JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2003, p. 5732–5734 Vol. 41, No. 12
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.12.5732–5734.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Evaluation of Two Miniaturized Systems, MicroScan W/A and BBL Crystal E/NF, for Identification of Clinical Isolates of *Aeromonas* spp.

L. Soler,[1] F. Marco,[2] J. Vila,[2] M. R. Chacón,[1] J. Guarro,[1] and M. J. Figueras[1]*

Unitat de Microbiologia, Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, 43201 Reus,[1] and Laboratori de Microbiologia, Institut d'Infeccions i Immunologia, Institut d'Investigació Biomèdica August Pi i Sunyer, Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona,[2] Spain

Received 3 July 2003/Returned for modification 22 August 2003/Accepted 2 September 2003

Fifty-two clinical strains and 22 type and reference *Aeromonas* strains, previously genetically characterized by 16S rRNA gene restriction fragment length polymorphism, were identified in parallel with the MicroScan Walk/Away and BBL Crystal Enteric/Nonfermenter systems. The former identified only 14.8% of the isolates correctly, and the latter identified only 20.3% correctly, which indicates that neither of these systems is useful for this purpose.

Members of the genus *Aeromonas* are rod-shaped gram-negative bacteria assigned to the family *Aeromonadaceae*. Thirteen species of the 15 included in the genus have been reported from human infections (9, 11). They include gastroenteritis, bacteremia, cellulitis, meningitis, peritonitis, and soft-tissue and bronchopulmonary infections (12, 13). However, the prevalence of the different species in clinical samples is not well known because the techniques routinely used for species identification are less than perfect (1, 4, 5). They are usually based on biochemical characteristics giving a false predominance of *Aeromonas hydrophila* (11, 22). When clinical strains are identified by molecular methods, *A. caviae* and *A. veronii* are more common than *A. hydrophila* (9, 15). Even though biochemical tests have proved to be less than accurate for *Aeromonas* identification (2, 7, 18), they are still broadly used. Recently, Abbott et al. (1) reported several biochemical schemes that can be useful for the phenotypic identification of *Aeromonas* spp. However, the use of these procedures in the clinical setting is difficult and, at present, laboratories still rely on easy-to-use miniaturized methods. Some of the most commonly used methods in clinical laboratories are the miniaturized BBL Crystal Enteric/Nonfermenter (E/NF; BBL Microbiology Systems, Cockeysville, Md.) and MicroScan Walk/Away (W/A; Dade MicroScan Inc., West Sacramento, Calif.) methods. We have evaluated the accuracy of these two methods in the identification of clinical *Aeromonas* isolates previously identified genetically by 16S rRNA gene (rDNA) restriction fragment length polymorphism (RFLP) (4, 10).

Fifty-two clinical isolates and 22 type and reference *Aeromonas* strains (Table 1 and 2) were included in this study. The isolates were grown on Trypticase soy agar (Difco, Barcelona, Spain) at 30°C for 24 h. These cultures were used to inoculate the BBL Crystal E/NF and MicroScan W/A Combo Negative 1S type panels, which were incubated at 36 ± 1°C. As recommended by the manufacturers, oxidase testing was performed as a complement for both systems, while the indole test was used to complement the BBL Crystal method. In the latter, the panel reading gave a 10-digit number that was compared to the corresponding database. A confidence rating (CR) of 0.6000 to 1.0000 was considered a correct identification (20). When the CR was <0.6000 but all the given options were *Aeromonas* species, the one with the higher CR was given as the valid identification. The chi-square test was used to compare the results obtained with both methods by using the Statistical Package for Social Sciences (SPSS 9.0 Inc., Chicago, Ill.). When the P value was <0.05, differences were considered statistically significant.

Of the 74 strains tested, the BBL Crystal method correctly identified 20.3% of them and MicroScan correctly identified 14.8% of them (Tables 1 and 2). When the 52 clinical *Aeromonas* isolates were tested, the former method identified 50 isolates (96%) correctly to the genus level and the latter identified 44 isolates (84.6%) correctly to the genus level. The difference was statistically significant ($P = 0.008$). At the species level, BBL Crystal correctly identified 14 (26.9%) and MicroScan correctly identified 10 (19.3%) of the 52 isolates (Table 2). The BBL Crystal method correctly identified 100% (10 of 10) of the *A. hydrophila* isolates with a CR of 0.8631 to 0.9993, 21.4% (3 of 14) of the *A. veronii* isolates with a coincident CR of 0.3604, and 5.2% (1 of 19) of the *A. caviae* clinical isolates with a CR of 0.7663. MicroScan also correctly identified all of the *A. hydrophila* isolates, although only to the *A. hydrophila* group level (Table 2). This is not acceptable because species other than *A. hydrophila* are included in this group and some of them have been isolated, although rarely, from clinical samples (1, 9, 15). Sixteen of the 19 isolates of *A. caviae* tested were incorrectly identified as *A. hydrophila* by the BBL Crystal method. This was probably due to the positive response in the lysine test, which was expected to be negative (3). Six of the 14 isolates of *A. veronii* were misidentified as *A. hydrophila*. This was due to their positive responses in the esculin hydrolysis test, which was expected to be negative (3).

* Corresponding author. Mailing address: Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Spain. Phone: 34-977759321. Fax: 34-977759322. E-mail: mjfs@fmcs.urv.es.

TABLE 1. Comparison of the BBL Crystal and MicroScan systems for the identification of 22 *Aeromonas* type and reference strains

Reference strain	BBL Crystal result	MicroScan result
A. hydrophila CECT[c] 839[T a]	*A. hydrophila*	*A. hydrophila* group
A. bestiarum CECT 4227[T]	*A. hydrophila*	*A. hydrophila* group
A. salmonicida LMG[d] 13451	*A. hydrophila*	*V. fluvialis*
A. salmonicida subsp. *salmonicida* CECT 894[T]	*Vibrio fluvialis*	NG[b]
A. salmonicida subsp. *masoucida* CECT 896	*A. hydrophila*	*A. hydrophila* group
A. salmonicida subsp. *achromogenes* CECT 895	*A. hydrophila*	*Pasteurella multocida*
A. salmonicida subsp. *smithia* NCIMB 13210	Misclassified gram-negative bacilli	*P. multocida*
A. caviae CECT 838[T]	*A. hydrophila*	*A. hydrophila* group
A. media CECT 4232[T]	*A. hydrophila*	*A. hydrophila* group
A. eucrenophila CECT 4224[T]	*A. hydrophila*	*V. fluvialis*
A. sobria CECT 4245[T]	*A. veronii*	*P. multocida*
A. veronii bv. sobria CECT 4246	*A. sobria*	*A. hydrophila* group
A. jandaei CECT 4228[T]	*A. hydrophila*	*A. hydrophila* group
A. veronii bv. veronii CECT 4257[T]	*A. hydrophila*	*A. hydrophila* group
Aeromonas sp. (GH11) strain CECT 4253	*V. cholerae*	*Pseudomonas fluorescens/putida*
Aeromonas group 501 CECT 5178	*A. hydrophila*	*A. hydrophila* group
Aeromonas group 501 CECT 4254	*Chromobacterium violaceum*	*V. damsela*
A. schubertii CECT 4240[T]	*A. hydrophila*	*A. hydrophila* group
A. trota CECT 4255[T]	*A. hydrophila*	*A. hydrophila* group
A. popoffii LMG 17541[T]	*A. hydrophila*	*V. damsela*
A. allosaccharophila CECT 4199[T]	*A. hydrophila*	*A. hydrophila* group
A. encheleia CECT 4342[T]	*A. hydrophila*	*V. parahaemolyticus*

[a] T, type strain.
[b] NG, numerous genera.
[c] CECT, Colección Española de Cultivos Tipo, Universidad de Valencia, Valencia, Spain.
[d] LMG, Culture Collection of the Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium.

In the case of MicroScan, most of the isolates of *A. caviae* and *A. veronii* were assigned to the *A. hydrophila* group.

Of the 22 type and reference strains tested, only the type strain of *A. hydrophila* was correctly identified to the species level by BBL Crystal and MicroScan (Table 1). In addition, 4 and 10 strains could not even be properly assigned to the genus *Aeromonas* by the respective methods.

TABLE 2. Comparison of the BBL Crystal and MicroScan systems for the identification of 52 clinical *Aeromonas* isolates identified by 16S rDNA RFLP

16S rDNA RFLP result	No. of strains tested	No. of strains, result	
		BBL Crystal	MicroScan
A. hydrophila	10	10, *A. hydrophila*	10, *A. hydrophila* group
A. caviae	19	16, *A. hydrophila* 1, *A. sobria* 1, *A. veronii* 1, *A. caviae*	17, *A. hydrophila* group 2, *Vibrio fluvialis*
A. veronii	14	6, *A. hydrophila* 4, *A. sobria* 3, *A. veronii* 3, *A. veronii* 1, *Burkholderia cepacia*	13, *A. hydrophila* group 1, *V. fluvialis*
A. media	4	4, *A. hydrophila*	3, *A. hydrophila* group 1, *V. fluvialis*
A. jandaei	2	1, *A. hydrophila* 1, *Vibrio cholerae*	1, *V. fluvialis* 1, *A. hydrophila* group
A. bestiarum	1	1, *A. hydrophila*	1, *A. hydrophila* group
A. salmonicida	2	2, *A. hydrophila*	1, *V. fluvialis* 1, *A. hydrophila* group

BBL Crystal and MicroScan incorrectly identified 60.8 and 63.5% of the 74 isolates as *A. hydrophila* and *A. hydrophila* group, respectively. If these results were correct, this would agree with those of Vivas et al. (21), who stated that this is the most common clinical species. However, by using the 16S rDNA RFLP method, we found that *A. hydrophila* only represented 8.1% of the 490 clinical isolates tested (unpublished data). Other authors have also confirmed this low occurrence of *A. hydrophila* in clinical samples (15, 16). This tendency of most commercial systems to identify clinical strains as *A. hydrophila* has led to an overestimation of the clinical relevance of this species (8, 15, 16, 17, 18) and has masked the true incidence of other species. This has been taken to such an extreme that in some recent papers, *A. hydrophila* has been considered the only important species (22).

To our knowledge, the study of Carnahan et al. (A. Carnahan, S. Lee, D. Watsky, and G. Thomas, Abstr. Annu. Meet. Am. Soc. Microbiol. 1994, abstr. C-245, p. 533, 1994) is the only one that has evaluated the BBL Crystal method for *Aeromonas* identification. There, all of the isolates were correctly identified to the genus level, in contrast to the 52% obtained with the commonly used API 20E system, which was also evaluated by the same authors.

The poor accuracy of MicroScan in identifying *Aeromonas* isolates to the species level in our study contrasted with the results of Vivas et al. (21). We tested isolates of all of the *Aeromonas* species, and only 19.3% of such isolates were correctly identified, while Vivas et al. (21) tested isolates of eight species and correctly identified 78.8% of them. An explanation for this discrepancy could be the fact that those authors com-

pared the results obtained with MicroScan with those obtained with other biochemical procedures, and in general, it has been demonstrated that biochemical identification methods are not reliable for this purpose (4, 6, 10, 14).

Of the 74 *Aeromonas* isolates tested in the present study, BBL Crystal and MicroScan recognized 8.1 and 21.6% of them, respectively, as not belonging to this genus (Tables 1 and 2). The tendency of miniaturized biochemical identification systems to confuse *Aeromonas* isolates with *Vibrio* isolates noticed in our study was already known (2, 19). In our case, MicroScan misidentified eight isolates (10.8%) as *Vibrio fluvialis*, results similar to those reported by Vivas et al. (21). More troubling is the fact that BBL Crystal misidentified two isolates as *Vibrio cholerae*, which is of special relevance because of the clinical significance of this microorganism. A method based on colony blot hybridization has recently been proposed to avoid misidentification as *Vibrio* spp. (7).

Application of the schemes proposed by Abbott et al. (1) could probably demonstrate similar limitations of the miniaturized systems evaluated here for *Aeromonas* identification. The drawbacks of commercial miniaturized biochemical systems for the identification of *Aeromonas* spp. lie mainly in inappropriate and incomplete databases that predate current taxonomy. For instance, the BBL Crystal database includes only *A. hydrophila*, *A. caviae*, *A. veronii*, and *A. sobria*, although none of these species was correctly identified in our study. Why the latter species is added to such databases is unclear since it is known that *A. sobria* has an environmental origin and is very rarely isolated from clinical samples (9, 13). Although *A. sobria* is the name classically used by clinical microbiology laboratories to refer to *A. veronii* bv. sobria (9), its use is no longer justified since it creates confusion. To further confuse, the BBL Crystal method identified the type strain of *A. sobria* as *A. veronii*, while a reference strain of *A. veronii* bv. sobria was identified as *A. sobria* (Table 1).

In summary, our results demonstrate that BBL Crystal and MicroScan are not useful systems for the identification of clinical *Aeromonas* isolates and highlight the need to develop systems that are more reliable.

This work was supported by grant FIS 99/0944 from the Spanish Ministry of Health, Fundació Ciència i Salut, and a fellowship from the Universitat Rovira i Virgili awarded to L. Soler and M. R. Chacón.

We thank the Colección Española de Cultivos Tipo (CECT) and the Belgium Coordinated Collection of Microorganisms (LMG) for kindly providing isolates.

REFERENCES

1. **Abbott, S. L., W. K. Cheung, and J. M. Janda.** 2003. The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. J. Clin. Microbiol. **41:**2348–2357.
2. **Abbott, S. L., L. S. Seli, M. Catino, M. A. Hartley, and J. M. Janda.** 1998. Misidentification of unusual *Aeromonas* species as members of the genus *Vibrio*: a continuing problem. J. Clin. Microbiol. **36:**1103–1104.
3. **Altwegg, M.** 1999. *Aeromonas* and *Plesiomonas*, p. 507–516. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
4. **Borrell, N., S. G. Acinas, M. J. Figueras, and A. Martínez-Murcia.** 1997. Identification of *Aeromonas* clinical isolated by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. J. Clin. Microbiol. **35:** 1671–1674.
5. **Carnahan, A. M., S. Behram, and S. W. Joseph.** 1991. Aerokey II: a flexible key for identifying clinical *Aeromonas* species. J. Clin. Microbiol. **29:**2843–2849.
6. **Castro-Escarpulli, G., M. J. Figueras, G. Aguilera-Arreola, L. Soler, E. Fernández-Rendón, G. O. Aparicio, J. Guarro, and M. R. Chacón.** 2003. Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. Int. J. Food Microbiol. **84:**41–49.
7. **Chacón, M. R., G. Castro-Escarpulli, L. Soler, J. Guarro, and M. J. Figueras.** 2002. A DNA probe specific for *Aeromonas* colonies. Diagn. Microbiol. Infect. Dis. **44:**221–225.
8. **Clark, N. M., and C. E. Chenoweth.** 2003. *Aeromonas* infection of the hepatobiliary system: report of 15 cases and review of the literature. Clin. Infect. Dis. **37:**506–513.
9. **Figueras, M. J., J. Guarro, and A. J. Martínez-Murcia.** 2000. Clinically relevant *Aeromonas* species. Clin. Infect. Dis. **30:**988–989.
10. **Figueras, M. J., L. Soler, M. R. Chacón, J. Guarro, and A. J. Martínez-Murcia.** 2000. Extended method for discrimination of *Aeromonas* spp. by 16S rDNA RFLP analysis. Int. J. Syst. Evol. Microbiol. **50:**2069–2073.
11. **Janda, J. M.** 2001. *Aeromonas* and *Plesiomonas*, p. 1237–1270. *In* M. Sussman (ed.), Molecular medical microbiology. Academic Press, San Diego, Calif.
12. **Janda, J. M., and S. L. Abbott.** 1996. Human pathogens, p. 151–174. *In* B. Austin, M. Altwegg, P. J. Gosling, and S. Joseph (ed.), The genus *Aeromonas*. John Wiley & Sons, Inc., New York, N.Y.
13. **Janda, J. M., and S. L. Abbott.** 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, diseases presentation, and unanswered questions. Clin. Infect. Dis. **27:**453–461.
14. **Kozińska, A., M. J. Figueras, M. R. Chacón, and L. Soler.** 2002. Phenotypic characteristics and pathogenicity of *Aeromonas* genomospecies isolated from common carp (*Cyprinus carpio* L.). J. Appl. Microbiol. **93:**1034–1041.
15. **Kuijper, E. J., A. G. Steigerwalt, B. C. I. M. Schoenmakers, M. F. Peeters, H. C. Zanen, and D. J. Brenner.** 1989. Phenotypic characterization and DNA relatedness in human fecal isolates of *Aeromonas* spp. J. Clin. Microbiol. **27:**132–138.
16. **Kuijper, E. J., and M. F. Peeters.** 1991. Bacteriological and clinical aspects of *Aeromonas*-associated diarrhea in The Netherlands. Experientia **47:**432–434.
17. **Overman, T. L., and J. K. Overley.** 1986. Feasibility of same-day identification of members of the family *Vibrionaceae* by the API 20E system. J. Clin. Microbiol. **23:**715–717.
18. **Overman, T. L., J. F. Kessler, and J. P. Seabolt.** 1985. Comparison of API 20E, API Rapid E, and API Rapid NFT for identification of members of the family *Vibrionaceae*. J. Clin. Microbiol. **22:**778–781.
19. **Park, T. S, S. H. Oh, E. Y. Lee, T. K. Lee, K. H. Park, M. J. Figueras, and C. L. Chang.** 2003. Misidentification of *Aeromonas* biovar sobria as *Vibrio alginolyticus* by the Vitek system. Lett. Appl. Microbiol. **37:**349–353.
20. **Peele, D., J. Bradfield, W. Pryor, and S. Vore.** 1997. Comparison of identifications of human and animal source gram-negative bacteria by API 20E and Crystal E/NF systems. J. Clin. Microbiol. **35:**213–216.
21. **Vivas, J., A. I. Sáa, A. Tinajas, L. Barbeyto, and L. A. Rodríguez.** 2000. Identification of motile *Aeromonas* strains with the Microscan WalkAway system in conjunction with the Combo Negative type 1S panels. Appl. Environ. Microbiol. **66:**1764–1766.
22. **Zhiyong, Z., L. Xiaoju, and G. Yanyu.** 2002. *Aeromonas hydrophila* infection: clinical aspects and therapeutic options. Rev. Med. Microbiol. **13:**1–12.