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DAIRY PRODUCTS AS A SOURCE OF POTENTIALLY PATHOGENIC AEROMONAS SPECIES AND THEIR IMPORTANT CHARACTERISTICS

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ABSTRACT

One hundred-fifty random samples of fresh cream, whipped cream and kareish cheese (50 each) were examined for incidence of Aeromonas species. Aeromonas species were detected in 28%, 18% and 32% of examined samples respectively as regards every species Aeromonas hydrophila was detected in 16%,8% and 16% of examined samples respectively, Aeromonas sobria was detected in 18%, 10% and 24% of examined samples respectively and Aeromonas caviae was detected in 14%, 14% end 20% of examined samples respectively Regarding to haemolysin production,100%, 92.31% and 41.67% of A.hydrophila, A. sobria and A.caviae produced haemolysin while 90%,61.54% and 41.67% of A.hydrophila,A.sobria and A.caviae were proved to produce enterotoxin as judged by fluid accumulation in rabbit ileal loop.Concerning the production of spoilage enzymes,Aeromonas hydrophila was dominating other Aeromonas species for proteolytic and lipolytic activities comprising 65% and 25% respectively.Regarding to antibiotic sensitivity against 9 antimicrobial agents A.hydrophila was 100% sensitive to chloramphenicol,tetracycline and neomycin while Aeromonas sobria was completely resistant to ampicillin,chloramphneicol and erythromycine and Aeromonans caviae was completely resistant to ampicilin and nalidixic.

INTRODUCTION

Aeromonas species are common inhabitants of fresh water environment and have been isolated from a wide range of fresh foods such as raw milk and milk products. Although it is not resistant to chlorine it is found in potable water and it is a transient component of the gut flora of humans and other animals ***(Adams and Moss, 1995)***.

Members of the genus Aeromonas can synthesize many extracellular enzymes including protease,lipase,chitinase and haemolysin***(Janda,1985)***. Several studies have suggested that at least two distinct haemolysins are produced by motile Aeromonads, an α-haemolysin, which is produced optimally at 22°C and causes incomplete haemolysis of erythrocytes and a β-haemolysin, which causes lysis of various species of erythrocytes and is obtained in highest yield from cultures grown at 37°C ***(Thelestam and Ljungh,1981)***.

Aeromonas species,particularly Aeromonas hydrophila and Aeromonas sobria produce other range of potential virulence enterotoxins including cytotoxic and cytotonic enterotoxins. Three cytotonic enterotoxins have been also described,which act like cholera toxin***(Adams and Moss,1995)***.

Aeromonas species are capable of growing and producing toxins at refrigeration temperature ***(Majeed et al.,1990 and Krovacek et al.,1991)***. The ability of Aeromonas species to adhere to and invade epithelial cells are among the other virulence factors produced by these organisms that will explain the pathogenesis of infection ***(Janda, 1991)***.

Two types of gastrointestinal illness have been attributed to A. hydrophila and A.sobria ***(Stelma, 1989)***. The most common type is a cholera-like illness,which is characterize by watery stools and mild fever and it is most commonly in children under five years old***(Adams and Moss,1995)***.

Aeromonas hydrophila is additionally associated with both diarrhoeal and extra-intestinal infection in human and it has become increasingly recognized as an enteric pathogens in recent years.Aeromonas species are common in a wide variety of foods including raw milk and milk products and Aeromonas hydrophila is the predominate species found in raw milk and dairy products.The presence of Aeromonas species in milk is of great concern because of their capability of growth at low temperature***(Ozbas et al., 2000).***

The increased availability of refrigerated ready-to eat foods offered by many food service sectors of the food industry,coupled with the known ability of Aeromonas species to grow at 4°C, has resulted in increased concern relative to public health hazards which may be associated with the consumption of these food ***(Beuchat, 1991)***.

In this study, besides isolation and identification of Aeromonas species from three common dairy products, haemolytic activity, enterotoxigenicity test, proteolytic and lipolytic activities and antibiotics sensitivity of the isolated strains were also conducted.

MATERIALS AND METHODS

One hundred-fifty random samples of fresh cream, Whipped cream and Kareish cheese (50 each) were collected from different supermarkets and dairy shops in Zagazig city, Egypt in clean, dry, tightly stoppered and sterile sampling jar. The collected samples were immediately transferred to laboratory and examined for presence of Aeromonas species.

I-Detection and identification of Aeromonas species:

Preparation of samples was carried out according to ***APHA (1992)***. From each previously prepared samples, 0.1 ml was inoculated onto a dry surface of starch ampicillin agar ***(Palumbo et al., 1985)*** using surface plating technique at which, duplicate plates were used for each dilution.

The inoculated plates were incubated at 30°C for 24 hours. After incubation, the plates were flooded with half-strength lugol's iodine solution and amylase positive colonies (yellow colonies, 1-2 m.m in diameter and showed clear zone of starch hydrolysis) were counted as presumptive Aeromonas species. Suspected colonies of each sample were picked up onto nutrient agar slants for further identification according to ***Popoff (1984)***.

II-Haemolytic activity (Rogulska et al., 1994):

B-haemolytic activity of 57 Aeromonas strains was studied using 5.0% sheep blood agar. B-haemolysin production was detected by presence of zone of haemolysis around the growth after being incubated at 30°C for 24 hours.

III- Enterotoxin production:

Using the technique recommended by ***Formal et al. (1961)***. The strains were tested as a whole cultures and stationary phase cultures were grown for 24 hours at 37°C in 500 ml Erylenmyer flasks containing 20 ml of brain heart infusion broth. All strains were tested in two ileal loops, each loop was in a different rabbit.

Each loop of about 10 cm length, was tied from one end after inoculation of broth culture, the other end was tied then hanged in physiological saline. Strains were considered to be positive for enterotoxin production when the ratio of fluids (ml) to length of loop (cm) was >1.0.

IV-Production of lipase and protease:

Aeromonas isolates were subcultured on nutrient agar plates and incubated at 30°C for 24 hours. Pure cultures were inoculated into nutrient broth and incubated overnight at 30°C before testing.

(a) Protease production *(APHA, 1992)*:

The overnight pure cultures were spot inoculated onto skim milk agar plates. The inoculated plates were incubated at 21±1°C for 72 hours and subsequently flooded with 10% acetic acid solution for one minute and the excess acid was decant. Colonies surrounded by clear zone were considered positive.

(b) Lipase production *(Harrigan and McCance, 1976)*:

A sugar-free nutrient agar with emulsified butter fat and Victoria blue as indicator was inoculated with overnight pure cultures followed by incubation at 25°C for 7 days. Bright blue colonies were considered positive.

V-Antibiotic sensitivity of the isolated strains:

The susceptibility of each strain to 9 antibiotics was carried out by disc diffusion agar method according to method recommended by ***National Committee for Clinical Laboratory Standard (1997)***. The used inoculum contained 105 cfu/ml by adjusting turbidity of broth culture to matcha 0.5 Mcfarland Barium sulphate standard tubes.

RESULTS

Table (1): Aeromonas count/gram in examined samples.

Dairy product	No. of examined samples	Positive samples		Max.	Average	Min.
		No.	%			
Fresh cream	50	14	28	$2.0x10^6$	$4.5x10^4$	$4.4x10^2$
Whipped cream	50	9	18	$6.2x10^3$	$3.4x10^4$	$4.0x10^2$
Kareish cheese	50	16	32	$5.0x10^7$	$4.5x10^5$	$0.2x10^2$

Table (2): Incidence of Aeromonas species in the examined samples (N=50).

Dairyproducts / Isolates	Fresh cream		Whipped cream		Kareish cheese		Total	
	No.	%	No.	%	No.	%	No.	%
A.hydrophila	8	16	4	8	8	16	20	13.33
A.sobria	9	18	5	10	12	24	26	17.33
A.caviae	7	14	7	14	10	20	24	16.00
Total	**24**	**16**	**16**	**10.67**	**30**	**20**	**70**	**46.66**

Table (3): Haemolytic activity & enterotoxigenicity test of Aeromonas strains.

Aeromonas strains	No. of tested strains	Haemolytic activity		Strains causing fluid accumulation		Range of fluid volume (ml/cm)
		No.	%	No.	%	
A.hydrophila	20	20	100.00	18	90.00	1.0 –1.9
A.sobria	26	24	92.31	16	61.54	1.1 – 2.0
A.caviae	24	10	41.67	10	41.67	1.0 – 1.8

Table (4): Proteolytic and lipolytic characteristics of Aeromonas species.

Aeromonas strain	No. of tested strains	Proteolytic activity		Lipolytic	
		No.	%	No.	%
A.hydrophila	20	13	65.00	5	25.00
A.sobria	26	8	30.77	2	7.65
A.caviae	24	9	37.50	2	8.33
Total	**70**	**30**	**42.86**	**9**	**12.86**

Table (5): Antibiotic resistance properties of isolated Aeromonas strains.

Aeromonas strains / Antimicrobial agents	A. hydrophila (n=20)		A. sobria (n=26)		A. caviae (n=24)	
	Sensitive strains		Sensitive strains		Sensitive strains	
	No.	%	No.	%	No.	%
Ampicillin	0	0.00	0	0.00	0	0.00
Streptomycin	16	80.00	10	38.44	1	4.16
Chloramphenicol	20	100.00	0	0.00	2	8.33
Erythromycin	0	0.00	0	0.00	2	8.33
Tetracyclcine	20	100.00	8	30.77	5	20.83
Neomycin	20	100.00	7	26.92	2	8.33
Nitrofuran	12	60.00	6	23.08	3	12.50
Sulphadiazine	13	65.00	3	11.54	1	4.16
Nalidixic acid	14	70.00	2	7.69	0	0.00

RESULTS & DISCUSSION

Regarding to Table (1), the obtained results point out the levels of Aeromona***s*** species in different milk products. Aeromonas species were detected in 28%, 18% and 32% of examined fresh cream, whipped cream and kareish cheese respectively. The average Aeromonas count was 4.5x104, 3.4x104 and 4.5x105 cfu/gram respectively.

Similar results were obtained by ***Saad (1991) and Abdel-Hakiem (2000)***. While higher figures were reported by ***Khalil (1997) and Nashwa and Isis(2001)***.The dairy products are considered as an important vehicle for the transmission of these organisms which are widely distributed in the environment.

It was clear from the results assembled in table (2) that A. hydrophila was detected in16%,8% and16%of examined fresh cream,whipped cream and kareish samples respectively. Nearly similar findings were reported by ***Knochel and Jeppenesen (1990),El-Prince(1998) and Abdel-Hakiem (2000)*** while ***Nashwa and Isis(2001)*** could detected A.hydrophila in lower percentages.

Presence of Aeromonas hydrophila in dairy products may be arised from contamination via natural sources as feeds, water, faeces, soil and milking equipment and this microorganism could contaminate udder via teats, then multiply and reach significant numbers and subsequently

discharge in milk. Moreover, transmission of motile Aeromonas species through a symptomatic carriers into food can also occur ***(El-Shenawy and Marth, 1990)***.

Regarding A. sobria and A. caviae were detected in 18% and 14%, 10% and 14% & 24% and 20% in examined dairy products respectively. These findings are nearly in accordance with results obtained by ***El-Prince (1998), Abdel-Hakiem(2000)and Nashwa and Isis(2001)***.Slightly higher percentages were declared by ***Khalil (1997)***.

Presence of Aeromonas species in examined dairy products could be attributed to bad quality of raw milk used, unsanitary manufacturing practices and/or improper methods of handling and distribution. Furthermore, storage condition of cream could also increase contamination, since Aeromonas species can grow at refrigeration temperature ***(Palumbo et al.,1986,Callister and Agger, 1987 and Tibana et al., 1987)***.

The low salt content, higher pH value and high moisture content of Kareish cheese might be implicated in increasing Aeromonas species population in examined samples ***(Palumbo,et al.,1986 and Santos et al.,1996)***. There are three types of human illness associated with Aeromonas species including extra-intestinal, wound infection and food-associated gastrointestinal infection that may be present as toxigenic rice water small intestinal diarrhoea, classical dysentery involving the large intestine or combination of both ***(Hobbs and Roberts, 1993)***.

The results assembled in table (3) declared that 100%, 92.31% and 41.67% of A.hydrophila, A.sobria and A.caviae produced haemolysin. Nearly similar findings were reported by ***Okrend et al.(1987) and Abdel-Hakiem (2000)***.

Recent attention has focused on the β-haemolysin of motile Aeromonas species because of its potential diagnostic and pathogenic significance. Several groups of workers have demonstrated a significant association between extracellular β-haemolysin production and development of enterotoxigenic like activity ***(Burke et al., 1982 and Turnbull, 1984)***.

The data of the present study(Table 3)indicate that 90%,61.54%and 41.67% of A. hydrophila A. sobria and A. caviae produced enterotoxin as judged by fluid accumulation in rabbit ileal loop. These findings are in agreement with that reported by ***Krovacek et al. (1992)***. It is clear that A. hydrophila and A.sobria caused significantly more fluid accumulation than A. caviae and that indicated higher enterotoxigenic activity, which explain

the less frequent detection of enterotoxigenic activity in A. caviae strains ***(Bruke et al., 1982, Majeed et al., 1989 and Barer et al., 1996)***.

Concerning the production of spoilage enzymes in table(4), Aeromonas hydrophila was predominating other Aeromonas species for proteolytic and lipolytic activities comprising 65% and 25% respectively. While A.caviae and A.sobria comprising 37.50%, 30.77% and 8.33, 7.65 for proteolytic and lipolytic activities, respectively.

These results agree with that reported by ***Abdel-Khalek (1997) and Abdel-Hakiem(2000)***. Extracellular proteinases and lipases of Aeromonas species are recognized to be the primary microbial spoilage enzymes of dairy products ***(Fairbairn and Law, 1986 and Stelma, 1989)***. Aeromonas species secrete thermostable proteinase which capable of lysis of milk protein ***(Richardson and Tewhaiti, 1978)***.

Table(5) shows the antibiotic sensitivity of Aeromonas species against 9antimicrobial agents and the results revealed that A.hydrophila was 100% sensitive to Chloramphenicol, Tetracycline and Neomycin, while it was 100% resistant to Ampicillin and erythromycin.

Aeromonas sobria was completely resistant to Ampicillin, Chloramphenicol and Erythromycin, while A. caviae was resistant to most of the antibiotics used. Similar findings were declared by ***Krovacek et al.(1992)***.

In conclusion, proper heat treatment, applying good sanitary manufacturing practices and proper methods of handling and distribution are extremely important to prevent consumers from being infected by Aeromonas species.

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منتجات الألبان كمصدر لميكروب الايروموناس المسبب للأمراض الكامنة وخصائصه الهامة

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تم تجميع 50 عينة من كل من القشدة الطازجة والقشدة المخفوقة والجبن القريش لدراسة مدى تواجد ميكروب الايروموناس وقد تم عزل ميكروب الايروموناس من 28% و18% و32% من عينات القشدة الطازجة والقشدة المخفوقة والجبن القريش على التوالى بينما تم عزل ميكروب الايروموناس هيدروفيلا من 16% و 8% و16% من العينات المفحوصة على التوالي أما بالنسبة للقدرة على إفراز الإنزيم المحلل لخلايا الدم فقد وجد أن 100% و92.30% و 41.67% من ميكروب الايروموناس هيدروفيلا والايروموناس سوبريا والايروموناس كافي على التوالي لهم القدرة على إفراز هذا الإنزيم بينما كان 90% و 61.54% و 41.67% من ميكروبات الايروموناس هيدروفيلا و الايروموناس سوبريا والايروموناس كافي كان لها القدرة على إنتاج السموم المعوية.

وعند دارسة قدرة ميكروبات الايروموناس على إفراز الإنزيمات المحللة لكلا من البروتينات والدهون فقد وجد أن ميكروب الايروموناس هيدروفيلا يفوق الأنواع الأخرى من الايروموناس فى القدرة على تحليل البروتينات والدهون.

وعند إجراء اختبار الحساسية للمضادات الحيوية فقد وجد أن كل ميكروبات الايروموناس هيدروفيلا كانت حساسة للمضادات الحيوية الآتية: الكلورامفينيكول والتتراسيكلين و النيوميسين ، بينما كانت جميع معزولات الايروموناس سوبريا مقاومة تماما لكل من الكلورامفينيكول والامبسلين والارثروميسين.