Alexandria Journal of Veterinary Sciences 2014, 40: 52-64
ISSN 110-2047
www.alexjvs.com

Enumeration and Characterization of Aeromonas spp. Isolated from Milk and Some Dairy Products in Sharkia Governorate Egypt

Nagwa I. Ahmed [1], Salah F. A. Abd El Aal[2], Madeha A. Ayoub[2], Magdy S. El Sayed [2]
[1] Animal Health Institute-Zagazig.
[2]Food Control Department Faculty of Veterinary Medicine, Zagazig University, Egypt.

Key words: Aeromonas; raw cow's milk; Domiati cheese; local plain yoghurt; laboratory pasteurized milk; acidity %; pH value; NaCl %.

ABSTRACT:

A survey was conducted to determine the prevalence of Aeromonas spp. in 75 random samples (25 each of raw cow's milk, local plain yoghurt and Domiati cheese) collected from different dairy shops, supermarket and street peddlers in Diarb Negm and Zagazig cities Sharkia Governorate, Egypt. Investigations involved proteolytic and lipolytic activities of isolated Aeromonas spp. and the effect of heat- treatment, acidity, pH and Sodium chloride concentration on prevalence of Aeromonas bacteria. Prevalence of Aeromonas spp. was proved in 32, 44 and 20.0% of examined raw cow's milk, local plain yoghurt and Domiati cheese samples with mean count of 9.8 $x10^3$, 1.4 $x10^5$ and 6.9 $x10^3$/ml, respectively. Identification of confirmed raw cow's milk isolates revealed that *A. trota*, *A. hydrophila*, *A. janda* and *A. caviae* were the predominant strains with percentages of 40, 25, 25 and 10.0% respectively. While local plain yoghurt isolates could be identified as *A. caviae*, *A. sobria, A. hydrophila*, *A. trota* and *A. schubertii* with percentages of 36.4, 22.7, 18.2, 13.6 and 9.1% respectively. Meanwhile identification of 10 confirmed Domiati cheese cultures revealed that the predominant strains were *A. hydrophila, A. caviae* and *A. trota* with percentages of 30, 50 and 20% respectively. All laboratory pasteurized milk samples revealed no count and there is marked decrease in the count of Aeromonas spp. as the acidity % of the examined raw cow's milk samples increase. While the count decrease when the pH value of the examined local plain yoghurt samples decrease and the NaCl % of the examined Domiati cheese samples increase. Characterization of isolated Aeromonas strains pointed that 50% of *A. hydrophila*, 60% of *A. caviae*, 40% of *A. sobria*, 53.8% of *A. Trota*, 100% of *A. janda* and 50% of *A. schubertii* were psychrotrophic. *A. hydrophila* exhibited proteolytic and lipolytic activities at the percentage of 41.7 and 16.7% respectively but in case of *A. caviae* strains the percentages were 46.7% and 20% respectively and with *A. trota* were 30.8 and 15.4% respectively. 60% of *A. sobria* and 100% of *A. janda* and *A. schubertii* strains showed proteolytic activity only. The public health importance and economic significance of existing microorganisms as well as the suggestive measures for improving the quality of raw milk and dairy products were discussed.

Corresponding Author: Dr. Salah Fathy Ahmed Abd El Aal (drsalah_aal@yahoo.com)

1-INTRODUCTION

The current practice of refrigerated storage of milk for prolonged periods before processing is selective for growth of psychrotrophs which may become the predominant microflora (Sørhaug, 1992). The most frequently encountered psychrotrophs are Gram-negative non-sporing rods belonging to the genera Pseudomonas Alcaligenes Achromobacter Aeromonas and Flavobacterium (Shan, 1994).

The organism is of great concern to microbiologists because of its

capability of growth at refrigerated temperatures. In humans they are most often associated with infections of wounds acquired near or in water or with diarrheal disease (Janda, 1991).

Motile Aeromonas have been reported as etiologic agents of several diseases in animals and humans (Mergraud, 1986). In humans gastroenteritis and septicemia in immunocompromized patients and cutaneous infections are the most common diseases (Atwegg and Geiss, 1989). Five types of diarrhea of Aeromonas related gastroenteritis: secretory (acute watery diarrhea often with vomiting) dysenteric (accompanied by blood and mucus in the stool) chronic (lasting longer than 10 days) choleric (rice water stools) and travelers (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 virulence factor (Janda and Duffey, 1988; Janda, 1991 and Sørhaug and Stepaniak, 1997).

Besides its increasing concern as a blood borne pathogen *A. hydrophila* could play important role in deterioration of food stored at refrigeration temperature because of its ability to grow and produce thermoresistant extracellular enzymes (lipase protease amylase and nuclease) which are capable of degrading important milk constituents and thus affect the quality of finished dairy products. (Beuchat, 1991 and Shan, 1994).

Isolation of Aeromonas spp. has been reported from raw milk yoghurt and cheese (Freitas *et al.,* 1993; Khalil, 1997; Abd El-hady and Halawa, 1999 and El-Shorbagy and Al-Ganzoury, 2002). However little is known about role of food in transmission of these pathogens so this study was undertaken as part of an effort to determine the role of food in the epidemiology of Aeromonas infections, the suitability of the selective media for recovery of the organism, the proteolytic and lipolytic activities of isolated Aeromonas spp. and the effect of heat treatment as well as effect of acidity, pH and Sodium chloride concentration on prevalence of Aeromonas bacteria. Moreover, the recognition of the economic and public health significance of Aeromonas spp.

2-MATERIALS AND METHODS

2.1.Collection of samples: Seventy five random samples (25 each of raw cow's milk, local plain yoghurt and Domiati cheese) were collected from different dairy shops, farms and street peddlers in Sharkia Governorate (Diarb Negm and Zagazig cities) Egypt. The collected samples were transferred to the laboratory in an insulated ice-box at 4°C with a minimum of delay.

2.2.Preparation of samples: The technique adopted was recommended by American Public Health Association "APHA"(1992). Each sample of raw cow's milk was subjected to Guaiac test for detection of heat- treatment before being examined (Schonberg, 1956)

2.3.Quality evaluation:

2.3.a.Determination of titratable acidity percentage of raw cow's milk using Standard method (James, 1995).

2.3.b.Determination of pH values of yoghurt using pH meter (Jenway, 3505 pH meter).

2.3.c.Determination of Sodium chloride content of Domiati cheese: By Nuclear Infra Red Chromatography model MPA Germany at the microanalytical laboratory Green Land Company, El-Asher Men Ramadan City, Egypt.

2.4.Bacteriological examination:

2.4.a.Preparation of serial dilution (APHA, 1992).

2.4.b.Enumeration and isolation of Aeromonas: 0.1 ml from each dilution were evenly spread onto pre-poured plates of Aeromonas selective agar medium (Oxoid) and incubated at 30^{o}C for 24 hours. The presumptive plates (translucent pinkish colonies with varying diameter from 0.5 – 3mm) were counted and calculated.

2.4.c.Identification of isolated

Aeromonas spp.: The presumptive colonies were streaked onto nutrient agar plates and incubated at 30°C for 24 hours. Identification was carried out according to Popoff (1984).

2.5. Determination of psychrotrophic, proteolytic and lipolytic activities of Aeromonas species (Harrigan and MacCance, 1976):

2.5.a.Preparation of isolates: Isolates were subcultured on nutrient agar plates and incubated at 30°C/24 hrs. Pure culture were inoculated into nutrient broth and incubated overnight at 30°C.

2.5.b.Psychrotrophic activity: (APHA, 1992): Isolates were inoculated onto standard plate count agar medium then incubated at 7°C for 10 days.

2.5.c.Proteolytic activity: Overnight culture was spot on standard plate count agar supplemented with 10% skim milk and incubated at 25°C/48 hrs then subsequently flooded with a 10% v/v acetic acid solution. Clear zones around the colonies after 1-minute exposure were considered positive.

2.5.d.Lipolytic activity (Oxoid, 1998): A sugar-free nutrient agar medium (pH 7.5) with emulsified butter fat and Victoria blue B as indicator was used. Overnight cultures were streaked on pre-poured and incubated at 25°C for up to 7 days. Bright blue colonies were regarded as lipase positive

2.6. Effect of heat- treatment on Aeromonas spp. in raw cow's milk: After sampling for sanitary and bacteriological examination raw cow's milk samples were subjected to heat - treatment (pasteurization) according to Robinson and Tamine (1983) where they heated at 63°C for 30 min. then cooled to 10°C within 30 min. Then the same procedures for detection of Aeromonas spp. were repeated and the results were recorded.

2.7.Statistical analysis: All the data analyzed using SPSS/PCT (Foster, 2001). Independent T-test was performed to evaluate differences.

3.RESULTS and DISCUSSION

Table (1): Statistical analytical results of acidity percent, pH value and NaCl content in examined samples (N=25 of each).

Type of test	Min.	Max.	Mean ±S.E.M.
Titratable acidity % of raw cow's milk	0.10	0.20	0.14 ± 0.006
pH value of yoghurt	3.20	4.70	4.03 ± 0.085
NaCl % of Domiati cheese	2.20	3.55	2.92 ± 0.069

Table (2): Statistical analytical results of *Aeromonas spp.* in examined samples (N=25 of each).

Examined samples	No. of examined samples	Positive samples		Min.	Max.	Mean	±S.E.M.
		No.	%				
Raw cow's Milk	25	8	32.0	2.0×10^3	3.0×10^4	9.8×10^3	3.2×10^3
Yoghurt	25	11	44.0	1.0×10^2	9.0×10^5	1.4×10^5	8.8×10^4
Domiati cheese	25	5	20.0	3.0×10^2	2.0×10^4	6.9×10^3	3.9×10^3

Table (3): Relation between titratable acidity % and count of Aeromonas spp. in positive raw cow's milk samples.

Acidity %	Count/ml.
0.1	3.0×10^4
0.1	1.0×10^4
0.11	12.0×10^3
0.11	12.0×10^3
0.11	6.0×10^3
0.12	4.0×10^3
0.12	2.0×10^3
0.13	2.0×10^3

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

pH value	Count/gm.
4.0	1.0×10^2
4.0	2.0×10^2
4.0	4.0×10^2
4.0	7.0×10^2
4.3	1.0×10^3
4.5	4.0×10^3
4.5	5.0×10^3
4.6	4.0×10^4
4.6	8.0×10^4
4.7	5.0×10^5
4.7	9.0×10^5

Table (5): Relation between NaCl % and count of Aeromonas spp. in positive Domiati cheese samples.

NaCl%	**Count/gm.**
3.0	2.0×10^4
3.2	12.0×10^3
3.4	1.2×10^3
3.4	1.0×10^3
3.55	3.0×10^2

Table (6): Frequency distribution of *Aeromonas spp.* Obtained from examined samples (N=25 of each).

Isolats	Raw cow's milk		Yoghurt		Domiati cheese	
	No. of samples	%	No. of samples	%	No. of samples	%
A. hydrophilia	2	8.0	2	8.0	2	8.0
A. caviae	2	8.0	4	16.0	2	8.0
A. sobria	0	0.0	3	12.0	0	0.0
A. trota	3	12.0	2	8.0	2	8.0
A. janda	3	12.0	0	0.0	0	0.0
A. schubertii	0	0.0	2	8.0	0	0.0

Table (7): Prevalence of *Aeromonas* isolates obtained from examined samples**.**

Isolats	Raw cow's milk		Yoghurt		Domiati cheese	
	No. of isolate	%	No. of isolate	%	No. of isolate	%
A. hydrophilia	5	25.0	4	18.2	3	30.0
A. caviae	2	10.0	8	36.4	5	50.0
A. sobria	0	0.0	5	22.7	0	0.0
A. trota	8	40.0	3	13.6	2	20.0
A. janda	5	25.0	0	0.0	0	0.0
A. schubertii	0	0.0	2	9.1	0	0.0
Total isolates	20	100.0	22	100.0	10	100.0

Table (8): Psychrotrophic, proteolytic and lipolytic activities of *Aeromonas spp.* isolated from examined samples**.**

Aeromonas spp.	No. of strains	Psychrotrophic growth		Proteolytic activity		Lipolytic activity	
		No.	%	No.	%	No.	%
A. hydrophilia	12	6	50.0	5	41.7	2	16.7
A. caviae	15	9	60.0	7	46.7	3	20.0
A. sobria	5	2	40.0	3	60.0	0	0.0
A. trota	13	7	53.8	4	30.8	2	15.4
A. janda	5	5	100.0	5	100.0	0	0.0
A. schubertii	2	1	50.0	2	100.0	0	0.0

3.1.Raw cow's milk:

Its high water activity moderate pH (6.4-6.6) and ample supply of nutrients makes milk an excellent medium for microbial growth. This demands high standards of hygiene in its production and processing a fact recognized in most countries where milk was the first food to be the focus of modern food hygiene legislation.

The results given in Table (1) showed that the acidity % of examined raw cow's milk samples was ranged from 0.10 to 0.20 % with an average of 0.14%. It is evident that all examined raw cow's milk samples which gave count did not exceed the normal level of acidity (0.12-0.16%); (APHA, 1992) as the maximum % was 0.13% (Table, 3). Titratable acidity % of milk has a greater importance where it is used for assessing its keeping quality. Higher acidity of milk may be attributed either to its contamination by lactic acid producing microorganisms or pathogenic microorganisms which ferment lactose and increase its acidity render it unmarketable due to off-taste and unfit for human consumption due to pathogens (APHA, 1992).

There is marked decrease in the count of Aeromonas spp. as the acidity % of the examined raw cow's milk sample increase the highest count was 3.0 $x10^4$ at acidity % of 0.10 while the lowest count was 2.0 $x10^3$ at acidity % of 0.13 (Table, 3). This may be attributed to all spoilage organisms were very sensitive to higher acidity as reported by Varnam and Evans (1991).

Aeromonads were identified in 8 (32.0%) of raw cow's milk samples which showed colony counts/ml varying from 2.0 $x10^3$ to 3.0 $x10^4$ with an average value of 9.8 $x10^3$ (Table, 2). The overall incidence of Aeromonas spp. was nearly similar to that reported by Ergullu (1978) Gogov *et al.* (1980); El-Gamal (1997) and Sami (1999). While Milliere and Veillet-Poncet (1979) and Banerjee and Black (1986) reported lower figures of raw cow's milk samples. Several surveys in Australia (Kirove *et al.,* 1993) and the United States (MaCrea *et al.,* 1993) also point to a high prevalence of the organisms in raw cow's milk 60% and 50% respectively. In Egypt higher incidence percentages of Aeromonas spp. were reported by Saad (1991); Hafez and Halawa (1993); Abdel-Khalek (1997); Khalil (1997); Moustafa (2000); Amer *et al.* (2005); Deeb (2005) and Korashy (2006). Existence of Aeromonas spp. in raw cow's milk is of great concern because of great capability of growth at low temperature further more Aeromonas may be present in milk at level not initially detectable and subsequently outgrow after prolonged refrigerated storage (Palumbo *et al.,* 1985b). Thus Aeromonas spp. in raw cow's milk stored at refrigerated temperature may reach high numbers which could cause milk borne illness.

8.0 % of examined raw cow's milk

samples were contaminated with *A. hydrophila* and *A. caviae* while 12.0 % of the samples were contaminated with *A. trota* and *A. janda* (Table, 6). Of 20 confirmed cultures isolated from raw cow's milk 8 (40.0 %) of the strains could be identified as *A. trota* followed by 5 (25.0 %) as *A. hydrophila*, 5 (25.0 %) as *A. janda* and 2 (10.0 %) as *A. caviae* (Table, 7). These results are in agreement with that reported by Ibrahim and Macrea (1991); El-Gamal (1997) and Khalil (1997). Higher results could be detected by Hussein (1999) and Moustafa (2000).

The role of raw milk as a vehicle of transmission causing milk borne diseases is well documented (Taylor *et al.,* 1979 and Varnam and Evans, 1991). The microorganisms invading cow's teats and udders causing mastitis (Bryan, 1983). These milk borne pathogens can reach significant numbers in mammary tissue and subsequently discharged into milk. These microorganisms are commonly present on farms (feed water feaces soil and equipment used for milking) and can thus contaminate the surface of the udder and teats of the cows and get into milk.

3.2.Yoghurt:

It is the best known of all cultured milk products and is the highest consumed in countries around the Mediterranean Sea due to its nutritional and therapeutic value.

The results reported in Table (1) indicated that the pH values in examined yoghurt samples ranged from 3.20 to 4.70 with a mean value of 4.03 ± 0.085.

It is evident that there is sharp decrease in the count of Aeromonas spp. as the pH value of the examined yoghurt sample decrease the highest count was 9 $x10^5$ at pH value of 4.7 while the lowest count was 1 $x10^2$ at pH value of 4 (Table, 4) this is in agreement with Ozbas and Aytac (1995). The low pH value of yoghurt creates 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 (Paulmbo and Buchanan, 1988 and Motlagh *et al.,* 1991). So the pH of yoghurt can be used to control the growth of Aeromonas hydrophila.

The revealed results in Table (2) showed that Aeromonas spp. could be detected in 11 (44.0%) of the examined yoghurt samples in counts ranged from 1.0 $x10^2$ to. 9.0 $x10^5$ with a mean value of 1.4 $x10^5$ ± 8.8 $x10^4$. Lower incidence was reported by Abdel Khalek *et al.* (1996); Sami (1999); El-Shorbagy and Al-Ganzoury (2002) and Deeb (2005). While higher count was reported by Korashy (2006).

A. caviae could be isolated from 4 (16.0%) of examined yoghurt samples followed by *A. sobria* 3 (12.0%) and *A. hydrophila, A. trota* and *A. schubertii* 2 (8.0%) of each Table (6). Of 22 cultures isolated from yoghurt samples 8 (36.4%) could be identified as *A. caviae*, 5 (22.7%) as *A. sobria*, 4 (18.2%) as *A. hydrophila*, 3 (13.6%) as *A. trota* and 2 (9.1%) as *A. schubertii* Table (7). Higher results could be detected by Sami (1999) and El-Shorbagy and El-Ganzoury (2002). Motlagh *et al.* (1991) tested the inhibitory properties of anti-microbial metabolites produced by starter culture bacteria against *A. hydrophila* and they found that diacetyl had some bactericidal effect against the tested strains. While Santos *et al.* (1996) found that *lactococcus lactis* had inhibitory activity against 3 strains of *A. hydrophila* and low pH was only partially responsible for the antagonism. Moreover, Palumbo *et al.* (1985b) reported that two out of ten strains would grow in brain heart infusion broth at pH 4.5 when incubated at 28^{o}C/20 days none grew at pH 4.5 when incubated at 5^{o}C/24 days and four out of ten grew at pH 5.5 8 at pH 6.5 and 9 at pH 7.2.

3.3.Cheese:

Cheese is extremely economical and popular food as more than 90% of the assimilated material being changed into body tissues or energy and because cheese contains all essential amino acids. It is considered the main protein supplement to farmers and most people in our country. Although cheese is an important food sometimes it may become unmarketable or even harmful if it is subjected to contamination during its production handling and storage.

Listed results in Table (1) reported that the NaCl % of tested Domiati cheese samples was ranged from 2.20 to 3.55 % with a mean value of 2.92 %. Amer (1982) found that Sodium chloride percent ranged from 2.22% to 8.21% with a mean value of 5.422±0.225.

The count of Aeromonas spp. was significantly decrease as the NaCl% of the examined Domiati cheese sample increase the highest count was 2.0 $x10^4$ at NaCl % of 3.0 while the lowest count was 3.0 $x10^2$ at NaCl % of 3.55 (Table, 5). Nearly similar finding detected by Knochel (1989) who reported that few strains tolerated 6% NaCl but generally Aeromonads don't tolerate concentration above 5% NaCl. So usual food preservative processes (pH< 5, Sodium chloride > 3.5%) (Palumbo *et al.,* 1985b). While Parvis *et al.* (1996) found that either increase in salt concentration (100 mM through 400 mM) or decrease (50 mM) resulted in decrease in survival of *Aeromonas hydrophila* when exposed to gradually increased concentrations of salt and the most effective dose for induction was 400 mM NaCl salt. Also Abu-Ghazaleh (2000) found that Sodium chloride (3%) significantly reduced the growth and 4% NaCl inhibited the growth of *A. caviae and A. sobria.* Meanwhile Vivekanandhan *et al.* (2003) examined the effects of salt concentration on *A. hydrophila* and stated that salt concentrations above 2% inhibited growth somewhat; some growth occurred at 4% NaCl concentration and no growth occurred at 5% though cells remained viable.

The white cheese manufacturing process itself does not appear to be deleterious to Aeromonas spp. Salt and pH are two traditional means of restricting the growth of food borne pathogens. In the absence of salt cells grew more slowly and to a lower population at pH 5.9 than at pH 6.1. Aeromonas spp. seems particularly sensitive to pH value below 6.0. The level of NaCl found in cheese may an explanation for the relative lack of growth of Aeromonas spp. in this product. Thus Aeromonas spp. are not likely to be organisms of concern in any food which has a pH below 6 or brine level of 3% or greater.

Table (2) declared that 5 (20%) out of 25 examined Domiati cheese samples contained Aeromonas spp. with count/ml varying from 3.0 $x10^2$ to 2.0 $x10^4$ and average value of 6.9 $x10^3$. Higher results were reported by Freitas *et al.* (1993); Abd El-Hady *et al.* (1996); El-Gamal (1997); Khalil (1997) and Amer *et al.* (2005).

Domiati cheese examined samples were contaminated with *A. hydrophila*, *A. caviae* and *A. trota* by percentages of 8.0 % while *A. sobria, A. janda* and *A. schubertii* failed to be isolated (Table, 6). Identification of the 10 confirmed cultures isolated from Domiati cheese revealed that 3 (30%) of the strains could be classified as *A. hydrophila* followed by 5 (50%) as *A. caviae* and 2 (20%) as *A. trota* (Table, 7).

3.4.Effect of heat -treatment (pasteurization) on Aeromonas spp.:

On studying the effect pasteurization on Aeromonas spp. in raw cow's milk samples we found that all laboratory pasteurized milk samples revealed no count and this may be attributed to its sensitivity to heat treatment. Nearly similar findings were reported by Melas *et al.* (1999) who found that none of the pasteurized milk samples yielded Aeromonas spp. and this

indicates that post processing contamination of these products may occur during and after production. Kirove *et al.* (2006) declared that pasteurization is effective at removing this contamination nevertheless around 4% of pasteurized milk samples contained potentially significant strains apparently introduced by subsequent handling of the milk. While Elionora and Malka (2007) found that during cold storage after milk collection psychrotrophic bacteria populations dominate the microflora and their extracellular enzymes mainly proteases and lipases resulting in the spoilage of dairy products.

3.5.Psychrotrophic, proteolytic and lipolytic activities of isolated Aeromonas spp.:

Currently Aeromonas has attracted attention primarily because of its ability to grow at chill temperatures prompting the concern that any threat it might pose will increase with the increase use of chilled foods.

Characterization of isolated Motile Aeromonas strains from examined samples pointed that 6 (50%) of *A. hydrophila*, 9 (60%) of *A. caviae*, 2 (40%) of *A. sobria*, 7(53.8%) of *A. Trota*, 1(100%) of *A. janda* and 1(50%) of *A. schubertii* were capable of forming normal colonies onto standard plate count agar at 7^{o}C (Table, 8). The psychrotrophic nature of motile Aeromonas spp. was reported by Palumbo *et al.* (1985a); Berrang *et al.* (1989) and Abd El-Khalek (1997). *A. hydrophila* exhibited proteolytic and lipolytic activity at the percentage of 41.7 and 16.7% respectively. 7 (46.7%) out of 15 *A. caviae* strains produce protease enzyme while 3 (20%) produce lipase enzyme. 60% of *A. sobria* strains showed proteolytic activity. *A. trota* showed proteolytic and lipolytic activity at the percentage of 30.8 and 15.4% respectively. 100% of *A. janda* and A. *schubertii* strains produce protease enzyme only (Table, 8).

Extracellular proteinases and lipases from psychrotrophic organisms are recognized to be the primary microbial spoilage enzymes of dairy products (Cousin, 1989 and Sørhaug and Stepaniak, 1991) where they can resist pasteurization (72^{o}C for 15s) and even ultra high temperature processing (UHT 138^{o}C for 2s or 149^{o}C for 10s). The lipases by hydrolyzing triglycerides cause flavor defects associated with fat breakdown in cream, butter, cheese and UHT products. Proteases are associated with bitterness in milk gelation of UHT sterilized milk and reduced yields of soft cheese. Most proteases can degrade caseins and are remarkably heat stable (Hantsis-Zacharov and Halpern, 2007). So psychrotrophs can cause about 10% lose in milk fats and proteins. Milking procedure may be contaminated from the teat surface the udder milking equipment and the milking parlor environment.

3.6.Public health hazardous

In foods competing flora and other factors such as salt concentration pH and growth atmosphere influence the growth of Aeromonas spp. (Kirove *et al.,* 1993; Parvis *et al.,* 1996; Zade *et al.* 1996; Abu-Ghazaleh, 2000; Papageorgiou *et al.,* 2006 and Pacheco and Galindo, 2010) but the ability of Aeromonas spp. to grow and produce virulence factors at low temperatures because of their psychrotrophic nature (Beuchat, 1991) strongly supports the notion that these organisms may reach significant numbers and might be potential causative agents of food borne disease (Wadstrom and Ljungh, 1991).

Aeromonas is widely distributed in nature and its frequent presence in virtually all food has become of great interest (Kirove *et al.,* 1993) especially in foods that require refrigeration. *Aeromonas hydrophila* is generally thought of as an aquatic spp. it is not restricted to that environment. It is commonly isolated from the faecal material of lower animals and has been implicated in outbreaks of bovine abortion

(Wholegemuth *et al.,* 1972) and diarrhea in piglets (Dobrescu, 1978). A small percentage of humans appear to be a symptomatic carriers to *A. hydrophila* this could have significance in regard to food handlers being a potential source of the microorganism.

Aeromonas species mainly *A. hydrophila* are becoming renowned as enteric pathogens of serious public health concern and potential human food poisoning as they acquire a number of virulence determinants that are linked with human diseases such as gastroenteritis soft-tissue muscle infections septicemia endocarditis peritonitis osteomyelitis septic arthritis meningitis eye and urinary tract infections and skin diseases Sachan *et al.* (2012). Aeromonas infections have been reported in both healthy and immunologically compromised hosts but commonly reported in immunocompromised hosts with approximately 50% mortality among patient with neoplastic disease (Janda and Duffey, 1988). Proper sanitary procedures are essential in the prevention of the spread of Aeromonas infections. Oral fluid electrolyte substitution is employed in the prevention of dehydration and broad-spectrum antibiotics are used in severe Aeromonas outbreaks Isoken *et al.* (2012).

4. CONCLUSION

Assessment of the obtained results allow us to conclude that Aeromonas spp. can contaminate raw cow's milk and dairy products and this condition is indicative of inadequate hygiene during production handling and storage. As presence of Aeromonads may lead at times to objectionable changes in raw milk especially when held at refrigeration temperature beside its public health significance. Therefore it seems necessary to stress on the importance of applying strict hygienic measures during production, handling and storage especially proper cleaning and sanitization of the milking and milk storage equipment.

In conclusion selection of high quality raw cow's milk and the importance of way of processing as well as the storage conditions of dairy products on its final hygienic status which may enhance or inhibit the microbial contaminants. So selling of dairy product should be strictly controlled with health authority to eliminate potentiality of occurring hazards arising from microbial contamination. The records maintained for the HACCP system should include a plan. Developing and implementing a quality assurance program requires an understanding of basic sanitation and management principles. It also requires an understanding of the hazards associated with the raw materials ingredients and processes in farm and inside the plant.

5.REFRENCES

Abd-El-Hady, H. M., El-Bagoury, M., Mohamed, H. A. 1996. Incidence of psychrotrophic food borne pathogens in soft and processed cheese. 3rd Vet. Med. Cong. Zagazig 8-10 Oct.

Abd El-Hady, H. M., Halawa, M. A. 1999. Surveillance of Aeromonas and Listeria species in some dairy products. J. Egypt Vet. Med. Ass. 59 (6): 1661-1670.

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

Abdel-Khalek, A., El-Gamal, A., El-Sherbini, M. 1996. Survival of Aeromonas hydrophila and Listeria monocytogenes in yoghurt. 3rdVet. Med. Cong. Zagazig 8-10 Oct. Fac. Vet. Med. Zag. Univ Egypt.

Abu-Ghazaleh, B. 2000. Effect of sodium chloride and citric acid on growth and toxin production by A. caviae and A. sobria at moderate and low temperatures. New Microbiol.; 23(4):433-440.

Amer, I. H. 1982. Microbiological studies on locally manufactured cheese in Zagazig markets. Ph. D. Thesis Fac. Vet. Med. Zagazig Univ Egypt.

Amer, I. H., Abd El Aal, S. F. A. , Mariam, M. E. 2005. Prevalence of psychrotrophs in milk sold in Sharkia Governorate. Zag. Vet. Med. J. 33 (1): 165-172.

APHA, 1992. Standard methods for the

examination of dairy products. 15th Ed. American Public Health Association New York.

Atwegg, M. , Geiss, H. K. 1989. Aeromonas as a human pathogen. Crit. Rev. Microbiol. 16: 253-286.

Banerjee, S. N., Black, W. A. 1986. Food poisoning psychrotrophic bacteria growing in pasteurized milk & milk products. Dairy and food sanitation 6 (11) 511. Dairy Sci. Abst. 49 (4) (1987).

Berrang, M. E., Brackett, R. E., Beuchat, L. R. 1989. Growth of Aeromonas hydrophila on fresh vegetables stored under a controlled atmosphere. Appl. Environ. Microbiol. 55: 2167-2171.

Beuchat, L. R. 1991. Behaviour of Aeromonas spp. at refrigeration temperature. Int. J. Food Microbiol. 13: 217-224.

Bryan, F. L. 1983. Epidemiology of milk-borne diseases. J. Food Prot. 46: 637-649.

Cousin, M. A. 1989. Physical and biochemical effects on milk components. In Enzymes of psychrotrophs in raw food (Mckellar R. C. ed.) pp. 205-225 CRC Press England.

Deeb, A. M. 2005. Incidence of aeromonas spp. in raw milk and some dairy products in Kafr El-Sheikh city and growth characteristics of Aeromonas hydrophila in yoghurt. Kafr El-sheikh Vet. Med. J. 3 (1): 53-64.

Dobrescu, L. 1978. Enterotoxigenic Aeromonas hydrophila from a case of piglet diarrhea. Zentrabl. Veternamed. (B): 25: 713-718.

Elionora, H. Z., Malka, H. 2007. Culturable Psychrotrophic Bacterial Communities in Raw milk and their proteolytic and lipolytic traits. J. Appl. Environ. Microbiol. 73(22): 7162-7168.

El-Gamal, A. M. 1997. Incidence of motile Aeromonas spp. in raw milk and some dairy products. Vet. Med. J. 45 (3): 353-360.

El-Shorbagy, I. M., Al Ganzoury, H. H. 2002. Incidence of Aeromonas species in raw milk and yoghurt in Zagazig city. J. Egypt Vet. Med. Ass. 62 (4): 229-233.

Ergullu, E. 1978. A study of incidence of Gram negative bacteria in commercial milk supplies. Dissertation Abstract International 38 (4): 605. Dairy Sci. Abst. 48 (7) (1980).

Foster, J. J. 2001. Data Analysis Using Spss For Windows: A Beginner's Guide (Second Edition) London Sage.

Freitas, A. C., Nunes, M. P., Milhomen, A. M., Ricciardi, I. D. 1993. Occurrence and characterization of Aeromonas spp. in pasteurized milk and white cheese in Riode Janeiro Brazil. J. of Food Prot. 56 (1): 62-65.

Gogov, I., Ilieva, K., Slavchev, G. 1980. psychrotrophic microorganism in raw and pasteurized milk. Veterinarno-meditsinki Nauki 17 (3): 100-106. Dairy Abst. 43 (11) (1981).

Hafez, N. M., Halawa, M. A. 1993. Incidence of Aeromonas hydrophila group in raw milk. Symposium of food pollution. 15-16 Nov. Fac. Vet. Med. Assuit Univ. Egypt.

Hantsis-Zacharov, E., Halpern, M. 2007. Culturable Psychrotrophic Bacterial Communities in Raw milk and their proteolytic and lipolytic traits. J. Appl. Environ. Microbiol. 73(22): 7162-7168.

Harrigan, W. F., MacCance, M. E. 1976. Laboratory methods in food and dairy microbiology. Academic Press London.

Hussein, M. F. (1999): Occurrence of Aeromonas hydrophila in milk and some dairy products in Assuit City. M.V.Sc. Thesis Fac. Vet. Med. Assuit Univ. Egypt.

Ibrahim, A., Macrae, I. C. 1991. Incidence of Aeromonas and Listeria spp. in red meat and milk samples in Brishane Australia. Int. J. Food Microbiol. 12 (2-3): 263-269. Dairy Sci. Abst. 54(1): 1992.

Isoken, H. I., Ehimario, U. I., Farhad, A., Mvuyo, T., Anthony, I. O. 2012. Emerging Aeromonas Species Infections and Their Significance in Public Health. The Scientific world J.2012(13).

Janda, J. M. 1991. Recent advances in the study of Taxonomy Pathogenicity and Infectious syndromes associated with the genus Aeromonas. Clin. Microbiol. Rev. 4: 397-410.

James, C. S. 1995. Analytical Chemistry of foods. 1st.Edn. Chapman and Hall New York.

Janda, J. M. , Duffey P. S. 1988. Mesophilic aeromonas in human disease: Current taxonomy laboratory identification and infectious diseases spectrum. Rev. Infect. Dis. 10 (5): 980-997.

Jenway, 3505 pH meter: Jenway bench PH /Mv meter for use in general and educational applications.

Khalil, N. G. 1997. Incidence of Aeromonas hydrophila in raw milk and some dairy products. Assuit Vet. Med. J. 37: 100-101.

Kirove, S. M., Hui, D. S., Hayward, L. J. 1993. Milk as a potential source of Aeromonas gastrointestinal infection. J. of Food Prot.

56(4): 306-312. CAB Abstracts.
Kirove, S. M., Hui, D. S., Hayward, L. J. 2006. Milk as a potential source of Aeromonas gastrointestinal infection. J. Food Prot. 56(4): 306-312.
Knochel, S. 1989. The suitability of four media for enumerating Aeromonas spp. from environmental samples. Letters in Appl. Microbiol. 9: 67-69.
Korashy, N. T. 2006. A study on Mesophilic Aeromonas in milk and some milk products in Port Said City. J. Appl. Sci. Res. 2(11): 1037-1041.
MaCrae, R. Robinson, R. K., Sadler, M. J. 1993. in encyclopedia of food science and food technology and nutrition. Harcourt biace Jovanovich publishers London New York.
Melas, D. E., Papageorgiou, D. K., Mantis, A. I. 1999. Enumeration and confirmation of Aeromonas hydrophila A. caviae and A. sobria isolated from raw milk and other milk products in Northern Greece. J. Food Prot. 62(5):463-466.
Mergraud, F. 1986. Incidence and virulence of Aeromonas on feces of children with diarrhea. Eur. J. Clin. Microbiol. 5: 311-316.
Milliere, J. B., Veillet-Poncet, L. 1979. Determination of caseolytic psychrotrophic bacteria in refrigerated raw milk. Lait 59 (581-582) 56-78. Dairy Food. Sci.. 41 (6).
Motlagh, A. M., Johsnson, M. C., Ray, B. 1991. Viability loss of food borne pathogens by starter culture metabolites. J. of Food Prot. 54 (11): 873-878.
Moustafa, A.H. 2000. Prevalence of Aeromonas spp. in milk and some dairy products in Dakahlia Governorate. Ph. D. Vet. Med. Tanta University Egypt.
Oxoid, 1998. The Oxoid Manual 6h ed. Published by Oxoid Limited London.
Ozbas, Z. Y., Aytac, S. A. 1995. Behaviour of Yersinia enterocolitica and Aeromonas hydrophila in yoghurt made with probiotic bacteria. Milchwissen Schaft 50(11): 626-629. Dairy Food Sci. (3) (1996).
Pacheco, F. P., Galindo, A. B. 2010. Microbial safety of raw milk cheeses traditionally made at pH below 4.7 and with other hurdles limiting pathogens growth.
Palumbo, S. A., Buchanan, R. L. 1988. Factors affecting growth or survival of Aeromonas hydrophila in foods. J. Food Safety 9:37-51.
Palumbo, S. A., Maxino, F., Williams, A. C., Bunchanan, R.L., Thager, D.W. 1985a. Starch –Ampicillin agar for the quantitative detection of Aeromonas hydrophila. Appl. Environ. Microbiol. 50: 1417-1030.
Palumbo, S. A., Morgan, D. R., Bunchanan, R.L. 1985b. Influence of temperature NaCl and pH on the growth of Aeromonas hydrophila. J. Food Sci. 50: 1417-1421.
Papageorgiou, D. K., Melas, D. S., Abrahim, A., Angelidis, A. S. 2006. Growth of Aeromonas hydrophila in the whey Cheeses Myzithra Manouri during storage at 4 and 12 degree C. J. Food Prot. 69(2): 308-314.
Parvis, A., Majibur, R., Shakhawat, B., Arifur, R., Ashraque, H. 1996. Acid sensitivity as affected by physicochemical stresses in Aeromonas hydrophila. JPn. J. Med. Sci Bio. 49(3):95-102.
Popoff, M. 1984. Genus Aeromonas in Bergey's manual of Systematic Bacteriology Vol. 1 (N. R. Krieg, J. G. Holt ed.) PP: 545-548 Williams , Wilkins Co. Baltimore MD.
Robinson, R. K., Tamine, A. Y. 1983. Microbiology of fermented milks: In dairy microbiology. Vol. 2 R. K. Robinson (ed.) Appl. Sci. Ltd. London.
Saad, N. 1991. Occurrence of Aeromonas hydrophila in raw milk. Assuit Vet. Med. J. 25 (50): 98-102.
Sachan, N., Agarwal, R. K., Singh, V. P. 2012. Study on outer membrane protein profile of Aeromonas strains using SPS page. Vet World J. 5 (3): 173-177.
Sami, E. M. 1999. Prevalence of Aeromonas spp. in milk and some dairy products in Sharkia Governorate. M.V.Sc. Thesis (Milk Hygiene) Fac. Vet. Med. Zagazig Univ.
Santos, G. A., Lopez Dias, T., Gracia Fernandez, M. C., Garcia Lopez, M. L., Otero, A. 1996. Effect of a lactic starter culture on the growth and protease activity of Aeromonas hydrophila. J. of Appl. Bacteriol. 80 (1): 13-18.
Schonberg, F. 1956. Milch kunde and Milch hygiene.7Auffage Verlarg M. and Scheber H. Hannover.
Shan, N. P. 1994. Psychrotrophs in milk: a review. Milchwissenschaft 19 (8): 432-437.
Sørhaug, T. 1992. Psychrotrophic Vs. total bacterial count in bulk milk samples. Vet. Med. Czech 47 (8): 227-233.
Sørhaug, T., Stepaniak, L. 1991. Microbial enzymes in the spoilage of milk and dairy products. In food enzymology (Fox P. F. ed.) Vol. 1 PP. 169-218 Elsevier Appl. Sci. London.
Sørhaug, T., Stepaniak, L. 1997.

Psychrotrophs and their enzymes in milk and dairy products: quality aspects. Trends in Food Sci. and Technol. (Vol. 8).

Taylor, P. R., Weinstein, W. M., Bryner, J. H. 1979. Campylobacter fetus infection in human subjects association with raw milk. Amer. J. Med. 66: 779-783.

Varnam, A. H., Evans, M. G. 1991. Food borne pathogens. Wolfe publishing Ltd. London.

Vivekanandhan, G., Savithamani, k., Lakshmanaperumalsamy, P. 2003. Influence of pH salt concentration and temperature on the growth of Aeromonas hydrophila. J. Environ. Biol. 24 (4): 373-379.

Wadstorm, T., Ljungh, A. 1991. Aeromonas and Plesiomonas as food and water borne pathogens. Int. J. Food Microbiol. 12: 303-312.

Wholegemuth, K., Pierce, R. L., KirBride, C. A. 1972. Bovine abortion associated with Aeromonas hydrophila. J. Amer. Vet. Med. Assoc. 160: 1001-1002.

Zade, N. N., Garg, S. R., Ashwani, K., Kumar, A. 1996. Survival and growth of Aeromonas hydrophila in meat and milk. Haryana Vetrinarian 35: 49-52.