Laboratory Identification of Aeromonads from Man and Other Animals

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Forty-eight isolates of aeromonads from clinical specimens were studied. The laboratory procedures employed and the results obtained are reported in the hope that they may be of assistance to other workers in the field of diagnostic microbiology. Capability of identifying these organisms in the laboratory will contribute to a better understanding of the role of this group of organisms in human disease as well as in diseases in other animals.

During the 6-year period from 1963 through 1968, 48 isolates of organisms of the aeromonas group (aeromonads) from a variety of human and animal sources were submitted to the Microbial Diseases Laboratory, California State Department of Public Health, for identification and confirmation. Members of this group are gram-negative, polar-flagellated rods, which are oxidase-positive and act on carbohydrates fermentatively. There have been some reports (2, 3, 5, 6, 11) in the medical literature of the last 10 years of human infections caused by the aeromonas group, including an excellent literature review and summary of 30 human cases by von Graevenitz and Mensch (11). The number of aeromonads being submitted to our laboratory for identification and confirmation suggest that infections caused by this group are more frequent than is currently recognized. Therefore, it seems appropriate to document our experience with these organisms.

MATERIALS AND METHODS

All organisms included in this study were isolated from clinical specimen materials by hospital laboratories, private clinical laboratories, or State Department of Agriculture Laboratories in the state of California. The isolates were submitted to the California State Microbial Diseases Reference Laboratory for confirmation or assistance in identification. The clinical sources are listed in Table 1. It is assumed that the isolates represent the organism considered etiologically related to an infectious process and not likely to be contaminants from the areas involved. However, extensive clinical investigations and isolation of the organism more than once from each patient would be necessary to strengthen this assumption. This information is not currently available on these isolates.

Upon receipt, a smear was prepared from each culture, Gram-stained, and examined microscopically.

Plates of Heart Infusion Agar (Difco) and Heart Infusion Agar with 5% sheep blood were streaked for isolation. After incubation for 18 to 24 hr at 37 C, the plates were examined by using a stereoscopic microscope to determine colony types and other growth characteristics. Single-colony pickings of each type of colony observed were made to Heart Infusion Agar slants and to Heart Infusion Broth. Gram-stained smears of these pickings were prepared from the agar slant after 18 to 24 hr of incubation at 35 to 37 C. The Heart Infusion Broth picking was used to inoculate the various other test media used.

Catalase tests were performed on agar-salant growth by flooding with H₂O₂ (3% by volume). A 1% solution of dimethyl-paraphenylenediamine oxalate (4) was used to determine the oxidase reaction of growth on Heart Infusion Agar. Indole was detected by growing the organism in 1% tryptone broth (Difco), extracting with xylene, and then adding Kovac's reagent (11). Simmons Citrate agar (Difco) was used to determine carbon utilization and Christensen Urea Agar (Difco) was used to determine urea hydrolysis. The ability to reduce nitrates to nitrates was determined by growing the test organism in Nitrate Broth (Difco) and adding the test reagents sulfanilic acid and α-naphthylamine in that order. Using MR-VP Medium (Difco), the Voges-Proskauer test was performed by the Levine, Epstein, and Vaughan (8) technique with the addition of α-naphthol.

Motility was observed in a semisolid medium, GI Medium (Difco). Production of pigment was noted on Loeffler slants (BBL). Proteolytic activity was measured on Loeffler slants, in litmus milk, and in gelatin stab cultures (12% gelatin in Heart Infusion Broth). The ability of the organisms to grow on MacConkey Agar (Difco) and SS Agar (Difco), both with added sucrose, was noted as well as their ability to grow in Heart Infusion Broth at room temperature (approximately 25 C), at 35 to 37 C, and at 45 C. The fermentative ability of these organisms was measured by the Hugh and Leifson (7) technique in OF Medium (Difco). Production of acid from individual carbohydrates was determined by using Ex.
tract Broth (BBL) containing 0.5% of the specific carbohydrate along with Andrade indicator. Broth carbohydrates were sterilized by filtration. Production of gas in selected carbohydrates was detected by the use of Durham tubes.

All tests were incubated at 35 to 37 °C (with the exceptions noted) and readings were made at the end of 1, 2, 3 to 6, and 7 days.

RESULTS AND DISCUSSION

A summary of the test results is reported in Table 2. Most of the 48 strains studied showed the general biochemical pattern reported for Aeromonas hydrophila by Ewing, Hugh, and Johnson (5). Results of the tests show these organisms to be a relatively homogeneous group.

Currently proposed classification of the aeromonas group would separate it into two genera, Aeromonas and Plesiomonas (1, 9), with the latter genus members being sensitive to the vibriostatic agent 2-3-diamino-6-7-diisopropylperidin. However, since none of the isolates studied was tested with the vibriostatic agent, we have followed the earlier classification and have placed all isolates in the Aeromonas genus (5).

Organisms of the aeromonas group are most frequently mistaken for members of the Enterobacteriaceae because of growth and biochemical characteristics shared with this family. We would agree with Gilardi (6) that performance of oxidase tests on all paracolon-like bacteria would result in more frequent identification of the aeromonas group from human sources. Oxidase-positive, gram-negative rods which are fermentative, are nitrate reducers, and exhibit the general biochemical characteristics associated with coliforms may be classified in this group.

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ADDITIONAL IN PROOF

Since the writing of this paper, the Manual of Clinical Microbiology, published by The American
Society for Microbiology (1970) and edited by John E. Blair, Edwin H. Lennette, and Joseph P. Truant, has been received. Chapter 17 written by Rudolph Hugh contains an excellent description and discussion of aeromonads.

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