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Rapid Automated Identification of Gram-Negative Bacilli from Blood Cultures with the AutoMicrobic System

RON B. SCHIFMAN* AND KENNETH J. RYAN

Department of Pathology, Arizona Health Sciences Center, University of Arizona, Tucson, Arizona 85724

Received 11 June 1981/Accepted 9 September 1981

Automated identification of gram-negative bacilli directly from blood culture bottles by using the AutoMicrobic System (AMS) was evaluated with a modified procedure for the AMS *Enterobacteriaceae*-plus nonfermenter identification card. A total of 150 strains were tested (44 clinical and 106 seeded) and compared with a conventional identification procedure. These strains included 107 *Enterobacteriaceae* and 43 oxidase-positive or glucose-nonfermenting, or both, organisms. AMS identifications on one of these strains were not interpretable owing to equal probability AMS identification values. Of the remaining 149 strains, 138 (92.6%) were correctly identified within 8 to 13 h of the first reading. Of 69 identifications analyzed after 6 h of incubation, 91% were correct. This procedure was found to be rapid, convenient, and nonlabor intensive and is recommended for presumptive identification of gram-negative bacilli in blood cultures.

The well-recognized clinical importance of bacteremia has stimulated the development of a number of rapid methods for direct identification of bacteria from blood cultures. Reported methods usually require centrifugation to obtain a pellet of bacteria from broth, which is then inoculated by hand into a series of biochemical tests. API 20E (3), BACTEC (10), Minitek (BBL Microbiology Systems, Cockeysville, Md.) (13), AutoMicrobic (11), and noncommercial identification systems (5, 12, 14, 16) have been used, with results available in 4 to 16 h. When compared with standard or commercial kit identification procedures, the AutoMicrobic system (AMS) is a more automated approach designed to provide microbial identification and susceptibility testing (1). The central feature of the system is a disposable plastic card with microwells containing biochemical, antibiotic, or control broths. Growth and biochemical reactions of organisms inoculated into these wells are periodically read, interpreted, and reported by the AMS without manual intervention. Cards have been developed by the manufacturer (Vitek Systems, Inc., Hazelwood, Mo.) for enumeration and identification of bacteria in urine (7, 12) and biochemical identification of *Enterobacteriaceae* (9) and yeast (13). Susceptibility testing applications are also available (8). The *Enterobacteriaceae*-plus (EBC+) card is a recent addition designed to identify clinically important oxidase-positive or glucose-nonfermenting, or both, organisms in addition to *Enterobacteriaceae* from isolated colonies. This paper describes a procedure which allows inoculation of the EBC+ card directly from blood cultures positive for gram-negative bacilli. This procedure, in conjunction with the expanded capacity of the EBC+ card, correctly identified 92.6% of the aerobic and facultative gram-negative bacilli encountered in clinical and seeded blood cultures within 8 to 13 h.

MATERIALS AND METHODS

Organisms. A total of 43 consecutive clinical blood cultures positive for aerobic and facultative gram-negative bacilli and 107 seeded blood cultures were included in the study (Table 1). One culture encountered during the clinical study contained both *Serratia liquefaciens* and *Enterobacter cloacae* organisms and was excluded. The 107 strains selected for the seeded culture study were recent clinical isolates maintained frozen in fetal calf serum at −70°C. These strains were selected to broaden the challenge to the AMS and included 11 strains with identifications not included in the AMS computer data base. These are listed as "other" in Table 1, footnote *a*.

Blood cultures. Blood specimens that were submitted for culture to the Clinical Microbiology Laboratories of the Arizona Health Sciences Center, Tucson, and that demonstrated growth of gram-negative bacilli were used for the clinical study. When growth was detected, 2 ml of well-mixed broth was aspirated aseptically into a 3-ml syringe and transferred to 10 ml of sterile distilled water. This mixture was allowed to incubate at 35°C for 10 min for red cell lysis and was then centrifuged at 1,000 × *g* for 10 min. The supernatant was discarded, leaving a button of bacteria and red cell stroma which was resuspended in AMS buffer to a McFarland turbidity standard of 1.0. This preparation was used as the inoculum for the usual AMS EBC+ procedure (see below).

For seeded blood cultures, 100-ml clinical blood culture bottles shown to be negative by routine laboratory procedures were used. No attempt was made to

TABLE 1. Direct AMS identification from positive blood cultures

Organism	No. of correct/identified species from following type of culture:		
	Clinical	Seeded	Errors (no.)
Escherichia coli	16/16	5/6	*Salmonella*
Klebsiella	8/9	9/11	*Enterobacter* (3)
Enterobacter	3/3	6/6	
Citrobacter		11/11	
Proteus	1/1	11/11	
Providencia		8/9	*Acinetobacter*
Salmonella		7/7	
Shigella		2/2	
Arizona		2/2	
Serratia	2/2	7/7	
Yersinia		2/3	Unidentified organism
Acinetobacter	3/3	4/4	
Aeromonas		5/5	
Pseudomonas aeruginosa	7/7	2/2	
Pseudomonas cepacia	2/2	2/3	*Pseudomonas fluorescens*
Pseudomonas maltophilia		3/3	
Vibrio		2/2	
Other[a]		9/12	*Acinetobacter, Pseudomonas cepacia* (2)

[a] Two *Pasteurella multocida*, three *Pseudomonas stutzerii*, two *Pseudomonas diminuta*, one *Pseudomonas putrifaciens*, two *Flavobacter*, and one *Alcaligenes* species (correct AMS identification = unidentified organism). A total of 98 and 91% of the species from clinical and seeded cultures, respectively, were correctly identified.

alter the usual patient-to-patient variation, except only bottles with enough red cell deposit to cover the bottom of the bottle were used. Bottles were inoculated with a straight wire taken from a light, single-colony, saline suspension. After overnight incubation, seeded bottles appeared to be the same as clinical positives and were processed in the manner described above.

AMS and EBC+ card. The AMS instrument has six components: diluent dispenser, filling module, reader-incubator, computer, cathode ray tube-keyboard module, and card sealer. The EBC+ card has 30 microwells containing 29 biochemical broths and a positive control broth. One end of a plastic transfer tube is attached to the EBC+ card injection port, and the other end is placed in a test tube containing the bacterial inoculum. This assembly is placed in the filling module, which mixes the inoculum and distributes it to the EBC+ card wells. The inoculated card is placed in the 35°C reader-incubator, and all wells are read automatically at 60-min intervals by light-emitting diodes and parallel-mounted photodetectors. A computer module analyzes these readings and controls the overall operation of the instrument. Final identifications are automatically printed 8 to 13 h after the first reading. Reports list the two most likely identifications, with probabilities expressed as percentage values. The EBC+ computer software allows early (nonfinal) identification status to be obtained on request via the cathode ray tube-keyboard module.

In addition to *Enterobacteriaceae*, the EBC+ card is designed to identify *Acinetobacter calcoaceticus, Pseudomonas aeruginosa, Pseudomonas cepacia, Pseudomonas maltophilia, Pseudomonas fluorescens, Aeromonas hydrophila, Plesiomonas shigelloides, Vibrio cholerae*, and *Vibrio parahaemolyticus*. This expanded spectrum is created by the addition to the EBC+ card of an oxidative glucose broth that determines the ability of the organism to oxidatively produce acid from glucose, a cetrimide broth that determines the ability of the organism to grow in the presence of the surfactant, cetrimide, and an acetamide broth that measures acetamide utilization by a change in pH. Finally, the EBC+ card allows a manually performed oxidase reaction to be indicated by placing a mark on the card before the initial reading.

Conventional biochemical tests. All organisms were identified to species level by standard methods (4, 6). For *Enterobacteriaceae, Aeromonas, Pasteurella*, and *Vibrio* species, glucose, mannitol, sucrose, lactose, adonitol, inositol, and dulcitol fermentation; malonate utilization; ornithine, lysine, and arginine decarboxylation; urea hydrolysis; citrate utilization; motility agar; DNA hydrolysis; lysine deamination, H_2S production; cytochrome oxidase; indole; and growth on MacConkey agar were included. Glucose-nonfermenting organisms were tested for motility; growth at 42°C; growth on MacConkey agar; oxidative or fermentative production of acid from glucose, mannitol, lactose, maltose, and sucrose (Hugh and Leifson); ornithine and lysine decarboxylation; indole; and pigment production.

Analysis. Direct AMS identifications which differed from the conventional result were considered to be incorrect except in the case of unidentified AMS responses. These were considered to be correct if the organism was not included in the AMS data base (e.g., *Alcaligenes odorans*) or incorrect if the organism was included in the data base (e.g., *Escherichia coli*). A total of 104 strains were tested with separate positive and negative oxidase markings. The accuracy of 4- and 6-h identifications was evaluated for 77 strains by using the AMS keyboard status function.

RESULTS

A total of 150 strains of gram-negative bacilli were tested. One strain of *Shigella* was excluded because it was identified as *Shigella* sp. and *Yersinia enterocolitica* with equal probability (47%). Of the remaining 149 strains, 138 (92.6%) were correctly identified (Table 1). The percentage of correct identifications was slightly better for clinical (98%) than seeded (91%) cultures, but seeded cultures contained a variety of organisms not commonly found in blood cultures. Only one error occurred in the clinical study. A strain of *Klebsiella pneumoniae* was misidentified as *Enterobacter aerogenes*. The same error occurred twice in the seeded study. In all three cases, the primary biochemical discrepancy was a false-positive ornithine decarboxylase reaction.

The remaining misidentifications occurred with organisms rarely encountered in blood cultures. One *Providencia alcalifaciens* strain was misidentified as *Acinetobacter calcoaceticus*. The major biochemical discrepancies were false-negative glucose and adonitol fermentation reactions. One strain of *Alcaligenes odorans* and one strain of *Alcaligenes faecalis* were incorrectly identified as *Pseudomonas cepacia*. One of five *Pseudomonas cepacia* strains tested was identified as *Pseudomonas fluorescens-putida* due to a false-negative tryptophan deaminase reaction. One of the two *Pasteurella multocida* strains tested was incorrectly identified as *Acinetobacter*. Besides the oxidase reaction, only the arginine and ornithine decarboxylase reactions were positive. One of two *Y. enterocolitica* strains tested was reported as an unidentified organism, although *Y. enterocolitica* is included in the AMS data base. Finally, one *Escherichia coli* strain was identified as *Salmonella* sp. When strains giving erroneous direct AMS results were retested either by the standard AMS procedure or as seeded blood cultures, they generally did not reproduce the previous error. Five of seven strains gave the correct result when retested.

Results for 77 strains analyzed at 4 and 6 h are shown in Table 2. At 4 h, 40 strains were correctly identified, 16 were misidentified, and 21 were not identified. At 6 h, 63 strains were correctly identified, 6 were incorrectly identified, and 8 were not identified. Two of the five misidentifications at 6 h occurred with *K. pneumoniae* strains that were called *Enterobacter aerogenes* at 6 and 8 h. Two *Citrobacter freundii* strains that were correctly identified at 8 h were reported as *Salmonella* at 6 h owing to a delayed *o*-nitrophenyl-β-D-galactopyranoside reaction. Finally, one *Escherichia coli* strain was misidentified as *Hafnia hafniae* and another as *Salmonella* at 6 h.

All but 3 of the 74 *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains tested with positive and negative oxidase markings were correctly identified even when the oxidase reaction was marked inappropriately. In two of these cases, the oxidase marking did not contribute to the misidentification. In the third case, a strain of *Y. enterocolitica* was identified correctly when the oxidase test was negative but was identified as *Shigella* when the oxidase test was marked positive. In contrast, only 11 of the remaining 31 strains were identified correctly when the oxidase marking was incorrect (Table 3). With the wrong oxidase marking, all five *Acinetobacter* strains tested were reported as unidentified organisms. A *V. parahaemolyticus*

TABLE 2. Direct AMS identification from positive blood cultures after 4- and 6-h incubation periods

Organism	Results from following period of incubation (h)[a]:			
	4		6	
	No. of correct/ identified species	No. of species not identified	No. of correct/ identified species	No. of species not identified
Escherichia coli	10/11	0	9/11	0
Klebsiella	9/12	2	10/12	2
Enterobacter	0/2	0	2/2	0
Citrobacter	6/9	2	9/11	0
Proteus	3/3	7	8/8	2
Providencia	0/1	8	6/6	3
Salmonella	2/4	0	4/4	0
Shigella	2/2	0	2/2	0
Arizona	0/2	0	2/2	0
Serratia	3/5	1	6/6	0
Aeromonas	4/4	0	4/4	0
Vibrio	1/1	1	1/1	1

[a] A total of 71 and 91% of the species from 4- and 6-h incubation periods, respectively, were identified correctly.

TABLE 3. Effect of oxidase marking on accuracy of direct AMS identification from blood cultures

Organism	No. of correct/identified species with indicated oxidase marking[a]:	
	Correct	Incorrect
Enterobacteriaceae	62/64	61/64
Pseudomonas aeruginosa	9/9	9/9
Pseudomonas cepacia	5/5	4/5
Other *Pseudomonas* spp.	8/8	3/8
Flavobacterium	2/2	0/2
Alcaligenes	0/2	0/2
Pasteurella	1/2	0/2
Acinetobacter	5/5	0/5
Aeromonas	5/5	0/2
Vibrio	1/2	0/2

[a] A total of 94 and 74% of the species from cultures with correct and incorrect oxidase markings, respectively, were identified correctly.

and a *Y. enterocolitica* species were identified as *Shigella*. *Aeromonas hydrophila* was classified as not identified. All other strains misidentified were called *Acinetobacter*.

DISCUSSION

These results demonstrate that the AMS EBC+ card can be used to directly identify aerobic and facultative gram-negative bacilli from the broth of positive blood cultures. A large variety of organisms were tested, including many which are uncommonly encountered in blood cultures. Overall, 92.6% of the 149 strains identified were correctly classified. This result is comparable to those from other reported direct identification procedures in which 83 to 99% agreement between conventional and rapid methods has been observed (3, 5, 10, 15, 16). In a recent study, Moore and co-workers at the University of California at Los Angeles (UCLA) achieved 95% identification accuracy with the AMS EBC+ card and seeded blood cultures (11). Our study differs from theirs primarily in the addition of a clinical trial and the use of a somewhat simpler inoculation procedure.

Of 20 *K. pneumoniae* strains tested, 3 were called *Enterobacter aerogenes* due to false-positive ornithine decarboxylase reactions. This was the only error in the 43 clinical blood cultures. This false-positive reaction may be related to material carried over from the particular blood sample, since concurrently inoculated EBC+ cards resulted in the same identification, but repeated testing with a seeded blood culture bottle from a different patient gave the correct answer. This error might be prevented by checking *Enterobacter* identifications with a hanging drop motility and reporting nonmotile strains as *Klebsiella-Enterobacter* pending standard identification. Current AMS software includes a modification designed to resolve this problem. The other misidentifications occurred with uncommonly encountered organisms such as *Providencia*, *Yersinia*, *Pasteurella*, and *Alcaligenes*.

Although 4-h identifications have been successful with the AMS (2), we were not able to achieve a satisfactory accuracy in 4 h when the inoculum was taken directly from a blood bottle. Only 45 of the 67 strains tested were identified at 4 h, for a 71% accuracy, but 6-h identifications were almost as accurate (91%) as the 8-h final results. If 6-h results are used, *Salmonella* identifications should be questioned, as two *Citrobacter* and an *Escherichia coli* species were called *Salmonella* at 6 h (Table 2) owing to a late *o*-nitrophenyl-β-D-galactopyranoside reaction.

The extended identification spectrum offered by the AMS EBC+ card was useful, but only if an oxidase result was available and marked on the card before insertion in the reader-incubator. *Enterobacteriaceae* and *Pseudomonas aeruginosa* could be identified without an oxidase, but the other organisms tested could not (Table 3). This is a potential limitation, since the oxidase result may not be known at the time a direct identification would normally be set up. This could be managed by leaving the oxidase unmarked and accepting only direct identifications of *Enterobacteriaceae* and *Pseudomonas aeruginosa* which would include most of the clinical positives. The UCLA group achieved 95% accuracy for all organisms by using an unmarked oxidase, but the proportion of oxidase-positive organisms other than *Pseudomonas aeruginosa* in their study was small (11). Another approach would be to set up two cards and match the oxidase to the appropriate card as soon as it could be obtained from a subculture or other plate.

In conclusion, we found this direct identification from blood cultures with the AMS to be accurate, rapid, and convenient for aerobic and facultative gram-negative bacilli in blood cultures. *Enterobacteriaceae* and *Pseudomonas aeruginosa* were reliably identified in 6 to 8 h without handling after the initial inoculation of the EBC+ card. Other organisms require correlation with a manual oxidase test for acceptable accuracy. As with all direct identification procedures, these results should be confirmed by standard methods to cover the occasional errors and complicating situations, such as polymicrobial bacteremia. Until more experience is gained with this approach, rare or unusual causes of bacteremia such as *Yersinia* or *Aeromonas* should be independently confirmed before issuing any reports. The AMS EBC+ card provides

a rapid and automated means of identifying the gram-negative rods most commonly found in blood cultures.

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