RESEARCH NOTE

Behaviour of *Aeromonas* spp. after Animal Passage

VC Almeida+ , MP Nunes

Laboratório de Zoonoses, Departamento de Microbiologia Médica, Instituto de Microbiologia, CCS-UFRJ,Caixa Postal 68040, 21941-590 Rio de Janeiro, RJ, Brasil

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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 instestines 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 supplyed by Fundação Oswaldo Cruz, Rio de Janeiro (*A. hydrophila* - T336). Those strains were maintend 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 eritrocytes 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 preciptation 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 Rosenburg et al. (1980 *Fems Microbiol Lett 9:* 29-33).

Strains for animal experiments were cultivated in 5ml of BHI (OXOID) at 28ºC for 5 hr. After this period, samples were centrifugated at 3000 x g by 10 min and ressuspended 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 enterotoxic and hemolytic despite the species and origin of strain. Such behaviour may be a result from the existence of a repression-derepression 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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+Corresponding author. Fax:55-21-270.8793
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periments, the changes in the hemolytic and enterotoxigenic behaviour also occurs after intravenous 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 expression of those characteristics despite of the inoculation route.

TABLE

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

Strains	Hemolitic activity	Enterotoxin	Autoagglutination	Hidrofobic capacity (%)
A. caviae (030)	-/-	-/-	SP^-PAB^-/SP^-PAB^-	58.0/62.5
Aeromonas sp. (057)	-/-	-/-	SP^+PAB^+/SP^+PAB^+	58.0/58.5
A. hydrophila (T336)	-/1024	-/+	SP^-PAB^-/SP^-PAB^+	70.0/10.5
A. trota (058)	8/8	-/-	SP^-PAB^-/SP^-PAB^-	12.0/22.5

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