Incidence of Motile Aeromonas Species in Aquatic Environments of Rio De Janeiro; Brazil

MAURO SIRIMACO NEVES, MARLY PAIVA NUNES and ILVAN DELGADO RICCIARDI*

Instituto de Microbiologia da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil

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ABSTRACT

Fresh and salt water samples collected in Rio de Janeiro city were analysed for the presence of motile Aeromonas species. Twenty-six out of 50 aliquots analysed (52%) were positive for Aeromonas. One hundred strains were isolated from both environments (A. caviae, 60%; A. veronii, 14%; A. hydrophila, 1%; A. sobria, 1%; and Aeromonas sp., 24%). Minimal tests such as oxidase, motility, sensitivity to the vibriostatic agent 2,4-diamino-6,7-diisopropylpteridine, fermentation and gas from glucose, acetoin from glucose (Voges-Proskauer), lysine decarboxylase, ornithine decarboxylase, and esculin hydrolysis were sufficient to classify the majority Aeromonas strains into species. No Aeromonas was found in nonpolluted waters but, in contrast, both fresh and salt polluted waters showed a high incidence of isolates. Most of the Aeromonas strains analysed produced hemolysin and/or heat-stable enterotoxin. The latter was produced by 73% of the A. veronii isolates.

The genus Aeromonas contains three motile species, A. hydrophila, A. sobria, and A. caviae, and only one nonmotile species represented by A. salmonicida (25). In last years, three new species were reported but not yet listed in the Bergey’s Manual: A. media, another nonmotile species (1), A. veronii, a motile and positive ornithine decarboxylase species (16), and A. schubertii, a motile and negative mannitol species (15). The motile Aeromonas species occur in water (1,4,14,19,28) and some are pathogenic for animals (23,29). They may occasionally infect humans causing several diseases such as gastroenteritis (8,10,11,13), septicemia in immunosuppressed patients (20), skin infections (16,18), endocarditis (6), meningitis (27), and osteomyelitis (22). Water has an important role in direct transmission of motile Aeromonas species which may also contaminate foods (3,5,24). Therefore, seafood, particularly fish, contaminated with these species could expand risks of foodborne diseases.

The purpose of this study was to investigate the presence of motile Aeromonas in water environments of Rio de Janeiro city using a simple scheme for the isolation and biochemical classification of species. In addition, virulence factors such as production of hemolysin and heat-stable enterotoxin were investigated in the isolates.

MATERIALS AND METHODS

Standard strains

Standard strains used were obtained from Pasteur Institut, Lille, France. A. hydrophila (CIP 76.14), A. sobria (CIP 74.33), A. caviae (CIP 76.16), and A. salmonicida (CIP 63.4).

Water samples

Two 1000 ml water samples were collected from each of five aquatic environments of Rio De Janeiro city (3 salt and 2 fresh) during January and February 1988. They were collected in 1000 ml sterile flasks and transported immediately in an ice bath to the laboratory and processed within one h. At the laboratory, from each 1000 ml water sample, five 100 ml aliquots were separated aseptically for attempt of Aeromonas isolation.

Isolation

Water samples were centrifuged at 10000 x g for 20 min at 4°C (9). The sediment was directly subcultured onto a selective medium for Pseudomonas-Aeromonas (21), GSP agar (Merck), containing ampicillin (10mg/l) and deoxycholate citrate agar (BBL). Plates were incubated at 25°C for 48 h.

Identification

Mucoid yellow colonies from GSP agar or mucoid pink (lactose positive) and colorless colonies (lactose negative) from deoxycholate citrate agar were submitted to oxidase, motility, sensitivity to the vibriostatic agent (2,4-diamino-6,7-diisopropylpteridine), and fermentation of glucose tests. Gram negative rods, motile, oxidase positive, glucose fermentative, and resistant to the vibriostatic agent were considered as Aeromonas spp. To further classify into species level, the following tests were used: gas production and acetoin from glucose, lysine and ornithine decarboxylase, and esculin hydrolysis (16,26).

Hemolysin assay

Hemolysin was performed according to the method described by Hugh (17).
Enterotoxin assay

Enterotoxin was assayed in the infant mouse system (7).

Analysis of coliforms

Water pollution was estimated through the total and faecal coliform counts by using a 5 tube Most Probable Number technique (APHA, 1980/2).

RESULTS AND DISCUSSION

One hundred Aeromonas strains were isolated from the ten water samples. Ninety-three strains were isolated from salt water environments, whereas only seven strains were isolated from fresh water (Table 1).

TABLE 1. Distribution of Aeromonas species isolated from salt and fresh water environments at Rio de Janeiro city.

<table>
<thead>
<tr>
<th>Sampling Local</th>
<th>Type of water</th>
<th>Species isolated</th>
<th>No. of strains isolated</th>
<th>Total coliforms 100 ml</th>
<th>Faecal coliforms 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tijuca National Park</td>
<td>Fresh</td>
<td>-</td>
<td>-</td>
<td>2.0 x 10^3</td>
<td>&lt;10^4</td>
</tr>
<tr>
<td>Boa Vista River</td>
<td>Fresh</td>
<td>A. caviae</td>
<td>3</td>
<td>1.82 x 10^5</td>
<td>1.7 x 10^4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. veronii</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barra da Tijuca</td>
<td>Salt</td>
<td>-</td>
<td>-</td>
<td>&lt;10^4</td>
<td>&lt;10^4</td>
</tr>
<tr>
<td>Oceanian Waters</td>
<td>Salt</td>
<td>A. caviae</td>
<td>19</td>
<td>4.0 x 10^5</td>
<td>1.78 x 10^6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. veronii</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aeromonas sp.</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. hydrophila</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. sobria</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. caviae</td>
<td>38</td>
<td>1.0 x 10^4</td>
<td>3.6 x 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. veronii</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aeromonas sp.</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eighty-three strains were isolated from GSP agar containing ampicillin (10 mg/1) in contrast with only seventeen strains isolated from deoxycholate citrate agar.

In our scheme for the isolation of motile Aeromonas we propose the utilization of GSP agar containing ampicillin (10mg/1). Ampicillin is an important factor that increases the selectivity of this medium since Aeromonas strains are generally resistant to this antibiotic. Another important factor associated with GSP agar is the yellowish tonality of colonies produced from the utilization of starch present in the medium by Aeromonas strains. In contrast, it was very difficult to identify Aeromonas colonies in deoxycholate citrate agar because Aeromonas strains may ferment or not the lactose present in the medium.

The most common species isolated were A. caviae (60%) and A. veronii (14%). On the other hand only one strain of A. hydrophila and A. sobria was isolated from salt water samples. The predominance of A. caviae among motile Aeromonas species was reported by several investigators in both water and clinical specimens (8,12,30). Strains that could not be distinguished with our biochemical scheme into species, were classified as Aeromonas spp.

The highest numbers of motile Aeromonas isolated were obtained in water environments where the coliform counts were also high. No motile Aeromonas was found in nonpolluted water environments (Table 1). These results agree with several reports which suggest that there is a positive correlation between the number of Aeromonas species and coliform counts (19).

The source of motile Aeromonas in Rio de Janeiro water environments is unknown. Whether they are originated from human or animal intestinal flora deserves further investigation. However, the main point that arises is the obvious public health implication of water resources contaminated with Aeromonas. In our laboratory, we recently isolated a strain of Aeromonas from an infected leg wound originated in a salt lake bath of a 26 year-old female (unpublished data).

The classification of motile Aeromonas into species is complex. Popoff and Véron (26) proposed some biochemical...
cal tests to classify *Aeromonas* spp. Gram negative rods, motile, oxidase positive, glucose fermentation, and resistance to the vibriostatic agent were considered as *Aeromonas* spp. To classify these strains into species, we used production of gas from glucose, production of acetoin from glucose (Voges-Proskauer), lysine and ornithine decarboxylase, and esculin hydrolysis tests *(16, 26).* According to this procedure we were able to settle seventy-six *Aeromonas* strains into species, whereas twenty-four strains could not. Additional studies should be performed to evaluate the possibility of new *Aeromonas* species among these strains.

Virulence factors produced by the isolates are given in Table 2. Isolates presenting both virulence factors (hemolysin and enterotoxin) were observed among all but one of the species identified. The exception, *A. sobria* is not relevant since we had only one isolation. On the other hand, it is relevant the detection of *A. veronii* enterotoxin-producing strains. As far as we know this characteristic of pathogenicity was not yet described for *A. veronii.*

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**REFERENCES**