Incidence of *Aeromonas* species isolated from different food sources and water.

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ABSTRACT

A total number of 350 random samples of raw milk, pasteurized milk, dairy products, tap water and water of udder wash were collected from markets in port- Said Province and examined for prevalence of ***Aeromonas*** species using starch ampicillin medium, The incidence of ***Aeromonas*** species isolated from raw milk, pasteurized milk, kariesh cheese, dmietta cheese, refrigerated white cheese, tap water and water of udder wash were 58%, 26%, 70%, 48%, 40%, 16% and 68%, respectively. The total number of mesophilic ***Aeromonas*** isolates from 350 examined samples was 202 isolates of which 104 (51.5%) isolates were ***A. hydrophila***, *60* (29.7%) isolates were ***A. caviae***, 25 (12.4%) isolates were ***A. sobria*** and 11 (5.4%) isolates were ***A. schubertii***. The highest number of isolates (42) were recovered from water of udder wash samples, while the lowest number of isolates were recovered from pasteurized milk and tap water samples. Result of antibiotic sensitivity test revealed high degree of sensitivity towards chloramphenicol, amikacin, ceftriaxone and ciprofloxacin, resistant to ampicillin and colistin sulphate. Result of agarose gel electrophoresis revealed that ***A. hydrophila*** , ***A. caviae*** and ***A. schubertii*** have only one plasmid DNA with molecular weight 2.8 kbp While ***A. sobria*** was negative for plasmid.

INTRODUCTION

Motile ***Aeromonades*** have been included in the list of bacterial pathogens ***(Janda et al, 1983*** *and* ***Holmberg et al, 1986****).* ***Buchanan (1984)*** indicated that the ***Aeromonades*** form a group of pathogens, which are emerging as food borne organisms of increasing importance.

Aeromonades are gram-negative rod shape, motile by single polar flagellum , can be isolated from a variety of food, including meat, poultry, milk, milk products, fish, shell fish and vegetables ***(Melas et al, 1999)***. And widely distributed in the aquatic environment, including raw and processed drinking water ***(Holmes et al, 1996).*** The presence of food borne pathogens in milk is due to direct contact with

contaminated sources in the dairy farm environment, excretion from the udder of an infected animal or contamination during the processing of milk products ***(Oliver et al, 2005)*** ***Aeromonas hydrophila*** is the most important species causing disease in humans. They can produce virulence factors including a relatively heat stable cholera-like enterotoxin and heat labile cytotoxic enterotoxin and is recognized as a potential cause of food associated out breaks of gastroenteritis and as etiological agent of acute diarrheal in particular among children ***(EL-Shenaway*** *and* ***Marth, 1990)***. Moreover, ***Aeromonas*** caused other human infection including septicemia, meningitis, wound and eye infection and urinary tract infection ***(Abbott and Janda, 2010).*** The aim of this work was isolation and identification of ***Aeromonas*** species from milk, milk products and water, Antibiotic sensitivity test for ***Aeromonas*** isolates, and investigating the plasmid profile of isolates by agarose gel electrophoresis.

MATERIALS AND METHODS

1-Samples:

A total of 350 samples of raw milk , pasteurized milk, milk products including (kareish cheese, damietta cheese and refrigerated white cheese), tap water and water of udder wash (50 samples for each) were randomly collected from supermarkets, street pedlars, dairy shops , markets and farm in Port-Said province.

All of the above samples were collected under aseptic condition and transferred immediately to Port Said bacteriology Lab in icebox.

2-Bacteriological examination:

a- Isolation and identification of *Aeromonas*:

Ten ml of each samples (10gm of kariesh cheese, damietta cheese and white refrigerator cheese) were homogenized with 90 ml alkaline peptone water (PH8.3) for 2 min, then incubated for 24hr at 30ºC ***(Villari et al, 2000)***. A loopful from alkaline peptone water was subsequently plated on the surface of starch ampicillin agar plate and incubated for 48 hr at 30ºc. Typical yellow colonies of ***Aeromonas*** species were purified on tryptone soya agar then stained by gram stain ***(A.P.H.A., 1992)*** and confirmed on the basis of the following test: Oxidase test, resistance to vibriostatic agent o/129, esculin hydrolysis, sugar fermentation and gas production, indole production and voges- proskaur test. Identification was performed on isolates according to the criteria of ***Krieg and Holt (1984)*** and Aerokey II of ***Carnahan et al (1991).***

b- Antibiotic Sensitivity test for the isolated ***Aeromonas*** from milk, some milk products and water was done by disc diffusion technique ***(Finegold and Martin, 1982).***

c- Plasmid profile technique was used for detection of ***Aeromonas*** plasmid and estimation plasmid

DNA molecular weight ***(Sambrook et al, 1989)***.

RESULTS AND DISCUSSION

Table (1): *Prevalence and distribution of Aeromonas species isolated from examined samples.*

Type of samples	No. of exam. samples	No of positive samples		No of *A.* isolates		*Aeromonas* species							
						A. hydrophila		*A. caviae*		*A. sobria*		*A. schubertii*	
		No	%	No	%	No	%	No	%	No	%	No	%
1-Raw milk	50	29	58%	35	70%	19	54.28 %	9	25.7%	4	11.4 %	3	8.5%
2- Pasteurized milk	50	13	26%	19	38%	9	47.3 %	5	26.3%	3	15.7 %	2	10.5 %
3-Kariesh cheese	50	35	70%	38	76%	19	47.5 %	13	32.5%	5	12.5 %	1	2.5%
4- Damietta cheese	50	24	48%	27	54%	15	55.5 %	7	25.9%	4	14.8 %	1	3.7%
5- Refrigerated White cheese	50	20	40%	24	48%	7	29.1 %	10	41.6%	5	20.8 %	2	8.3%
6-Tap water	50	8	16%	15	30%	8	35.3 %	7	46.6%	-	-	-	-
7- Water of udder wash	50	34	68%	42	84%	27	64.2 %	9	21.4%	4	9.5%	2	4.7%
Total	350	163	46.6 %	200	57.1%	104	51.5 %	60	29.7%	25	12.4 %	11	5.4%

Bacteriological Examination of 350 samples of raw milk, pasteurized milk and milk products including kariesh cheese, damietta cheese and refrigerated white cheese, tap water and water of udder wash.(50 samples for each) as shown in Table (1) revealed prevalence of ***Aeromonas*** species in the collected samples: 29 (58%) out of 50 raw milk samples were positive for ***Aeromonas*** species. While 13 (26%), 35 (70%), 24 (48%), 20 (40%) of pasteurized milk, kariesh cheese, damietta cheese, and refrigerated white cheese, respectively, were positive for isolation of ***Aeromonas*** species. The highest positive samples were observed in those from water of udder wash 34 (68%) while the lowest positive sample were recorded in tap water 8 (16%). This higher percent in water of udder wash could be attributed to the wide presence of the organism in nature, in feeds, water, faeces, soil and equipment used for milking. In addition, water used for washing the udder and milking equipment is consider as a significant source of contamination and presence of motile ***Aeromonads*** in high level in raw milk because the organism can contaminate the udder via the teat, then multiply in mammary tissue

and subsequently discharged in milk ***(EL-Shemawy and Marth, 1990)***.

The presence of mesophilic ***Aeromonas*** species in the pasteurized milk might be due to inefficient pasteurization or post pasteurization contamination during packaging of pasteurized milk as a result of unhygienic conditions during manufacture ***(Yucel et al, 2005)***.The evidence of ***Aeromonas*** species in kariesh cheese samples due to the fact that milk used in manufacture of kariesh milk usually didn't exposed to boiling or any other heat treatment. In addition due to its low salt content. In addition ***Bomo et al (2004)*** attributed the presence of ***Aeromonas*** species in water to its colonization of drinking water distribution system and production of biofilms that increase ***Aeromonas*** resistance to antimicrobial or disinfectants.

The result tabulated in Table (1) demonstrated the total number of ***Aeromonas*** isolates and frequency distribution of ***Aeromonas*** species among different sample. 35 isolates of ***Aeromonas*** were recovered from raw milk samples identified as higher percent of ***A. hydrophila*** (19) (54.28%) while lower percent of ***A. schubertii*** (3) (8.5%). Nearly similar results were recorded by ***Melas et al (1999)*** *and* ***Henedak (2002)***. In contrary ***Akan et al (1996)*** could isolate ***A. hydrophila*** in 65.3% followed by 30.4% of ***A. sobria*** and ***A. caviae*** in 4.3% from raw milk samples.

Concerning to incidence of ***Aeromonas*** species isolated from pasteurized milk as shown in Table (1). It was recognized that ***A. hydrophila*** occupied the first position 47.3% while ***A. schubertii*** which could be isolated only in 10.5%. This result is nearly in agreement with those reported by **Abou-** ***Ayana and Gamal EL Deen (2010).***

Examination of kariesh cheese samples revealed isolation of 40 isolates: 47.5% belong to ***A. hydrophila***, 32.5% belong to ***A. caviae***, 12.5% belong to ***A. sobria*** and 2.5% only for ***A. schubertii***. These findings are similar to those reported by ***Effat et al (2000)*** and ***Nahla (2006)***. While lower incidence was reported by ***EL-Prince (1998)*** *and* ***Enany et al (2004)***.

Results of damietta cheese revealed that ***A. hydrophila*** (55.5%) was the most predominate species in the isolated samples. followed by ***A.caviae*** (25.9%) then ***A. sobria*** (14.8%). While the least frequently occurring species was ***A. schubertii*** (3.7%). On the other hand ***EL-Prince (1998)*** could isolate ***A. caviae*** followed by ***A. hydrophila*** , ***A. sobria*** from damietta cheese.

Regarding to incidence of ***Aeromonas*** species from refrigerated white cheese. As shown in Table (1) ***A. caviae*** showed highest incidence (41.6%) followed by ***A. hydrophila*** (29.1%), ***A. sobria*** (20.8%) and ***A. schubertii*** (8.3%).

The results of incidence of ***Aeromonas*** species in Table (1) revealed that ***Aeromonas*** isolates that could be recorded from tap water samples distributed were between ***A. hydrophila*** (35.3%) and ***A. caviae*** (46.6%). These results nearly are in agreement with those reported by ***Burke et al (1984 a&b)*** *and* ***Maria et al. (2008)***. In contrary ***Manuel et al (2009)*** could isolate ***A. caviae*** and ***A. media*** only.

Concerning to incidence of ***Aeromonas*** species in water of udder washing, 42 ***Aeromonas*** isolates were identified as ***A. hydrophil***a (64.2%), ***A. caviae*** (21.4%), ***A. sobria*** (9.5%) and *A.* ***schubertii*** (4.7%).

Abeyta and Wekell (1988) reported that ***A. hydrophila*** is commonly present in farms, feeds, water, faeces, soil and equipement used for milking, thus it

The antibiotic susceptibility pattern of ***Aeromonas*** strains isolated from all samples revealed that all isolates were sensitive to chloramphenicol and amikacin in 100% and resistant to ampicillin and colistin sulphate. The least frequent sensitivity was recorded with erythromycin. The result agreed with ***Awan et al (2009), Yucel et al (2005)*** *and* ***Nagar et al (2011)***. In contrary these results disagree with ***Altwegg and Greiss (1989)*** who recorded that ***Aeromonas*** strains were resistant to chloramphenicol. This study indicated that different isolates of ***Aeromonas*** strains varied in their sensitivity to antibiotics (Ciprofloxacin, Doxycycline hydrochloride, erythromycin and trimethoprim sulfamethoxazole).

Results of agarose gel electrophoresis of plasmid DNA extracted from four strains of ***Aeromonas*** in Suez Canal biotechnology lab indicated that ***A. hydrophila, A. caviae*** and ***A. schubertii*** have only one plasmid DNA with molecular weight 2.8 kbp. While ***A. sobria*** was negative for plasmid. These results are nearly similar with those reported by ***Son et al (1997)*** and ***Abulhamd (2009)***. The presence of plasmids may present a potential public health hazard. Thus, the presence of plasmids in clinically important bacteria increases their virulence.

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الملخص العربى

تم جميع ٣٥٠ من عينات اللبن الخام ومنتجاته والماء من مدينة بورسعيد ، أخضعت للكشف عن وجود بكتريا الايروموناس، استخدمت Alkaline peptone water كبيئة تعزيزية لنمو الميكروب حيث تم التحضين على ٢٨ درجة مئوية لمدة ٤٨ ساعة واستخدمت للعزل بيئة Starch ampicillin agar حيث تم التحضين على ٢٨ درجة مئوية لمدة ٢٤ ساعة.

وقد أسفرت النتائج عن التالى :-

١) كانت نسبة تواجد الايروموناس فى اللبن الخام، اللبن المبستر، الجبنة القريش، الجبنة الدمياطى، الجبنة الثلاجة، ماء الحنفية وماء غسيل الضرع ٥٨%، ٢٦%، ٧٠%، ٤٨%، ٤٠%، ١٦%، ٦٨% على التوالى.

٢) دلت النتائج على مدى تواجد ميكروب الايروموناس على starch ampicillin agar حيث تم عزل أكثر من نوع من عترات الايروموناس من عينات اللبن الخام، اللبن المبستر، الجبنة القريش، الجبنة الدمياطى، الجبنة الثلاجة، ماء الحنفية وماء غسيل الضرع وكانت كالأتى: من العترة الأولى (هيدروفيلا) ٥٤,٢٨%، ٤٧,٣%، ٤٧,٥%، ٥٥,٥%، ٢٩,١%،٣٥,٣%، ٦٤,٢% على التوالى . ومن العترة الثانية (كافى) ٢٥,٧%، ٢٦,٣%، ٣٢,٥%، ٢٥,٩%، ٤١,٦%، ٤٦,٦%، ٢١,٤% على التوالى. بينما العترة الثالثة (سوبريا) لم يتم عزلها من ماء الحنفية وعزلت من باقى العينات كالأتى ١١,٤ %، ١٥,٧%، ١٢,٥%، ١٤,٨%، ٢٠,٨%، ٩,٥% على التوالى. أيضا العترة الرابعة (شوبرتى) لم يتم عزلها من ماء الحنفية وتم عزلها من باقى العينات بنسبة ٨,٥%، ١٠,٥%، ٢,٥%، ٣,٧%، ٨,٣%، ٤,٧% على التوالى.

٣)ولقد تم دراسة حساسية لكل عترات الايروموناس المعزولة من عينات الألبان وبعض منتجاته مستخدما المضادات الحيوية المختلفه فى المعمل وكانت النتائج كالتالى: أظهرت درجة حساسية عالية لكل أميكاسين وكلورامفينكول. بينما العترات أظهرت مقاومة عالية لكل من الأمبيسلين والكلوستين سالفيت. كما أوضحت هذه النتائج إلى درجات مختلفة للحساسية إلى كل من سيفتريكسون ، سيبروفلوكساسين ، ديكسوسيكلين هيدروكلوريد، الإريثروميسين والسلفا ميثاكساسول/ ترايميثوبريم.

٤) كما أشارت نتائج دراسة البلازميد إلى وجود بلازميد واحد له نفس الحجم 2800.15bp فى كل من عترات الايروموناس الأولى والثانية والرابعة أما بالنسبة للعترة الثالثة وهى الايروموناس سوبريا فإنها خالية من البلازميد .