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## **INCIDENCE OF AEROMONAS HYDROPHILA GROUP IN RAW MILK AND SOME DAIRY PRODUCTS IN ASSIUT CITY**

(With 2 tables)

By  
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"مدى إنتشار مجموعة ميكروب الايرومونات هايدروفيليا فى اللبن الخام  
وبعض منتجات الألبان بأسسيوط"

نوال غبريال خليل

تضمنت الدراسة فحص ١٣٥ عينة من اللبن الخام والجبن القريش والاييس كريم كل منها ٤٥ عينة، بهدف التعرف على وجود ميكروب الايرومونات هايدروفيليا بالزرع على المستنبتات البكتيرية المختلفة. وأظهرت النتائج تواجد ميكروب الايرومونات هايدروفيليا فى ٦٦,٧٪، ٥١,١٪، ٤٠٪ فى كل من عينات اللبن الخام والجبن القريش والاييس كريم على التوالي بمتوسطات ١٠×٢١، ١٠×٩، ١٠×٢٨ فى العينات السابق ذكرها على التوالي. كما تم تصنيف مجموعة ميكروب الايرومونات هايدروفيليا الى ايرومونات هايدروفيليا، ايرومونات كافيائى، ايرومونات سوبريا بنسب مئوية ٢٦,٦٧٪، ٣٥,٥٦٪، ٤,٤٤٪ على التوالي من اللبن الخام وبنسب ٢٢,٢٢٪، ١٥,٥٦٪، ١٣,٣٣٪ على التوالي من الجبن القريش، ١٧,٧٨٪، ٢٠٪، ٢,٢٢٪ من الأيس كريم على التوالي. كما تم دراسة الخواص المرضيه لكل من ايرومونات هايدروفيليا وأيرومونات سوبريا. ولقد نوقشت الأهمية الصحية والإجراءات الواجب إتباعها لمنع تلوث اللبن ومنتجاته بهذا الميكروب.

### **SUMMARY**

135 samples of raw milk, kareish cheese and ice cream (45 samples of each) were examined for presence of *Aeromonas hydrophila* group, by using enrichment and plating procedures. *Aeromonas* species were detected in 66.7%, 51.1% and 40% of examined raw milk, kareish cheese and ice cream samples, with an average counts  $21 \times 10^5$ ,  $9 \times 10^7$  and  $28 \times 10^{10}$  in the examined samples respectively. *Aeromonas hydrophila* could be detected in 26.67%, 22.22% and

17.78% of raw milk, kareish cheese and ice cream respectively. *A. caviae* could be detected in 35.56%, 15.56%, and 20% from raw milk, kareish cheese and ice cream respectively. *A. caviae* could be detected in 35.56%, 15.5%, and 20% from raw milk, kareish cheese and ice cream respectively. *A. sobria* isolated from 4.44%, 13.33% and 2.22% from the same samples respectively. Haemolytic and proteolytic activity of *A. hydrophila* and *A. sobria* were studied. The public health importance as well as recommended sanitary measures were discussed.

*Key words: Aeromonas hydrophila in raw milk and dairy products*

## INTRODUCTION

*Aeromonas hydrophila* group, is a member of vibriaceae family, facultative anaerobic, gram negative rods. This group consists of *A. hydrophila*, *A. caviae* and *A. sobria*. The *A. hydrophila* group is collectively referred to as motile aeromonads or mesophilic Aeromonas (A.P.H.A., 1992). Motile aeromonads are wide spread in the environment and cause food related illness and become of concern to food microbiologist as it has been isolated from variety of foods, including raw milk (Palumbo *et al.*, 1985a, and Greenway, 1988).

The organism is capable to grow at refrigerated temperature, and has been observed as a part of microflora of milk, fish, poultry and meat (palumbo *et al.*, 1985a,b). Increasing interest concerning the possible role of species of *A. hydrophila* group as a cause of human gastroenteritis, both clinical and laboratory investigations have suggested that the species is a significant enteric pathogen (Hazen *et al.*, 1978; Gracey, *et al.*, 1982 and Burke, *et al.*, 1983). The spoilage potential and pathogenicity of the organism have been correlated to its ability to secrete several extracellular virulent products including enterotoxins, cytotoxins, haemolysine, lipase and proteases (Trust and Chipman, 1979 and Ljungh and Wadstrom, 1983).

The fatality rate of patients affected with *A. hydrophila* group may reach to 61% (Davis *et al.*, 1978).



The aim of this study were to determine the prevalence of motile *Aeromonas* species in raw milk, kareish cheese and ice cream samples and it's role as a public health hazard.

## MATERIAL and METHODS

A total of 135 samples of raw milk and milk products including kareish cheese and ice cream (45 samples of each), were collected from governmental farms, street pedlars, super markets, dairy shops, markets and groceries in Assiut city.

All samples were dispatched to the laboratory in clean, dry and sterile containers with a minimum of delay.

### Preparation of samples:-

Milk samples were tested for heat treatment and shaken thoroughly while kareish cheese were grind well in strile mortar, on the other hand ice-cream samples were melted in a thermostatically controlled water bath ( $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) for 15 minutes and well mixed (Richardson, 1985).

### Enrichment procedure:-

10 ml. of milk, ice cream and 10 gm. of cheese were added to 90 ml of Trypticase Soy broth containing 10 ug Ampicillin/ml and blended for 2 min., then incubated at  $28^{\circ}\text{C}$  for 20-24 hrs.

### Enumeration and Identification:-

After incubation the enrichment cultures were serially diluted up to  $10^{-6}$  in case of milk and cheese, and up to  $10^{10}$  in case of ice-cream, 0.1 ml from prepared dilutions was spread over MacConkey Manitol Ampicillin agar with a sterile bent glass rod, and incubated at  $28^{\circ}\text{C}$  for 20-24 hrs. (Fathi and Moustafa., 1991). The numbers of isolated *Aeromonas* were estimated and typical red colonies were picked up to triple sugar iron agar and nutrient a gar slants. After over night incubation at  $28^{\circ}\text{C}$  , a few drops of a 1% solution of N.N. dimethyl-p-phenylene- diamine monohydrochloride were added to the growth of the nutrient agar slants to determine the oxidase reaction.

Colonies which proved to be oxidase and manitol positive were differentiated to *A. hydrophila*, *A. sobria* and *A. caviae* by their glucose fermentation and esculin hydrolysis reactions according to Okrend et al., 1987 and Palumbo et al., 1985a.

The identified strains of *A. hydrophila* and *A. sobria* were evaluated for the haemolytic activity by 5% horse blood agar and

proteolytic activity on agar with 15% gelatin, according to Rogulska et al., 1994.

## RESULTS

Results are tabulated in two Tables (1, 2).

## DISCUSSION

Increasing recovery of *A. hydrophila* as a food-borne and human pathogen was detected. In recent years, investigations into the cause of human gastroenteritis have resulted in increased concern about *A. hydrophila* as a possible cause of diarrheal disease in man. At the same time the role of *A. hydrophila* as a food borne pathogen is not full understood, whereas present information suggested that because of its ubiquitous nature and psychrotrophic characteristic, this organism is a common contaminant in numerous food (Faghri et al., 1984; and Hood, et al., 1984).

In the present study *Aeromonas* species were detected in 30 out of 45 examined raw milk samples (66.7%) with the count range from  $65 \times 10^4$  up to  $42 \times 10^5$  with the mean value of  $21 \times 10^5$  CFU/ml table (1). Nearly similar results (70% and 60%) were reported by Hafez and Halawa (1993) and Ibrahim and Macrae (1991) respectively.

Lower finding (2.44%, 9.7%, 9.17% and 9.9%) were reported by Banerjee and Block (1986) Ergullu (1978), Kumar et al. (1978) and kielweing (1971) respectively.

Raw milk is well-known as an important source of pathogenic microorganisms, especially those which are widely distributed in nature such as *Aeromonas hydrophilla* group. Members of such group can contaminate the udder via the teat, then multiply, reach significant numbers in mammary tissue and subsequently be discharged in milk (El-Shenawy and Marth 1990). So presence of motile aeromonads in a high level in raw milk samples is indicative to the neglected hygienic measures of milk production and distribution.

*Aeromonas* species were detected in 23 out of 45 kareish cheese samples examined (51.1%), the count ranged from  $50 \times 10^3$  to  $18 \times 10^7$  with mean of  $9 \times 10^7$  CFU/gm table (1).

Freitas et al., 1993 reported incidence of 32% from soft cheese, lower incidence was reported by kielweing (1975).



No available data about this study in Egypt could be traced, and kareish cheese is considered in our country the main protein supplement to farmers and average class population. So the presence of *Aeromonas* in this level could cause food borne illness.

*Aeromonas* species isolated from 18 out of 45 ice-cream samples examined, with an incidence of 40% the count range from  $30 \times 10^4$  to  $56 \times 10^{10}$  with mean of  $28 \times 10^{10}$  CFU/ml table (1).

Knochel and Jeppesen (1990) reported 28% isolation rate of *Aeromonas* species from ice-cream, and this is some what similar to our results. However, Brezinova (1976) reported lower incidence, while Pintor et al., (1991) could not be isolate *Aeromonas* species from pasteurized ice-cream.

Ice-cream is one of the most popular milk products especially in summer in our country, and results of this study indicate that *Aeromonas* species may be present in raw milk or contaminate it during preparation of ice-cream. Also, storage of the product at refrigeration temperature, may result in high numbers of *Aeromonas* which could cause food borne illness.

Callister and Agger (1987) suggested that environmental isolates of *Aeromonas* species from sources routinely kept at low temperature are more adapted than others to competitive growth at low temperature, and results in high numbers of aeromonas.

It is evident from the results given in table (2) that *A. hydrophila* could be detected in 12 raw milk samples (26.67%).

Similar results (27.5%) were recorded by Hafez and Halawa (1993) while higher findings (60% and 40%) were reported by Ibrahim and Macrae (1991) and FDA (1985) respectively.

*A. Caviae* and *A. Sobria* could be detected in 35.56% and 4.44% of examined raw milk samples respectively table (2) and this is nearl similiary to those 40% and 2.5% which reported by Hafez & Halawa (1993).

Also *A. hydrophila* isolated from 22.22% of examined kareish cheese table(2). Nearly similar incidence 29.9% was reported by Freitas, et al.; (1993).

*A. Caviae* and *A. Sobria* could be isolated in 15.56% and 13.33% from kareish cheese respectively.

Lower incidence (7.9% and 3.8%) were respectively reported by Freitas et al., (1993).

From Table (2) *A. hydrophila* isolated from 8(17.78%) of 45 samples of ice-cream; *A. Caviae* from (9) samples and *A. Sobria* from one sample only.

Knochel & Jeppesen (1990) reported that *A. hydrophila* was the dominant species isolated from ice-cream.

Presence of *A. hydrophila* group in raw milk, cheese and ice-cream should not be ignored even, if the population is extremely small, since the pathogen can grow at refrigeration temperatures, despite the presence of large numbers of competing organisms and attain numbers which can cause illness (Abeyta and Wekell, 1988; Palumbo *et al.*, 1985a,b and Palumbo 1986).

In this study 22 out of 30 (73.3% of isolated *A. hydrophila* produce haemolysine while in case of *A. Sobria* 8 out of 9 (88.9%) produce haemolysine. Concerning proteolytic activity, 19 out of 30 (63.3%) and 6 out of 9 (66.7%) of *A. hydrophila* and *A. Sobria* respectively were produce protease.

Rogulska *et al.*, (1994) that reported haemolytic and proteolytic activity of *A. hydrophila* and *A. Sobria* as markers of pathogenicity.

In conclusion *A. hydrophila* groups particularly *A. hydrophila* and *A. Sobria* represents new food borne pathogens, which can not be ignored with the high isolation rate of such organisms, in this work. So the best way to overcome this problem is preventing the raw milk from contamination and good processing and handling of the finished products.

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**Table (1):** Statistical analytical results of *Aeromonas* species/ml or g in examined samples.

Type of samples	No. of samples exam.	Positive samples			count		
		No.	%	Min.	Max.	Average	
Raw milk	45	30	66.7	65x10 <sup>3</sup>	42x10 <sup>5</sup>	21x10 <sup>5</sup>	
kareish cheese	45	22	51.1	50x10 <sup>3</sup>	18x10 <sup>7</sup>	9x10 <sup>7</sup>	
Ice-cream	45	18	40	30x10 <sup>4</sup>	56x10 <sup>10</sup>	28x10 <sup>10</sup>	

**Table (2):** Incidence of *Aeromonas hydrophila* group in examined samples.

Isolates	Type of samples						Total
	Raw milk		Kareish cheese		Ice-cream		
	No.	%	No.	%	No.	%	
<i>A. hydrophila</i>	12	26.67	10	22.22	8	17.78	30
<i>A. Caviae</i>	16	35.56	7	15.56	9	20	32
<i>A. Sobria</i>	2	4.44	6	13.33	1	2.22	9

