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PREVALENCE OF *AEROMONAS HYDROPHILA* IN SOME DAIRY PRODUCTS AVAILABLE IN ASSIUT CITY

(With 7 Tables)

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انتشار الايروموناس هيدروفيلا في بعض منتجات الألبان في مدينة أسيوط

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تم فحص عدد ١٧٥ عينة من الجبن الدمياطي (٥٠) ، زبد الطهي (٥٠) والآيس كريم (٧٥) تم جمعها عشوائيا من أماكن مختلفة في مدينة أسيوط. وقد تم فحص العينات لمعرفة مدى تواجد وعدد ميكروب الايروموناس هيدروفيلا في هذه المنتجات. كذلك تم تصنيف عترات الايروموناس هيدروفيلا المعزولة وتم التعرف على خاصيتها الممرضة. وباستخدام مستنبت ال TSA تم عزل هذا الميكروب من ٣٦ ، ٨٠ ، ٤٦.٧ % من عينات الجبن الدمياطي ,زبد الطهي والآيس كريم على التوالي . وقد كانت أغلب العينات وهي ٣٨.٩ ، ٦٧.٥ ، ٣١.٤% من المنتجات المفحوصة تحتوى على اعداد من الميكروب تراوحت من 10^{2} – 10^{4} ، 10^{6} – 10^{8} ، 10^{4} – 10^{6} ميكروب لكل جرام أو مل على التوالي. وبتصنيف عترات الايروموناس اتضح ان الايروموناس هيدروفيلا , الكافي والسوبريا تواجد في ١٤، ٢٠ ، ٢% من عينات الجبن الدمياطى على الترتيب . بينما تواجدت هذه العترات في زبد الطهي بنسب مئوية هي ٤٦، ٢٦ ، ٨ على التوالي. بينما تواجدت عترات الايروموناس هيدروفيلا , الكافي والسوبريا في ١٧.٣ ، ٢٢.٧ ، ٦.٧ % من عينات الآيس كريم على الترتيب. وبالكشف عن العترات الممرضة من ميكروبات الايروموناس المعزولة اتضح أن ٧١.٤ ، ٧٨.٣ ، ٦١.٥ % من عترات الهيدروفيلا المعزولة من الجبن والزبد والآيس كريم على الترتيب كانت لها خاصية ممرضة. بينما ٢٣.١ ، ٥.٩ % من عترات الايروموناس كافي المعزولة من زبد الطهي والآيس كريم كانت ممرضة في حين لم تكن كذلك بالنسبة للعترات المعزولة من الجبن الدمياطي. أيضا تبين أن ٧٥ ، ٦٠ % من عترات الايروموناس سوبريا المعزولة من زبد الطهي والآيس كريم كانت ممرضة. وكانت العترة الوحيدة المعزولة من الجبن الدمياطي لها خاصية ممرضة وقد ناقش البحث الشروط الصحية لمنع تلوث هذه المنتجات بميكروب الايروموناس وكذلك مدى خطورته الصحية .

SUMMARY

One hundred and seventy five random samples of Damietta cheese (50), cooking butter (50) and ice cream (75) were collected randonly from different localities in Assiut City for enumeration and isolation of *Aeromonas hydrophila* species and for detection of their pathogenicity. Aeromonas species could be detected using TSA agar plates in 36, 80 and 46.7% of the examined Damietta cheese, cooking butter and ice cream samples with highest frequency distributions of 38.9, 67.5 and 31.4% within the range of 10^2-10^4, 10^6-10^8 and 10^4-10^6 organisms/g or ml, respectively. Identification of *Aeromonas* species proved that *A.hydrophila, A.caviae* and *A.sobria* were found in 14, 20 and 2% of the examined Damietta cheese samples, respectively. Cooking butter proved to contain *A.hydrophila, A.caviae* and *A.sobria* in percentages of 46, 26 and 8, respectively. While, ice cream samples were contaminated by these organisms in percentages of 17.3, 22.7 and 6.7 of the samples, respectively. Detection of pathogenicity of the isolated *Aeromonas* species revealed that 71.4, 78.3 and 61.5% of the isolated *A.hydrophila* recovered from Damietta cheese, cooking butter and ice cream, respectively were pathogenic depending on their haemolysin production. However, 23.1 and 5.9% of the isolated *A.caviae* detected in cooking butter and ice cream samples, respectively were pathogenic, while 75 and 60% of A.sobria recovered from those products, respectively were pathogenic. Damietta cheese samples yielded no pathogenic strains of A.caviae, while the only strain recovered from such product was pathogenic. The public health hazard and the recommended measures to prevent contamination by this organism were discussed.

Key words: *Aeromonas, cheese, butter, ice cream*

INTRODUCTION

Milk products provide a high favourable media for multiplication of different types of microorganisms including *Aeromonas hydrophila* which has been implicated in many cases of food borne gastroenteritis. *Aeromonas hydrophila* consists of three species, *A.hydrophila, A.caviae* and *A.sobria*, these species collectively referred to as motile or mesophilic aeromonads.

Milk and milk products including cheeses, butter and ice cream have been survyed by several investigators for the occurrence of *A.hydrophila* (Knochel and Jeppesen, 1990; Freitas et al., 1993; Hafez, 1996; Khalil, 1997 and El-Prince, 1998). These organisms cause gastroenteritis and several diseases such osteomylitis (Lopez et al., 1968); Septicemia (Ketover et al., 1973); meningitis (Qadri et al., 1976); endocarditis (Davis et al., 1978); skin infection (Joseph et al., 1979); cellulitis, wound infections, pneumonia, urinary tract infections, endocarditis and ear infections (Koneman et al., 1994).

Aeromonas hydrophila produces a number of potential virulence factors including enterotoxins, cytotoxins, haemolysins, lipases and proteases. Therefore it posses a highly significant public health problem as well as economic importance (Trust and Chipman, 1979). This work was done to secure the occurrence of such organism in some dairy products including Damietta cheese, cooking butter and ice cream.

MATERIAL and METHODS

1- Collecting samples:

Fifty random samples each of Damietta cheese and cooking butter and 75 random samples of ice cream were collected from different places in Assiut City. The samples were collected in clean dry sterile containers, and transferred to laboratory with a minimum of delay, where they were prepared for bacteriological examination according to A.P.H.A (1985).

2- Enumeration of *Aeromonas hydrophila* species:

Surface plating technique of 0.1 ml of prepared product was done over a dry surface of trypticase soy ampicillin (TSA) agar (FAO, 1979). The suspected colonies after 24h incubation at 28-30°C (pale yellow colonies) were counted. A significant number of the suspected colonies were picked up on agar slants and incubated at 28-30°C for 48h. For further identification.

3- Isolation of Aeromonas hydrophila species:

Trypticase soy broth tubes containing 10 µg/ml ampicillin were inoculated by 1ml of the prepared sample and incubated 24h at 28-30°C. Loopfuls from the incubated broth tubes were streaked onto Trypticase soy ampicillin agar (TSA) and incubated at 28-30°C for 48 h. Suspected colonies were picked up for further identification.

4- Identification of isolated strains:

The isolated orgainsms were identified biochemically according to Popoff and Veron (1976) including Gram's stain; Motility test, Voges-Proskauer test; Indol production; Gelatin liquification; Sugar fermentation; Oxidation fermentation test and Asculine broth hydrolysis.

5- Detection of haemolysis:

Detection of haemolysis was carried out according to Finegold and Martin (1982).

RESULTS

The obtained results are recorded in Tables 1-7

DISCUSSION

The results summarized in Table 1 showed that 18 out of 50 Damietta cheese samples (36%) were contaminated by *A.hydrophila*. The majority of the positive samples (38.9%) contained the organism in numbers ranged from 10^2-10^4/g. About 27.7% of the positive samples had counts within the range of 10^6-10^8/g, while 6 positive samples were equally distributed (16.7%) among counts less than 10^2/g and counts of 10^4-10^6/g, respectively.

The obtained results are in agreement with those recorded by Freitas et al. (1993) and lower incidence were obtained by Hafez (1996) and El-Prince (1998). Regarding the data presented in Table 2 *Aeromonas* species could be differentiated into *A.hydrophila* which existed in 14% of the examined Damietta cheese samples. While, *A.caviae* and A. sobria were detected in 20 and 2% of the examined samples, respectively. It is obvious from these results that *A.hydrophila* and *A. caviae* are the predominant species found in the examined Damietta cheese samples and this could be attributed to the fact that such species are the most prevalent ones in the environment and commonly found in contaminated food (Hafez, 1996).

The data recorded in Table 3 revealed that 80% of the examined cooking butter samples were contaminated by *Aeromonas* species. Most of positive samples (67.5%) had counts ranged from 10^6-10^8/g. The rest of the positive samples distributed within the range of 10^2-10^4, 10^4-10^6 and $>10^8$ organisms/g in percentages of 5, 25 and 2.5%, respectively. Aeromonas specie recovered from the examined samples as shown in

Table 4 could be identified into *A.hydrophila, A.caviae* and *A.sobria* in 46, 26 and 8% of the samples, respectively. The presence of *Aeromonas* species in cooking butter could be attributed to the fact that the organisn is widely distributed in nature and its existance depends on the effectiveness of sanitary measures and the hygienic supervision during processing, handling and distribution. Also, one of the most important factors affecting cooking butter contamination is the way of storage and the freezing temperature. (Saad et al., 1994).

The results of Table 5 point out that 46.7% of the examined ice cream samples proved to have Aeromonas species. The highest frequency distribution (31.4%) had counts ranged from 10^4-10^6 / ml, out of the positive samples 28.6% were contaminated by the organism in counts less than 10^2/g. However, 25.7% of the positive samples had counts ranged from 10^6-10^8/ml. Table 6 indicates that *A.hydrophila* existed in 17.3% of the examined ice cream samples while, *A.caviae* was detected in 17 (22.7%) of ice cream samples. *A.sobria* organisms contaminated 6.7% of the examined samples. The obtained results are in agreement with those reported by Knochel and jeppesen (1990) and Khalil (1997).

The high incidence of *Aeromonas* species in the examined ice cream samples could be attributed to contamination of raw milk used (Hafez and Halawa, 1993), using contaminated milk powder, low quality ingredients, using of polluted water supplies, lack of hygienic supervision during processing and handling, besides the absence of pasteurization especially in case of small scale produced ice cream.

The summarized data collected in Table 7 reveal the pathogenicity of isolated *Aeromonas* straing recovered from the examined products depending on their haemolysin production. The pathogenic strains of *A.hydrophila, A. caviae* and *A.sobria* recovered from Damietta cheese were, respectively 71.4, 0, and 100% of the isolated organisms, while in case of cooking butter the pathogenic strain were 78.3, 23.1 and 75% of the isolated *Aeromonas* organisms, respectively. In case of ice cream, 61.5, 5.9 and 60% of *A.hydrophila, A.caviae* and *A.sobria* were pathogenic, respectively.

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Table 1: Incidence and frequency distribution of the examined Damietta cheese samples based on their Aeromonas count/g.

No. of Examined Samples	Positive samples		Distribution of positive samples		
	No.	%	Range	Frequency	
				No.	%
50	18	36	<100	3	16.7
			10^2-10^4	7	38.9
			10^4-10^6	3	16.7
			10^6-10^8	5	27.7
			>10^8	-	-
			Total	15	100

Table 2: Incidence of different Aeromonas recovered from the examined Damietta cheese samples.

No. of Examined Samples	Positive samples					
	A.hydrophila		*A.caviae*		*A.sobria*	
	No.	%	No.	%	No.	%
50	7	14	10	20	1	2

Table 3: Incidence frequency distribution of the examined cooking butter samples based on their Aeromonas count/g.

No. of Examined Samples	Positive samples		Distribution of positive samples		
	No.	%	Range	Frequency	
				No.	%
50	40	80	<100	-	-
			10^2-10^4	2	5.0
			10^4-10^6	10	25.0
			10^6-10^8	27	67.5
			>10^8	1	2.5
			Total	40	100

Table 4: Incidence of different Aeromonas recovered from the examined cooking butter samples

No. of Examined Samples	Positive samples					
	A.hydrophila		*A.caviae*		*A.sobria*	
	No.	%	No.	%	No.	%
50	35	46	13	26	4	8

Table 5: Incidence and frequency distribution of the examined ice cream samples based on their Aeromonas count/ml.

No. of Examined Samples	Positive samples		Distribution of positive samples		
	No.	%	Range	Frequency	
				No.	%
75	35	46.7	<100	10	28.6
			10^2-10^4	2	5.7
			10^4-10^6	11	31.4
			10^6-10^8	9	25.7
			>10^8	3	8.6
			Total	35	100

Table 6: Incidence of different Aeromonas recovered from the examined ice cream samples.

No. of Examined Samples	Positive samples					
	A.hydrophila		*A.caviae*		*A.sobria*	
	No.	%	No.	%	No.	%
75	13	17.3	17	22.7	5	6.7

Table 7: Pathogenic Aeromonas strains recovered from the examined samples depending on their haemolysin production.

Product	*A.hydrophila*			*A.caviae*			*A.sobria*		
	No. of Isolated Strains	Haemolytic strains		No. of Isolated strains	Haemolytic strains		No. of Isolated strains	Haemolytic strains	
		No.	%		No.	%		No.	%
Damietta cheese	7	5	71.4	10	-	-	1	1	100
Cooking butter	23	18	78.3	13	3	23.1	4	3	75
Ice-cream	13	8	61.5	17	1	5.9	5	3	60
Total	43	31	72.1	40	4	10	10	7	70