

Suicide Phenomenon in Mesophilic Aeromonads as a Basis for Species Identification

HASSAN NAMDARI AND EDWARD J. BOTTONE*

Department of Microbiology, The Mount Sinai Hospital, One Gustave L. Levy Place, New York, New York 10029-6574

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The acetic acid-mediated suicide phenomenon in mesophilic aeromonads in conjunction with tests for aerogenicity and esculin hydrolysis served as the basis for species identification. Of 210 *Aeromonas* isolates tested at 30°C, 88 were identified as *Aeromonas hydrophila* (nonsuicidal, aerogenic, esculin positive), 52 were identified as *A. sobria* (suicide variable, aerogenic, esculin negative), and 70 were identified as *A. caviae* (suicidal, anaerogenic, esculin positive). These identifications paralleled those achieved by biochemical criteria.

To assess the true clinical significance of three mesophilic *Aeromonas* species (*Aeromonas hydrophila*, *A. sobria*, and *A. caviae*) as incitants of gastrointestinal tract disturbance and their potential for invasive disease in appropriate settings, species identification must be achieved (5). To date, however, this task is not easily accomplished in clinical microbiology laboratories, and many authors refer to these mesophilic species as *Aeromonas hydrophila* complex (2, 3). This designation reflects both the lack of clear-cut criteria for differentiating, especially among *A. sobria* and *A. hydrophila* isolates, and the overall taxonomic uncertainties surrounding the number of species which make up mesophilic aeromonads (8).

To facilitate species identification, which often requires evaluation of numerous biochemical tests (3), various investigators have sought to identify a particular species marker. Thus, Janda and Motyl (4) have proposed cephalothin susceptibility as a phenotypic marker for *A. sobria*, and Figura and Guglielmetti (1) used the CAMP reaction, assessed aerobically and anaerobically, to distinguish *A. sobria* (CAMP positive aerobically only) from *A. hydrophila* (CAMP positive aerobically and anaerobically); *A. caviae* is CAMP negative. Each of these techniques, while useful, may, however, be subject to biologic variation.

Towards the use of species-specific biologic phenomena for the rapid differentiation of mesophilic aeromonads, we propose the utilization of the suicide phenomenon in conjunction with tests for aerogenicity and esculin hydrolysis. The suicide phenomenon in *Aeromonas* species is expressed when strains are grown in broth media containing 0.5% glucose. Supplied glucose suppresses the tricarboxylic acid cycle, which results in acetate accumulation and cell death (6, 7).

A total of 210 *Aeromonas* isolates, collected from environmental and clinical sources, were tested (Table 1). Ten *A. caviae* strains were provided by J. M. Janda. The isolates were initially characterized to the species level by the criteria of Popoff and Veron (8) and Janda (3). Nutrient broth (Difco Laboratories) containing 0.5% glucose and 0.0015% bromocresol purple (Sigma Chemical Co.) was dispensed (6 ml) into screw-cap tubes (16 by 125 mm) to which were added inverted (Durham) tubes (6 by 50 mm). The suicidal tendency and aerogenicity of the strains were assessed by

inoculating this medium individually with the *Aeromonas* isolates grown on 5% sheep blood agar (BBL Microbiology Systems). After 24 h of incubation at 30°C, suicidal strains spontaneously pelleted, leaving a clear overlying medium, whereas nonsuicidal cultures appeared uniformly turbid. Glucose fermentation and aerogenicity were noted by a change in the bromocresol purple indicator to yellow, with accumulation of gas bubbles in the Durham tube. Hydrolysis of esculin was determined by inoculating slants of blood agar base (Difco) supplemented with 1.0 g of esculin and 0.5 g of ferric citrate per liter and observing for the appearance of a black precipitate after 24 h of incubation at 30°C.

Of the 210 *Aeromonas* strains, 88 were identified as *A. hydrophila*, 52 were identified as *A. sobria*, and 70 were identified as *A. caviae*. At 30°C, 98.9% of *A. hydrophila* strains were nonsuicidal, 96.6% were aerogenic, and 100% hydrolyzed esculin (Table 2). Of *A. sobria* isolates, 21% were suicidal, 92.3% were aerogenic, and 92.3% were esculin negative. *A. caviae* strains were uniformly suicidal and anaerogenic, and 83% hydrolyzed esculin. These identifications paralleled those achieved by biochemical criteria (5). When one test characteristic in the conventional system did not conform to species identification, species identification was achieved by comparing multiple biochemical characteristics. These guidelines were also adhered to when strains were identified on the basis of the suicide phenomenon, aerogenicity, and esculin hydrolysis.

By using the suicide phenomenon as a basis, a schema was developed for the rapid species identification of clinical and environmental isolates of mesophilic aeromonads (Fig. 1). Isolates that are suicidal and anaerogenic and hydrolyze esculin are uniformly *A. caviae*. Lack of hemolytic activity on sheep blood agar may augment this identification. *A. hydrophila* isolates may be rapidly distinguished by their

TABLE 1. *Aeromonas* isolates from clinical and environmental sources

<i>Aeromonas</i> species	Total no.	No. from source				
		Clinical			Environmental	
		Stool	Wound	Other	Fresh water	Sewage
<i>A. hydrophila</i>	88	18	8	26	34	2
<i>A. sobria</i>	52	5	4	14	22	7
<i>A. caviae</i>	70	26	2	8	4	30

* Corresponding author.

TABLE 2. Species identification of *Aeromonas* isolates on the basis of suicide phenomenon, aerogenicity, and esculin hydrolysis

<i>Aeromonas</i> species	No. tested	No. (%) of strains		
		Suicidal	Aerogenic	Esculin positive
<i>A. hydrophila</i>	88	1 (1.1)	85 (96.6)	88 (100)
<i>A. sobria</i>	52	11 (21)	48 (92.3)	4 (7.7)
<i>A. caviae</i>	70	70 (100)	0 (0)	58 (83)

lack of suicidal activity, marked aerogenicity, and rapid esculin hydrolysis. *A. sobria* isolates, while overlapping in the three tested characteristics with *A. hydrophila*, may still be readily differentiated from this species when the percentages of all three characteristics are assessed together. Of *A. sobria* isolates, 79% were nonsuicidal, 92.3% were aerogenic, and only 7.7% were esculin positive, as contrasted to 100% for *A. hydrophila*.

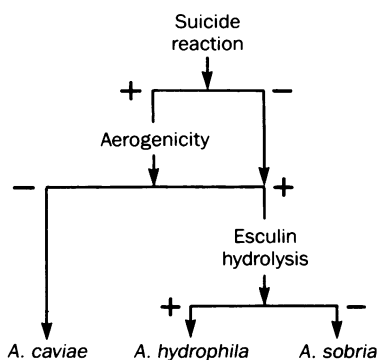


FIG. 1. Flow diagram for species identification of mesophilic *Aeromonas* species.

The suicide phenomenon in *Aeromonas* species is a stable and reproducible characteristic among clinical and environmental mesophilic aeromonads and is optimally demonstrated in static glucose broth culture at 30°C. The phenomenon, distinguished by spontaneous pelleting (autoagglutination), in conjunction with evaluation of aerogenicity and esculin hydrolysis, enables the rapid differentiation of mesophilic *Aeromonas* species without resort to multiple biochemical studies. The three tests require only two tubes of medium, are easy to assess visually, and are inexpensive. Species identification parallels that of more extensive biochemical testing and allows for the rapid assessment of the clinical significance of an isolate, especially when the isolate has been recovered from a gastrointestinal tract specimen of a symptomatic patient (5).

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