RESEARCH NOTE

Behaviour of *Aeromonas* spp. after Animal Passage

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The *Aeromonas* genus have been considered as important infectious agents in humans and animals (JM Janda 1991 *Clin Microbiol Reviews* 4: 397-410). The biological characteristics such as, toxins production (e.g. hemolysins, cytotoxins and enterotoxins) and also cell-associated features, that appear to play important role in infectious processes in humans and animals, have been studied in the attempt of elucidate patogenicity of the different *Aeromonas* species (Janda *loc. cit*.). Evidences indicate that the animal passage may influence in the expression of biological characteristics in many organisms such as, *Plesiomonas shigelloides* (SC Sanyal et al. 1980 *J Med Microbiol* 13: 401-409) and *Vibrio cholera* O1 biotype El Tor (A Tikoo et al. 1994 *J Med Microbiol* 40: 246-251). DV Singh and SC Sanyal (1992 *J Med Microbiol* 37: 262-267) reported that passage through rabbit intestines may control the expression of the genes responsible for toxins production in *Aeromonas* spp.

In this communication we report on the alteration of the hemolytic and enterotoxigenic character and also surface characteristics in one strain of *Aeromonas* isolated from environment, after animal passage by endovenous route.

Four samples of *Aeromonas* were used in this study, three from polluted estuary water (*A. caviae* - 030, *Aeromonas* sp. - 057, *A. trota* - 058) and one from drain treatment station supplied by Fundação Oswaldo Cruz, Rio de Janeiro (*A. hydrophila* - T336). Those strains were maintained in nutrient agar (NA) with 1% of NaCl (FW Hickman-Brenner et al. 1987 *J Clin Microbiol* 25: 900-906) at room temperature.

All samples were tested with regar to hemolysin production, through β hemolysis zones around the colonies in rabbit blood agar with 5% (v/v) of erytrocytes and the haemolitic activity was analyzed with the metodology described by M Cumberbatch et al. (1979 *Infect Immun* 23: 829-837). The suckling mouse test (WA Dean et al. 1972 *Infect Dis* 125: 407), was used for enterotoxin detection, the autoagglutination capacity for self pelleting (SP) and for precipitation after boiling (PAB) as described by JM Janda et al. (1987 *Infect Immun* 55: 3070-77). The hidrofobic profile by phase partitioning with hydrocarbon solvents described by M Rosenberg et al. (1980 *Fems Microbiol Lett* 9: 29-33).

Strains for animal experiments were cultivated in 5ml of BHI (OXOID) at 28°C for 5 hr. After this period, samples were centrifugated at 3000 x g by 10 min and resuspended in sterile saline (0.85%).

Six groups of two male albinic mouses BALB/C, 4-6 weeks old, were inoculated by endovenous route with 0.5 ml of the bacterial suspension with 10^{10} cells per ml. The control group received 0.5 ml of sterile saline (0.85%). All strains were recovered from spleen 24 hr after inoculation.

It was observed that, from the four enviroment strains, just *A. hydrophila* T336, from the drain water station treatment, that had not produced toxins in the early tests, became hemolytic with high titre and produced enterotoxin after inoculation in mouse (Table). The autoagglutination capacity expressed by SP,PAB* phenotype (did not make self pellet and precipitate after boiling) considered by Janda (*loc. cit*) as a virulence marker for *Aeromonas* was observed in *A. hydrophila* T336 over the increase of it’s hidrofobic capacity after animal passage.

According to Singh (*loc. cit*) strains of *Aeromonas* which did not produce toxins during the initial experiments became toxigenic after one to three consecutive passages through rabbit intestines, thus suggesting that *Aeromonas* are potentially enterotox and hemolytic despite the species and origin of strain. Such behaviour may be a result from the existence of a repression-depression phenomenon controlling expression of a toxin gene, depending on a passage through the gut of a susceptible host. However, according to our ex-

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periments, the changes in the hemolytic and enterotoxigenic behaviour also occurs after intra-
venous inoculation together with alterations in the autoagglutination capacity and hidrofobicity, as seen with *A. hydrophila* T336. It was observed that the animal passage may influence in the expres-
sion of those characteristics despite of the inocula-
tion route.

**TABLE**
Characteristics of *Aeromonas* spp. strains before and after animal passage

<table>
<thead>
<tr>
<th>Strains</th>
<th>Hemolitic activity</th>
<th>Enterotoxin</th>
<th>Autoagglutination</th>
<th>Hidrofobic capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. caviae</em> (030)</td>
<td>/-</td>
<td>/-</td>
<td>SP^-PAB^-PAB^-</td>
<td>58.0/62.5</td>
</tr>
<tr>
<td><em>Aeromonas</em> sp. (057)</td>
<td>/-</td>
<td>/-</td>
<td>SP^-PAB^-PAB^-</td>
<td>58.0/58.5</td>
</tr>
<tr>
<td><em>A. hydrophila</em> (T336)</td>
<td>-/1024</td>
<td>/-</td>
<td>SP^-PAB^-PAB^-</td>
<td>70.0/10.5</td>
</tr>
<tr>
<td><em>A. trota</em> (058)</td>
<td>8/8</td>
<td>/-</td>
<td>SP^-PAB^-PAB^-</td>
<td>12.0/22.5</td>
</tr>
</tbody>
</table>

- negative results ; + positive results; SP: self pelleting ; PAB: precipitation after boiling; %: adherence percentage face to xilen apolar solvent