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Proficiency of Clinical Laboratories in Spain in Detecting Vancomycin-Resistant *Enterococcus* spp.

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Studies in a variety of U.S. clinical laboratories have demonstrated difficulty in detecting intermediate and low-level vancomycin-resistant enterococci (VRE). The misclassification of "at least intermediate resistant isolates" as vancomycin susceptible may have both clinical implications and a negative impact on measures to control the spread of VRE. No published study has assessed the ability of clinical laboratories in Europe to detect VRE. So, the apparent low prevalence of VRE in European hospitals may be, in part, secondary to the inability of these laboratories to detect all VRE. In an effort to assess European laboratories' proficiency in detecting VRE, we identified 22 laboratories in Spain and asked them to test four VRE strains and one susceptible enterococcal strain from the Centers for Disease Control and Prevention collection. Each organism was tested by the routine antimicrobial susceptibility testing method used by each laboratory. Overall, VRE were correctly identified in 61 of 88 (69.1%) instances. The accuracy of VRE detection varied with the level of resistance and the antimicrobial susceptibility method. The high-level-resistant strain (*Enterococcus faecium*; MIC, 512 μg/ml) was accurately detected in 20 of 22 (91.3%) instances, whereas the intermediate-resistant isolate (*Enterococcus gallinarum*; MIC, 8 μg/ml) was accurately detected in only 11 of 22 (50%) instances. Classification errors occurred in 27 of 88 (30.9%) instances. Misclassification as vancomycin susceptible was the most common error (16 of 27 [59.3%] instances). Our study shows that the participating Spanish laboratories had an overall acceptable proficiency in detecting VRE but that a substantial proportion of VRE isolates with low or intermediate levels of resistance were not detected. We recommend that studies be conducted to validate laboratory proficiency testing as an important step in the prevention and control of the spread of antimicrobial resistance.

Enterococci are major nosocomial pathogens and have been isolated from 9% of nosocomial bloodstream, 12% of surgical site, and 16% of urinary tract infections reported by U.S. hospitals participating in the hospital-wide component of the National Nosocomial Infections Surveillance (NNIS) system (13). In the face of the increasing incidence of high-level resistance to penicillin and aminoglycosides, enterococci resistant to all three antimicrobial agents (penicillin, aminoglycosides, and vancomycin) with activity against enterococci pose a serious challenge not only for clinicians but also for health care institutions, because numerous nosocomial outbreaks have been reported (10, 11). Moreover, enterococci may be a reservoir for resistance genes for other gram-positive organisms, including *Staphylococcus aureus*; in vitro studies have shown that the *vanA* gene coding for vancomycin resistance can be transferred from enterococci to *S. aureus* (14). Prevention and control of the spread of vancomycin-resistant enterococci (VRE) are therefore major national and international public health challenges. Specific guidelines and recommendations for preventing the spread of VRE were published in 1995 by the Centers for Disease Control and Prevention (CDC) and its Hospital Infection Control Practices Advisory Committee (3). Despite this, 40% of hospitals participating in NNIS reported the detection of one or more isolates of VRE in 1996, and the proportion of enterococci resistant to vancomycin at hospitals participating in NNIS has continued to increase to 22.6.% among intensive care unit patients and 16.5% among nonintensive care patients in 1997 (4). The first step in controlling the spread of VRE is its early detection. Nevertheless, detection of the intermediate- and low-level resistance exhibited by strains with the *vanB* and *vanC* phenotypes is not consistently done by automated commercial methods (15). Different studies in the United States and Argentina have shown that only 16 to 27% of these isolates are correctly identified (6, 16).

Little is known about the epidemiology of VRE in Europe. Several hospital-based reports suggest low prevalence rates of VRE in clinical specimens (9, 17). In Spain, an annual nationwide point prevalence study demonstrated a stable prevalence rate of approximately 10% for enterococcal nosocomial infections; however, no data on VRE were collected (8). During 1994 and 1995, in three hospitals in Madrid, Spain, vancomycin resistance was found among 8 of 100 (8%) enterococcal isolates cultured from blood (1). In 1994, the European Glycopeptide Susceptibility Survey presented unpublished data suggesting problems in the testing of the susceptibilities of various gram-positive isolates to glycopeptides (7). However, no isolates with low-level or intermediate- to low-level vancomycin resistance were tested, and no final results or information on the laboratory susceptibility test methods used have been published. To date, no study of the proficiency of detection of VRE in clinical laboratories in Spain has been conducted. In the study described here, we sought to assess the ability of clinical laboratories in Spain to detect VRE.

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TABLE 1. Characteristics of enterococcal study isolates, 1996

Organism no. and species	MIC (μg/ml), interpretation by NCCLS criteria[a]			Vancomycin phenotype
	Vancomycin	Penicillin	Ampicillin	
1. *E. faecium*	512, R	32, R	4, S	VanA
2. *E. faecium*	64, R	>256, R	64, R	VanB2
3. *E. faecalis*	16–32, I-R	4, S	1, S	VanB
4. *E. gallinarum*	8, I	2, S	1, S	VanC1
5. *E. faecalis*	1–4, S	1–4, S	0.5–2, S	

[a] R, resistant; S, susceptible; I, intermediate.

MATERIALS AND METHODS

Bacterial strains. Five enterococcal isolates were obtained from the CDC strain collection and were coded as organisms 1 through 5, respectively. The isolates included two *Enterococcus faecium* isolates, one *Enterococcus faecalis* isolate, and one *Enterococcus gallinarium* isolate, with each isolate having one of the four most common vancomycin-resistant phenotypes. In addition, *E. faecalis* ATCC 29212, which is susceptible to vancomycin, penicillin, and ampicillin, was included (Table 1).

The isolates were inoculated onto nutrient agar slants (Becton Dickinson Microbiology Systems, Cockeysville, Md.), incubated for 24 h, and distributed to the participating clinical laboratories along with standardized susceptibility test result forms. The participating laboratories were blinded as to the species and the antimicrobial susceptibility patterns of the isolates. Each participant was instructed to test the five isolates for their susceptibilities to vancomycin by their routine laboratory procedures and to report the zone size or MIC. Additionally, the participating laboratories were asked to provide hospital characteristics, the number and proportion of enterococci and isolates of VRE detected in the preceding year, the routine antimicrobial susceptibility method(s) used, and whether the E test and agar screening tests were routinely used. After completion of testing, the forms were completed and returned to CDC for data entry and analysis. Testing at CDC was by National Committee for Clinical Laboratory Standards (NCCLS) reference methods (12). The MIC and/or zone size results from participants and CDC were compared. Testing errors were classified as either very major, major, or minor errors. A very major error occurred when the CDC method determined that an organism was resistant to an antimicrobial agent and the method used by the participant reported that it was susceptible to that agent. A major error occurred when the CDC method found that an organism was susceptible and the method used by the participant found that it was resistant. A minor error occurred when the CDC method determined that an organism was susceptible or resistant to an antimicrobial agent and the method used by the participant reported that it was intermediate or when the CDC method determined that an organism was intermediate and the participant reported that it was resistant or susceptible.

Reference methods. Disk diffusion testing at CDC was performed as described previously (16). Isolates were defined as susceptible, intermediate, or resistant by using NCCLS criteria (12). The strains were tested for the presence of the *vanA*, *vanB*, and *vanC* genes by PCR reaction as described previously (5).

RESULTS

Participating hospitals' characteristics. Of 57 hospital laboratories contacted, 27 (47%) agreed to participate in the study. Five participants did not provide the MIC or zone size; data from these laboratories were excluded from the analysis (Table 2). Overall, the participating hospitals were small (<400 beds; $n = 10$) or medium (400 to 600 beds; $n = 7$) in size. Of 14 hospitals, 8 (57%) were not affiliated with a university. All participants routinely tested enterococcal isolates from blood for vancomycin resistance, and the majority routinely tested enterococcal isolates from urine for vancomycin resistance. Participating hospitals used a variety of vancomycin susceptibility testing methods (Table 3). The β-lactamase test was routinely performed in 11 of 22 (50%) laboratories. In contrast, agar screening was never used, and the E test was used very infrequently (1 of 22 [4.5%]). In 1994, 7,469 enterococci were isolated at the participating laboratories. The median prevalence rate of VRE for the 20 participants reporting these data was 0.25% (range, 0 to 9%). In 10 of these 20 (50%) hospitals, no VRE isolates had been detected. VRE were not significantly more likely to be isolated at hospitals with >600 beds than at hospitals with ≤600 beds.

Vancomycin resistance. Overall, the participating laboratories accurately determined vancomycin resistance in 61 of 88 (69.3%) instances. This rate varied with the level of vancomycin resistance (Table 3). Organism 1, with high-level resistance (VanA phenotype), was detected in 20 (90.9%) instances, whereas organism 2, with low-level resistance (VanB2 phenotype), and organism 4, with intermediate-level resistance (VanC phenotype), were correctly detected in only 13 (59.1%) and 11 (50%) instances, respectively. Organism 3, with intermediate- to low-level resistance (VanB phenotype), was correctly identified in 17 (77.3%) instances. Proficiency was higher if we considered detection of "at least intermediate level of resistance" as accurate (21 of 22 [95.4%] instances for organism 2 and 14 of 22 [63.6%] instances for organism 4).

Categorical errors in the detection of vancomycin resistance occurred in 27 of 88 (30.7%) categorical errors (Table 4). Minor errors were the most common categorical error for all enterococcal strains (24 of 27 [88.9%] instances). For 13 of 24 (54.2%) of these errors, the organism was misclassified as vancomycin susceptible, for 8 of 24 (33.3%) of these errors the organism was misclassified as intermediate resistant, and for 3 of 24 (12.5%) of these errors the organism was misclassified as resistant. Very major errors occurred among 3 of 27 (11.1%) errors. Therefore, misclassification of isolates with at least an intermediate level of resistance as vancomycin susceptible accounted for 16 of 27 (59.3%) of the errors and occurred in 16 of 88 (18.2%) instances.

Proficiency in determination of vancomycin resistance also varied with the antimicrobial susceptibility testing method used (Tables 3 and 5). However, only three methods (Pasco [Difco Laboratories, Detroit, Mich.] and Microscan Walkaway and

TABLE 2. Characteristics of hospital participants, Spain, 1996

Characteristic	No. of respondents (response rate [%])	No. (%) of hospitals
Noncategorical variables[a]		
No. of licensed beds	22 (100.0)	
Rate of VRE prevalence	20 (90.9)	
Categorical variables		
University affiliation	14 (63.6)	6 (42.9)
Medium or small size[b]	22 (100.0)	15 (68.2)
Population >50,000	13 (59.1)	12 (92.3)
β-Lactamase test	22 (100)	11 (50.0)
Agar screening test	10 (45.5)	0 (0.0)
E test	22 (100.0)	1 (4.5)
Routine antibiotic susceptibility testing		
Blood		
Vancomycin	22 (100)	22 (100.0)
Ampicillin	22 (100)	22 (100.0)
Penicillin	20 (90.9)	19 (95.0)
Aminoglycosides	20 (90.9)	19 (95.0)
Urine		
Vancomycin	22 (100)	19 (86.4)
Ampicillin	22 (100)	22 (100)
Penicillin	21 (95.4)	15 (71.4)
Aminoglycosides	20 (90.9)	13 (65.0)

[a] The medians (ranges) for number of licensed beds and rate of VRE prevalence were 473 (96 to 1,250) and 0.25 (0 to 9), respectively.
[b] Medium or small size is <600 beds.

TABLE 3. Distribution of concordant rates of participating laboratories: CDC results by organism and susceptibility testing method, vancomycin susceptibility tests, 1996

Organism no.	MIC (μg/ml)[a]	Total no. (%) concordant results (n = 22)[b]	No. (%) of hospitals that used the following antimicrobial susceptibility test method[c]:				
			Walkaway (n = 9)	Pasco (n = 5)	Kirby-Bauer (n = 2)	Autoscan (n = 3)	Others (n = 3)[c]
1	512	20 (91)	9 (100)	5 (100)	2 (100)	3 (100)	1 (33)
2	64	13 (59)	7 (78)	2 (40)	1 (50)	2 (67)	1 (33)
3	16–32	17 (77)	9 (100)	3 (60)	1 (50)	3 (100)	1 (33)
4	8	11 (50)	6 (67)	1 (20)	1 (50)	2 (67)	1 (33)
5	2	23 (100)	9 (100)	5 (100)	2 (100)	3 (100)	3 (100)

[a] MIC, actual MIC of vancomycin for each organism, as determined by CDC.
[b] Percent is number of results concordant with CDC results/number of tests performed for each organism.
[c] Others included Vitek (n = 1), Sceptor b-b (n = 1), and Placas CIM sensitive (n = 1).

Microscan Autoscan [Dade International, Inc., Microscan Division, Haywood, Calif.]) were used by at least three laboratories. All methods had difficulties detecting low and intermediate levels of resistance. However, Microscan users had consistently better results for every isolate. For organisms 2 through 4, Microscan Walkaway users accurately determined the MIC in 22 of 27 (81.5%) instances, Microscan Autoscan users accurately determined the MIC in 7 of 9 (77.7%) instances, and Pasco users accurately determined the MIC in 6 of 15 (40%) instances. In addition, errors were consistently distributed among Pasco users but not among Microscan users. Only one of five (20%) Pasco users had no errors at all, whereas seven of nine (77.7%) Microscan Walkaway users and two of three (66.6%) Microscan Autoscan users had no errors at all. Moreover, errors within only onefold dilution were more common among Microscan users than among Pasco users (Microscan Walkaway, two of five [40%] of the errors; Microscan Autoscan, one of two [50%] of the errors; and Pasco, three of nine [33.3%] of the errors). Finally, we examined the distribution of errors among the 10 of 20 (50%) hospitals from which no VRE were reported in 1996. The clinical laboratories from these hospitals misclassified enterococcal isolates with at least an intermediate level of vancomycin resistance as vancomycin susceptible in 8 of 40 (20%) instances; in only 2 of the 8 (25%) errors was the Microscan system used. In contrast, in the clinical laboratories from the 10 hospitals that reported the detection of VRE in 1996, misclassification of the organisms as vancomycin susceptible occurred in 6 of 40 (15%) instances. This difference, however, was not statistically significant. Of these 10 hospitals, 8 (80%) used the Microscan system.

DISCUSSION

The laboratory serves as the first step in the prevention and control of the spread of antimicrobial resistance. To accurately detect antimicrobial agent-resistant strains, proficiency is essential. Previous studies have documented difficulties in detecting in the clinical setting strains of enterococci with low or intermediate levels of vancomycin resistance (6, 7, 16). Misclassification of an isolate as susceptible (minor errors for intermediate vancomycin-resistant strains and very major errors) has serious implications for both the clinical management of patients and the adequacy of any antimicrobial resistance surveillance system. On the other hand, misclassification of an isolate as at least intermediate resistant (major errors and minor errors for resistant isolates) has less serious consequences for the clinical management of patients but overestimates the number of isolates with at least an intermediate level of resistance. The control of the spread of VRE will be more difficult if more isolates are misclassified as vancomycin susceptible. However, overestimation of the number of VRE isolates adds expenses for unnecessary prevention and control measures.

Overall, the Spanish clinical laboratories participating in this study correctly identified VRE in 61 of 88 (69.3%) instances. These results are better than those obtained in the New Jersey (58.5%) or Argentine-U.S. (60%) studies (6, 16). Similar to those studies, proficiency varied by level of vancomycin resistance, and most participating laboratories had difficulty in detecting isolates with low and intermediate levels of resistance. Improvement was noted, however, in the detection of vancomycin resistance in isolates with the VanB2 and VanB phenotypes. Previously reported rates of detection of these organisms have ranged from 29 to 50 and 38 to 50%, respectively. These rates are lower than the rates of 59.19 and 77.3%, respectively, in the present study. Results with the organism of the VanB phenotype are particularly reassuring, because the proportion of these clinical isolates is increasing (2). Misclassification of vancomycin-resistant isolates as vancomycin susceptible occurred in 16 of 88 (18.2%) instances, which represents a definitive improvement compared to the rates in former studies performed in the United States and Argentina (approximately 30% rate of misclassification as vancomycin susceptible in both countries). Our results are even better, if we consider only the results of methods used by at least three participants (8 of 68 [11.8%] instances). Misclassification to vancomycin susceptible accounted for the majority of errors (16 of 27 [59.3%] errors). However, half of these occurred with organism 3 (*E. gallinarum* phenotype VanC), which accounts for only 5 to 10% of clinical isolates and which, to date, has not been implicated in nosocomial outbreaks (2, 5). Thus, the impact of these misclassification errors on the total number of VRE missed is minimal. Very major errors are worrisome. In this

TABLE 4. Distribution of categorical error rates by microorganism tested for vancomycin susceptibility, 1996

Organism no.	MIC (μg/ml)[a]	No. (%) of the following[b]:			
		Total errors	Very major error	Major error	Minor error
1	512	2 (9.1)	1 (4.3)	0 (0)	1 (4.3)
2	64	9 (40.9)	2 (8.6)	0 (0)	7 (30.4)
3	16–32	5 (22.7)	0 (0)	0 (0)	5 (22.7)
4	8	11 (50.0)	0 (0)	0 (0)	11 (50.0)
5	2	0 (0)	0 (0)	0 (0)	0 (0)

[a] MIC, actual MIC of vancomycin for each organism, as determined by CDC.
[b] Percent is number of errors/number of participating laboratories.

TABLE 5. Distribution of reported result rates and categorical errors by susceptibility testing method, vancomycin susceptibility tests, 1996

Susceptibility test method	Total errors[a]	No. of the following:		
		Very major error	Major error	Minor error
Walkaway	5/45 (11.1)	0	0	5
Pasco	9/25 (36.0)	0	0	9
Kirby-Bauer	3/10 (30.0)	1	0	2
Autoscan	2/15 (13.3)	0	0	2
Other	8/15 (53.3)	2	0	6

[a] Data indicate number of results discordant with CDC results/total number of tests performed by each susceptibility test method (percent).

study, they were seen in 3.4% of the instances. They tended to occur by methods used by a very small number of laboratories.

All comparisons between methods must be made with caution. This study was designed to evaluate proficiency in the detection of vancomycin resistance by participating clinical laboratories and not to evaluate the proficiencies of the diagnostic methods. Therefore, we did not collect information on innoculum size, incubation time, or the controls used at these facilities to standardize the test procedures, but rather, we asked the participants to test the isolates by their routine laboratory techniques. Differences in those factors might explain differences in performance.

Automated MIC determination methods were most commonly used by participating Spanish clinical laboratories (20 of 22 [90.9%]). All methods had difficulty in detecting intermediate or low levels of resistance. However, the accuracy varied by the method. When the analysis was limited to methods used by at least three laboratories, the Microscan Walkaway and Microscan Autoscan systems yielded the most accurate and consistent results. For tests with isolates with at least an intermediate level of resistance, their overall error rates were 5 of 36 (13.9%) and 2 of 12 (16.7%) instances, respectively. Half of their errors were within onefold dilution, and no isolate with vancomycin resistance mediated by *vanA* and *vanB* was misclassified as susceptible. Moreover, of 10 participating laboratories with no errors, 9 (90%) used these methods. Finally, errors by Microscan users were clustered among 5 of 12 (41.6%) participants, which suggests some differences in local factors not related to the method. When compared with the study performed in New Jersey (16), our results for Microscan users were much better (Walkaway error rate, 13.9 versus 50.5%; Autoscan error rate, 16.7 versus 51.9%). Improvements in the software may explain this improved proficiency. In contrast, the broth-based method, i.e., the Pasco system, performed poorly at the five participating laboratories that used this method. The Pasco system has previously been reported to be highly accurate, and as stated above, this difference in performance might be partly explained by differences in local factors such as innoculum size, incubation time, or the controls used at these facilities.

The rates of incidence or prevalence of VRE in Spain and most countries in Europe are unknown. The very few published studies suggest low VRE prevalence rates. In our study, the participating laboratories reported very low annual prevalence rates (median, 0.25%; range, 0 to 9%), with 10 of 20 (50%) participants reporting no VRE isolates. However, those hospitals reporting no VRE isolates misclassified enterococcal isolates with at least an intermediate level of vancomycin resistance as vancomycin susceptible in 8 of 40 (20%) instances. Therefore, VRE prevalence rates may be underestimated at the participating laboratories.

There are several limitations to our study. First, the participating laboratories are not a representative sample of all clinical laboratories in Spain. Therefore, we cannot estimate the magnitude of underreporting of the prevalence of VRE related to inadequate proficiency in laboratory detection in Spain. Second, because some methods were used by only a small number of participants, any comparison between methods must be made with great caution.

In conclusion, the Spanish laboratories that participated in this study showed an overall acceptable proficiency in detecting VRE and provided more accurate results than those provided by other laboratories in similar studies in other countries (6, 16). However, our results suggest the possibility of a substantial underestimation of VRE prevalence rates as a result of an inability to detect low and intermediate levels of vancomycin resistance. As in previous studies, all antimicrobial susceptibility testing methods demonstrated difficulties in detecting isolates with intermediate and low levels of vancomycin resistance. However, the current Microscan Walkaway and Microscan Autoscan methods demonstrated improved proficiency compared to those demonstrated in former studies and to those of the other methods used in this study. Moreover, our study documented that a substantial proportion of the errors clustered in a few laboratories; it is hoped that feedback of our results will enhance the proficiencies of those laboratories. It is encouraging that most errors were those with limited clinical significance. We recommend that studies be conducted to validate laboratory proficiency testing as an important step in the prevention and control of antimicrobial resistance.

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APPENDIX

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