Differentiation of Motile and Mesophilic Aeromonas Strains into Species by Testing for a CAMP-Like Factor

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Motile and mesophilic Aeromonas strains can presumptively be differentiated into species in 18 to 24 h by testing the isolates for the production of a CAMP-like factor. Aeromonas hydrophila strains were positive either aerobically or anaerobically, Aeromonas sobria strains were positive only aerobically, and Aeromonas caviae strains were always negative.

Motile and mesophilic Aeromonas species are well-known as a cause of generalized infections in immunocompromised patients. These microorganisms are also considered primary intestinal pathogens, even though the results of some studies do not entirely confirm acceptance of this conclusion (1).

The differentiation of Aeromonas isolates into species is generally done by biochemical tests, some of which require up to 5 days before they can be read. In certain circumstances, early identification of aeromonads to the species level is requested, as in the case of strains isolated from feces of patients at risk for bacteremia. Most of the strains isolated from bacteremic subjects are in fact Aeromonas hydrophila and Aeromonas sobria. The third motile and mesophilic Aeromonas species, Aeromonas caviae, is seldom isolated in extraintestinal infections, and even when isolated from patients with diarrhea, it is not considered to be of etiological importance since it only rarely shows phenotypic characteristics of enteropathogenicity.

We found that the three mesophilic Aeromonas species can be differentiated in only 18 to 24 h by testing the isolates for the production of a CAMP-like factor. A total of 69 Aeromonas strains were tested: 26 strains of A. hydrophila (8 strains isolated from the stools of children, 15 strains from animals, 2 strains from water samples, and A. hydrophila ATCC 7966), 8 strains of A. sobria, and 35 strains of A. caviae (isolated from feces of children). The genus identification of these strains was done by API 20E or API 20NE systems. The species identification was done by methods reported previously (1). The procedure used for the CAMP-like factor test was as follows. Aeromonas strains were streaked perpendicular to a streak of a beta-lysin-producing Staphylococcus aureus (ATCC 25923). Two plates of nutrient agar containing 5% sheep blood were used for each test (Fig. 1). Plates were incubated at 37°C overnight, one plate aerobically, the other anaerobically. Streptococcus agalactiae ATCC 13813 was used as a positive control.

A. hydrophila strains produced the CAMP-like factor either aerobically or anaerobically, A. sobria strains produced it only aerobically, and A. caviae strains did not produce it at all (Fig. 1).

The reaction was detectable with different agar bases, including Columbia, brucella, Trypticase soy, and Mueller-Hinton, but with Columbia agar it was more marked. No CAMP-like phenomenon was observed with horse blood.

To ensure that the test was specific for Aeromonas species, we also tested one strain of Vibrio cholerae (Inaba 569 B; Sclavo Collection, Siena, Italy), one clinical isolate of Plesiomonas shigelloides, one isolate of Vibrio fluvialis,

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three clinical and two environmental strains of *Vibrio parahaemolyticus* (kindly given by S. Matsusaki, Yamaguchi, Japan), *Pseudomonas aeruginosa* ATCC 27853, and two clinical isolates of hemolytic *Escherichia coli*.

No CAMP-like reaction was detectable with any of these strains, except for *V. fluvialis* and two *V. parahaemolyticus* strains, which showed a faint enhancement of beta-hemolysis of *Staphylococcus aureus* only aerobically. However, these strains (as well as all other vibrios and *Plesiomonas* sp.) can be easily differentiated from *Aeromonas* species by testing the suspected colonies for their susceptibility to the vibriostatic compound 0/129; this can be done on the same plate used for the CAMP-like reaction test.

All aeromonads tested in this study were resistant to 0/129 (no zone of inhibition occurred near the disk), but all vibrios and *Plesiomonas shigelloides* were susceptible. We think, therefore, that the test is a simple and useful one for an early (although presumptive) identification of aeromonads by species.

**LITERATURE CITED**