Evaluation of two miniaturized systems, MicroScan W/A and BBL Crystal E/NF, for the identification of clinical isolates of *Aeromonas* spp.

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Evaluation of two miniaturized systems, MicroScan W/A and BBL Crystal E/NF, for the identification of clinical isolates of *Aeromonas* spp.

Fifty-two clinical strains and 22 type and reference strains of *Aeromonas* were identified in parallel with the MicroScan W/A and the BBL Crystal E/NF systems. Isolates had been previously genetically identified by 16S rDNA-RFLP. Discrimination to species level was very poor. MicroScan identified correctly only 19.3% of the isolates and BBL Crystal only 26.9%.

Thirteen species of a total of 15 included in the genus *Aeromonas* have been reported from human infections (7). They include gastroenteritis, bacteriemia, cellulitis, meningitis, peritionitis, soft-tissue and broncho-pulmonary infections (10; 11). However, the prevalence of the different species in clinical samples is not well known because the techniques used for species identification are unreliable (9). They are usually based on biochemical characters giving a false predominance of *A. hydrophila* (9). When clinical strains are identified by molecular methods, the species *A. caviae* and *A. veronii* bt sobria are more common than *A. hydrophila* (7; 11). Even though biochemical tests have proved to be less than absolutely accurate for *Aeromonas* identification (1; 6; 16), they are still broadly used. Some of the commonest used methods at clinical laboratories are the miniaturized BBL Crystal Enteric/Nonfermenter (E/NF) (Crystal; Becton Dickinson Microbiological Systems, Cockeysville, Md) and the MicroScan Walk/Away (W/A) (Dade MicroScan Inc., West Sacramento, Calif.). We have evaluated the accuracy of these two methods to identify clinical isolates of *Aeromonas*, previously identified genetically by 16S rDNA-RFLP (3; 8).

Fifty-two clinical isolates and 22 type and reference strains of *Aeromonas* (Table 1 and 2) were included in the study. The isolates were growth on Trypticase Soy Agar (Difco; Barcelona, Spain) at 30°C for 24 h. Pure 24 h cultures were used to inoculate the BBL Crystal E/NF and the MicroScan W/A Combo Negative 1S type panels. As recommended by the manufacturers, oxidase was performed as complementary test for both systems while indole test was used to complement the BBL Crystal. In the case of BBL Crystal, the reading of the panel gave a 10-digit number that was compared to the corresponding database. A confidence rating of 0.6000 to 1.0000 was considered a positive identification (17). When this confidence

rating was <0.6000, but all the given options were species of *Aeromonas*, the one with a higher confidence rating was given as valid identification. The chi-square test was used to compare the results obtained with both methods, using the Statistical Package for Social Sciences (SPSS 9.0 Inc., Chicago, USA). When $p<0.05$, they were considered statistically significant.

From the 22 type and reference strains of *Aeromonas* tested, only the type strain of *A. hydrophila* was correctly identified at species level by BBL Crystal and MicroScan (Table 1). The former method identified correctly at genus level 50 (96%) and the latter 44 (84.6%) of the 52 clinical strains tested. This difference was statistically significant ($p=0.008$). All results of *Aeromonas* identification appeared as 'A. hydrophila group' with the latter method. With BBL Crystal, 100% of the *Aeromonas* isolates were correctly identified to the genus level, contrasting with the 52% obtained with the commonly used system APE-20E (4). BBL Crystal and MicroScan only identified correctly at species level 14 (26.9%) and 10 (19.3%) of the isolates, respectively (Table 2). The BBL Crystal, identified correctly 100% (10/10) of the *A. hydrophila* with a confidence rating (CR) of 0.8631-0.9993, 21.4% (3/14) of the *A. veronii* with a coincident CR of 0.3604 and 5.2% (1/19) of the *A. caviae* clinical isolates with a CR of 0.7663. However the MicroScan only identified correctly the *A. hydrophila* ('A. hydrophila' group) isolates (Table 2).

The incorrect identification of 16 *A. caviae* clinical isolates as *A. hydrophila* by the BBL Crystal was due to a positive response for the lysine test, which is expected to be negative (2), nevertheless, 14 of such isolates were identified in second option as *A. caviae*, with a very low confidence rating (0.0035-0.3962). The misidentification of 6 *A. veronii* isolates as *A. hydrophila* was due to their positive responses to aesculin hydrolisis test, which is expected to be negative (2). In the case of MicroScan, the most confusing biochemical test was Voges-Proskauer.

BBL Crystal and MicroScan wrongly identified 71.4% and 85.7%, respectively, of the isolates as *A. hydrophila*. If these results were correct, this would agree with Vivas et al. (18) who after identifying the isolates with MicroScan stated that this is the most common clinical species. However, when identifying clinical isolates with molecular methods, *A. hydrophila* is not the most prevalent species (9; 11). Using the 16S rDNA RFLP method we found that *A. hydrophila* only represented a 8.1% of the total (n=490) of isolates tested (unpublished data). This tendency of most commercial systems to identify clinical strains as *A. hydrophila* has lead to an overestimation of the

clinical relevance of this species and has masked the true incidence of other *Aeromonas* spp. (13; 14; 15; 16).

The poor accuracy of MicroScan in identifying *Aeromonas* at species level in our study contrasted with the results of Vivas et al. (18). We tested isolates of all the species of *Aeromonas* and only 19.3% of them were correctly identified, while Vivas et al. (18) tested isolates from 8 species, and found that 78.8% of them were correctly identified. An explanation for this discrepancy could be the fact that these authors confirmed identification using biochemical procedures which has been repeatedly demonstrated that they are not reliable for this purpose (3; 5; 8; 12).

From the total of 74 isolates here tested (type and reference strains plus clinical isolates), BBL Crystal and MicroScan identified 8.1% and 21.6% of them, respectively, as not belonging to this genus (Table 1 and 2). The tendency of biochemical identification miniaturized systems to confuse *Aeromonas* with *Vibrio* noticed in our study was already known (1). In our case MicroScan misidentified 8 isolates (10.8%) as *Vibrio fluvialis*, similar results than those reported by Vivas et al. (18) which was of 8%. It is worth of mentioning that BBL Crystal misidentified two isolates as *Vibrio cholerae*, which is of special relevance due to the pathogenic meaning of this microorganism. Modern methods based on colony blot hybridization have been proposed to avoid the misidentification of *Aeromonas* as *Vibrio* (6).

The drawbacks of commercial biochemical miniaturized systems for the identification of *Aeromonas* spp. lie mainly in their inappropriate and incomplete databases. For instance, the database of BBL Crystal includes the species *A. hydrophila*, *A. caviae*, *A. veronii* and *A. sobria*, although none of these species were correctly identified. Why the latter species is added in the database is unclear since is known that *A. sobria* has an environmental origin and it is very rarely isolated from clinical samples (7; 11). Maybe an explanation lies in the fact that *A. sobria* is the name classically used by clinical microbiology laboratories to refer to *A. veronii* bt sobria (7). To increase this confusion the BBL Crystal identified the type strain of *A. sobria* as *A. veronii*, while a reference strain of *A. veronii* bt sobria was identified as *A. sobria* (Table 1).

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

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TABLE 1. Comparison of the BBL Crystal and MicroScan systems for the identification of 22 *Aeromonas* type and reference strains identified by 16S rDNA-RFLP.

16S rDNA-RFLP	BBL Crystal	MicroScan
A. hydrophila CECT 839[Ta]	*A. hydrophila*	'A. hydrophila' group
A. bestiarum CECT 4227[T]	*A. hydrophila*	'A. hydrophila' group
A. salmonicida LMG13451	*A. hydrophila*	*V. fluvialis*
A. salmonicida subsp salmonicida CECT 894[T]	*Vibrio fluvialis*	N.G[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 bt sobria CECT 4246	*A. sobria*	'A. hydrophila' group
A. jandaei CECT 4228[T]	*A. hydrophila*	'A. hydrophila' group
A. veronii bt veronii CECT 4257[T]	*A. hydrophila*	'A. hydrophila' group
Aeromonas sp (GH11) CECT 4253	*V.cholerae*	*Ps. 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]Type strain; [b]N.G. Numerous genera; CECT Colección Española de Cultivos Tipo, Universidad de Valencia, Valencia, Spain; LMG, Culture Collection of the Laboratorium voor Microbiologie Gent, Universiteit Gent, Ghent, Belgium.

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