Evaluation of the VITEK 2 System for Rapid Direct Identification and Susceptibility Testing of Gram-Negative Bacilli from Positive Blood Cultures

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This study explores the possibility of combining the BacT/Alert Microbial Detection System with the VITEK 2 system to achieve rapid bacterial identification and susceptibility testing. Direct inoculation of bacterial suspension to the VITEK 2 ID-GBN card and AST-NO09 card was made by differential centrifugation of blood cultures of organisms with gram-negative enteric bacillus-like morphology. A total of 118 strains were investigated; of these, 97 (82.2%) strains were correctly identified to the species level and 21 (17.8%) strains were not identified; by comparing the results with those of the reference method of API identification systems using a pure culture, it was found that no strain had been misidentified. Among the 21 strains with no identification, 13 (61.9%) strains were nonfermenters. The direct-identification reporting time of VITEK 2 was 3.3 h. Direct testing of susceptibility to 11 antibiotics, i.e., amikacin, cefepime, cefazidime, ciprofloxacin, gentamicin, imipenem, meropenem, netilmicin, piperacillin, piperacillin-tazobactam, and tobramycin, was also performed by using the broth microdilution (MB) method according to the NCCLS guidelines as a reference. After comparing the MICs of the VITEK 2 system with those obtained by the MB method within ± twofold dilution, it was determined that the 1,067 organism-antibiotic combinations had an overall correct rate of 97.6% (1,041 combinations). The rates of susceptibility to the 11 antibiotics ranged from 88.7 to 100%, respectively. Only two (0.2%) and four (0.4%) combinations of the susceptibility tests gave very major errors (i.e., reported as sensitive by the VITEK 2 system but shown to be resistant by the MB method) and major errors (i.e., reported as resistant by the VITEK 2 system but shown to be sensitive by the MB method), respectively. The reporting time for the direct testing of susceptibility against the 11 antibiotics for 97 blood culture isolates by the VITEK 2 system ranged from 3.3 to 17.5 h. Compared with conventional methods that require 1 or 2 days, this method can make same-day reporting possible and thus permit better patient management.

The detection of bloodstream infections is one of the most important tasks performed by the clinical microbiology laboratory. Rapid bacterial identification and susceptibility testing not only improve patient therapy and outcome, but also reduce costs (1, 17, 18). Both automated blood culture systems and automated systems for identification and susceptibility testing of bacteria have been on the market for a number of years (8). The VITEK 2 system (BioMérieux) is a new automated bacterial identification and susceptibility testing system that uses fluorescence-based technology. Previous studies showed that this system could give reliable identification and susceptibility results with pure bacterial cultures (5, 6, 10). This study explores the possibility of combining these systems to achieve rapid identification and susceptibility testing by direct inoculation from positive blood cultures.

MATERIALS AND METHODS

The growth of microorganisms in blood cultures was screened by the BacT/Alert Microbial Detection System (Organon Teknika). This is an automated test system capable of incubating, agitation, and continuously monitoring aerobic and anaerobic media inoculated with specimens from patients suspected of having bacteremia. This study was carried out during normal working days between 21 July and 15 December 2002 in the Prince of Wales Hospital, Hong Kong, People’s Republic of China. The aerobic bottle of the positive blood culture set that consisted of an aerobic and an anaerobic bottle was used for direct identification and susceptibility testing using the VITEK 2 system. Only the blood cultures showing gram-negative enteric bacillus-like morphology by microscopy were investigated. All blood cultures with anaerobic bacteria or polymicrobial growth and all pediatric blood cultures were excluded. The positive blood cultures underwent further identification and susceptibility tests in the routine clinical laboratory for comparison.

A 5-ml sample from the positive blood culture bottle was centrifuged at 160 × g for 5 min to pellet blood cells. The supernatant was then centrifuged at 650 × g for 10 min to pellet bacteria. The turbidity of the bacterial suspension was adjusted with VITEK Densichek (bioMérieux) to match the McFarland 0.5 standard in 0.45% sodium chloride. Afterward, the VITEK 2 ID-GBN card, the AST-NO09 card, and the bacterial suspension were manually loaded into the VITEK 2 system (10). The VITEK 2 system reported the results automatically with software release 2.01.

Standard strains including Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls. Identification of a pure overnight culture by API 20E, API 20NE (BioMérieux), or other standard biochemical tests (13) was used as the reference method. The results of susceptibility testing for bacterial isolates by the VITEK 2 direct susceptibility method were compared with those of broth microdilution (MB) method (MIC-2000 System; Dynatech, McLean, Va.) using pure cultures according to NCCLS guidelines (14). The 11 antibiotics tested were amikacin, cefepime, cefazidime, ciprofloxacin, gentamicin, imipenem, meropenem, netilmicin, piperacillin, piperacillin-tazobactam, and tobramycin. Susceptibility discrepancies were reported as very major discrepancies (i.e., sensitive with the VITEK 2 system but resistant by the reference method), major discrepancies (i.e., resistant with the VITEK 2 system but sensitive by the reference method) or minor discrepancies (i.e., susceptible or resistant with the VITEK 2 system and intermediate by the reference test or vice versa). Only pure cultures were retested by the reference methods if discrepancies occurred.

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RESULTS

Identification. A total of 118 bacterial strains with gram-negative enteric bacillus-like morphology from positive blood cultures were investigated. Ninety-seven (82.2%) strains were correctly identified to the species level, 21 (17.8%) strains were not identified, and no strain was found to have been misidentified after their identities were verified with the reference systems (Table 1). Among the 21 unidentified strains, 13 (61.9%) were nonfermenters and 6 were E. coli. The identities of these 21 discrepant strains remained unchanged after being reconfirmed by the same reference method on pure culture. The direct-identification reporting time of the VITEK 2 was 3.3 h after incubation.

Antimicrobial susceptibility testing. Among the 118 isolates, only 97 isolates that had acceptable, good, very good, or excellent identification were evaluated for the direct susceptibility testing. The unidentified strains and strains with low discrimination were excluded. There was a high percentage of MIC agreement between the VITEK 2 system and the MB method, ranging from 88.7 to 100% for the 11 antibiotics (Table 2). The VITEK 2 system reported 1,041 (97.6%) correct organism-antibiotic combinations of 1,067 combinations within ± twofold dilution compared with the MB method. The discrepancy rates for ciprofloxacin, piperacillin, and piperacillin-tazobactam were slightly higher than those for other drugs. After retesting the pure cultures of the isolates with discrepant organism-antibiotic combinations by the MB method, the MICs of drugs with major discrepancies (ciprofloxacin, netilmicin, piperacillin, and piperacillin-tazobactam) or very major discrepancies (cefepime and piperacillin-tazobactam) were reconfirmed. The direct susceptibility testing report time of the VITEK 2 system ranged from 3.3 to 17.5 h after incubation.

DISCUSSION

Bacteremia caused by nonfastidious aerobic gram-negative bacilli is common in Hong Kong, and the antimicrobial susceptibility profiles of these bacteria vary widely (9). The possibility of combining an automated blood culture system with an automated identification and susceptibility testing system by direct inoculation from positive blood cultures has been studied by several groups of investigators, but their results vary (3, 7, 15, 16). The VITEK 2 direct identification testing of gram-negative bacilli had a correct identification rate of 82.2% for the positive aerobic blood cultures with a single type of organism; none of the results gave a wrong identification. Twenty-one strains (17.8%) were not identified, among which six were E. coli; one half of the results showed unidentified organisms with low discrimination, and the other half showed unidentified organisms. This direct-identification rate was slightly lower than that obtained by comparing results of the VITEK 2 systems with those of standard API identification systems on pure isolates (5, 6, 10). This might be because the traces of blood components such as blood cells and fibers could not be separated completely from the bacterial pellets, which may have affected the biochemical reactions involved in the VITEK 2 identification process. In future studies, including more washing steps may improve the rate of identification. There was a higher percentage of nonidentification in the non-Enterobacteriaceae group, which had shown similar results in previous studies (5, 6, 10). The slower rate of metabolism of nonenteric bacteria may cause weaker fluorescent biochemical reactions in the reaction wells of the VITEK 2 GNB cards; therefore, some nonenteric bacteria may not be identifiable by this system.

Compared with the results of the MB method within ±1 twofold dilution, the results of the VITEK 2 direct susceptibility testing had a high agreement rate ranging from 88.7 to 100% for the 11 antibiotics tested (1,067 organism-antibiotic combinations). The overall correct rate was 97.6%. These results are similar to those found previously by other investigators working on pure cultures with the VITEK 2 method (5, 6, 10). Imipenem had the lowest MIC agreement (84.2%) in a...
previous study (10) but a 100% agreement in this study. Five antimicrobial agents, including amikacin, ceftazidime, gentamicin, imipenem, and meropenem showed no discrepancy in the direct testing results. Susceptibility testing for the 11 antibiotics had an overall increase in accuracy with a higher percentage in MIC agreement than this in the previous study on pure isolates. Direct susceptibility testing using the disk method was found to be useful, providing results up to 24 h sooner (2, 12). However, the reliability of the direct disk susceptibility assay may be difficult to establish due to the fact that the techniques have not been well standardized (4, 11). Susceptibility results usually require to be reconfirmed after obtaining a pure culture.

The VITEK 2 system had an overall reliable performance, except for some minor problems. For example, there were memory button and test card autoloader failures, and the differential centrifugation necessary to obtain the bacterial pellet is quite labor intensive. However, the direct identification results take only 3.3 h, which is much faster than conventional identification results, as the latter are usually reported 2 days after the blood cultures are positive. The direct susceptibility results were available within 3.3 to 17.5 h after incubation.

The direct method from positive blood cultures cannot totally replace the approved methods of identification and susceptibility. Further evaluation of gram-positive and fastidious organisms are needed. Over two-thirds of our patients have gram-negative bacteremia; this rapid method may allow proper antimicrobial treatments almost 1 or 2 days earlier than the conventional method. This definitely decreases the turnaround time for the clinical laboratory and thus provides better patient management.

REFERENCES