

Study of some virulence factors of *Aeromonas* Spp. isolated from stool samples of children with diarrhea

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Summary

Three hundred samples of stool were collected from children, who are suffering from diarrhea, during the period from October to December 2016 at Fatima Al Zahra and Shaheed al-Sadr hospitals. All bacterial isolates have been submitted to the cultural, microscopical, biochemical examinations, and Vitek 2 system identification to identify *Aeromonas* isolates. All the isolates under study were tested for their ability to the production of certain factors associated with virulence. It has been concluded that 12 (4%) of collected samples were positive to *Aeromonas* Spp. The virulence factors of *Aeromonas* spp. were detected and showed that all the isolates have the ability to produce haemolysin, protease, lipase, phospholipase, gelatinase enzymes, and biofilm on Congo red agar.

Keywords: Diarrhea, Virulence factors, *Aeromonas* Spp.

Introduction

It had been documented that *Aeromonas* Spp. received increasing concern as an opportunistic pathogen since it is linked with diarrhea and extra intestine infections in human mainly in children and peoples with impaired immune system (1 and 2). *Aeromonas* Spp. is negative for gram reaction, motile, facultative anaerobic, rod, and positive for oxidase bacteria of the lately allocated Aeromonadaceae family (3 and 4). These bacteria related with two kinds of gastroenteritis, the first one is similar to cholera which characterized by rice- watery diarrhea and the other is dysenteric type that causes blood and mucus appearance in the stool. Dysenteric gastroenteritis is the most severe one (5). The pathogenic mechanisms of these bacteria are complicated and multifactorial (6), as it was attributed to many virulence factors, like cell components: lipopolysaccharide, proteins of outer membrane, pili and flagella, kind III secretion system which play an important role in adhesion, moreover extracellular elements like lipase, phospholipase, haemolysin, protease, and biofilm formation that appear to play a main role in the pathogenesis (7). The objective of this study is identification of *Aeromonas* spp. from patients suffering from diarrhea and detection of some their virulence factors such as: haemolysin, lipase, protease,

gelatinase, phospholipase enzymes, and biofilm formation.

Materials and Methods

A total of (300) stool samples were collected between October and December 2016 from children suffering from diarrhea, before antibacterial therapy, in Fatima Al Zahra Hospital and Shaheed Al-Sadr Hospital. The characteristics morphology of the colonies were verified on the *Aeromonas* isolation agar (HiMedia) supplemented with ampicillin for initial identification of *Aeromonas* Spp. (8). Bacterial smear was prepared from each isolate and stained by Gram stain for initial microbial identification of *Aeromonas* Spp. (9). Biochemical tests of *Aeromonas* Spp. were tested for oxidase, catalase, Simmons Citrate, Kligler Iron agar, and Indole tests (8).

Vitek 2 system (Biomerieux, France) is used to verify the identification of *Aeromonas* isolates in this study. This examination was performed at Al-Shaheed Al-Sadr Hospital. The strains were tested for β -hemolytic activity on blood agar base contain 5% of blood. A loopful from the bacterial growth was cultured on blood agar by streaking way, incubated at 37°C for 24 hrs. The existence of a clear zone around the colonies is a sign of hemolytic activity (10). Heart infusion agar was prepared and autoclaved, and then addition of 1% of tween 20 that sterilized by

0.04 μm Millipore, after that the medium poured into sterile plates. Turbid zone around colonies considered a positive result of lipase (11).

Skim milk agar was used to check protease production. A loopful from bacterial growth was cultured on skim milk agar by streaking, incubated at 37°C for 24 hrs. The incidence of a clear zone around the colonies indicates protease production (12). The specific media for detection phospholipase, was inoculated with a single colony of bacterial culture from nutrient agar, incubated for 24-72 hrs. at 37°C. The presence of opaque zone around the colonies considered a positive result of phosphates (12). Gelatinase was checked by the conventional method. The tubes of medium containing 1.3 g of gelatin for each 100 ml of nutrient medium were inoculated with a single colony of bacterial growth, then incubated at 37°C for 24 hrs. The gelatin liquefaction was tested by comparing with a control tube. The gelatin was liquefied as the result of gelatinase production (11).

Results and Discussion

Out of three hundred (300) stool samples there were 12 (4%) samples positive for *Aeromonas* bacteria. The current result of this study agrees with Lobna (12) that reported 3.15% (11 isolates) of *Aeromonas* from 349 stool samples in Babylon province. Furthermore, it also agreed with other studies, 2.7% (13 isolates) from 479 stool samples in Babylon province (13), 3.12% (4 isolates) from 128 stool samples in Nigeria (14), and 6.6% (27 isolates) from 408 stool samples were positive for *Aeromonas* Spp. in Brazil (15). These isolates are oxidase positive and gram negative. The colonies of *Aeromonas* Spp. appear as pale on the MacConkey agar because it is unable to ferment lactose sugar with diameter ranged from (2-3) mm, these typical characteristics as described previously by others (16 and 17). Accordingly, *Aeromonas* Spp. consists of straight, bacilli with rounded ends, it occurs singly or in pairs, and rarely as short chains. The biochemical tests of *Aeromonas* Spp. showed its ability to grow (Alk/Acid) on kligler iron agar and positive for simmone's citrate, indole, catalase,

and oxidase tests but does not produce urease and H₂S. These biochemical characters were agreement those characteristics mentioned by many authors (18-20). Moreover, the vitek 2 system confirmed the positive results obtained from the prior tests (Table, 1). Other studies (21 and 22) were used vitek 2 system as effective biochemical tests for identification of *Aeromonas* Spp. The analytical profile index had showed percentage probability of 93% of identification.

The isolated *Aeromonas* Spp. were positive for haemolysin production, type beta (β - haemolysin) (Fig. 1 A). These results were noticed in (23), the later authors noted that the fourteen isolates of *Aeromonas hydrophila* were β -haemolytic and two isolates were not, also there were similar findings observed by others (24). There were some reports were indicated that β -haemolysin, which is produced from *Aeromonas* Spp., has a close relationship between production of toxins in the cell-producing enzyme, and a toxin called cytotoxic factors (25 and 26). Also, these isolates have the ability to produce biofilm on Congo red agar (Fig. 1 B). Similar results were observed in Brazil (27) they stated that the all isolates of *Aeromonas hydrophila* produce biofilm. Biofilm production is attributed to persistent of infections and in charge of up to 30 % of *Aeromonas* gastroenteritis infections (28). *Aeromonas* spp. had the ability to produce protease and lipase as shown in (Fig.2 A and B). The isolated *Aeromonas* have the ability to produce protease when inoculated on skim milk agar for 24 hrs. at 37°C and also have the ability to break fats by lipase enzyme when inoculated on Tween agar. These results were agreeing with others (29 and 30). The *Aeromonas* spp. are lipase producers, also this agreement with others (31). The bacterial isolates were able to produce phospholipase and gelatinase enzymes as shown in (Fig. 3 A and B). Gelatinase acts on hydrolysis of gelatin, which leads to the loss of gels as it converted it to liquid material (32). The isolated *Aeromonas* Spp. from clinical cases, produce phospholipase which is involved in different pathogenic processes related to intestinal harm (33 and 34).

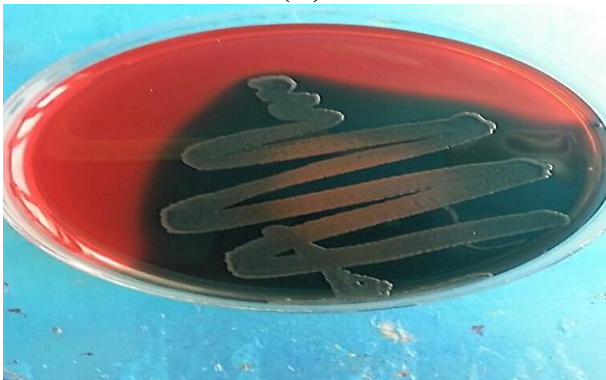
Table 1: Vitek 2 system reactions of one isolates of *Aeromonas* Spp.

Identification Information		Analysis Time:	6.00 hours	Status:	Final
Selected Organism		93% Probability	Aeromonas		
ID Analysis Messages		Bionumber:	5627713150500211		

Biochemical Details																	
2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			



(A)



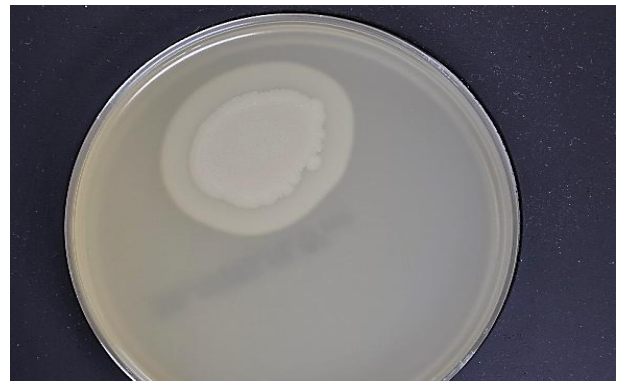
(B)

Figure 1: (A) β -haemolysis by *Aeromonas* spp. (B) - Biofilm production by *Aeromonas* Spp.

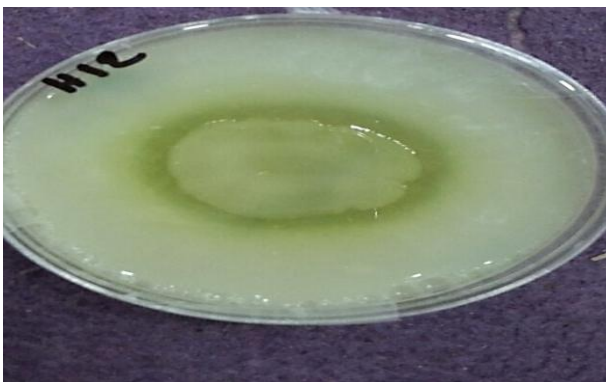


(B)

Figure 2: (A) Protease production by *Aeromonas* spp. (B) Lipase production by *Aeromonas* Spp.



(A)



(A)



(B)

Figure 3: (A) Phospholipase production. (B) Gelatinase production by *Aeromonas* Spp.

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دراسة بعض عوامل الضراوة لبكتريا *Aeromonas* Spp. المعزولة من براز الأطفال المصابين بالإسهال

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الخلاصة

جمعت 300 عينة من أطفال يعانون من الإسهال من مستشفى الزهراء للولادة ومستشفى الشهيد الصدر العام في مدينة بغداد وذلك للمدة من من تشرين الأول ولغاية كانون الأول 2016. زرعت العينات على أوساط زرعية مختلفة لغرض التحري عن جراثيم *Aeromonas* Spp. وذلك عن طريق الفحوصات المجهرية والكيموحيوية فضلاً عن استعمال نظام 2 vitek. واختبرت عزلات *Aeromonas* Spp. في الكشف عن قابليتها على إنتاج بعض عوامل الضراوة. أظهرت نتائج الدراسة أن نسبة عزل الجراثيم كانت 4% (12) كما لوحظ أن جميع هذه العزلات لها القابلية على إنتاج السم الحال للدم الهيمولاييسين والخميرة الحالة للبروتين البروتينيز والخميرة الحالة للدهون اللايبينز والخميرة الحالة للدهون المرتبطة بالفوسفور الفوسفولاييبينز والخميرة الحالة للجيلاتين الجيلاتينيز فضلاً عن قدرتها على تكوين الغشاء الحيوي (البايوفلم).

الكلمات المفتاحية: الإسهال، عوامل الضراوة، بكتريا *Aeromonas*.