Antimicrobial susceptibility testing of vancomycin-resistant Enterococcus by the VITEK 2 system, and comparison with two NCCLS reference methods

Intetsu Kobayashi, Hiroe Muraoka, Takako Iyoda, Minoru Nishida, Miyuki Hasegawa and Keizou Yamaguchi

1 Chemotherapy Division, Mitsubishi Kagaku Bio-Clinical Laboratories Inc., 3-30-1 Shimura, Itabashi-ku, Tokyo 174-8555, Japan
2 Department of Microbiology, Toho University of Medicine, Tokyo, Japan

We evaluated the automated VITEK 2 system (bioMérieux) for antimicrobial susceptibility testing of vancomycin-resistant Enterococcus (VRE). The results obtained with the VITEK 2 system were compared to those obtained using two NCCLS reference methods. The VITEK 2 system produced MICs for penicillin G, erythromycin and vancomycin that were very similar to those of the reference agar-dilution test with all results being within a twofold dilution. When MICs of teicoplanin for these isolates were measured by the agar-dilution method and VITEK 2 system, there was one ‘very major’ error and seven ‘minor’ errors. There were no ‘major’ errors for any of the antibiotics tested. When the results obtained by the micro broth-dilution method were compared with those obtained by the VITEK 2 system, there was one ‘very major’ error for teicoplanin by the VITEK 2 system, as was the case with the agar-dilution method. There were two ‘minor’ errors for erythromycin and seven ‘minor’ errors for teicoplanin. There were no ‘major’ errors for any of the antibiotics tested. The 35 VRE strains identified phenotypically by the VITEK 2 Advanced Expert System included nine of Enterococcus faecalis and 23 of Enterococcus faecium. Neither Enterococcus avium nor Enterococcus hirae were identified. A total of 32 phenotypes were classified into 22 VanA and 10 VanB strains. PCR genotyping demonstrated 23 vanA+ and nine vanB+ strains. There were differences between the VITEK 2 system results and those of PCR. Overall, 54·3 % of the test results were obtained within 7 h. All MIC values for the 35 VRE isolates were determined within 13 h of completing incubation. The VITEK 2 system is a simple method for accurately detecting vancomycin-resistant strains of Enterococcus and can be used to rapidly determine MICs.

INTRODUCTION

Vancomycin-resistant Enterococcus (VRE) has increasingly been implicated as a causative pathogen in nosocomial infections (CDC, 1993; Murray, 1995; Tokars et al., 1999). Vancomycin-resistant strains of Enterococcus are generally resistant not only to vancomycin but also to various types of commercially available antibiotics, and can cause severe infections in compromised hosts (French, 1998). Rapid identification of the infectious bacterial strain and its susceptibility to antibiotics are necessary from both the clinical and the economic viewpoint.

The VITEK 2 system is a recently developed automated method for rapid bacterial identification and antimicrobial susceptibility testing. The usefulness of the VITEK 2 system for identifying Enterococcus species has already been reported (van den Braak et al., 2001; Garcia-Garrote et al., 2000). However, different genotypes (vanA, vanB) were found to encode either high, intermediate or low levels of resistance glycopeptides, mainly in Enterococcus faecalis and Enterococcus faecium (Perichon et al., 1997; Fines et al., 1999). The difficulties encountered by several automated susceptibility tests in accurately detecting bacterial resistance to vancomycin have also been described (van den Braak et al., 2001; Sahm & Olsen, 1990).

The present study was designed to evaluate the ability of the VITEK 2 system to determine VRE susceptibility.

METHODS

Test strains. Thirty-five isolates of VRE species, including nine Enterococcus faecalis, 23 Enterococcus faecium, two Enterococcus avium and one Enterococcus hirae, obtained from clinical specimens (urine, faeces and blood) of patients with infections in several Japanese
hospitals were identified by the VITEK 2 system between March 1999 and September 2000.

**Antimicrobial agents.** The antimicrobial agents used in the reference micro broth-dilution panels and agar-dilution method were penicillin G, erythromycin, vancomycin and teicoplanin. All antibiotics were purchased from Sigma except for teicoplanin which was supplied by a pharmaceutical company (Aventis Pharma) as a standard powder with a known potency.

These antimicrobial agents were selected on the basis of antimicrobial agents which can be measured by the VITEK 2 system card according to NCCLS guideline M7-A5 (NCCLS, 2000a).

**VITEK 2 system susceptibility tests.** Antimicrobial susceptibilities of the test organisms were determined using the VITEK 2 system (software version 1.02) (bioMérieux) according to the manufacturer's recommendations.

The test organisms from colonies grown on Trypticase Soy agar (Becton Dickinson) after 18 h incubation were suspended in sterilized physiological saline to 0.5 McFarland standards. The bacterial suspension was used to fill the Antimicrobial Susceptibility Testing F516 card, which was then inserted into the incubator-reader of the VITEK 2 system.

**Reference susceptibility tests.** Susceptibility tests were also performed using two reference methods: the micro broth-dilution and agar-dilution with Mueller–Hinton broth and agar. These tests were performed according to NCCLS M7-A5 guidelines (NCCLS, 2000a) and M100-S10 guidelines (NCCLS, 2000b), respectively. The MICs were interpreted using the recommended NCCLS thresholds.

**Discrepant MIC values.** The interpretive category errors were estimated for each drug based on the following definitions: ‘very major’ error, susceptible by the VITEK 2 system but resistant by the agar and/or broth reference method; ‘major’ error, resistant by the VITEK 2 system but susceptible by the agar and/or broth reference method; ‘minor’ error, intermediately resistant by either the VITEK 2 system or the agar and/or broth reference method and either susceptible or resistant by the other method.

**Amplification of vanA and vanB genes by PCR.** PCR was used to detect vanA and vanB genes as previously described by Clark et al. (1993). The control organisms used for PCR were E. faecalis ATCC 51299 (vanB-positive), E. faecium C.I. (clinical isolate; vanA-positive) and E. faecalis ATCC 29212 (negative control).

### RESULTS AND DISCUSSION

The MICs of the four antibiotics for 35 vancomycin-resistant isolates of *Enterococcus* were determined using the VITEK 2 system and the two reference methods (Table 1).

The MICs of penicillin G for these isolates were measured by the VITEK 2 system, micro broth-dilution or agar-dilution, allowing comparisons of the results obtained by these three methods. The MICs of penicillin G by the three methods were ≧16 μg ml⁻¹ (resistant region) for 28 strains and ≦8 μg ml⁻¹ (susceptible region) for seven strains. The results obtained by the three methods were the same for all strains. The majority of MICs for erythromycin in the resistance region (MIC ≧8 μg ml⁻¹) determined using the VITEK 2 system were the same as those determined by the reference methods. All 35 VRE were clearly demonstrated to be...
resistant to vancomycin using the three methods, and no differences were observed between the VITEK 2 system and the reference method in MIC distribution patterns.

Teicoplanin MICs in the resistance region (MIC $\geq 32 \mu g/ml^{-1}$) were demonstrated in 24 of the 35 strains using the reference methods, whereas 18 strains were determined to be resistant to teicoplanin using the VITEK 2 system. Furthermore, one and six of the 35 strains were intermediately resistant when MICs were determined using the reference methods and the VITEK 2 system, respectively. The teicoplanin MICs for the other 11 strains (31.4 % of the 35 strains) were 0.5–1 $\mu g/ml^{-1}$, in the susceptibility region when determined by the VITEK 2 system, but these MICs showed a broad distribution between concentrations of 0.5 and 4 $\mu g/ml^{-1}$ when determined by the micro broth-dilution method and between 0.25 and 8 $\mu g/ml^{-1}$ when determined by the agar-dilution method. Although MICs obtained by the VITEK 2 system and the two reference methods generally agreed, the prevalences of ‘very major’, ‘major’ and ‘minor’ errors in the MICs obtained by the VITEK 2 system were determined (Table 2). With the agar-dilution method there was one ‘very major’ error and seven ‘minor’ errors for teicoplanin while there were no major errors with the VITEK 2 system. There were no errors for any of the other antibiotics.

When the MICs obtained by the micro broth-dilution method were compared with those obtained by the VITEK 2 system, there was one ‘very major’ error with the VITEK 2 system for teicoplanin, as was the case for the agar-dilution method, but there were no ‘major’ errors. However, there were seven ‘minor’ errors for teicoplanin and two ‘minor’ errors for erythromycin.

The vanA- and vanB-mediated resistance of the 35 vancomycin-resistant isolates of Enterococcus was analysed according to the method of Clark et al. (1993) after PCR amplification; the results obtained were then compared with the phenotypes of the isolates determined by the VITEK 2 system. The strain determined to be neither vanA$^+$ nor vanB$^+$ by both methods was an E. hirae, and two E. avium strains could not be identified by the VITEK 2 system alone. Thirty-two strains were identifiable by both methods. Of these, the strains identified by VITEK 2 system phenotyping included 22 VanA and 10 VanB, while PCR genotyping identified 23 vanA$^+$ and nine vanB$^+$ strains. The MIC of teicoplanin against the E. faecium strain (no. 14) was 1 $\mu g/ml^{-1}$ by the VITEK 2 system and the phenotype of this strain was determined to be VanB. The MICs of teicoplanin against this strain were 32 $\mu g/ml^{-1}$ by the agar- and broth-dilution methods. PCR showed the genotype of this strain to be vanA$^+$.

The times required to obtain the final MICs of vancomycin for the 35 vancomycin-resistant isolates of Enterococcus are shown in Table 3. Overall, 54.3 % of the test results were obtained within 7 h. MIC values for all 35 isolates were determined within 13 h of incubation.

VITEK 2 systems have recently been adopted by many microbiological laboratories for rapid identification and the determination of antimicrobial susceptibilities of various types of pathogens, including VRE (van den Braak et al., 2001; Funke et al., 1998; Garcia-Garrote et al., 2000). The prevalence of VRE infections in compromised hosts has become a serious problem (Jochimsen et al., 1999), and treatment options are limited (Saraiva et al., 1997). To control the transmission of VRE and outbreaks of cross-infection within hospitals and the community, several technical problems involving laboratory tests must be overcome. Given the current situation with VRE infections, it is necessary to confirm the speed and accuracy of the VITEK 2 system in determining MICs for VRE. Furthermore, rapid detection of resistant phenotypes in VRE isolates from patients is essential for prompt and effective antibiotic treatment.

Table 3. Times required to obtain the final results of susceptibility testing in 35 VRE isolates using the VITEK 2 system

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>No. isolates (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>6–7</td>
<td>14 (40.0)</td>
</tr>
<tr>
<td>8–9</td>
<td>13 (37.1)</td>
</tr>
<tr>
<td>10–11</td>
<td>2 (5.7)</td>
</tr>
<tr>
<td>12–13</td>
<td>1 (2.9)</td>
</tr>
</tbody>
</table>

*Number and percentage of isolates whose testing results were available at the indicated times of incubation.

Table 2. Interpretive category errors of the VITEK 2 system in susceptibility testing of 35 VRE isolates compared to that of the reference methods

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. errors/agar-dilution</th>
<th>No. errors/broth-dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘Very major’</td>
<td>‘Major’</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
We evaluated the ability of the VITEK 2 system to determine the antimicrobial susceptibility of recent VRE isolates using penicillin G, erythromycin, vancomycin and teicoplanin as test antibiotics. The strains which were determined to be resistant to penicillin G, erythromycin and vancomycin by the VITEK 2 system were also resistant according to both reference methods employed. There were differences between the MICs of these antibiotics by the VITEK 2 system and the reference methods, but susceptible strains were not identified as being resistant and resistant strains were not indicated to be susceptible.

There was one ‘very major’ error and seven ‘minor’ errors when the MICs of teicoplanin were calculated using the VITEK 2 system, as compared with the reference method results. The failure of VITEK 2 to determine resistance to teicoplanin has already been reported (van den Braak et al., 2001; Garcia-Garrote et al., 2000). Some vancomycin-resistant strains of Enterococcus were phenotypically classified as VanB by the VITEK 2 system in the study by van den Braak et al. (2001).

In the present study, there was one ‘very major’ error in the teicoplanin MIC for one strain (VRE no. 14), i.e. the MIC was 1 μg ml⁻¹ using the VITEK 2 system but 32 μg ml⁻¹ by the two reference methods. This error was considered to be the same as that reported by van den Braak et al. (2001). However, the VITEK 2 system detected VRE accurately and distinguished them from the 11 teicoplanin-susceptible strains; the VITEK 2 system results corresponded well with those obtained using the two reference methods.

The VITEK 2 system is easy to use and provides accurate results in detecting resistance of Enterococcus species to penicillin G, erythromycin and glycopeptides; this system can also be used to determine the antimicrobial susceptibility of Enterococcus including vancomycin-resistant isolates. One of the major advantages of the VITEK 2 system is the significant reduction in handling time as compared with conventional test procedures.

A 24 h incubation period is needed to determine the MICs of vancomycin or teicoplanin for VRE by either the micro broth-dilution or the agar-dilution method according to NCCLS guidelines (2000b). However, the MICs of these antibiotics for 35 VRE isolates were determined within 13 h by the VITEK 2 system, with the phenotypes of all 35 isolates being simultaneously determined during this period. The VITEK 2 system promises to expedite work in clinical microbiological laboratories. Furthermore, the VITEK 2 system may play an important role in the investigation of nosocomial VRE infections.

REFERENCES


