vancomycin-resistant enterococci (VRE). In a previous evaluation (26), we found that the Vitek system with computer software version 6.1 had an unacceptably low level of sensitivity (72%) for VRE detection. In addition, we evaluated the ability of the Vitek GPI card to correctly identify the species within the genus *Enterococcus*.

We selected 374 clinical enterococcal isolates so as to include a large number of VRE (67%) with a variety of glycopeptide resistance phenotypes. The majority of VRE were from cultures of blood, urine, and wound specimens from patients in New York City hospitals, whereas susceptible strains were mostly from oncology patients in Princess Margaret Hospital, Toronto, Ontario, Canada. The control organisms used in the study were *E. raffinosis* ATCC 49427, *E. mundtii* ATCC 43186, *E. avium* ATCC 9274, *E. durans* ATCC 19432, *E. faecalis* ATCC 29212, *E. faecium* 228-3, *E. faecium* 228 (8), and *E. faecium* NYC 2491 (26). Organisms were stored at −70°C in buffered glycerol and were subcultured twice prior to further testing.

Vancomycin (Sigma Chemical Co., St. Louis, Mo.) and teicoplanin (Marion Merrel Dow Inc., Cincinnati, Ohio) MICs were determined by the agar dilution methodology of the National Committee for Clinical Laboratory Standards (17, 18), and identification of enterococci to the species level was performed by using the conventional biochemical methods described by Facklam and colleagues (3, 4). The organisms were tested by using the Vitek GPI and GPS-TA cards in accordance with the manufacturer's instructions. The data accumulated by the Vitek reader system were interpreted by the revised computer software, version 7.1. Vitek results were compared with those obtained by reference methods, and from these comparisons we calculated sensitivities, specificities, and error rates (16) for the software version. For the purposes of the sensitivity and specificity calculations, all strains determined to be in the intermediate vancomycin category, either by agar dilution or by the Vitek system, were combined with those isolates determined to be resistant by these systems. For the purpose of the present study, the vancomycin resistance phenotypes of Quintiliani et al. (20) were used. However, phenotypic class B was modified to include all teicoplanin-susceptible VRE, regardless of the MICs for the isolates, with the obvious exception of *E. gallinarum* and *E. casseliflavus* isolates, both of which we included in class C.

Conventional methods identified the enterococci as follows: 234 *E. faecium*, 112 *E. faecalis*, 6 *E. gallinarum*, 3 *E. mundtii*, 2 *E. casseliflavus*, 2 *E. durans*, and 1 each of *E. hirae*, *E. raffinosis*, and *E. avium*. We were unable to identify 12 VRE by the schema of Facklam and colleagues (3, 4), the results of which are presented in Table 1. These isolates may possibly be variants of *E. faecium* (as in biotype 1) or previously unrecognized enterococci (biotypes 2 and 3), because each strain differed from the "closest fit" by at least three of the biochemicals tested. Future homology studies with reference strains will be required to resolve the identification of these isolates. Unfortunately, the preva-

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TABLE 1. Conventional biochemical test results for the 12 unidentifiable vancomycin-resistant enterococcal isolates

Test	Result for strains of biotype (no. of strains):		
	1 (10)	2 (1)	3 (1)
Pyrrolidonyl arylamidase	+	+	+
Leucine aminopeptidase	+	+	+
o-Nitrophenyl β-D-Galactosidase	+	+	+
Bile esculin	+	+	+
Mannitol	+	+	+
Sorbitol	+	+	+
Sorbose	−	−	−
Arabinose	+	−	−
Raffinose	+	+	−
Sucrose	+	+	−
Pyruvate	−	−	−
Glycerol	+	+	+
Arginine	+	+	−
Pigment	−	−	−
Motility	−	−	−

TABLE 2. Species distribution of the vancomycin resistance phenotypes among the 374 enterococci tested

Species (no. of isolates)	No. of isolates in the following vancomycin-resistant phenotypic classes[a]:			No. of vancomycin-susceptible isolates (122)
	A (219)[b]	B (25)	C (8)	
E. faecium (234)	198	22	0	14
E. faecalis (112)	7	3	0	102
E. mundtii (3)	2	0	0	1
E. gallinarum (6)	0	0	6	0
E. casseliflavus (2)	0	0	2	0
Other *Enterococcus* spp. (5)	0	0	0	5
Unidentified strains (12)	12	0	0	0

[a] Class A, vancomycin MIC, ≥128 μg/ml; teicoplanin resistant; class B, vancomycin MIC, 16 to 512 μg/ml; teicoplanin susceptible; class C, vancomycin MIC 8.0 to 32 μg/ml; teicoplanin susceptible.
[b] Values in parentheses are total number of isolates.

lence of such strains is not known, because few laboratories identify enterococci to the species level.

The interpretation of the Vitek GPI card by version 7.1 was found to be acceptable for both *E. faecium* and *E. faecalis*, because 229 of 234 (98%) and 111 of 112 (99%) of these species, respectively, were correctly identified. Of the other 28 enterococcal isolates, only one *E. durans* and one *E. avium* were identified. The remaining isolates were reported either as "unidentified" (20 of 28) or were misidentified as *E. faecium* (6 of 28). The six misidentified isolates included one strain each of *E. casseliflavus* and *E. mundtii* and four of the enterococci that we were unable to identify to the species level. It should be noted that the Vitek data base includes only the following species: *E. avium*, *E. casseliflavus*, *E. casseliflavus* or *E. gallinarum*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, and *Enterococcus* species. However, the data base could not even identify these unlisted isolates as *Enterococcus* species; instead, they were either misidentified or reported as "unidentified." In addition, since Vitek is unable to detect motility or yellow pigment, this immediately excludes the identification of *E. gallinarum*, *E. casseliflavus*, and *E. mundtii*. Therefore, when using this system, it would be prudent to suspect that one of these species is an enterococcal isolate if the vancomycin MIC for the isolate is 8.0 μg/ml, and in such cases, the minimum requirements for further identification should be a tube motility test in semisolid medium incubated at 30°C for up to 72 h as well as the close examination of colonies for the production of a yellow pigment, which is best detected with a cotton swab (3, 4). Conversely, if a yellow pigment is detected, motility as well as MIC testing should be performed.

The resistance phenotypes, as determined by agar dilution susceptibility testing of vancomycin and teicoplanin, are summarized in Table 2. Class A VRE, which are known to be mediated by the *vanA* gene cluster (2, 13, 27), appear to be the most prevalent VRE (87%) in the New York City area (12). A source of great concern is the presence of class A resistance, as confirmed by *vanA* probing (data not shown), in the two isolates of *E. mundtii* and those VRE which we were unable to identify to the species level. To the best of our knowledge, high-level vancomycin resistance in such strains has not been reported previously. These results and those presented in other reports (5, 6, 10) indicate a slow, but ongoing interspecies transfer of the *vanA* gene. Less common were class B VRE (10%), 40% of which were found to be highly resistant to vancomycin; the vancomycin MICs for these organisms were ≥256 μg/ml. Isolates of this phenotype have not previously been noted among VRE isolated in New York City, but they may be similar to VRE with this phenotype reported in other parts of the United States (23, 24) and to those enterococci with high-level vancomycin resistance that harbor the *vanB* gene, as demonstrated by Quintiliani et al. (20). The only isolates for which vancomycin MICs were within the vancomycin-intermediate range were the six *E. gallinarum* and the two *E. casseliflavus* strains (class C); (vancomycin MICs for all of these isolates were 8.0 μg/ml) and one strain each of *E. faecium* and *E. faecalis* (vancomycin MICs, 16 μg/ml; teicoplanin susceptible).

The sensitivity of the GPS-TA card for the detection of vancomycin resistance was found to be 98%, and the specificity was found to be an acceptable 95%. The error rates generated by the card were as follows: there were four very major errors among class A *E. faecium* isolates, resulting in very major error rates of 1.8% for *E. faecium* and 1.6% overall. The minor errors included two *E. faecalis* isolates that were susceptible by agar dilution but intermediate by testing with the Vitek system (on repeat Vitek testing, both isolates were susceptible), one *E. faecium* and one *E. faecalis* were intermediate by agar dilution but were called susceptible by Vitek, and one *E. faecalis* that was resistant by agar dilution was called intermediate by Vitek. These resulted in minor error rates of 0.4% for *E. faecium* and 3.5% for *E. faecalis*, with an overall minor error rate of 1.3%. No major errors were incurred, and there were no errors among isolates of other enterococcal species. The low-level vancomycin resistance of *E. gallinarum* and *E. casseliflavus* was detected by the GPS-TA card in all cases. It appears that the vancomycin MICs fall within the intermediate category for very few *E. faecalis* or *E. faecium* strains (<1% of vancomycin-resistant isolates in the present study). The use of a screen plate containing 6 μg of vancomycin per ml detects such borderline isolates (26).

In conclusion, we found evidence of a variety of vancomycin-resistant phenotypes among the New York City enterococcal isolates, although the majority of strains were of

the class A resistance phenotype. We also found class A vancomycin resistance in two isolates of *E. mundtii* as well as in a number of strains that appeared to be either variants of existing species or isolates of novel enterococcal species. Identification of *E. faecium* and *E. faecalis* by the GPI card was found to be acceptable, although for species other than these, conventional methods are required for correct species identification. Detection of vancomycin resistance by the Vitek GPS-TA card in enterococci has been markedly improved by the updated software version 7.1 and should now be considered acceptable. Because the universal susceptibility of the enterococci to vancomycin can no longer be presumed, it is vitally important that laboratory procedures accurately identify resistant strains. If routine susceptibility testing is not performed on all enterococcal isolates, such as those from urine and wound specimens, it may be prudent to implement the use of the vancomycin screen plate (26).

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