INCIDENCE OF AEROMONAS SPECIES IN RAW MILK AND SOME DAIRY PRODUCTS IN KAFR EL-SHEIKH CITY AND GROWTH CHARACTERISTICS OF AEROMONAS HYDROPHILA IN YOGHURT

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ABSTRACT

75 random samples of raw milk, yoghurt and cooking butter (25 of each) were collected from different localities, in Kafr El-Sheikh city, to be examined for the presence and any countable population of ***Aeromonas*** *species.* ***Aeromonas*** *species could be isolated from 8%, 44% and 36% from raw milk, yoghurt and cooking butter by direct plate surface technique while could be isolated from 12, 44 and 48% of the examined sample by using enrichment broth medium. A.* ***hydrophila*** *could by isolated from 4%, 24% and 16% of raw milk, yoghurt and cooking butter,* ***A. caviae*** *could isolated form 4, 24 and 24%,* ***A. sobria*** *could be isolated form 12% of examined yoghurt samples only,* ***A. trota*** *could be isolated from 8% of each raw milk & yoghurt samples while 4% from cooking butter,* ***A. janda*** *could be isolated from 4% of examined raw milk samples only and* ***A. schuberttii*** *could be isolated from 4% of examined yoghurt samples.*

The pH of yoghurt has significant effect on growth of aeromonas hydrophila as the pH decrease. The count of ***A. hydrophila*** *decrease and the organism is completely inhibited as the pH reach 3.9.*

INTRODUCTION

Aeromonas are ubiquitous inhabitants of natural waters, both fresh and salt, where they infect animals. In humans, they are most often associated with infections of wounds acquired near or in water or with diarrheal disease ***(Janda, 1991).***

Five diarrhea presentations for aeromonas-related gastroenteritis: Secretory (acute watery diarrhoea, often with vomiting), dysenteric (accompanied by blood and mucus in the stool), chronic (lasting longer than 10 days), choleric ("rice water" stools), and traveler's ***(Janda and Duffey, 1988)***.

Aeromonas-hydrophila produces a heat-labile enterotoxin and a heat-stable cytotoxic enterotoxin. Adherence mediated by pili may also serve as a virulence factor ***(Janda, 1991 and Junda & Duffey, 1988)***.

Besides its increasing concern as a food borne pathogen, *A. hydrophila* could play an important role in deterioration of food stored at refrigeration temperature because of its ability to grow and produce extracellular enzymes (lipase, protease, amylase and nuclease) at low temperature ***(Beuchat, 1991)***.

Isolation of *Aeromonas* spp. has been reported from raw milk, butter, yoghurt, soft cheese and ice cream ***(Freitas et al., 1993; Khalil, 1997; Abd El-Hady and Halawa, 1999 and El-Shorbagy and Al-Ganzoury, 2002)***.

The goal of the present investigation is to estimate the prevalence of *Aeromonas* species in raw milk, yoghurt and cooking butter in Kafr El-Sheikh city.

MATERIAL AND METHODS

1. Collection of samples:

Seventy five random samples(25 raw milk,25 yoghurt and 25cooking butter)were collected from different dairy shops and groceries in Kafr El-Sheikh city. Collected samples were transferred to the laboratory with a minimum of delay for sanitary,chemical and microbiological examinations.

2. Preparation of samples:

Samples were prepared as the technique described by ***APHA (1992)***.

3. Sanitary examination:

A) Determination of titratable acidity of milk *(A.P.H.A., 1985)*.

B) Determination of pH values of yoghurt.

The pH values of yoghurt samples were measured by pH meter (Jenway, 3505 pH meter).

4. Determination of sodium chloride content of Butter (ISO 1738-1980).

5. Microbiological examination:

A) Preparation of serial dilution *(A.P.H.A., 1992)*:

B) Enumeration of *Aeromonas* spp.:

a- 100 µl from each dilution were evenly spread onto pre-poured plates of aeromonas selective agar (Oxoid) and incubated at 30°C/24 hrs. The presumptive plates were counted and calculated.

b- Isolation and confirmation of *Aeromonas* spp. by using enrichment broth medium. 25 ml or g. of each sample were added to 225 ml of Ampicillin broth (TSB plus 30 mg/L ampicillin) and incubated at 30°C for 24 hrs. ***(APHA, 1992)***. Five colonies presumed to be Aeromonas were streaked onto tryptone soya agar (TSA) slants, incubated at 30°C/24 hrs. for complete confirmation according to ***(Popoff, 1984)***.

6. Survival of *Aeromonas hydrophilia* in yoghurt:

A) Bacterial strains:

1. A mixture of active culture of *Streptococcus thermophillus* (14486) and *Lactobacillus bulgaricus* (11842); (1: 1) was used as starter.

2. Stock culture of *Aeromonas hydrophilla* was prepared in alkaline peptone water.

B) Treatment:

Yoghurt was manufactured by the method of ***Robinson*** and ***Tamino (1983)*** as follows: Raw cow's milk for yoghurt manufacture was pasteurized at 63^{o}C for 30 min., cooled to 10^{o}C within 30 min., then inoculated with the yoghurt culture (2%) and mixed thoroughly. Two batches of yoghurt were prepared; one batch was used as control and the other was inoculated with the test organism to provide an inoculum level of 4×10^{7}/ ml milk.Both batches were kept at 4^{o}C.Samples were taken from the curd and then after24 h till96h after curd formation and measured for pH value and *Aeromonas hydrophilla* counts.

DISCUSSION

The results given in Table(1)show that the acidity of raw milk samples tested were within the normal limits which ranged from 0.11 to 0.19 with a mean value of 0.15 ± 0.021.

Aeromonads were identified in12% of milk samples using enrichment broth medium.Only 8%of the milk samples were positive using direct plate surface technique and showing colony counts varying from 1×10^{5} to 4×10^{5} CFU/ml with an average value of 2.5×10^{5} CFU/ml.(Table 2&3).

Nearly similar results could be detected by ***Enas (1999)*** while ***Moustafa (2000)*** found higher incidence.

Aeromonads are commonly present in farm (feed, water, soil, and equipments used for milking) and can thus contaminate the surface of udder, teats and get into milk. Hence the role of raw milk as a vehicle of transmission causing milk borne disease is well documented ***(Varnam and Evans, 1991)***.

4% of milk samples were contaminated with *A.hydrophila,A.caviae* and *A. janda* while 8% of the samples were contaminated with *A. trota* (Table 5). Of 28 confirmed cultures isolated from raw milk, 14(50%) of the strains could be classified as *A. trota* followed by 10 (35.8%) as *A. hydrophilia* and 2 (7.1%) as *A. caviae* and *A. janda* (Table 6).

Higher results could be detected by ***Hussein (1999)*** and ***Moustafa (2000)***.

The results reported in Table(1)indicate that the pH values in examined yoghurt samples ranged from 3.70 to 4.25 with a mean value of 4.01 + 0.03.

The revealed results in Table (2 and 3) showed that *Aeromonas* spp. could be detected in 11 (44%) of the examined yoghurt samples in counts ranged from 1×10^2 to 3.5×10^5 with a mean value of $4 \times 10^4 + 3 \times 10^4$. Lower incidence could be reported by ***Sami(1999)*** and ***El-Shorbagy and Al-Ganzoury (2002)***.

There is a marked decrease in the count of *Aeromonas* spp. as the pH value of the examined yoghurt sample decrease, the highest count 3.5×10^5 was at pH value of 4.25 while the lowest count 1×10^2 was at pH value of 3.81 (Table 4). This is in agreement with ***Aykut Aytac and Yesim Özbas (1994)***.

The low pH value of yoghurt creates an undesirable environment for the growth of most spoilage microorganisms, where *Aeromonas* spp. are reported to be sensitive for pH value below 6.0. On the other hand, starter bacteria can produce diacetyl which had some bactericidal activity against *Aeromonas* ***(Plumbo and Buchanan, 1988; Motlagh et al.,1991 and Varnam and Evans, 1991)***.

Each of *A. hydrophila* and *A. coviae* could be isolated from 6 (24%) examined yoghurt samples followed by *A. sobria* 3 (12%), *A. trota* 2 (8%) and *A. schuberttii* 1 (4%) (Table 5). Of 74 cultures isolated from yoghurt samples, 37.8% could be classified as *A. caviae,* 32.5% as *A. hydrophila*, each of *A. sobria* and *A. trota* by 13.5% and 2.7% *A. schuberttii* (Table 6). Higher results could be detected by ***Enas (1999) and El-Shorbagy and Al-Ganzoury (2002)***.

The results obtained in Table (1) show that sodium chloride percent in cooking butter ranged from 0.5-1% with a mean value 0.9 ± 0.03.

Aeromonads were detected in 36% of cooking butter samples examined by direct technique with count ranged from 1 x 10^3 to 6.1 x 10^4 cfu/g and with a mean value of 1.4 x 10^4 $\pm$ 6.2 x 10^3 c.f.u/g (Table 2), while the incidence was 48% of examined samples after enrichment (Table 3).

From the results of analysis, it is noticed that there was no relation between the incidence of *Aeromonas* spp. and NaCl% of samples as the NaCl% is less than the legal requirement of NaCl% for cooking butter 3% according to the ***Egyptian standard (1976)***.

The lower recovery with direct method than with enrichment was explained by ***MacRae et al. (1993)*** who reported that *Aeromonads* can be inhibited by microorganisms present in the same sample because *Aeromonads* are poor competitor.

A. caviae was detected in 6(24%)samples followed by *A. hydrophila* 4 (16%) and *A. trota* 1 (4%) of 46 Aeromonas isolates 56.5% were identified as *A. caviae*, 39.1 *A. hydrophila* and 4.4% *A. trota.*

Lower results could be detected by ***Abd El-Hady and Halawa (1999)***, while higher results were reported by ***Hussein (1999)***.

High incidence of *Aeromonas* in cooking butter may be a consequence of contaminated milk as the cooking butter is made from raw sower cream which produced under unsanitary conditions ***(Palumbo, 1987)***.

Regarding to Table (7 and Fig. 1) *Aeromonas hydrophila* failed to be detected in yoghurt after 72 h of storage at refrigeration temperature. Their count decreased from 4 x 10^7 to 0 parallel to decrease in pH value from 5.7 to 3.9. These results are supported by ***Aykut Aytac and Yesim Özbas (1994)*** who stated that *A. hydrophila* was completely inhibited in control and acidophilus yoghurt after 5 days of storage.

So the pH of yoghurt can be used to control the growth of *A. hydrophila*.

In conclusion, selection of high quality raw milk and the importance of way of processing as well as the storage condition of dairy products on its final hygienic status, which may enhance or inhibit the microbial contaminants present. So selling of dairy product should be strictly controlled with health authority to eliminate potentiality of occurring hazards arising from microbial contamination.

Table (1): Statistical analytical results of sanitary tests and NaCl content in examined samples.

Type of test	Min.	Max.	Mean ± S.E.M.
Titratable acidity of raw milk	0.11	0.19	0.15 ± 0.021
pH value of yoghurt	3.70	4.25	4.01 ± 0.03
NaCl % of Butter	0.5	1	0.9 ± 0.03

- N.B. No. of examined sample 25 each.

Table (2): Statistical analytical results of *Aeromonas* spp. in examined samples using direct plate surface technique.

Type of test	No. of examined samples	+ve sample		Count/ml or g		
		No.	%	Min.	Max.	Mean ± SE
Raw milk	25	2	8	1×10^5	4×10^5	$2.5 \times 10^5 \pm 1.1 \times 10^5$
Yoghurt	25	11	44	1×10^2	3.5×10^5	$4 \times 10^4 \pm 3 \times 10^4$
Cooking butter	25	9	36	1×10^3	6.1×10^4	$1.4 \times 10^4 \pm 6.2 \times 10^3$

Table (3): Prevalence of *Aeromonas* spp. in examined samples using enrichment broth medium.

Types of samples	No. of examined samples	Frequency	
		+ve samples	%
Raw milk	25	3	12
Yoghurt	25	11	44
Cooking butter	25	12	48

Table (4): Relation between pH values and count of *Aeromonas* spp.in positive yoghurt samples.

pH value	Count CFU/g
4.25	3.5×10^5
4.20	7×10^4
4.16	5.9×10^3
4.16	5.9×10^3
4.11	5×10^3
4.06	2.7×10^3
4.05	1×10^2
3.98	1×10^2
3.96	1×10^2
3.92	1×10^2
3.81	1×10^2

Table (5): Incidence of different motile *Aeromonas* spp. in dairy products analysed.

Type of samples	No. of examined samples	No. of samples with											
		A. hydrophilia		*A. caviae*		*A. sobria*		*A. trota*		*A. janda*		*A. schubertii*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	25	1	4	1	4	0	0	2	8	1	4	0	0
Yoghurt	25	6	24	6	24	3	12	2	8	0	0	1	4
Cooking butter	25	4	16	6	24	0	0	1	4	0	0	0	0

Table (6): Frequency distribution of isolated *Aeromonas* spp. from examined dairy products.

Type of samples	No. of isolates	No. of samples with											
		A. hydrophilia		*A. caviae*		*A. sobria*		*A. trota*		*A. janda*		*A. schubertii*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	28	10	35.8	2	7.1	0	0	14	50	2	7.1	0	0
Yoghurt	74	24	32.5	28	37.8	10	13.5	10	13.5	0	0	2	2.7
Cooking butter	46	18	39.1	26	56.5	0	0	2	4.4	0	0	0	0

Table (7): Effect of storage and pH-value in the survival of *Aeromonas hydrophila* in yoghurt.

Storage (h)	pH	Count c.f.u/g
Initial count	6.2	4×10^7
Curd	5.7	8×10^7
24 h	5.0	1×10^6
48 h	4.5	7×10^3
72 h	4.2	5 x 10
96 h	3.9	0

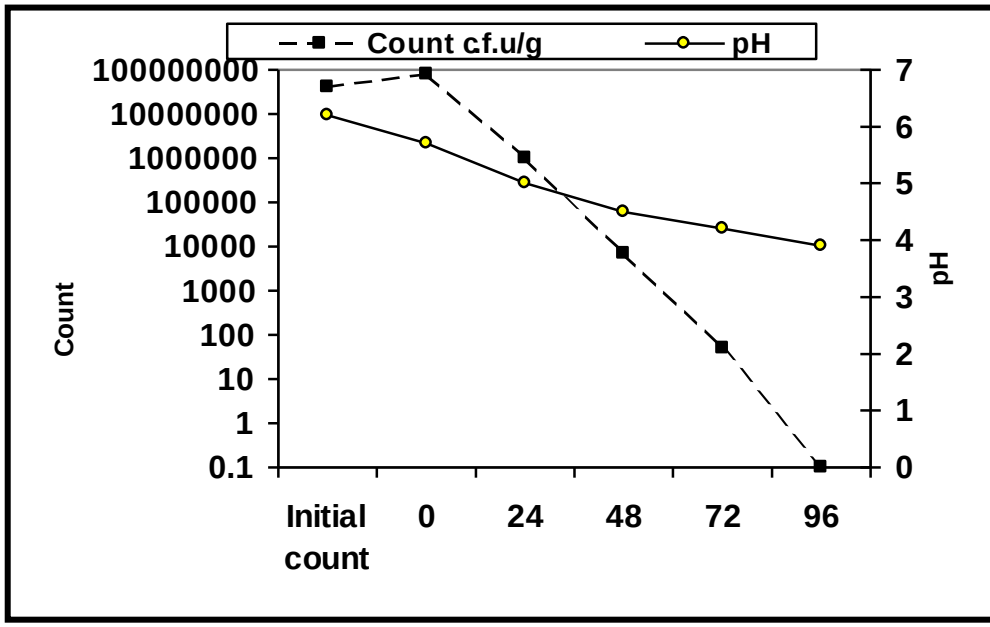

Fig. (1): Effect of storage and pH value on the survival of *A. hydrophila* in yoghurt.

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مدى تواجد ميكروب الايروموناس فى الحليب الخام وبعض منتجات الألبان فى مدينة كفر الشيخ
ونمو ميكروب الايروموناس هيدروفيلا فى الزبادى

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تم تجميع 75 عينة من الحليب الخام ، الزبادى وزبد الطبخ (25 من كل منتج) من أماكن مختلفة من أسواق مدينة كفر الشيخ لفحصها لمدى تواجد ميكروبات الايروموناس. وقد تم عزل ميكروبات الايروموناس من 8% ، 44% ، 36% من الحليب الخام ، الزبادى وزبد الطبخ باستخدام الفرد السطحى على المستنبت بينما تم عزلها بنسبة 12% ، 44% ، 48% باستخدام طريقة الإنماء الغير مباشر فى عينات الحليب الخام ، الزبادى وزبد الطبخ.

تبين من النتائج أن ميكروب الايروموناس هيدروفيلا تم عزله من 4 ، 24 ، 16% من الحليب الخام ، الزبادى وزبد الطبخ ، أما بالنسبه لميكروب الايروموناس كافى فقد تم عزله بنسبة 4 ، 24 ، 24% من نفس العينات على التوالى وبالنسبه لميكروب الايروموناس سوبريا فقد تم عزله من عينات الزبادى فقط بنسبة 12% وقد تم عزل ميكروب الايروموناس تروتا من 8% من كل من عينات الحليب الخام والزبادى وبنسبة 4% من عينات الزبد أما بالنسبة لميكروب الايروموناس جاندا فقد تم عزله من الحليب الخام فقط بنسبة 4% وأخيرا ميكروب الايروموناس سكبرتى فقد تم عزله من الزبادى فقط بنسبة 4%.

هذا وقد تم دراسة تأثير تركيز أيون الهيدروجين pH فى الزبادى على نمو ميكروب الايروموناس هيدروفيلا مع حفظ المنتج فى درجة حرارة الثلاجة وقد وجد أن pH لها تأثير فعال على نمو هذا الميكروب وكلما تناقص تركيز أيون الهيدروجين تناقص عدد هذا الميكروب حتى اختفى بعد 96 ساعة من الحقن وعند تركيز pH 3.9.

وقد تمت مناقشة الأهمية الصحية لوجود تلك الميكروبات فى منتجات الألبان تحت الدراسة.