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Identificación de cepas de Aeromonas de origen clínico con perfiles fenotípicos atípicos

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RESUMEN

Un total de 47 cepas de *Aeromonas* aisladas de pacientes con gastroenteritis fue analizado para 40 caracteres fenotípicos y para la evaluación de la taxonomía numérica basada en 27 pruebas discriminatorias. Se evidenció que los aislamientos clínicos mostraron una relativa distancia fenotípica y los grupos de cepas que mostraron perfiles atípicos fueron comparados con las especies tipos, mediante los actuales esquemas de identificación.

Descriptores DeCS: AEROMONAS/aislamiento & purificación; GASTROENTERITIS; FENOTIPO.

During the last years aeromonads have been increasingly reported as causative agents of human disease, ranging from acute gastroenteritis (AGE) to wound infections or even disseminated infection 1. At the same time, the taxonomy of genus *Aeromonas* has undergone major revisions on the basis of phenetic analysis and DNA-DNA hybridization studies. As a genus, it has been proposed to be moved from the family Vibrionaceae to a separate family (Aeromonadaceae) to reflect the relevant differences existing between aeromonads and the other Vibrionaceae. Concerning species definition, the original classification of aeromonads into four species, *Aeromonas hydrophila, Aeromonas sobria, Aeromonas salmonicida,* has been thoroughly revised, and several additional species are currently recognized as clinically relevant.[1]

Aeromonas strains analyzed in this study included 47 recent clinical isolates and 7 reference strains. Clinical strains were from Cuban children aged under 5 suffering from *Aeromonas*-associated AGE. *Aeromonas*-associated AGEs were defined as those AGE cases in which *Aeromonas* strains were isolated from faeces in absence of any other bacterial, viral or parasitic enteropathogen commonly encountered in that area. Clinical strains were preliminarily identified as beloging into genus *Aeromonas* on the basis of being oxidase-positive glocuse-fermenting Gram-negative rods, resistant to the vibriostactic agent O/129, and unable to grow in the presence of 6.5 % NaCl. Reference strains analyzed in this study included *A. hydrophila* ATCC 7966^T, *Aeromonas veronii bv. sobria* ATCC 9071^T, *Aeromonas veronii bv. veronii* ATCC 35624^T, *Aeromonas caviae* ATCC 15468^T, *Aeromonas jandaei*

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ATCC 49568^T, *Aeromonas trota* ATCC 49657^T, and *Aeromonas schubertii* ATCC 43700^T.[2]

NUMERICAL TAXONOMY ANALYSIS

Numerical taxonomy analysis on the basis of the 27 different phenotypic characters which resulted variable among the strains studied. The similarity coefficient used for numerical taxonomy analysis was the simple matching one, and cluster analysis was performed by the average linkage method (unweighted pair group using mathe-matical average).[3]

During a six-months surveillance study on pediatric diarrhoeas performed at eight different districts located in the island of Cuba, 47 cases of *Aeromonas*-associated AGE were encountered. The 47 strains isolates from these cases were identified as belonging into *Aeromonas* genus on the basis of their being oxidase-positive glucose-fermenting Gram-negative rods, resistant to the vibriostactic agent G/129 and unable to grow in the presence of 6.5 % NaCl.

It should be noted that one these strains actually turned out to be resistant to O/129 when using a 10 μg diskette, while it appeared to be susceptible when using a 150 μg diskette. Since this strain did not grow in the presence of 6.5 % NaCl and the results of the subsequent phenetic analysis showed that its profile was overall consistent with those of other *Aeromonas* strains, it was considered to be an aeromonad.

This finding suggest that susceptibility to O/129 using the 150 μg diskette should not be considered a criterion sufficient for exclusion of a clinical isolate from beloging to genus *Aeromonas*.

Each of the 47 clinical strains was tested for the 40 phenotypic characters. The same characters were also tested in the type strains of the following clinically relevant species: *A hydrophila, A. veronii* (both *bv. sobria* and *bv. veronii*), *A. jandael , A trota,* and *A. schubertii*.

Considering the results of the relevant test for species identification according to the Aerokey II identification sheme, all the reference strains were correctly identified and, among the clinical strains, 19 (41 %) were identified as *A. caviae*, 16 (34 %) as *A. veronii biovar sobria*, 9 (19 %) as *A. hydrophilia*, 2 (4 %) as *A. trota*, and 1 (2 %) as *A. jandaei*.

Twenty-seven of the 40 phenotypic characters evaluated in this study appeared to be variable among different strains and were used to perform a numerical taxonomy analysis.

Cluster 1 included 18 strains with a similarity value within 78 %. It gathered the *A. caviae* type strains and 17 additional clinical strains identified as *A. caviae* according to Aerokey II. The boundary of this cluster at a similarity value of 78 % was actually due to the presence of an atypical galactose-negative and melibiose-positive clinical strain. All other strains of this cluster, in fact, showed a similarity value within 82 %. Cluster 2 included 10 strains with a similarity value within 81 %. It gathered all the 9 clinical isolates that were identified as *A. hydrophila* according to Aerokey II, and a gas-negative clinical isolate which was identified as *A. caviae* by Aerokey II. Interestingly, cluster 2 linked the *A. hydrophila* type strain (line 2) only at a similarity value of 67 %, while linking cluster 3 at a similarity value of 80 %. Cluster 3 included 3 strains with a similarity value within 85 %. It gathered the *A. veronii bv. sobria* reference strain and two clinical isolates, of which one was identified as *A. veronii bv. sobria* according to Aerokey II, while the other (which was lysine decarboxylase-and Voges-Proskauer-positive), but also esculin-positive and gas-negative) was identified as *A. caviae* by Aerokey II. Cluster 4 included 13 strains with a similarity value within 85 %. It gathered 13 clinical isolates identified as *A. veronii bv. sobria* according to Aerokey II, but it linked cluster 3, containing the *A. veronii bv. sobria* type strain at a similarity value of only 67 %. Li nes 5 and 6 represented two clinical isolates identified as *A. veronii bv. sobria* according to Aerokey II, which linked the *A. veronii bv. veronii* type strain (line 4) at a similarity value of 83 % and 77,5 % respectively. Both of these strains were ornithine decarboxylase-negative but, apart from being esculin-negative and indole-Voges-Proskauer-, and sucrose-positive, appeared to be clearly separated from either cluster 3 or 4. Cluster 5 included two clinical isolates identified as *A. trota* according to Aerokey II, which linked, at a similarity value of 81 %, another clinical strain identified as *A. jandaei* according to Aerokey II. This latter isolate was fairly atypical since it was Voges-Proskauer-positive and ampicillin-resistant like *A. jandaei*, cephalotin-resistant similarly to *A. trota*, and also arabinose- and sucrose-positive, unlike both of these species. These three strains appeared to be relative dissimilar from both the *A. trota* (line 7, linked at a similarity value of 72.5 %) and the *A. jandaei* (line 3, linked at a similarity value of 69 %) type strains. Finally, the *A. schubertii* type strain appeared to be very dissimilar from all the other strains, linking them at a similarity value of 40 %[4] (fig.).

Results of the phenetic analysis, therefore, indicated that, in the areas covered by this study, aeromonads isolated from cases of pediatric AGE were distributed within a relatively large phenetic space and included several strains with atypical phenotypic profiles. In particular, two relatively large clusters of strains appeared to be present which, although to currently established identification schemes based on phenetic data, exhibited

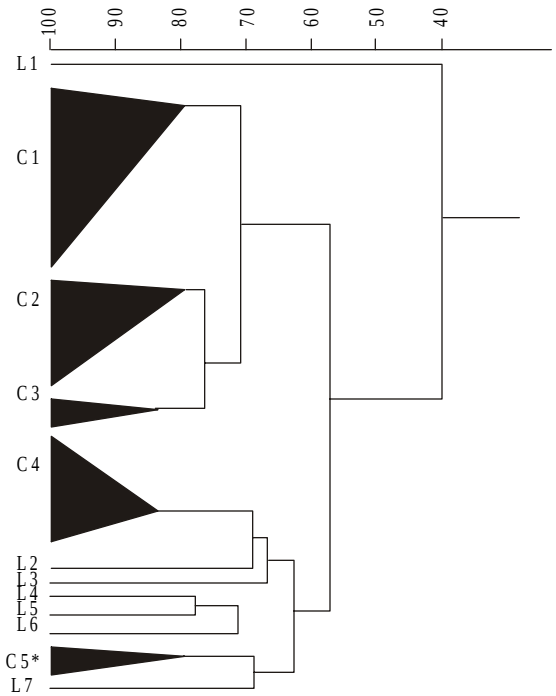

Fig. Similitary value within *Aeromonas*

a clear-cut phenotypic separation from the respective type strains. Members of cluster 2 appeared to be actually more similar to the *A. veronii bv. sobria* type strain than to the *A. hydrophila* one and, by comparing their biochemical profiles to that of the *A. hydrophila* type strain it was noticed that, unlike the latter, all of them were able to produce acid from galactose and amygdalin but not from arabinose, and most of them were unable to degrade glycine and to utilize citrate. On the other hand, members of cluster 4 appeared to be actually more similar to the *A. hydrophila* type strain than to the *A. veronii bv. sobria* one and, comparing their biochemical profiles to that of the *A. veronii bv. sobria* type strain, it was noticed that, unlike the latter, all of them were able to the above clusters, also other strains were found for which the phenotypic profiles were rather distant from those of the type strains of species to which they were assigned according to the Aerokey II identification sheme.[5]

All these "atypical" isolates would deserve further studies to clarify their taxonomic position and to revise, accordingly, identification criteria based on phenetic data.

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SUMMARY

A total of 47 strains of *Aereomona*s isolated from patients with gastroenteritis was analized for 40 phenotypical characters and for evaluating the numeric taxonomy based on 27 discriminatory tests. It was proved that the clinical isolates showed a relative phenotypical distance and the groups of strains that had atypical profiles were compared with the type species by the present identification schemes.

Subject headings: AEROMONAS/isolation & purification; GASTROENTERITIS; PHENOTYPE.

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