Assiut Vet. Med. J. Vol. 39 No. 78, July 1998.

Dept. of Food Hygiene,
Fac. of Vet. Med., Assiut University
Head of Dept. Prof. Dr. M.K.Moustafa

INCIDENCE AND CHARACTERIZATION OF AEROMONAS SPECIES IN DOMIATI AND KAREISH CHEESE SOLD IN ASSIUT PROVINCE

(With 5 Tables)

By

ENAS EL-PRINCE

(Received at 27/6/1998)

مدى تواجد وخصائص الايروموناس فى الجبن الدمياطي والقريش بمدينة أسيوط

ايناس البرنس

تضمنت هذه الدراسة فحص عدد مائه عينة من الجبن الدمياطى والجبن القريش المصنع محليا بواقع ٥٠ عينه لكل منهما وذلك بعد تجميعها من محلات الألبان والباعة الجائلين والفلاحين بأسيوط ، وذلك لاستبيان وجود الايروموناس. وقد تبين من الفحص أن ١٤% و١٦% من عينات الجبن الدمياطى تحتوى على الميكروب وذلك باستخدام طريقة الفرد السطحى على مستنبتى TSA, MMA، وكان متوسط العدد في العينات ١x١٠ ٤ ,١ x١٠ ٤ / جرام على كلا المستنبتين على التوالى ، بينما فى عينات الجبن القريش كانت نسبة تواجده ٦٦% ، ٦٤% بمتوسط قدره ٥ x ١٠ ٣ ، ٩ x١٠ ٤ / جرام على المستنبتين على التوالى . وهذه النتائج دلت على عدم وجود فرق معنوى واضح بين المستنبتين المستخدمين. وقد تم عزل ١٦ عترة من الجبن الدمياطى تم تصنيفها إلى ١١ (٧ر٦٨ %) ايروموناس كافى وهى أعلى نسبه ثم ٣ (٨ر١٨ %) ايروموناس هيدروفيلا ثم ٢(٥ر١٢%) ايروموناس سوبريا. بينما فى الجبن القريش تم عزل ٣٨ عترة و كانت على التوالى ١٥(٥ر٣٩ %) ، ١٥ (٥ر٣٩ %) ، ٨(٢١ %). وبالاضافه لما سبق تمت دراسة الخواص الممرضة لكل من الايروموناس هيدروفيلا والايروموناس كافى والايروموناس سوبريا، وأسفرت النتائج على أن عدد العترات ذات الخواص الممرضة فى الجبن الدمياطى أقل بكثير منها فى الجبن القريش. وقد ناقش البحث أهمية الميكروب من الناحية الصحية والاقتصادية وكذلك الشروط الواجب توافرها واتخاذها لمنع تلوث الجبن بهذا الميكروب لدرء خطره حفاظا على صحة المستهلك.

SUMMARY

A total of 100 random samples of locally manufactured Domiati and Kareish cheese (50 from each) were collected from dairy shops, groceries and farmers houses in Assiut province. The samples were examined for enumeration and isolation of Aeromonas species as well as to evaluate their haemolytic activity and degree of virulence. The Aeromonas species could be isolated from 14% and 16% of the examined Domiati cheese samples using MacConkey mannitol ampicillin agar (MMA) and Trypticase soy ampicillin agar (TSA) with an average count of 1×10^4 and 1×10^4/g, respectively. However, in case of Kareish cheese , the percentages of positive samples were 66% by using MMA medium and 64% by using TSA medium , as well as the average counts were 5×10^3 and 9×10^4/g, respectively. A. caviae was the most common species isolated from Domiati cheese (68.7%) followed by A. hydrophila (18.8%) and A. sobria (12.5%). While in case of Kareish cheese, A. hydrophila and A. caviae were the predominant species comprising (39.5%), while A. sobria comprising (21.0%) of the total isolates. Concerning the haemolytic activity of the isolated species recovered from Domiati cheese samples, one of each of A. hydrophila and A. sobria and 2 of A. caviae had the ability to produce haemolysin. While 10, 2 and 3 strains of A. hydrophila, A. caviae and A. sobria isolated from Kareish cheese were positive for haemolysin, respectively. The public health hazard of Aeromonas species and the suggestive measures for improving the quality of Domiati and Kareish cheeses were discussed.

Key Words: *Aeromonas species, Domiati cheese, Kareish cheese.*

INTRODUCTION

Genus Aeromonas contains two well separated groups. The first group consists of a single, psychrotrophic, non-motile species, A. salmoncida, which is not a pathogen of man (Popoff, 1984), while the second group consists of mesophilic motile strains. Three species were described by Popoff et al. (1981), including A. hydrophila, A. sobria and A. caviae. In humans, the organisms are recognised as the cause of disseminating infections in the immunocompromised patients (Ellison and Mostow, 1984) and gastroenteritis (Wadstrom and Ljungh, 1991).

Gastrointestinal infections due to Aeromonas are of two distinct types, a cholera-like and dysentry-like illness. Moreover, it can produce a wide range of extraintestinal infections as septicaemia and meningitis (Davis et al., 1978 and Ellison and Mostow, 1984), cellulitis, wound infections and other unusual manifestations include eye infections, pneumonia, urinary tract infections, hepatobiliary, endocarditis and ear infections (Koneman et al., 1994). On the other hand, Aeromonas produces a number of potential virulence factors including a heat labile (beta haemolysin) and a heat-stable cytotoxic enterotoxins, as well as, many extracellular enzymes as protease, amylase, lipase and nuclease (Eley, 1996). Thus, it posses a highly significant public health problem, as well as, it is of economic importance.

In recent years, Aeromonas has received increasing attention as an agent of foodborne diarrhoeal disease in otherwise healthy people (Gracey et al., 1982 and Palumbo et al., 1985a). Isolation of these bacteria has been reported from a variety of food of animal origin including raw milk (Kielwein et al., 1969, Palumbo et al., 1985a, Saad, 1991, Hafez and Halawa, 1993, Kirov et al., 1993 and Abdel-Khalek, 1997) and pasteurized milk (Freitas et al., 1993 and Bahout, 1997) .Also, A. caviae was isolated from 4.7% of ice cream samples in Wales (Hunter and Burge, 1987).

In case of, white cheese, Freitas et al. (1993) could isolate Aeromonas strains from 32% of the examined samples. They recorded that unclassified strains were the most frequent isolates (61.5%) followed by A. hydrophila (26.9%), A. caviae (7.6%) and A. sobria (3.8%). Moreover, only A. hydrophila and A. sobria showed high rate of production of haemolysin, cytotoxin and staphylolytic activity. In addition, it has been reported that 13.3% of white soft cheese samples contain Aeromonas species which represented mainly by 33.3% A. hydrophila and 66.7% A. caviae (Hafez, 1996). Unfortunately, these species were able to grow during cheese making of farm house-made Villalon cheese during storage at 4°C (Santos et al., 1996). Moreover, Aeromonas species could be detected and isolated from samples of raw milk, ice cream as well as Kareish cheese sold in Assiut City in percentages of 66.7, 40 and 51.1%, respectively and the haemolytic and proteolytic activity of the isolated strains were also investigated (Khalil, 1997).

The ability of aeromonads to grow at low temperature is of great concern, particularly in foods which are stored under refrigeration. This explains that Aeromonas could be a potential foodborne pathogen and

dairy products may represent an important vehicle of its transmission (Freitas et al., 1993). Therefore, the purpose of this study was to investigate the occurrence of Aeromonas species in Domiati and Kareish cheese sold in Assiut City markets and farmers houses as well as to examine the ability of the isolated strains to produce exotoxin (haemolysin) and the degree of virulence.

Material and Methods

A total of one hundred random samples of locally manufactured Domiati and Kareish cheese (50 from each) were collected from dairy shops, groceries and farmers houses in Assiut province. The samples were dispatched to the laboratory in sterile plastic bags without delay to be examined bacteriologically for the presence of Aeromonas species.

1- ***Enumeration of Aeromonas species***:-

Numbers of Aeromonas species were determined by using surface plating technique, where 10 g of each sample was aseptically transferred to 90 ml of peptone water 1 % and blended for 2 min. in a Stomacher (Lab Blender Model 400, Seward Medical Limited, London, UK). The prepared samples were serially diluted up to 10^{-6} using 1% peptone water and spread on two different selective media, MacConkey mannitol ampicillin (MMA) agar and Trypticase soy ampicillin (TSA) agar, using a bent glass rod. The plates were incubated at 28°C for 24 h. The typical red colonies on (MMA) agar or pale yellow colonies on (TSA) agar were estimated as Aeromonas species and picked into triple sugar iron (TSI) agar and nutrient agar slants for further confirmation and identification (Okrend et al., 1987).

2- ***Identification of Aeromonas species***:-

Presumptive aeromonads were identified by biochemical tests according to Koneman et al. (1994). The isolated species were differentiated as described by Okrend et al. (1987) . In addition, the identified strains were evaluated for haemolytic activity of 5% sheep blood agar (Rogulska et al., 1994) as well as detection of virulence by slide agglutination test (Mittale et al., 1980).

RESULTS

The obtained results were recorded in Tables 1-5.

Table 1. Count of Aeromonas species/g of examined Domiati cheese samples

Media Used	No. of examined samples	Positive samples		Count/g		
		No.	%	Min.	Max.	Average
MacConkey mannitol ampicillin agar	50	7	14	2×10^2	1×10^5	1×10^4
Trypticase-soy ampicillin agar	50	8	16	3×10	8×10^4	1×10^4

Table 2. Count of Aeromonas species /g of examined Kareish cheese samples

Media Used	No.of examined samples	Positive samples		Count/g		
		No.	%	Min.	Max.	Average
MacConkey mannitol ampicillin agar	50	33	66	4×10	5×10^4	5×10^3
Trypticase soy ampicillin agar	50	32	64	3×10^2	2×10^6	9×10^4

Table 3. Frequency distribution of isolated Aeromonas species in examined cheese samples.

Type of cheese	No. of isolated strains	A.hydrophila		A.caviae		A.sobria	
		No.	%	No.	%	No.	%
Domiati cheese	16	3	18.8	11	68.7	2	12.5
Kareish cheese	38	15	39.5	15	39.5	8	21.0

Table 4. Detection of haemolysin and virulence of Aeromonas species recovered from the examined Domiati cheese samples.

Species	No. of strains examined	Haemolysin positive strains	Virulence	
			High	Low
A. hydrophila	3	1	0	3
A. caviae	11	2	0	11
A. sobria	2	1	2	0

Table 5. Detection of haemolysin and virulence of Aeromonas species recovered from the examined Kareish cheese samples.

Species	No. of strains examined	Haemolysin positive strains	Virulence	
			High	Low
A. hydrophila	15	10	5	10
A. caviae	15	2	0	15
A. sobria	8	3	6	2

DISCUSSION

Table 1 revealed that 14 and 16% of the examined Domiati cheese samples contained Aeromonas species with average counts of 1 x 10^4/g using MMA and TSA agar, respectively. While in the examined Kareish cheese (Table 2), 66 and 64% of samples were positive for Aeromonas with average counts of 5 x 10^3 and 9 x 10^4 /g on both media, respectively. The obtained average counts are lower than that recorded by Khalil (1997). The sensitivity of TSA in comparison with MMA medium was studied. MacConkey's agar was chosen for comparison since it is capable of growing most of gram-negative microorganisms of the enteric and associated groups. No practical difference among both media was observed in the ability to propagate Aeromonas and this substantiate what have been stated by Okrend et al. (1987). Also, the results in Table 1 and 2 established that Aeromonas species were found in Kareish cheese in relatively high incidence as compared with that in Domiati cheese. This could be attributed to the high salt content in Domiati cheese or due to some other formulation parameters which may prevent the growth of aeromonads (Palumbo et al., 1985b and Okrend et al., 1987).

Sixteen isolates were recovered from Domiati cheese samples as showed in Table 3. A. caviae was the most common species isolated 11 (68.7%) followed by A. hydrophila 3 (18.8%) and A. sobria 2 (12.5%). These findings are in accordance with other reports which also found high Aeromonas numbers in food of animal origin (Palumbo et al., 1985 a, Okrend et al., 1987 and Majeed et al., 1989). In contrast, Freitas et al. (1993) and Hafez (1996) stated lower incidence of aeromonads recovered from white soft cheese. Concerning Kareish cheese samples, 38 isolates were recovered. Isolated species were predominantly (39.5%) A. hydrophila and A. caviae and (21.0%) A. sobria. The obtained results were slightly higher than that recorded by Khalil (1997). The low salt content, high pH value, high moisture content and temperature abuse during transport result in high levels of Aeromonas species in examined samples (Palumbo et al., 1985 b and Santos et al., 1996).

It is evident from the data represented in Table 4 that, only one of each of A. hydrophila and A. sobria and two of A. caviae had the ability to produce haemolysin and only the 2 strains of A. sobria were high virulent in examined Domiati cheese samples. These results disagreed with those reported by Okrend et al. (1987), Palumbo et al. (1989) and Freitas et al. (1993). They pointed out that haemolysin was detected in 100% of A. hydrophila strains recovered from retail poultry, beef, pork and white cheese. The low percentages for virulence in examined Domiati cheese may be due to the small number of isolates. On the other hand, many of aeromonads isolated from Kareish cheese were haemolysin producers as illustrated in Table 5. A. hydrophila and A. sobria were more virulent than A. caviae. These findings support the results of Freitas et al. (1993). Also, Kirov et al. (1993) established that the majority of raw milk isolates were not enterotoxigenic and produce no or only small amount of haemolysin. Most studies to date have identified A. hydrophila and A. sobria as the primary enteropathogenic species (Abeyta et al., 1994), however, A. caviae has been implicated in some cases of diarrhoeal disease (Namdari and Bottone, 1990). In addition, B. haemolytic strains of aeromonads are assigned to A. hydrophila and A. sobria, although haemolytic strains of A. caviae have been also found (Deodhar et al., 1991).

The results of this study coincide that, the presence of Aeromonas species in Domiati and Kareish cheeses must be regarded a public health hazard because these microorganisms produce number of potential virulence factors. Also, they can withstand stressful conditions as they survive low temperature. Since Domiati and Kareish cheese are

the main protein supplement to farmers and average class-population in Egypt, it could, if contaminated, be major causes of foodborne illness. Therefore, strict hygienic measures and pasteurization of milk used for cheese manufacturing should be recommended to avoid contamination by Aeromonas microorganisms.

ACKNOWLEDGMENT

The author wishes to express sincere thanks to Prof. Dr. Nagah, M. Saad , Prof. of Milk Hygiene, Fac. of Vet. Med., Assiut Univ. for her constant help and advice during this work.

REFERENCES

Abdel-Khalek, A. (1997): Enumeration and characterization of Aeromonas species isolated from farm bulk milk in Mansoura, Egypt. Alex. J. Vet. Sci., 13 (2): 35-40.

Abeyta, C.; Palumbo, S.A. and Stelma, G.N. (1994): Aeromonas hydrophila group, ch.1. In Y.H. Hui, J.R. Gorham,K.D. Murrell, and D.O. Cliver (ed.), Foodborne disease handbook. Diseases caused by bacteria. Marcel Dekker, Inc., New York.

Bahout, A. A. (1997): Incidence of Aeromonas species in pasteurized milk. Alex. J. Vet. Sci., 13 (2): 31–34.

Davis, W.A.; Kane, J.G. and Garagusi, V.F. (1978): Human Aeromonas infection: a review of the literature and a case report of endocarditis. Medicine, 57: 267-277.

Deodhar, L.P.; Saraswathi, K. and Varudkar, V. (1991): Aeromonas spp. and their association with human diarrheal disease. J. Clin. Microbiol., 29: 853-856.

Eley, A.R. (1996): Microbial food poisoning . 2nd ed., Chapman & Hall , London, UK.

Ellison, R.T. and Mostow, S.R. (1984): Pyogenic meningitis manifestations during therapy for Aeromonas hydrophila sepsis. Arch. Inter. Med., 144: 2078-2079.

Freitas, A. C.; Nunes, M. P.; Milhomem, A. M. and Ricciardi, I. D. (1993): Occurrence and characterization of Aeromonas species in pasteurized milk and white cheese in Rio de Janeiro, Brazil. J. of Food Prot., 56 (1): 62 – 65.

Gracey, M.; Burke, V. and Robinson, J. (1982): Aeromonas associated gastroenteritis. Lancet., 2 : 1304–1306.

Hafez, N. M. (1996): Prevalence and survival of Aeromonas hydrophila group in white soft cheese. Vet. Med. J., Giza. 44 (2): 163–167.

Hafez, N. M. and Halawa, M. A. (1993): Incidence of Aeromonas hydrophila group in raw milk. The 14th, symposium on food pollution, Fac. of Vet. Med. Assiut Univ., pp.13–17.

Hunter, P. R. and Burge, S. H. (1987): Isolation of Aeromonas caviae from ice cream. Lett. Appl. Microbiol., 4:45 – 46.

Khalil, N.GH. (1997): Incidence of Aeromonas hydrophila group in raw milk and some dairy products in Assiut City. Assiut Vet. Med. J., 37 (73): 100-108.

Kielwein, G.; Gerlach, R. and Johne, H. (1969): Prevalence of Aeromonas hydrophila in raw milk. Arch. Lebensmittel Hyg., 20 : 34.

Kirov, S. M.; Hui, D. S. and Hayward, L. J. (1993): Milk as a potential source of Aeromonas gastrointestinal infection. J. Food Prot., 56 (4): 306 – 312.

Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C. and Winn, W.C. Jr. (1994): Introduction to diagnostic microbiology. J.B. Lippincott Company, pp. 117-123.

Majeed, K.; Egan, A. and MacRae, I.C. (1989): Enterotoxigenic aeromonads on retail lamb meat and offal. J. Appl. Bacteriol., 67: 165-170.

Mittale, R. K.; Lalonde, G.; Leblance, D.; Olivier, G. and Lallier, R. (1980): Aeromonas hydrophila in rainbow trout: relation between virulence and surface characteristics. Can. J. Microbiol., 26 : 1501 – 1503.

Namdari, H. and Bottone, E.J. (1990): Microbiological and clinical evidence supporting the role of Aeromonas caviae as a pediatric enteric pathogen. J. Clin. Microbiol., 28: 837-840.

Okrend, A.J.G.; Rose, B.E. and Bennett, B. (1987): Incidence and toxigenicity of Aeromonas species in retail poultry, beef and pork. J. Food Prot., 50 (6): 509-513.

Palumbo, S.A.; Bencivengo, M.M.; Carral, F. D.; Williams, A.C. and Buchanan, R.L. (1989): Characterization of the Aeromonas hydrophila group isolated from retail foods of animal origin. J. Clin. Microbiol., 27: 854-859.

Palumbo, S.A.; Maxino, F.; Williams, A.C.; Buchanan, R.L. and Thayer, D.W. (1985a): Starch-ampicillin agar for the quantitative detection of Aeromonas hydrophila. Appl. Environ. Microbiol., 50 (4): 1027-1030.

Palumbo, S.A.; Morgan, D.R. and Buchanan, R.L. (1985 b): Influence of temperature, NaCl and pH in the growth of Aeromonas hydrophila. J. Food Sci., 50: 1417-1421.

Popoff, M. (1984): Aeromonas. In Bergey's manual of systematic bacteriology, Vol.1 Krieg, N.R. and Holt, J.G.(ed.). Williams and Wilkins: Baltimore and London.

Popoff, M.; Cognault, C.; Kinedjian, M. and Lemelin, M. (1981): Polynucleotide sequence relatedness among motile Aeromonas species. Curr. Mic., 159: 1629-1631.

Rogulska, A.; Antychowicz, J. and Zelazny, J. (1994): Haemolytic and proteolytic activity of Aeromonas hydrophila and Aeromonas sobria as markers of pathogenicity for carp (Cyrinas corpiol). Medycyna Weterynaryina, 50 (2): 55-58.

Saad, N. (1991): Occurrence of Aeromonas hydrophila in raw milk. Assiut Vet. Med. J., 25 (50): 98–102.

Santos, J.A.; Lopez-Diaz, T.M.; Garcia-Fernandez, M.C.; Garcia-Lopez, M.L. and Otero, A. (1996): Villalon, a fresh ewe's milk spanish cheese, as a source of potentially pathogenic Aeromonas strains. J. Food Prot., 59 (12): 1288-1291.

Wadstrom, T. and Ljungh, A. (1991): Aeromonas and Plesiomonas as food and waterborne pathogens. Int. J. Food Microbiol., 12:303–312.