JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1999, p. 4186–4188 Vol. 37, No. 12
0095-1137/99/$04.00+0

Identification of *Hafnia alvei* with the MicroScan WalkAway System

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Received 28 January 1999/Returned for modification 5 June 1999/Accepted 21 August 1999

***Hafnia alvei* is a gram-negative facultatively anaerobic bacillus that belongs to the family *Enterobacteriaceae*. This organism is a causative agent of intestinal disorders and is found in different environments. *H. alvei* has received increased clinical attention as a cause of different infections in humans. This study was performed to compare the MicroScan WalkAway automated identification system in conjunction with the new MicroScan Combo Negative type 1S panels with conventional biochemical methods for identification of 21 *H. alvei* strains. The MicroScan WalkAway system was found capable of correctly identifying 20 of the 21 strains tested.**

Hafnia alvei is a gram-negative facultatively anaerobic bacillus that belongs in the family *Enterobacteriaceae*. It is suspected to cause a variety of intestinal disorders, including gastroenteritis (2, 21, 24, 28, 35). *H. alvei* has also been isolated from various mammals (29), fish (10, 26), birds (11, 20), soil, water (4, 28, 30), and a number of foods (6, 8, 14, 23, 32). *H. alvei* possesses several different virulence mechanisms, which are similar or identical to those of other gram-negative enteropathogens (3). In humans, *H. alvei* is a recognized cause of a number of illnesses, including pneumonia (13), meningitis (17), abscesses (1), and septicemia (16).

Recently, automated systems have been developed to identify gram-negative bacteria (9, 15, 18, 19, 22, 27, 33), but the reports about the evaluation of these systems did not include a large number of *H. alvei* strains. The MicroScan WalkAway (Dade MicroScan, Inc., Sacramento, Calif.) is an automated, commercially available system for rapid identification and susceptibility testing of gram-negative bacilli and has received favorable reports relative to the identification of these bacteria (5, 11, 15, 22, 25). MicroScan has recently marketed MicroScan Combo Negative type 1S panels. The panels are designed to identify to the species level aerobic or anaerobic facultative gram-negative bacilli. The system uses fluorogenic substrates and a pH indicator to detect bacterial enzymatic activity. The purpose of this study was to evaluate the ability of the MicroScan WalkAway system in conjunction with the new Combo Negative type 1S panels to identify *H. alvei* strains.

Bacterial strains. Twenty-one *Hafnia alvei* strains were selected for testing (Table 1). The strains were identified with the MicroScan WalkAway system and were tested in parallel by standard reference procedures (31). The strains were routinely cultured on Trypticase soy agar (TSA; Cultimed) at 37°C for 24 h and stored on TSA slants at 4°C under mineral oil and frozen at −70°C with 15% glycerol.

MicroScan panels. Conventional MicroScan Negative Combo type 1S panels were inoculated with the strains by the turbidity standard technique. The panels were incubated for 24 h at 35°C within the MicroScan WalkAway system. All procedures were performed according to the manufacturer's directions (7).

Comparison of biochemical tests. The following biochemical tests were performed: D-glucose, sucrose, D-sorbitol, raffinose, L-rhamnose, L-arabinose, myoinositol, D-adonitol, and melibiose; urease; hydrogen sulfide (H_2S) production; indole production; decarboxylation of lysine and ornithine; arginine dihydrolase; tryptophan deaminase (TDA); esculin hydrolysis; Voges-Proskauer (VP); utilization of citrate; *o*-nitrophenyl-β-D-galactopyranoside (ONPG); and OF-glucose.

Results from comparison of different assay systems in testing important biochemical characteristics for the identification of *H. alvei* strains are listed in Table 2.

The MicroScan identification patterns for the 20 strains correctly identified as *H. alvei* were positive for the fermentation of D-glucose and L-arabinose, decarboxylation of lysine and

TABLE 1. Origin and sources of *H. alvei* strains used in this study

Strain	Origin	Source[a]
X1	Human enteritis	T. G. Winstanley
F4319	Human enteritis	T. G. Winstanley
C-34	*Oncorhynchus mikiss*	J. L. Muzquiz
187/95	*Gallus domesticus*	F. Real
OR-1	Sweet cream	L. A. Rodríguez
1967-82	Human enteritis	CDC
4256-83	Human blood	CDC
842-81	Human gall bladder	CDC
4094-83	Human sputum	CDC
9760	Unknown	ATCC
13337	Unknown	ATCC
11-69	Human feces	PCM
30-65	Unknown human origin	PCM
1187	Human gastric fluid	PCM
7-68	Human feces	PCM
25-65	Human feces	PCM
23-65	Human feces	PCM
7-67	Human feces	PCM
14-67	Human feces	PCM
537	Unknown	CDC
19-68	Lizard	PCM

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TABLE 2. Comparison between MicroScan WalkAway system and conventional laboratory tests to evaluate important biochemical characteristics for identification of *H. alvei* strains

	No. of results/ no. tested with MicroScan		Result by:		No. (%) of results with correlation by both methods
	Positive	Negative	Conventional biochemical test	*Bergey's Manual*[a]	
Glucose	21/21	0/21	+	+	21/21 (100)
Sucrose	0/21	21/21	−	−	21/21 (100)
Sorbitol	0/21	21/21	−	−	21/21 (100)
Raffinose	0/21	21/21	−	ND	21/21 (100)
Rhamnose	19/21	2/21	+[b]	+	21/21 (100)
Arabinose	20/21	1/21	+[c]	+	21/21 (100)
Inositol	0/21	21/21	−	−	21/21 (100)
Adonitol	0/21	21/21	−	−	21/21 (100)
Melibiose	0/21	21/21	−	−	21/21 (100)
Urease	16/21	5/21	−[d]	−	5/21 (23.8)
SH_2	0/21	21/21	−	−	21/21 (100)
Indole	0/21	21/21	−	−	21/21 (100)
Lysine	21/21	0/21	+	+	21/21 (100)
Arginine	4/21	17/21			19/21 (80.9)
	0/21	21/21	−	−	21/21 (100)[e]
Ornithine	21/21	0/21	+	+	21/21 (100)
TDA	0/21	21/21	−	ND	21/21 (100)
Esculin	6/21	15/21	−[f]	−	21/21 (100)
VP	18/21	3/21	−	(+)	3/21 (14.2)
Citrate	20/21	1/21	−	−	1/21 (4.7)
ONPG	9/21	12/21	+[g]	+	10/21 (47.6)
OF-glucose	21/21	0/21	+	+	21/21 (100)

[a] Characteristics given in *Bergey's Manual of Determinative Bacteriology*, 9th ed. (13a): −, 0 to 10% positive; (+), 76 to 89% positive; +, 90 to 100% positive; F, fermentative; ND, not determined.
[b] Strains 11-69 and 14-67 are negative.
[c] Strain ATCC 9760 is negative.
[d] Strains OR-1, 11-69, ATCC 13337, 4094-83, and 842-81 are negative.
[e] MicroScan results using the manual reading.
[f] Strains 537, 11-69, ATCC 13337, 842-81, 1187, and 14-67 are positive.
[g] Strain OR-1 is negative.

ornithine, and utilization of citrate and OF-glucose and were negative for fermentation of sucrose, D-sorbitol, raffinose, myoinositol, D-adonitol, and melibiose; hydrogen sulfide (H_2S) production; indole production; and TDA; and were variable for fermentation of L-rhamnose, urease, arginine dihydrolase, esculin, VP, and ONPG.

The MicroScan WalkAway system was able to identify 20 of 21 of the *H. alvei* strains tested (95%), and only 1 strain, *H. alvei* 14-67, was misidentified as a rare biotype. Strain 14-67 was negative in the L-rhamnose and urease tests, the same as strain 11-69, but in the case of strain 14-67, the MicroScan system interpreted the urease test as positive, which produced a misidentification as a rare biotype. In the case of strain 11-69, the urease test was negative, and the final identification was *H. alvei*, with a probability of 99.9%.

Initially, the MicroScan system classified as a rare biotype strains 1967-82, F-4319, 30-65, and 7-68. The arginine test was initially interpreted as positive, but after a subsequent manual reading of the panels by specialized personnel in the center, it was recorded as negative. Therefore, the final identification was *H. alvei*, with a probability of 99.9%.

Although *H. alvei* infections are relatively rare, their clinical importance is well documented. More accurate and reliable methods with which to rapidly identify these organisms need to be developed. Some studies on the reliability of automated systems have shown good results, but insofar as the *H. alvei* strains are concerned, only small numbers were evaluated (34, 36, 37), with the unique exception of Kelly et al. (12), who, using 38 *Hafnia alvei* strains, obtained results similar (92%) to those in our study.

On the whole, the MicroScan WalkAway system in conjunction with the new Combo Negative type 1S panels proved to be very useful and reliable in identifying *H. alvei* strains of different origins. This system correctly identified 20 of the 21 strains tested in this study (95%). In 16 of 21 tests analyzed, the correlation between the MicroScan system and conventional tests was 100%. Occasionally, the erroneous test results were important enough to result in a misidentification. Discrepancies might be expected because conventional tests may represent standard tubed media read after overnight incubation, thus accepting some loss of precision and accuracy for the sake of convenience. Such procedures could not represent appropriate reference methods for evaluating automatic systems. The low percentages of correlation in some tests (urease, 23.8%; VP, 14.2%; citrate, 4.7%; and ONPG, 47.6%) did not seem to affect the final identification, although they should be considered if other gram-negative bacteria are tested.

The arginine dihydrolase test presented interpretation difficulties. This test caused a misidentification of one strain as a rare biotype on the MicroScan system. Therefore, it is recommended that this test be read manually in the event of a positive result in the automatic reading.

Generally, the L-rhamnose test is positive for *H. alvei*, although there are a few samples that are L-rhamnose negative (strains 11-69 and 14-67). When the L-rhamnose test is negative, special attention should be paid to the urease test, because a positive urease result gives a rare biotype in the MicroScan system, while a positive L-rhamnose result always gives the identification of *H. alvei*, independent of the urease test.

In conclusion, the results of this study confirm that the MicroScan WalkAway system, in conjunction with the new MicroScan Combo Negative type 1S panels, is reliable for identification of *H. alvei* strains.

We thank J. Michael Janda for critical review of the manuscript. The assistance of the staff of the Clinical Microbiology Laboratory at the Hospital Cristal Piñor Ourense is greatly appreciated. We thank Cultimed-Spain for providing culture media.

L. A. Rodríguez is the recipient of a University of Vigo grant.

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