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Proficiency of determination of vancomycin susceptibility in enterococci by clinical laboratories in Taiwan

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Eighty clinical microbiology laboratories in Taiwan were evaluated for proficiency in the determination of vancomycin susceptibility of enterococci. Each laboratory was given 1 vancomycin-sensitive isolate and 3 vancomycin-resistant enterococci (VRE) isolates to determine the levels of vancomycin resistance. Among a total of 240 tests performed, 153 (63.8%) correctly determined the levels of vancomycin resistance of the survey isolates. Seventy eight (98%) of the 80 laboratories accurately identified the high level of vancomycin resistant isolates [*Enterococcus faecalis*; minimum inhibitory concentration (MIC) >256 μg/mL]. Seventy two laboratories (90%) correctly determined the level of vancomycin resistance of another VRE with a vancomycin MIC of 64 μg/mL. Only 3 of the 80 laboratories correctly determined the intermediate-level vancomycin resistant isolates (*Enterococcus casseliflavus*; MIC = 8 μg/mL). Eight laboratories reported the vancomycin-susceptible isolate as being vancomycin-resistant or of intermediate susceptibility. This survey demonstrated that clinical microbiology laboratories in Taiwan are proficient in detecting high-level but not low-level VRE, suggesting a need to improve their proficiency in VRE detection.

Key words: *Enterococcus*, laboratories, vancomycin resistance

Enterococci are the second most frequent nosocomial pathogen and the third most common cause of hospital-acquired bacteremia in the United States [1]. Enterococci pose a serious threat to health care institutions because of the increasing occurrence of high-level resistance to penicillin and vancomycin. Vancomycin-resistant enterococci (VRE) harbor various vancomycin resistance genes (*van*A, *van*B, *van*C, etc.) and may serve as a reservoir of antibiotic resistance genes for other bacteria. The *van*A gene of VRE has been shown to be transferable to *Staphylococcus aureus* in vitro [2], and such transfer has also been found in clinical isolates [3].

Clinical microbiology laboratories (labs) are the first line of defense against the spread of VRE infections. It is crucial that these labs are able to rapidly and accurately detect VRE. Unfortunately, commercially available kits for detection of intermediate- and low-level resistant VRE do not perform consistently [4]. A survey conducted in the United States and Argentina showed that only 16 to 27% of VRE isolates are correctly identified in terms of their levels of vancomycin resistance [5,6]. VRE were first reported in Taiwan in 1995 [7], and many cases of VRE-associated clinical infections have been reported since then from several different medical centers [8-11]. To assess the proficiency of clinical microbiology labs in Taiwan in detecting VRE, we conducted a proficiency survey in VRE detection in 80 different labs.

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Materials and Methods

Bacterial strains

Three VRE isolates from our previous study [12] were coded as Organism 1 (*Enterococcus faecalis*), Organism 2 (*Enterococcus faecium*), and Organism 3 (*Enterococcus casseliflavus*), respectively, and a vancomycin-sensitive *E. faecalis* (ATCC 29212) was coded as Organism 4 (Table 1). All isolates were inoculated on blood agar plates, incubated for 24 hours, and then distributed to the participating labs. These labs were instructed to determine the level of vancomycin susceptibility of the isolates and report the results on the provided susceptibility test form. This survey was initiated in December 2000 and completed by April 2001.

Table 1. Characteristics of the enterococci isolates used in this survey

Organism no. and species	Minimum inhibitory concentration (μg/mL)[a]			*van* gene
	Vancomycin	Ampicillin	Gentamicin	
(1) *E. faecalis*	512 (R)	0.75 (S)	>256 (R)	*vanA*
(2) *E. faecium*	64 (R)	64 (R)	>256 (R)	*vanB2*
(3) *E. casseliflavus*	8 (I)	0.5 (S)	>256 (R)	*vanC2*
(4) *E. faecalis*	1-4 (S)	0.5-2 (S)	1-4 (S)	-

Abbreviations: R = resistant; S = susceptible; I = intermediate

[a]Criteria are based on the National Committee for Clinical Laboratory Standards guidelines [13].

Participating hospitals

Clinical microbiology laboratories from 80 different hospitals in Taiwan participated in this proficiency survey, including 13 tertiary-care teaching hospitals (>500 beds, Type I), 36 medium size hospitals (250 to 500 beds, Type II), and 31 smaller hospitals (<250 beds, Type III). All labs were instructed to use the disk-diffusion method [14] for vancomycin susceptibility detection.

Error types

Test errors were classified as very major, major, or minor. A very major error was defined as reporting of a resistant isolate as susceptible. If a susceptible isolate was reported as resistant, it was considered as a major error. A minor error was defined as reporting of a susceptible or resistant isolate as intermediate resistant or if an intermediate resistant isolate was reported as resistant or susceptible (Table 2).

Results and Discussion

Proficiency in the determination of vancomycin resistance was based on the use of the disk-diffusion method in all participating labs. Among a total of 240 (3×80) tests, 153 (64%) correctly determined the level of vancomycin resistance of the survey isolates. This proficiency rate of 64% is similar to that of the survey conducted in New Jersey (59%) [5] and another one conducted in Argentina (60%) [6]. The finding is also similar to previous reports [5,6] that the proficiency rate is higher for high-level VRE. Organism 1 was a very high-level resistant [minimum inhibitory concentration (MIC) >256 μg/mL, VanA phenotype] VRE and was correctly identified by 78 labs (98%). Organism 2 (*E. faecium*) was a high-level resistant (MIC = 64 μg/mL, VanB2 phenotype) VRE and was correctly identified by 72 (90%) labs. The proficiency rate was slightly less for Organism 2 than for Organism 1. The observation that most labs correctly determined the level of vancomycin resistance of Organism 2 is important since vanB2 VRE represent the majority of VRE isolated in Taiwan with a prevalence of 69% [11]. Surprisingly, only 3 labs (4%) correctly identified Organism 3 (*E. casseliflavus*; MIC = 8 μg/ml, VanC2 phenotype) as an intermediate-level resistant (VanC2 phenotype) VRE (Table 2). Therefore, the majority of labs had difficulty

Table 2. Results of vancomycin susceptibility tests in participating laboratories

Organism MIC (R/I/S)	Susceptibility reported	Number of laboratories (error type)[a]			Total (%)
		Type I	Type II	Type III	
(1) *E. faecalis* (*van*A)	R	13	35	30	78 (98%)
>256 μg/mL (R)	I	0	0	0	0
	S	0	1 (VM)	1 (VM)	2 (3%)
(2) *E. faecium* (*van*B2)	R	13	31	28	72 (90%)
64 μg/mL (R)	I	0	2 (MI)	0	2 (3%)
	S	0	3 (VM)	3 (VM)	6 (8%)
(3) *E. casseliflavus* (*van*C2)	R	0	1 (MI)	1 (MI)	2 (3%)
8 μg/mL (I)	I	0	0	3	3 (4%)
	S	13 (MI)	35 (MI)	27 (MI)	75 (94%)
(4) *E. faecalis* (ATCC25912)	R	0	0	1 (MJ)	1 (0.1%)
1-4 μg/mL (S)	I	0	3 (MI)	4 (MI)	7 (9%)
	S	13	33	26	72 (90%)

Abbreviations: MIC = minimum inhibitory concentration; R = resistant; S = susceptible; I = intermediate

[a]Error type: VM, very major; MJ, major; MI, minor.

determining the intermediate level of vancomycin resistance; 75 of 80 labs (94%) identified the intermediate resistant isolate as vancomycin-sensitive and 2 labs identified it as high-level vancomycin resistant (Table 2). Only 2 labs correctly identified it as intermediate resistant to vancomycin. Since this type of VRE accounts for only 5 to 10% of VRE isolates and has not been implicated in nosocomial outbreaks [15,16], the impact of misidentification of this type of VRE in infection controls may be minimal.

Eighty seven of 240 tests (36%) were incorrect, including 8 (9%, 8/87) very major errors that incorrectly identified a high-level resistant isolate as susceptible and 79 (91%, 79/87) minor errors (Table 2). Of the 79 minor errors, 75 misclassified an intermediate resistant isolate as susceptible, 2 misidentified a high-level resistant isolate as intermediate resistant, and the other 2 misidentified an intermediate resistant isolate as high-level resistant. Misclassification of vancomycin-resistant (both high and intermediate levels) as vancomycin-susceptible enterococci occurred in 8 labs (Table 2).

Although this is an undesirable error, this error rate (10%) is lower than that of previous reports which was approximately 30% [5,6]. Identification of a sensitive isolate as resistant would have serious adverse consequences because it may lead to unnecessary use of antibiotics. Fortunately, only 1 lab (a Type III lab) made this major mistake and the lab was notified of this error. Disk quality control measures were suggested in order to prevent it happening again. Seven minor errors (3 Type II and 4 Type III labs) occurred in the identification of the vancomycin-susceptible isolate. These errors may have occurred due to incorrect concentration of the inoculum or inappropriate incubation environment. The observation that all 13 Type I labs correctly identified all isolates indicates the importance of continuing education as all these 13 labs are in teaching hospitals.

Eight labs made errors in the identification of vancomycin-sensitive *E. faecalis* (ATCC 29212), including 1 major error that determined it as resistant and 7 minor errors which identified it as intermediate resistant (Table 2).

In conclusion, the labs which participated in this study showed an overall acceptable proficiency in the determination of the levels of vancomycin resistance of VRE. Several labs had an unsatisfactory proficiency. These results indicate the need for program to improve the proficiency of VRE testing in Taiwan.

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