Rapid identification and susceptibility testing using the VITEK® 2 system using culture fluids from positive BacT/ALERT® blood cultures

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Background and Purpose: In order to reduce the turnaround time for laboratory diagnosis of bacteremia, the efficacy of identification and antimicrobial susceptibility testing using samples taken directly from positive BacT/ALERT® standard aerobic and standard anaerobic blood culture bottles was evaluated.
Methods: 160 positive blood culture bottles were examined and incubated at 35°C in 5% carbon dioxide for 4-24 h, and an aliquot of the culture fluid was Gram stained. Samples containing Gram-negative bacilli were inoculated on VITEK® 2 ID-GNB (identification-Gram-negative bacilli) and AST (antimicrobial susceptibility testing)-GN04 cards, and those containing Gram-positive cocci were inoculated on ID-GPC (identification-Gram-positive cocci) and AST-P526 cards. The same samples were also examined by the standard method, involving subculture from positive BacT/ALERT standard blood culture bottles.
Results: Eighty seven of 97 Gram-negative bacilli (89.7%) and 21 of 63 Gram-positive cocci (33.3%) were correctly identified to the species level. For antimicrobial susceptibility testing, the direct method had an overall error rate of 5.4% for Gram-negative bacilli, with 0.9% very major, 0.9% major, and 3.6% minor discrepancies compared to the standard method. The overall error rate in antimicrobial susceptibility testing for the 13 *Staphylococcus* spp. was 10.3%, with 6.0% very major, 2.6% major, and 1.7% minor discrepancies.
Conclusion: These data suggest that VITEK 2 cards inoculated with samples taken directly from positive Bact/ALERT blood culture bottles would provide acceptable identification and antimicrobial susceptibility testing results for Gram-negative bacilli, but not for Gram-positive cocci. Compared to the standard method, the direct method would reduce turnaround time by at least 24 h.

Key words: Bacterial typing techniques; Gram-positive bacteria; Gram-negative bacteria; Microbial sensitivity tests; Reagent kits, diagnostic

Introduction

Rapid detection, identification, and antimicrobial susceptibility testing of bacteria from blood are crucial in patient management. Among the several methods currently used in clinical laboratories, culture is the most sensitive one for the detection of bacteria in blood samples. However, blood cultures require at least 4-24 h of incubation time and an additional 24-48 h for biochemical or immunological tests to identify bacteria and determine their susceptibility to antimicrobial agents. The time required to obtain a culture result is much longer in situations of low bacteria counts or infection with slow-growing bacteria. Faster reporting of bacterial identification and susceptibility results will have both clinical and financial benefits [1-5]. Both automated blood culture and automated identification and susceptibility testing systems have been available for a number of years [6,7]. This study investigated the possibility of combining these

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two automated systems to achieve rapid identification and susceptibility testing by inoculation of the two systems using samples taken directly from positive blood culture bottles.

Methods

Culture methods

This study was carried out from November 3, 2004 to April 13, 2005 in the Tri-Service General Hospital, Taiwan, Republic of China. The presence of bacteria in blood cultures was screened by the BacT/ALERT® Microbial Detection System (bioMérieux, Marcy l'Etoile, France). The VITEK® 2 system (bioMérieux) was used for identification and antimicrobial susceptibility testing of bacteria grown in standard aerobic blood culture bottles. All Gram-negative bacilli and Gram-positive cocci were investigated; yeast, anaerobic bacteria and cultures with mixed growth were excluded. For comparison with the VITEK 2 system, all positive blood cultures underwent conventional identification and susceptibility testing processes. In total, 160 consecutive positive aerobic blood cultures were analyzed, including 97 cultures with Gram-negative bacilli and 63 with Gram-positive cocci.

Identification and antimicrobial susceptibility testing

For direct identification of bacteria from blood culture bottles, 3 mL culture fluid was removed from a blood culture bottle that had been incubated at 35°C for 4-24 h and centrifuged at 150 g for 10 min to pellet blood cells. Two mL of the resulting supernatant was mixed with 1 mL of 0.45% saline to lyse residual red blood cells, and bacteria in this suspension were pelleted by centrifugation at 1000 g for 10 min. The bacterial pellet was resuspended in 0.45% saline to 0.5-0.63 McFarland units as determined by the VITEK Densichek colorimeter (bioMérieux). Since Gram-positive cocci grow more slowly in blood culture bottles, 9 mL of culture fluid was used in order to obtain sufficient bacteria. If no bacterial pellet was observed after centrifugation, 1.5 mL of 0.45% saline and 3 mL of Brain-Heart Infusion (BHI) broth were added. The tube was the incubated at 37°C with shaking.

After 2 h of incubation, bacteria were pelleted and resuspended in 0.45% saline to the required McFarland units. Approximately 2 mL of this suspension was automatically loaded into the VITEK 2 ID-GNB (identification-Gram-negative bacilli) and AST

(antimicrobial susceptibility testing)-GN04 cards (for Gram-negative bacilli) and VITEK 2 ID-GPC (identification-Gram-positive cocci) and AST-P526 cards (for Gram-positive cocci), using the VITEK 2 system with the 2.01 release software. The VITEK 2 ID and AST systems reported results within 3 and 2.5-16.25 h, respectively.

For identification and susceptibility testing of bacteria by the standard method, approximately 0.1 mL of culture fluid from a blood culture bottle was inoculated onto blood agar and chocolate agar plates and incubated at 35°C in 5% carbon dioxide overnight. Bacteria were then suspended in 0.45% saline to a McFarland unit value of 0.5-0.63 and then loaded into appropriate VITEK identification and antimicrobial susceptibility testing cards, as described above. Reference strains including *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as controls.

Statistical analysis

Bacterial identification and susceptibility data from the direct method were compared to those obtained from the standard method. Only isolates correctly identified by the standard method were evaluated. Organisms reported as not identified by the standard method were excluded from the study. In the VITEK 2 ID system, *Klebsiella pneumoniae* subsp. *pneumoniae* (*planticola/terrigena*) and *K. pneumoniae* subsp. *ozaenae* were considered identical and reported as *K. pneumoniae*. Results obtained from the direct method were grouped into three different categories: correctly identified, misidentified, and not identified.

All Gram-negative bacilli were tested for susceptibility to 10 antibiotics: ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, imipenem, ciprofloxacin, gentamicin, amikacin, and trimethoprim-sulfamethoxazole. Gram-positive cocci were tested against 9 antibiotics: ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, trimethoprim-sulfamethoxazole, teicoplanin, and vancomycin. Discrepancies in susceptibility testing between the two methods were reported as very major, major, and minor. Very major discrepancies were those determined as sensitive by the direct method but resistant by the standard method. Major discrepancies were those reported as resistant by the direct method but sensitive by the standard method. Minor discrepancies were those determined to be susceptible or resistant by the direct method and intermediate by

the standard method or vice versa. Only pure cultures were retested by the standard methods if discrepancies occurred.

Results

Identification of Gram-negative bacilli

Of the 97 Gram-negative bacilli, 79 enterobacteria and 18 non-fermenters were identified by the direct method (Table 1). Eighty seven isolates (89.7%) were correctly identified to the species level, five isolates (5.2%) were reported as various non-fermenting Gram-negative bacilli, one isolate (1.0%) was not identified, and four isolates (4.1%) were misidentified compared to the standard method. The unidentified isolate was *Citrobacter amalonaticus*, and the four misidentified isolates were *Enterobacter agglomerans*, *K. pneumoniae*, *Aeromonas* spp. and *P. aeruginosa*. The VITEK 2 system reported results within 3.0 h after inoculation.

Identification of Gram-positive cocci

Of the 63 Gram-positive cocci, 3 enterococci, 2 *Micrococcus* spp., 44 staphylococci, and 14 streptococci were identified by the direct method (Table 2). Twenty one isolates (33.3%) were correctly identified to the species level, nine isolates (14.3%) were not identified, and 33 isolates (52.4%) were misidentified

compared to the standard method. The nine isolates not identified included 2 *Enterococcus faecalis*, 3 *S. aureus*, 1 group D *Streptococcus*, and 3 *Streptococcus viridans* isolates. The 33 misidentified isolates included 11 *S. aureus*, 1 *Staphylococcus epidermidis*, 1 *Staphylococcus hominis*, 2 *Staphylococcus saprophyticus*, 11 coagulase-negative *Staphylococcus*, 2 *Streptococcus* group B, 3 *Streptococcus* group D, and 2 *Streptococcus pneumoniae* isolates. Most of the misidentified isolates (45%, 15 of 33) were *Kocuria* spp. or *Micrococcus luteus*. The VITEK 2 ID system reported results within 2.5 h after inoculation.

Antimicrobial susceptibility testing of Gram-negative bacilli

Of the 87 correctly identified Gram-negative bacilli, 6 were excluded from antibiotic susceptibility testing due to inconsistent identification or susceptibility patterns. Therefore, 81 Gram-negative bacilli were tested for 10 antimicrobial agents with a total of 810 susceptibility tests. There was a high degree (84.0-98.8%) of agreement between the two methods. Overall, the direct method reported 767 tests (94.7%) correctly (Table 3). The discrepancy rate (16%) for ampicillin was slightly higher than those of other antibiotics (1.2-8.6%). The reporting time for the direct method ranged from 5.25 to 16.25 h after inoculation.

Table 1. Identification of Gram-negative bacilli using VITEK® 2 ID-GNB (identification-Gram-negative bacilli) cards inoculated with culture fluids from positive blood culture bottles

Species	Number of isolates				
	Tested	Correctly identified	VNGNB	Unidentified	Misidentified
Enterobacteriaceae (79)					
Citrobacter amalonaticus	1			1	
Escherichia coli	44	44			
Escherichia coli (ESBL)	1	1			
Enterobacter agglomerans	1				1
Enterobacter cloacae	2	2			
Enterobacter cloacae (ESBL)	1	1			
Klebsiella pneumoniae	20	19			1
Klebsiella oxytoca	1	1			
Salmonella spp.	4	4			
Serratia marcescens	3	3			
Aeromonas spp.	1				1
Non-fermenters (18)					
Acinetobacter baumanii	5	3	2		
Chryseobacterium indologenes	1	1			
Pseudomonas aeruginosa	11	7	3		1
Stenotrophomonas maltophila	1	1			
Total	97	87	5	1	4

Abbreviations: VNGNB = various non-fermenting Gram-negative bacilli; ESBL = extended-spectrum beta-lactamase

Table 2. Identification of Gram-negative bacilli using VITEK® 2 ID-GPC (identification-Gram-positive cocci) cards inoculated with culture fluids from positive blood culture bottles

Species	Number of isolates			
	Tested	Correctly identified	Unidentified	Misidentified
Enterococci (3)				
Enterococcus faecalis	3	1	2	
Micrococcus spp. (2)	2	2		
Staphylococci (44)				
Staphylococcus aureus	21	7	3	11
Staphylococcus epidermidis	4	3		1
Staphylococcus auricularis	1	1		
Staphylococcus haemolyticus	1	1		
Staphylococcus hominis	1			1
Staphylococcus saprophyticus	2			2
Coagulase-negative *Staphylococcus*	14	3		11
Streptococci (14)				
Streptococcus Group B	2			2
Streptococcus Group D	4		1	3
Streptococcus pneumoniae	2			2
Steptococcus viridans group	6	3	3	
Total	63	21	9	33

Antimicrobial susceptibility testing of Gram-positive cocci

Of the 21 correctly identified Gram-positive cocci, 7 isolates, including 2 *Micrococcus* spp., 1 *Staphylococcus auricularis*, 1 *Staphylococcus haemolyticus* and 3 *S. viridans* group isolates were excluded from antibiotic susceptibility testing due to inconsistent direct identification and susceptibility patterns. Therefore, only 13 *Staphylococcus* spp. were assayed for susceptibility to 9 antimicrobial agents, with a total of 117 tests. The concordant rates between the two methods ranged from 76.9 to 100.0% (Table 4). Overall, the direct method reported 105 tests (89.7%) correctly. The

discrepancy rate (23.1%) for clindamycin was slightly higher than those for other drugs (0.0-15.4%). The direct method reported susceptibility results within 6.5 to 11.25 h after inoculation.

Discussion

In this study, direct identification and antimicrobial susceptibility testing of bacteria using samples from BacT/ALERT standard aerobic and standard anaerobic blood culture bottles was investigated. Eighty seven of 97 Gram-negative bacilli (89.7%) and 21 of 63 Gram-positive cocci (33.3%) were correctly identified

Table 3. Agreement in antimicrobial susceptibility testing between direct and standard methods for Gram-negative bacilli using VITEK® 2 AST (antimicrobial susceptibility testing)-GN04 cards

Antibiotic	Number of isolates (n = 81) [%]			
	Category agreement	Minor discrepancy	Major discrepancy	Very major discrepancy
Ampicillin	68 (84.0)	5	3	5
Aztreonam	75 (92.6)	6	0	0
Ceftazidime	77 (95.1)	4	0	0
Ceftriaxone	77 (95.1)	3	0	1
Cefepime	79 (97.5)	0	1	1
Imipenem	80 (98.8)	1	0	0
Ciprofloxacin	74 (91.4)	7	0	0
Gentamicin	78 (96.3)	1	2	0
Amikacin	80 (98.8)	1	0	0
Trimethoprim-sulfamethoxazole	79 (97.5)	1	1	0
Total tests (n = 810)	767 (94.7)	29 (3.6)	7 (0.9)	7 (0.9)

Table 4. Agreement in antimicrobial susceptibility testing between direct and standard methods for *Staphylococcus* spp. using VITEK® 2 AST (antimicrobial susceptibility testing)-P526 cards

Antibiotic	Number of isolates (n = 13) [%]			
	Category agreement	Minor discrepancy	Major discrepancy	Very major discrepancy
Clindamycin	10 (76.9)	1	1	1
Ciprofloxacin	12 (92.3)	0	0	1
Erythromycin	11 (84.6)	0	1	1
Gentamicin	11 (84.6)	1	0	1
Oxacillin	11 (84.6)	0	1	1
Penicillin	12 (92.3)	0	0	1
Trimethoprim-sulfamethoxazole	12 (92.3)	0	0	1
Teicoplanin	13 (100.0)	0	0	0
Vancomycin	13 (100.0)	0	0	0
Total tests (n = 117)	105 (89.7)	2 (1.7)	3 (2.6)	7 (6.0)

to the species level compared to the standard method of identification/susceptibility testing using bacteria subcultured on agar plates from blood culture bottles. This correlation rate for Gram-negative bacilli is similar to those of other studies (82-96%) [6,8-12]. As in previous studies, there was also a higher percentage of non-identification in non-*Enterobacteriaceae* isolates [13-15]. This may be due to the slower metabolic rate of non-enteric bacteria, resulting in weaker fluorescence in biochemical reactions of the VITEK 2 GNB cards.

It has been reported that an overall category error rate of <10% is acceptable for antimicrobial susceptibility testing, including <1.5% very major and <3% major errors [16]. In our study, the overall error rate was 5.4% for Gram-negative bacilli, with 0.9% very major, 0.9% major, and 3.6% minor errors. The overall error rate in the 117 antibiotic susceptibility tests for *Staphylococcus* spp. was 10.3%, with 6.0% very major, 2.6% major and 1.7% minor errors. For Gram-negative bacilli, most (3.6%) of the errors were on susceptibility testing for penicillin and cephalosporins. The error rates for ciprofloxacin, imipenem, gentamicin, and trimethoprim-sulfamethoxazole were much lower (1.8%), with no very major errors and only 1.4% (11/810) minor and 0.4% (3/810) major errors. For Gram-positive cocci, however, there was a large number of major errors, and only the susceptibility tests for vancomycin and teicoplanin had no errors.

Previous studies have identified two main sources of errors in identification/susceptibility testing by the direct method as mixed cultures and non-standardized inoculum size [9,12,17]. In the present study, the purity of a positive blood culture was verified by Gram staining and subculture. Gram staining allows determination of monomicrobial blood cultures. However, it has been reported that 6 to 9% of specimens determined to be monomicrobial by Gram staining were later found to be polymicrobial during subculture [9,12,17]. In this study, specimens that yielded more than one isolate after subculture were excluded from analysis, and our data were based only on monomicrobial blood cultures. Therefore, the first source of error could be excluded.

The second source of error is inoculum size. To obtain a standardized inoculum for the direct method, we used the Densichek VITEK colorimeter to produce the required McFarland suspension units. In this study, bacterial number for direct inoculation of Gram-positive cultures was usually not sufficient with 3 mL samples from positive blood culture bottles. It was necessary to centrifuge at least 3 mL × 3 tubes to obtain sufficient numbers of bacteria to reach the required VITEK 2 McFarland density or to inoculate BHI broth for further incubation. Seven of the 63 Gram-positive cocci from positive bottles required prior incubation in BHI broth for 2 h. Also, 16% (15 of 63) Gram-positive cocci were identified as *Kocuria* spp. or *M. luteus* by the VITEK 2 ID-GPC system. The low correlation rates between the direct and standard method in identification and antimicrobial susceptibility testing of Gram-positive cocci could be due to blood cells and traces of blood culture broth interfering with the colorimetric measurements in the VITEK 2 system [13,18].

Since only correctly identified isolates were evaluated in the antimicrobial susceptibility testing, the error rates may be underestimated in this study. Although it seems unlikely that the direct method could totally replace the standard identification/susceptibility testing methods, we found that the direct method is reliable in

identification/susceptibility testing of Gram-negative bacilli. Since a major cause of bacteremia is Gram-negative bacilli, the direct method can nevertheless benefit patient management as it can shorten the turn-around time of bacterial identification and susceptibility testing to one day.

Acknowledgments

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