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ORIGINAL ARTICLE

Virulence factors *of A. caviae* strains isolated from acute diarrheic disease in Cuba

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ABSTRACT. Fifty *Aeromonas caviae* strains from intestinal infection in different Cuban provinces were identified by the Aerokey II method and virulence factors were investigated. The strains did not produce haemolysins but other exoenzymes such as proteases, lipases, and DNases; additionally, all isolates adhered to the HEp-2 cell line by the Carrello method and this did not correlate with other virulence factors presence which demonstrates that the haemolysin phenotypic expression is not necessary for these strains to be pathogenic and that pathogenicity is multifactorial, each strain expressing at least one virulence factor.

Key words: *A. caviae*, virulence factors.

RESUMEN. Se identificaron 50 cepas de *Aeromonas caviae* aisladas de infección intestinal en diferentes provincias de Cuba con el método Aerokey II y se investigaron los factores de virulencia. Las cepas no producen hemolisinas pero sí otras exoenzimas como proteasas, lipasas y DNasas además todos los aislamientos se adhieren a la línea celular HEp-2 con el método de Carrello y ésta no correlacionó con la presencia de otros factores de virulencia, lo que demuestra que la expresión fenotípica de la hemolisina no es necesaria para que éstas sean patógenas y que la patogenicidad es multifactorial expresando cada cepa al menos un factor de virulencia.

Palabras clave: *A. caviae*, factores de virulencia.

INTRODUCTION

Members of the *Aeromonas* genus are widely distributed through nature and are considered important fish and other cold-and warm-blooded organisms pathogens since their first isolation in 1893.[1] For some years the significance of this genus as a human diarrhea-causing agent was controversial, but currently this fact is not in doubt.[11]

Several studies have demonstrated that the *Aeromonas* species pathogenic mechanism is complex and probably multifactorial since species of this genus hold a great variety of virulence factors.[9] However, the particular combination of such factors to explain their etiological role in human disease is still unknown. *Aeromonadales* produce exoenzymes which are important virulence associated factors, involving: proteases, lipases, cytotoxin, chitinases, DNases, amylases, and haemolysins; as well as adhesins.[8,10]

Some studies suggest the expression of virulence factors is linked to the strain source and to the culture conditions. *Aeromonas hydrophila* and *Aeromonas veronii* bt sobria present total hemolysis zones as hemolysis patterns, while *Aeromonas caviae* is not hemolytic according to some authors.[6,14]

However, in the last years beta hemolytic *A. caviae* strains isolations in several countries have been reported[4,13,15] and given the significance of this aerolysin as a virulence factor, the isolation of cytolysin producing strains from clinic samples of patients with acute diarrheic disease is of interest because by the virulence factors determination it can be assessed which factors are being expressed during the disease course.

MATERIAL AND METHODS

A total of 50 *Aeromonas* spp. strains, isolated from stools of 5 years old children with acute diarrheic disease (ADD) were studied. Samples were originary from all the country Hygiene and Epidemiology Centers (HEC), submitted to the "Laboratorio Nacional de Referencia Enfermedades Diarréicas Agudas" of the "Pedro Kourí" Institute (IPK), between 1998-1999 and were identified as *A. caviae* by the Aerokey II scheme.[3] 7 virulence factors were investigated: haemolysis,[2] CAMP-like factor,[5] lecitinase, gelatinase, and DNase[1] activities, hemagglutination,[12] and adherence to the HEp-2 cell line.[4]

RESULTS

Results obtained in the determination of virulence associated factors for the 50 *A. caviae* isolated strains are shown in Table 1. The 50 strains were negative to hemolysis (100%), and did not exhibit cAMP factor in aerobic nor anaerobic conditions (100%). Gelatinase activity was present in 29 strains (58%), lecitinase in 44 strains (88%), and DNase in 33 strains (66%).

From the 50 investigated strains, only 4 were positive for hemagglutination with group O human erythrocytes,

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Table 1. Virulence factors in *A. caviae* strains.

Strain No.	Isolation precedence Cuban Province	Virulence Factor Determined						
		Hemolysis	CAMP Factor	Gelatinase	Lecitinase	DNase	Hemagglutination	Adherence
1	Ciego de Avila	-	-	-	+	-	-	M
2	Matanzas	-	-	-	+	-	-	M
3	Guantánamo	-	-	-	-	+	-	H
4	Santiago de Cuba	-	-	-	+	-	+	H
5	Matanzas	-	-	-	+	-	-	H
6	Sancti Spiritus	-	-	-	+	-	-	H
7	Las Tunas	-	-	+	-	-	-	H
8	Granma	-	-	-	+	-	-	H
9	Guantánamo	-	-	-	+	-	-	H
10	Santiago de Cuba	-	-	-	+	+	-	H
11	Villa Clara	-	-	-	+	-	-	H
12	Sancti Spiritus	-	-	-	-	+	-	H
13	Isla de la Juventud	-	-	-	+	+	-	H
14	Provincia La Habana	-	-	-	+	+	-	H
15	Pinar del Río	-	-	-	+	+	-	H
16	Guantánamo	-	-	+	+	-	-	H
17	Ciudad Habana	-	-	+	-	+	-	H
18	Santiago de Cuba	-	-	+	+	-	-	H
19	Las Tunas	-	-	+	+	-	+	H
20	Holguín	-	-	+	+	-	-	H
21	Granma	-	-	+	+	-	-	H
22	Las Tunas	-	-	-	+	+	-	H
23	Santiago de Cuba	-	-	+	+	-	-	H
24	Santiago de Cuba	-	-	-	+	+	-	H
25	Santiago de Cuba	-	-	-	+	+	-	H
26	Camagüey	-	-	-	+	+	-	M
27	Santiago de Cuba	-	-	-	+	+	-	H
28	Isla de la Juventud	-	-	+	+	+	-	H
29	Matanzas	-	-	+	+	+	+	H
30	Santiago de Cuba	-	-	+	+	+	-	H
31	Isla de la Juventud	-	-	+	+	+	-	H
32	Granma	-	-	+	+	+	-	M
33	Las Tunas	-	-	+	+	+	-	L
34	Villa Clara	-	-	+	+	+	-	H
35	Holguín	-	-	+	+	+	-	H
36	Pinar del Río	-	-	+	+	+	-	H
37	Camagüey	-	-	+	+	+	-	H
38	Matanzas	-	-	+	+	+	-	H
39	Santiago de Cuba	-	-	+	+	+	-	H
40	Guantánamo	-	-	+	+	+	-	H
41	Isla de la Juventud	-	-	+	+	+	-	H
42	Ciudad Habana	-	-	+	+	+	-	H
43	Santiago de Cuba	-	-	+	+	+	-	H
44	Matanzas	-	-	+	+	+	-	H
45	Camagüey	-	-	+	+	+	-	H
46	Santiago de Cuba	-	-	+	+	+	+	H
47	Las Tunas	-	-	+	+	+	-	H
48	Santiago de Cuba	-	-	+	+	+	-	M
49	Ciego de Ávila	-	-	-	-	-	-	H
50	Camagüey	-	-	-	-	-	-	L

H: High adherence (> 20 bacteria per cell), **M:** Moderate adherence (5 – 20 bacteria per cell), **L:** Low adherence (< 5 bacteria per cell), **-** no expressed factor, **+** expressed factor.

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which in turn were D-mannose sensitive; and also adhered to the HEp-2 cell line.

The 46 remaining strains did not hemagglutinate human red cells and adhered to the HEp-2 cell line at different extents which were denominated as high, moderate, and low adherence level, 39 strains were highly adherent, 5 moderately adherent, and 2 showed a low adherence level.

From the 50 investigated strains, 38 (76%) exhibited 3 or more virulence factors, 10 (20%) showed 2 factors, and 2 (4%) showed only one virulence factor.

DISCUSSION

A great variety of work referent to the *Aeromonas* genus enteropathogenicity has been done[6,7,14] and it has been attributed to the exoenzymes, exotoxins, and adhesins production, although in isolates from clinic samples the exact mechanism of each virulence associated factor is unknown.[8]

In Cuba, there were isolated 50 *A. caviae* strains from stools of children with acute diarrhea which produced exoenzymes and adhesions. The determination of such virulence associated factors in the 50 strains indicated that none of them produced hemolysins; but there were important exoenzymes such as: proteases, lipases, and DNases. Furthermore, all were adherent to the HEp-2 cell line and adherence was independent from human erythrocyte hemagglutination which demonstrates that the hemolysin phenotypic expression is not necessary for them to be pathogens and that pathogenicity is multifactorial, being expressed at least one virulence factor in each strain.

CONCLUSIONS

Strains identified as *A. caviae*, isolated from patients with Acute Diarrheic Disease in Cuba, have at least one virulence factor, which supports the fact that their pathogenicity is multifactorial. The HEp-2 cells adherence assay is a useful model to investigate enteropathogenicity in this strains. Their ability to adhere to the HEp-2 cell line was independent from the hemagglutination observed in human erythrocytes, suggesting that there are probably differences at bacterial ligand level.

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