

## INCIDENCE OF MOTILE AEROMONAS SPP IN RAW MILK AND SOME DAIRY PRODUCTS

L-GAMAL A. M.

Animal Health Research Institute-Mansoura

### SUMMARY

A total of 150 samples, 50 each of raw milk, pasteurized milk and white soft cheese from different dairy farms; retail shops and supermarkets in Mansoura City, El-Dakahlia province, Egypt, were examined for the presence of motile *Aeromonas* spp. The detection of the organisms by quantitative methods providing more reliable results compared to qualitative method. Motile *Aeromonas* occurred in 5.3% and 6% of examined samples using direct plating method and enrichment method respectively. The incidence of motile *Aeromonas* spp. was 6%; 6% and 16% samples of raw milk; pasteurized milk and white soft cheese, respectively using enrichment method. The most common *Aeromonas* isolated was *Aeromonas hydrophila* which comprised 46.2% of the isolates, whilst, *A. Caviae*; *A. Sobria* and not classified strains, represented 29.5%; 9.8% and 4.5% respectively. All *Aeromonas* isolates were uniformly resistant to ampicillin; pincillin and erythromycin and sensitive to other tested antibiotics with different activity percentage. The presence of motile *Aeromonas* spp in raw milk

and some dairy products may represent an important vehicle of its transmission.

### INTRODUCTION

*Aeromonas* species have been strongly implicated in many countries as causative agents of gastroenteritis, particularly in children, the elderly, and immunocompromised patients (Kirov and Hayward, 1993).

*Aeromonas* are ubiquitous in nature, being readily isolated from a variety of sources. While *Aeromonas* species are generally thought of as aquatic organisms, *aeromonas* strains identical to diarrhoea-associated strains are readily isolated from a wide variety of retail foods (Fricker and Tompsett, 1989; Palumbo et al., 1989 and Kirov et al., 1990) Moreover, many of these strains are able to grow at refrigeration temperatures (Palumbo and Buchanan, 1988 and Beuchat, 1991).

The purpose of the present study was to investigate the occurrence of motile *Aeromonas*

spp in rae milk, pasteurized milk and white soft cheese as well as search for its antibiotic sensetivity.

## MATERIAL AND METHODS

### Sampling:

One hundred and fifty random samples of raw milk, pasteurized milk and white soft cheese (Each of 50) were collected from different dairy farms, retail dairy shops and supermarkets in the suburbs of Mansoura city, el-Dakahlia province, egypt. All samples were dispatched directly to the laboratory, where they were kept chilled and investigated for occurrence of motile *Aeromonas* spp.

Preparation and handling of samples were done according to Standard Methods for the examination of Dairy products (Richardson, 1985).

### Isolation of *Aeromonas* spp.

1- Direct method :0.1 ml of each sample after preparation was directly surface plated onto Starch ampicillin agar plates (SA) (Palumbo et al., 1985) and incubated at 28°C for 24 hours. Plates showed large (3-5mm) honey yellow to yellow

colonies with a surrounding clear zone after treatment with half-strength lugol's iodine solution were picked up as presumptiv *Aeromonas* spp.

2- Enrichement method: 25 ml of each sample was added to 225 ml Ampicillin broth (TSB plus 30 mg/L Ampicillin) and incubated at 28°C for 24h. A Loophul of the enrichment culture was streaked onto starch ampicillin agar plates and examined for the presence of presumptive *Aeromonas* colonies after 24 hour incubation at 28°C. The presumptive *Aeromonas* colonies were streaked onto blood agar for purification and verified as motile *Aeromonas* (Popoff, 1984). The isolates were identified according to Hickman-Brenner et al., 1987); and Kirov et al., (1983).

### Antibiotic sensitivity:

Antibiotic snstivity test of the *Aeromonas* isolates were performed by the disc method developed by Bauer et al., (1966). Antibiotic discs were obtained from Bio-ADWIC abuzaabal Egypt.

## RESULTS

Results are presented in Tables (1 and 2).

## RESULTS

Results are presented in Tables (1 and 2).

**Table (1)** : Incidence of motile *Aeromonas* spp in raw milk and some milk products.

Dairy product	No. of samples	Incidence			
		Direct plating method		Enrichement method	
		No. positive	%	No. positive	%
Raw milk	50	4	8	13	26
Pasteurized milk	50	2	4	3	6
White soft cheese	50	2	4	8	16
Total	150	8	5.3	24	16

**Table (2)** : Frequency distribution of isolated motile *Aeromonas* spp.

Dairy products	No. of isolates	A. Hydrophila		A. Caviae		A. Sobria		Not classified	
		No	%	No	%	No	%	No	%
Raw milk	65	34	52.3	21	32.3	5	7.7	5	7.7
Pasteurized milk	25	9	36	12	48	4	16	0	0
White soft cheese	42	18	42.9	6	14.3	4	9.5	14	33.3
Total	132	61	46.2	39	29.5	13	9.8	19	14.5

**Table (3)** : Antibiogram of *A. hydrophila*, *A. sobria* and *A. caviae* isolated from milk and some dairy products.

Antibiotic discs and their potency	A. hydrophil (20)		A. sobria(6)		A. caviae(12)	
	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent
Chlormphincol (30ug)	20	100	0	0	10	83.3
Neomycin (30ug)	7	35	3	50	12	100
Gentamycin (10ug)	13	65	6	100	10	83.3
Erythromycin (15ug)	0	0	0	0	0	0
Trimethoprim-sulphame-thoxazole (1.25ug-23.75ug)	10	50	5	83.3	11	91.6
Streptomycin (30ug)	16	80	0	0	2	16.6
pencillin (10ug)	0	0	0	0	0	0
Ampicillin (10ug)	0	0	0	0	0	0
Naladixic acid (30ug)	14	70	4	66.6	9	75
Tetracyclin (30ug)	12	60	5	83.3	3	25
Amoxycillin (25ug)	0	0	1	16.6	0	0

\* Nernber of tested isolates.

## DISCUSSION

Results given in Table (1) indicate that the incidence of motile *Aeromonas* spp in relation to the method of isolation was greatly varied. The detection of the organisms by enrichment method providing more reliable results compared to direct plating method. Out of 150 samples of dairy products 8 (5.3%) were positive by direct plating method, meanwhile with enrichment method 24 (16%) sample were positive. Nearly similar results were given by Schweizer et al., (1995) who found that the incidence of *Aeromonas hydrophila* in raw milk was higher by using enrichment method than by direct plating method. Moreover, Palumbo et al., (1985) mentioned that motile *Aeromonas* spp may be present in milk at levels not initially detectable and subsequently out grow after enrichment or prolonged refrigerated storage.

It is likely that the use of enrichment method for isolation of motile *Aeromonas* spp will be required for optimal recovery of this bacteria.

### 1- Raw milk:

Motile *Aeromonas* spp was detected in (13) 26% of raw milk samples by using enrichment method. This finding finding nearly simulate that obtained by Ibrahim and Macrae (1991). while lower findings were reported by Kielwein et al. (1969), Schweizer et al., (1995) and El-Said (1996) and higher findings were reported by Food and Drug Administration "FDA" (1985) and Kirov et al., (1993). On the contrary Krovacek et al. (1992) failed to isolated motile *Aeromonas* spp

from tested raw milk samples.

The distribution of isolated *Aeromonas* spp isolates) from raw milk samples were identified: *A. hydrophila* 34 (52.3%); *A. caviae* 21 (32.3%); *A. sobria* 7 (17.7%) and not classified strain (7.5%) (Table 2) these findings substantiate have been reported by Ibrahim and Macrae (1991) and Kirov et al., (1993).

It seems that the frequent occurrence of *hydrophila* and *A. caviae* in the environment water (Neves et al., 1990 and Schubert 1991) animal faeces containing organisms (Gray et al. 1990) or milker handlers symptomatic or asymptomatic (Kirov 1993), give ample opportunity for contamination of milk.

### 2- Pasteurized milk:

It is evident from table (1) that the incidence of motile *Aeromonas* spp in pasteurized milk samples was 6% using enrichment method which coincides with that of Walker and Brooks (1993). Greenaway (1988) and Freitas et al. (1993) recorded high results, while lower incidence was obtained by Kirov et al. (1993).

*Aeromonas caviae* was the predominant species found in pasteurized milk which comprised 48% of isolates, whilst *A. hydrophila* and *A. sobria* represented 36% and 16% of isolates respectively. (Table 2). Nearly similar results were given by Freitas et al. (1993). On the contrary Kirov et al. (1993) mentioned that the predominant species recovered from pasteurized milk was *A. sobria* (42.9%).

Aeromonads are not heat-resistant and has a decimal reduction time (Dvalue) of between 2.2 and 6.6 min at 48°C (Palumbo et al., 1987) or 0.17 min at 55°C (Condon et al., 1992). Consequently pasteurization can be relied upon to destroy the strains of Aeromonads. Therefore the presence of these species in the pasteurized milk imply post pasteurization contamination.

### 3- White soft cheeses:

The incidence of motile Aeromonas spp in examined white soft cheese was 16% using enrichment method. (Table 1). Relatively few surveys have verified by incidence of Aeromonads in white soft cheese. Freitas et al. (1993) could isolate motile Aeromonas spp from 32% samples of not aged white cheese. While a lower contamination rate 2% was reported in study of Walker and Brooks (1993). In Egypt, Abd El-Rahman and Ahmed (1988) succeeded to isolate Aeromonas hydrophila from soft cheese.

Aeromonas hydrophila was the major species found in white soft cheeses which represented 42.9% of isolates, followed by non classified strains 33.3%, A. Caviae 14.3% and A. Sobria 9.5% (Table 2). Contrast result was reported by Freitas et al. (1993) who found that non classified strains were the most frequent isolates 61.5%.

### Antibiotic sensitivity of isolates:

The results recorded in Table (3) reveal that all aeromonads isolates were showed uniform resistant to ampicillin; penicillin and erythromycin. These findings are similar to those

reported by Gilardi (1983); Villuendas et al. (1991) and Krovacek et al. (1992). Conversely Rahim and Kay (1988) found that 7.5% of 52 aeromonas isolates were sensitive to ampicillin.

The obtained data revealed that A. hydrophila was sensitive to chloramphenicol, neomycin, gentamicin, trimethoprim sulphathiazole, streptomycin, naladixic acid and tetracycline with activity percentage of 100%; 35%; 65%; 50%; 80%; 70% and 60% respectively. While it was resistant to erythromycin; ampicillin, penicillin and amoxicillin. These findings agree with those reported by Overman (1980); Fass and Barnishan, (1981) who reported that all tested isolates were susceptible to chloramphenicol and they were resistant to penicillin; erythromycin; cephalosporins and polymyxins. In contrary, Altwegg and Geiss (1989) indicated that the environmental isolates of A. hydrophila were resistant to chloramphenicol and tetracycline.

With regard to antibiotic susceptibility of A. Sobria, the obtained results showed that it was sensitive to neomycin; gentamicin; trimethoprim-sulphathiazole; naladixic acid, tetracycline and amoxicillin with activity percentage of 50%, 100%, 83%, 83.3%, 66.6%, 83.3% and 16.6% respectively. While it was resistant to chloramphenicol, erythromycin, penicillin, ampicillin and streptomycin which are in agreement with that of Krovacek et al. (1992).

Conversely results were reported by Bornemann (1989) who found that 72% of 50 strains of A. Sobria were resistant to ampicillin and 8% to chloramphenicol. Regarding to antibiotic

sensitivity of *A. Caviae*, the obtained data agree with the finding reported by Hassan (1991).

### Public health significance of isolates:

It is generally accepted that some strains of motile *Aeromonas* species are enteropathogens. Such strains possess virulence associated properties such as the ability to produce enterotoxin, cytotoxin, haemolysins and an array of proteases and/or invasive ability (Kirov 1993). Some of these strains are able to grow in food under refrigeration (Beuchat 1991). Two types of gastrointestinal illness have been attributed to *A. hydrophila* and *A. sobria*. The first and most common is cholera like illness. Both types of diarrhoea are usually mild and self-limited (Stelma, 1989). Janda (1991) and Kirov (1993) reported that *A. sobria* was the most spp. often associated with more severe gastrointestinal illness.

In conclusion, the present study shows that species of *Aeromonas* can be isolated from raw milk, pasteurized milk and white soft cheese with various isolation rates. Furthermore its presence in raw milk and some dairy products should be regarded as potential health threat particularly for children and immunocompromised individuals.

### REFERENCES

Abd El-Rahman. H.A. and Ahmed, A.A.H. (1988): Incidence and level of occurrences of proteolytic microorganisms in some selected foods. *Assiut Vet. Medical J.* 19: 38, 72-78.

- Altwegg, M. and Geiss H.K. (1989): *Aeromonas* as a pathogen. *Crit. rev. Microbiol.* 16, 253-286.
- Bauer, A. W.; Kirbee, W.M.; Serris J.C. and Turck (1966): Antibiotic sensitivity testing by standard disc method. *Am. J. of Clin. Path.* 45; 493-496.
- Beuchat, L.R. (1991): Behaviour of *Aeromonas* species at refrigeration temperatures. *Int. J. Food Microbiol.* 217-224.
- Bornemann H. (1989): Occurrence of antibiotic resistant strains of *Aeromonas* in fermented cheese. Inaugural-Dissertation fachbereich Veterinar Medizin. 146.
- Condon, S.; Garcia, M.L.; Otero, A. and Sala; F.J. (1990): Effect of culture age, pre-incubation at low temperature and pH. on the thermal resistance of *Aeromonas hydrophila*. *J. Appl. Bacteriol.* 72, 322-326.
- El-Said, e.I. (1996): study of proteolytic psychrotrophic microflora in raw Buffalo's milk. Master. Thesis Faculty of Vet. Med. Zagazig Univ., Egypt.
- Fass R.J. and Barnishan J. (1981). In vitro susceptibility of *A. hydrophila* to 32 antimicrobial agents. *Antimicrobial Agent and Chemother* 19,327-358.
- Food and drug administration (1985): Pathogen Surveillance Sampling of *Aeromonas hydrophila* in Food Food Safety Compliance Program 7303-030.
- Freitas, A.C.; Nunes, M.P.; Milhomem, A.M. and Ricciardi, I.d. (1993): Occurrence and characterization of *Aeromonas* spp. in pasteurized milk and white cheese in Rio De Janeiro, Brazil. *J. of food prot.* 56, 62-65.
- Fricker C.r. and Tompsett S. (1989): *Aeromonas* spp. in foods; a significant cause of food poisoning. *Int, J. Food Microbiol*, 9, 17-23.
- Gilardi G.L. (1983): *Aeromonas* and *Plesiomonas*. *clinical Microbiol. Newsletter* 5. 49-51.
- Gray, s.J. Sticker, D.J. and Bryant, T.N. (1990): The incidence of virulence factors in mesophilic *Aeromonas* spp. isolated from animals and their environment. *Epidemiol. Infect.* 105, 277-294.

- Greenaway, C. (1988): *Aeromonas*-A future concern for the dairy and food industries. pp 73-75. In *The dairy technologist*, Food Research Institute, Victoria, Australia.
- Hassan Z. (1991): Some study on motile aeromonads in fresh water fish with special emphasis on their toxigenic profile. M.V.Sc. Thesis (Fish disease Fac. Vet Med. Uni. of Alexandria).
- Hickman-Brenner, F.W., Macdonald, K.L., Steigerwalt, A.G., Fanning, G.R.; Brenner, D.J. and Farmer, J. III (1987): *Aeromonas veronii* a new ornithine decarboxylase positive species that may cause diarrhoea. *J. Clin. Microbiol.* 25, 900-906.
- Ibrahim, A. and MacRae, I.C. (1991): Incidence of aeromonas and *Listeria* spp. in red meat and milk samples in Brisbane, Australia. *Int. Food Microbiol.* 12, 263-270.
- Jonda J.M. (1991): Recent advance in the study of the taxonomy, pathogenicity and infectious syndromes associated with the genus *Aeromonas*. *Clin. Microbiol. rev.* 4, 397-410.
- Kielwein, G., Gerlach, r. and Johne, H. (1969): Prevalence of *A. hydrophila* in raw milk. *Arch. Lebensmittel Hyg.* 20, 34-38.
- Kirov, S.M. (1993): The public health significance of *Aeromonas* spp. in foods. *Int. J. Food Microbiol.* 20, 179-198.
- Kirov, S.M. and Hayward L.J. (1993): Virulence traits of *Aeromonas* in relation to species and geographic region. *Aust. J. Med. Sci.* 14, 54-58.
- Kirov, S.M., Anderson, M.J., and McMeekin, t.A. (1990): A note on *Aeromonas* spp. from chickens as possible food-borne pathogens. *J. appl. Bacteriol.* 68, 327-334.
- Kirov, S.M., Hui, D.S. and Hayward, L.J. (1993): Milk as a potential source of *Aeromonas* gastrointestinal infection. *Food. Prot.* 56, 306-312.
- Krovacek, K.; Faris, A.; Baloda, S.B.; Peterz, M. Lindberg. T. and Mansson, I. (1992): Prevalence and characterization of *Aeromonas* spp. isolated from foods in uppsala, Sweden. *J. Food Microbiol.* 9, 29-36.
- Neves, M.S.; Nunes. M.P. and Ricciadi I.D. (1990): Incidence of motile *Aeromonas* species in a aquatic environments of Rio de Janeiro, Brazil *J. food Prot.*, 53: 78-80.
- Overman t.L. (1980): Antimicrobial susceptibility of *A. hydrophila*. *Antimicrob. Agents, Chemother.* 17, 612-614.
- Palumbo, S.A. and Buchanan, R.I. (1988): Factors affecting growth or survival of *Aeromonas hydrophila* in foods. *J. Food Safety* 9, 37-51.
- Pulumbo, S.A., Bencivengo M.M.; Delcorral F.; Williams A.C. and Buchanan. R.L. (1989): Characterization of the *Aeromonas hydrophila* group isolated from retail Foods of animal origin. *J. Clin. Microb.* 27, 854-859.
- Palumbo, S.A.; Maxino, F.; Williams, A.C.; Buchanan r.L. and thayer, D.W. (1985): Starch-Ampicillin agar for the Quantitative detection of *Aeromonas hydrophila*. *Appl. and Environ. Microbiol.* 50, 1027-1030.
- Polumbo, S.A.; williams, A.C.; buchanan, R.L. and Phillips, J.G. (1987): Thermal resistance of *Aeromonas hydrophila*. *J. Food prot.* 50, 761-764.
- Popoff, M. (1984): Genus III. aeromonas. In N.R. Krieg and J.G. Holt (Eds). *Bergey's Manual of systematic Bacteriology*. Vol. I. williams and Wilkins, Boltimore pp 545-548.
- Rahim Z. and Kay B.A. (1988): Incidence of *Aeromonas* and *Plesiomonas shigelloides* during a diarrhoeal epidemic in Bangladesh. *J. Diarrhoeal Dis res.* 6, 144-145.
- Richardson, G.H. (1985): Standard methods for the examination of dairy products 15<sup>th</sup> American Public Health Association (APHA). Washington, D.C.
- Schubert, R.H. (1991): Aeromonads and their significance as potential pathogens in water. *Soc. Appl. Bacteriol. Symp. Ser.* 20, 131S-135S.

Schweizer R., Kaderli, M. and Spahr, U. (1995): *Aeromonas hydrophila* in swiss raw milk  
Schweizerische-Milchwirtschaftliche-Forschung. 24:1, 9-11.

Stelma, G.N. JR. (1989): *Aeromonas hydrophila*. In foodborne Bacterial pathogens ed. Doyle, M.P. pp. 1-19, New York: Marcel Dekker.

Villuendas, M.C.; Esteban A.; Lopez-Gomez, Goytumo, B.; Serrano M. and Moles B. (1993): *Aeromonas* en infecciones extraintestinales. Rev. Espanola de Microbiologia Clinica 9, 446-450.

Walker, S.J. and Brooks, J. (1993): Survey of the incidence of *Aeromonas* and *Yersinia* species in retail foods. J. Control. J. 4-1, 34-40.