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Evaluation of the MicroScan Urinary Combo Panel and API 20E System for Identification of Glucose-Nonfermenting Gram-Negative Bacilli Isolated from Clinical Veterinary Materials

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Many isolates of glucose-nonfermenting gram-negative bacilli (NFB) cultured from clinical veterinary specimens are not identified because of the large number of identification tests required. We evaluated two commercial identification systems to determine if they could accurately identify NFB isolated from animals. Of 182 strains of NFB, the MicroScan Urinary Combo Panel (MicroScan, Inc., Campbell, Calif.) correctly identified 72%, and the API 20E system (Analytab Products, Plainview, N.Y.) correctly identified 74%. Of the 118 strains of the three most common species of NFB isolated from animals, the MicroScan Urinary Combo Panel identified 86% correctly, and the API 20E system identified 92% correctly. The use of either of these systems could improve the accuracy of identification of NFB from clinical veterinary materials.

Glucose-nonfermenting gram-negative bacilli (NFB) isolated from clinical veterinary specimens often are not completely identified because of the large number of identification tests required. Since many NFB have not been identified, little is known about their role in animal diseases. We recently found that almost 10% of all clinical veterinary specimens submitted for culturing yielded NFB isolates (5). The complete identification of NFB isolated from animals would yield valuable information about the clinical role of these organisms.

Commercial identification systems would be very useful in identifying NFB if they could accurately identify strains of animal origin. The data bases of these systems are based mostly on human strains, and differences between biotypes of human and veterinary isolates of NFB may exist. The purpose of this study was to determine the accuracy of two commercial systems, the MicroScan Urinary Combo Panel (MS) (MicroScan, Inc., Campbell, Calif.) and the API 20E system (API) (Analytab Products, Plainview, N.Y.), in the identification of NFB isolated from clinical veterinary materials.

MATERIALS AND METHODS

Bacterial strains. Nonfermenting strains used were identified by conventional methods described elsewhere (5). Identifications made by conventional methods were considered correct for purposes of comparison. A total of 182 strains of NFB, representing 26 species, were tested with each system. Clinical veterinary isolates accounted for 160 strains, and 22 strains of human origin (provided by G. L. Gilardi, Hospital for Joint Diseases, New York, N.Y.) were also tested. Each isolate was streaked onto a blood agar plate and then incubated for 18 to 24 h. The growth was checked for purity and served as the source of the inoculum for both systems.

MS. The MS contains a set of 24 miniaturized identification tests combined with a minimum inhibitory concentration system for several antimicrobial agents in a microtiter plate. The MS comes frozen and is thawed just before inoculation. The MS was inoculated, incubated, and interpreted according to the manufacturer's instructions.

The MS was inoculated with approximately 10^5 colony-forming units per well and then incubated aerobically at 35°C. Positive reactions were recorded at 24 h. At 48 h, reagents were added to tests requiring them, and all tests were interpreted. The tests and substrates used in the MS to identify NFB are urease production, indole production, H_2S production, lysine and ornithine decarboxylase, arginine dihydrolase, tryptophan deaminase, Voges-Proskauer, esculin hydrolysis, utilization of citrate, acetamide, malonate, and tartrate, cetrimide tolerance, *o*-nitrophenyl-β-D-galactopyranoside, glucose (oxidation-fermentation), nitrate reduction, starch hydrolysis, susceptibility to penicillin (4 μg/ml), kanamycin (4 μg/ml), colistin (4 μg/ml), nitrofurantoin (64 μg/ml), and tobramycin (4 μg/ml), and oxidase. Positive tests were used to code a profile number for each isolate. This profile number, as listed in the MicroScan data base, determined the identity of the isolate.

API. The API consists of a plastic strip containing 20 tubules that accommodate 21 identification tests. For strains of NFB, four additional tests, glucose (oxida-

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TABLE 1. Accuracy of the MS in identifying NFB from animals

Organism (no. of strains tested)	No. (%) of strains that were			
	Correctly identified	Within a spectrum of identification	Incorrectly identified	Not identified
Pseudomonas aeruginosa (50)	44 (88)	0 (0)	3 (6)	3 (6)
P. fluorescens (3)	1 (33)	1 (33)	1 (33)	0 (0)
P. putida (5)	3 (60)	1 (20)	1 (20)	0 (0)
Acinetobacter calcoaceticus biotype *anitratus* (25)	25 (100)	0 (0)	0 (0)	0 (0)
A. calcoaceticus biotype *lwoffii* (25)	19 (76)	0 (0)	1 (4)	5 (20)
Bordetella bronchiseptica (18)	13 (72)	0 (0)	5 (28)	0 (0)
Pseudomonas pseudoalcaligenes (8)	1 (13)	6 (75)	1 (13)	0 (0)
P. maltophilia (5)	3 (60)	0 (0)	0 (0)	2 (40)
P. stutzeri (6)	4 (67)	1 (17)	1 (17)	0 (0)
P. alcaligenes (5)	2 (40)	0 (0)	3 (60)	0 (0)
Moraxella phenylpyruvica (4)	0 (0)	0 (0)	4 (100)	0 (0)
CDC M-5 (3)	3 (100)	0 (0)	0 (0)	0 (0)
Moraxella nonliquefaciens (1)	1 (100)	0 (0)	0 (0)	0 (0)
M. osloensis (1)	1 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas putrefaciens (1)	1 (100)	0 (0)	0 (0)	0 (0)
Alcaligenes faecalis (3)	1 (33)	1 (33)	1 (33)	0 (0)
A. odorans (2)	2 (100)	0 (0)	0 (0)	0 (0)
CDC IIf (3)	2 (67)	0 (0)	0 (0)	1 (33)
Flavobacterium meningosepticum (1)	1 (100)	0 (0)	0 (0)	0 (0)
CDC Ve-2 (3)	1 (33)	0 (0)	1 (33)	1 (33)
Pseudomonas testosteroni (2)	0 (0)	0 (0)	2 (100)	0 (0)
CDC Ve-1 (1)	0 (0)	0 (0)	1 (100)	0 (0)
CDC Va-1 (1)	0 (0)	0 (0)	1 (100)	0 (0)
Pseudomonas cepacia (1)	1 (100)	0 (0)	0 (0)	0 (0)
Achromobacter xylosoxidans (1)	1 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas acidovorans (1)	1 (100)	0 (0)	0 (0)	0 (0)
P. diminuta (3)	0 (0)	0 (0)	3 (100)	0 (0)
Total (182)	131 (72)	10 (5)	29 (16)	12 (7)

tion-fermentation), oxidase, motility, and growth on MacConkey medium, were performed and used to code a profile number. The API (including the additional tests) was inoculated, incubated, and interpreted according to the manufacturer's instructions.

The API (including the additional tests) was incubated aerobically at 35°C. Positive tests were recorded at 24 h of incubation. Necessary reagents were added at 48 h, and all tests were interpreted then. The tests and substrates used in the API to identify NFB are *o*-nitrophenyl-β-D-galactopyranoside, arginine dihydrolase, lysine and ornithine decarboxylase, utilization of citrate, H_2S production, urease production, tryptophan deaminase, indole production, Voges-Proskauer, gelatin liquefaction, oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose, oxidase, nitrate reduction, glucose (oxidation-fermentation), motility, and growth on MacConkey medium. Positive tests were used to code a profile number for each isolate. This profile number, as listed in the Analytab Products data base, determined the identity of the isolate.

Interpretation of the identifications. The identity of an isolate determined by each system was compared with that determined by conventional methods. The identifications were classified into four categories: (i) correct; (ii) one of a spectrum of identifications, but not the first choice; (iii) incorrect; and (iv) no identification listed.

An identification was considered to be correct if the same species as identified by conventional methods was listed as the first choice in the data base. For the MS, the identification was also considered correct if it was listed as a part of the spectrum of identifications but could be separated from the other identifications listed by additional characteristics given in the data base. The only additional characteristics considered were motility, colony morphology, and those for any test that could be determined directly from the MS. Since the API required tests in addition to those on each strip to determine a profile number, no other tests were done to confirm the identity of an isolate even when additional tests were suggested in the profile index.

The spectrum of identifications category was used when the correct species identification was listed but was not the first choice. This category was also used when the correct genus identification was given but when no species were listed. An isolate was considered incorrectly identified if it was wrongly identified and was not given as an alternative choice. The no identification listed category was used when the profile number of the isolate did not appear in the data base of the system.

RESULTS

The results of MS testing are shown in Table 1. This system correctly identified 72% of the

TABLE 2. Accuracy of the API in identifying NFB from animals

Organism (no. of strains tested)	No. (%) of strains that were			
	Correctly identified	Within a spectrum of identification	Incorrectly identified	Not identified
Pseudomonas aeruginosa (50)	45 (90)	0 (0)	0 (0)	5 (10)
P. fluorescens (3)	0 (0)	3 (100)	0 (0)	0 (0)
P. putida (5)	1 (20)	4 (80)	0 (0)	0 (0)
Acinetobacter calcoaceticus biotype *anitratus* (25)	20 (80)	0 (0)	0 (0)	5 (20)
A. calcoaceticus biotype *lwoffii* (25)	25 (100)	0 (0)	0 (0)	0 (0)
Bordetella bronchiseptica (18)	18 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas pseudoalcaligenes (8)	0 (0)	8 (100)	0 (0)	0 (0)
P. maltophilia (5)	5 (100)	0 (0)	0 (0)	0 (0)
P. stutzeri (6)	4 (67)	1 (17)	1 (17)	0 (0)
P. alcaligenes (5)	0 (0)	5 (100)	0 (0)	0 (0)
Moraxella phenylpyruvica (4)	0 (0)	0 (0)	4 (100)	0 (0)
CDC M-5 (3)	2 (67)	1 (33)	0 (0)	0 (0)
Moraxella nonliquefaciens (1)	0 (0)	0 (0)	0 (0)	1 (100)
M. osloensis (1)	1 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas putrefaciens (1)	1 (100)	0 (0)	0 (0)	0 (0)
Alcaligenes faecalis (3)	3 (100)	0 (0)	0 (0)	0 (0)
A. odorans (2)	1 (50)	1 (50)	0 (0)	0 (0)
CDC IIf (3)	2 (67)	1 (33)	0 (0)	0 (0)
Flavobacterium meningosepticum (1)	1 (100)	0 (0)	0 (0)	0 (0)
CDC Ve-2 (3)	2 (67)	1 (33)	0 (0)	0 (0)
Pseudomonas testosteroni (2)	0 (0)	2 (100)	0 (0)	0 (0)
CDC Ve-1 (1)	1 (100)	0 (0)	0 (0)	0 (0)
CDC Va-1 (1)	0 (0)	0 (0)	1 (100)	0 (0)
Pseudomonas cepacia (1)	1 (100)	0 (0)	0 (0)	0 (0)
Achromobacter xylosoxidans (1)	1 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas acidovorans (1)	0 (0)	1 (100)	0 (0)	0 (0)
P. diminuta (3)	0 (0)	2 (67)	0 (0)	1 (33)
Total (182)	134 (74)	30 (16)	6 (3)	12 (7)

182 nonfermenting strains. It misidentified 16% of these isolates and did not identify 7% of the NFB. This system correctly identified 86% of the most common NFB, *Pseudomonas aeruginosa, Acinetobacter calcoaceticus*, and *Bordetella bronchiseptica*. It had the most difficulty identifying the inert species of NFB.

The API correctly identified 74% of the 182 strains (Table 2). It misidentified 3% of these isolates and did not identify 7% of the NFB. This system correctly identified 92% of the three most common NFB (see above). It had the most difficulty identifying the inert species of NFB and the fluorescent pseudomonads other than *P. aeruginosa*.

DISCUSSION

Since it is a new product, there are no reported evaluations of the MS for identification of human or veterinary NFB. It correctly identified 72% of the 182 strains. This is higher accuracy than our diagnostic laboratory obtained with a small number of conventional identification tests.

The API has been extensively evaluated for identification of NFB from humans (1, 4, 6–10). This system has been reported to identify 64% of animal strains of *Pasteurella multocida* and 20% of animal strains of *Pasteurella haemolytica* (3). Collins and Swanson reported that the API identified 62% of non-*Enterobacteriaceae* strains, including some nonfermenting strains from animals (2). In this study, 74% of 182 isolates were correctly identified.

The NFB most commonly isolated from animals are *P. aeruginosa, A. calcoaceticus*, and *B. bronchiseptica* (5). These three organisms make up 86% of the nonfermenters cultured from clinical veterinary specimens. Of 118 strains of the three most common NFB, the API identified 92%, and the MS identified 86%. Both systems tested would be accurate enough for routine identification of NFB isolated from clinical veterinary materials.

The purpose of this study was to determine if these commercial systems could accurately identify NFB isolated from animals. At present the identification of most NFB in clinical veterinary laboratories is incomplete. The use of either system could improve the accuracy of identification of NFB in clinical veterinary specimens, and improved identification of such

isolates would help to clarify the role of NFB in animal diseases.

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