PSYCHROTROPHS, ESPECIALLY PSEUDOMONAS SPP IN RAW MILK

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ABSTRACT

Total psychrotrophic count and Pseudomonas count of raw milk samples obtained from bulk tanks of farm and cooled small tanks in different dairy shops from Damietta City were determined. Isolated Pseudomonas bacteria were identified. The mean psychrotrophic count(cfu/ml) and Pseudomonas count of raw milk samples collected from farms and different dairy shops were ($7.2x10^4 \pm 1.28$ & $3.3x10^7 \pm 1.34$) and ($2.8x10^4 \pm 1.23$ & $1.7x10^7 \pm 1.31$), respectively. Pseudomonas spp. were identified by using API20NE system. In farm milk Ps. fluorescens, Ps. cepacia and Ps.putida represented 52.4, 38.1 and 9.5%, respectively. But, in market raw milk represented 63, 16 and 21%, respectively. Ps. fluorescens was the most prominent Pseudomonas spp. in raw milk which causes spoilage of milk and dairy products due to the action of extracellular heat resistant lipases and proteinases enzymes.

INTRODUCTION

The refrigeration of raw milk in farms and dairy plants as a method of preservation has improved the quality of raw milk and dairy products. Where, the growth of mesophilic microflora was minimized. Unfortunately, long refrigeration favoured the growth of psychrotrophic bacteria (**Burdova et al., 2002**). The psychrotrophs are group of microorganisms that are able to grow at low temperatures causing spoilage of fluid milk and some other dairy products (**Kornacki and Gabis., 1990**).

The psychrotrophs found in milk are environmental, originating from soil; water; vegetation; uncleaned teat or udder and improperly cleaned milking; storage and transporting equipment. Furthermore, post pasteurization contamination may happen at the filling operation (**Eneroth et al., 1998**).

Psychrotrophs seem to become the newest thorn in dairy industry. As, the serious problems related to milk spoilage not due to themselves because most of them are thermolilable microorganisms, which are inactivated by pasteurization temperature. But, they produce thermoresistant proteolytic and lipolytic enzymes which can degrade the important component of milk. Consequently, a qualitative risk during milk processing and cause the spoilage of final products during storage (**Vyletelová et al., 1999 and Chen et al., 2003**). So, contamination with psychrotrophs is one of the most important points in determining

milk quality as documented by **SmithWell and Kailaspthy (1995)**.

Pseudomonas species ranks with the most significant psychrotrophic bacteria constitute the predominant microorganisms limiting the shelf life of processed fluid milk at 4°C (**Gilmour and Rowe, 1990**). Besides, their rapid growth in refrigerated milk, pseudomonas species produce heat stable extracellular proteases, lipases and lecithinase which causing organoleptic defects in milk and milk products **(Champagne et al., 1994; Sorhaug and Stepaniak , 1997)**.

Pseudomonas aeruginosa has been recognized as a potential human pathogen and constituted potential hazards to both human and animal health **(Jay, 2000)**. Also, it has been implicated in many types of infections and food poisoning out breaks **(Grover and Srinivasan, 1988)**. Beside that it is multiple drug resistant bacteria MDR which considered a potent biological hazard as there are possibilities for resistance genes to be spread to human beings via food **(Meyer, 2005)**.

Therefore, the present study was undertaken to evaluate total bacterial count, psychrotrophic count and Pseudomonas count in raw milk (farm raw milk and market raw milk), the incidence of pseudomonas species in raw milk and identification of isolated pseudomonas species.

MATERIAL AND METHODS

Sample collection

A total 50 random samples of raw milk (250ml) were collected. 25 each of market raw milk, farm raw milk. All samples were aseptically collected and transferred directly to laboratory in an insulated ice box with a minimum of delay to be immediately examined bacteriologically.

Sample preparation

At the commencement of the analysis, each sample was prepared using the procedure described in A.P.H.A. (1992). 25 ml of milk sample was transferred to 225ml of sterile tryptone soya broth and mixed to obtain 1/10 dilution food homogenate.

Enumeration of total psychrotrophic count by applying Modified Rapid Method (MRM), according to **(Abdel - Khalek, 1991)**. On modified standard plate count agar medium with crystal violet 2mg/L and penicillin G.sodium 20.000 I.U/L. The plates incubated at 32°C for 2 days. Colonies were counted and PBC/ ml was recorded.

Enumeration of total Pseudomonas count, **(Roberts, et al., 1995)**. Using surface method of enumeration on Pseudomonas agar base containing glycerol (10ml/L) and the selective agents cetrimide (10mg/L), cephaloridine (50mg/L) and fucidin (10mg/L). The plates were incubated at 25 - 30°C for 2 days.

Identification and characterization of isolates.

The different colonies were selected from Pseudomonas agar base plates and after purification. They streaked onto nutrient agar plates and incubated at 30°C/2 days. Identification of isolates were carried out on bases of morphological, cultural and biochemical characteristics as described by **(Forbes et al., 2003)**. Further phenotypic characterization

was conducted using API20NE identification kits **(bioMérieux, 2006)**.

The data was analyzed by ANOVA with comparison of difference between means of the treatments at the significance level of $P< 0.05$ using SPSS programme (Statistical Package for Social Science, version 10.00). Before the statistical analysis the values of PC and Pseudomonas count were logarithmically transformed in order to approach normal distribution.

RESULTS & DISCUSSION

Total psychrotrophic count (PC) of raw milk.

The mean PC (log10 cfu/ml) of milk samples collected from farm bulk tanks and small cooled tanks in different dairy shops were 4.86 ± 0.11log10 cfu/ml and 7.53 ± 0.13 log10cfu/ml, respectively (table 1). There was a significant ($P < 0.05$) difference between the PC of milk samples collected from farm and dairy shops. **Lee and lin (2007)** reported similar finding for farm bulk tank milk. In similar studies performed by **Duangpan and Suriyaphan, (2009)** regarding the psychrotrophic count (PC) of farm raw milk were lower compared to these results.

The mean PC of market milk is nearly similar to that reported by **Sabry (2001)**, while relatively lower counts were obtained by **Awad et al., (2005)** and **Dan et al., (2008)**. Psychrotrophic count (PC) in market milk was greatly higher than PC in farm milk. The high PC in market milk may attributed to the long time handling of milk at ambient temperature like milking and transport to different milk vendors. In addition to, maintenance of low temperature during transport and / or storage of raw milk in large dairy shops. This low temperature favors the growth of psychrotrophic bacteria.

Total pseudomonas count of raw milk.

The load with bacteria from genus Pseudomonas was variable, mean value of Pseudomonas Count in farm raw milk was 4.45±0.09 log10 cfu/ml. But, mean value of Pseudomonas count in market raw milk 7.25±0.12 log10 cfu/ml (table 2).

The mean Total pseudomonas count of farm milk was lower than those reported by **El-Said, (1996).** While, it was higher than those reported by **Demasures et al., (1997)**. But, the mean Total pseudomonas count of market raw milk was higher than those reported by **EL-Kholy et al., (2008)** and **Dan et al., (2008)**.

The relatively high count met within this work declare to what extent the raw milk is exposed to contamination during milking, handling in dirty equipment or produced under undesirable condition or carelessness of milker and contact with infected water. In addition to a universal distribution of genus Pseudomonas resulted from the capacity of Pseudomonas spp. to adapt various conditions **(Palleroni, 1992)**.

There was a significant difference ($P<0.05$) between pseudomonas count of market raw milk and pseudomonas count of farm raw milk.

Pseudomonas count of market milk was

greatly higher than that of farm milk. The Pseudomonas count of market milk might be attributed to the long period between milking and sampling. In addition to, cold storage during transport and storage of raw milk. On the other hand, Genus Pseudomonas includes species with the shortest generation interval at 0 to 7°C and the lowest theoretical growth temperature (-10°C), which rank its species with typical psychrotrophic agents (**Sorhaug, 1992**).

In addition to, **Almudena et al., (1995)** investigated that pseudomonas spp. after cold incubation showed an average 10- fold higher growth at 7°C, 1000- fold more proteolytic activity and 280- fold more lipolytic activity than those found before the incubation.

Incidence of Pseudomonas spp. in raw milk.

Pseudomonas spp. isolated from 56% of examined farm raw milk samples. Out of 21 isolates, 11, 8 and 2 isolates were identified as Ps.fluorescens, Ps.cepacia and Ps.putida representing 52.4, 38.1 and 9.5%, respectively (table 3, 4).

Pseudomonas spp. isolated from 92% of examined market raw milk samples. Out of 38 isolates, 24, 6 and 8 isolates were identified as Ps.fluorescens, Ps.cepacia and Ps.putida representing 63, 16 and 21%, respectively (table 3, 4).

Ps. fluorescens is the most predominant Pseudomonas spp. in both farm raw milk which agrees with the results of **Mousaa et al., (2008)** and market raw milk which agree with the findings has been reported by **Dunstall et al., (2005) and Polyanskii et al., (2005)**.

In conclusion, psychrotrophic bacteria highly contaminate raw milk specially; Pseudomonas Spp. which indicates inadequate hygiene measures during production, handling and storage of raw milk at dairy farms. Therefore, strict hygienic measures should be imposed for milk production, handling and storage. Great care must be taken while handling raw milk to avoid spoilage. Rapid cooling of raw milk has reduced effect on the growth of contaminating bacteria.

Table (1): Statistical analytical results of total psychrotrophic count of examined raw milk (farm raw milk, market raw milk) samples.

Product	No. of examined samples	Positive samples		Min	Max	Mean	SED
		No.	%				
Farm raw milk	25	25	100	3.49 (3×10^3)	5.96 (9.1×10^5)	4.86* ($7.2\times10^{4*}$)	0.11 (1.28)
Market raw milk	25	25	100	6.08 (1.2×10^6)	9.86 (4.6×10^8)	7.53* ($3.3\times10^{7*}$)	0.13 (1.34)

Analysis based on logarithms to base 10 of counts (log10 cfu/ml). Anti log values (cfu/ml) are shown in brackets. Means bearing different superscripts differ significantly (P<0.05).

Table (2): Statistical analytical results of total *Pseudomonas* count of examined raw milk (farm raw milk, market raw milk) samples.

Product	No. of examined samples	Positive samples		Min	Max	Mean	SED
		No.	%				
Farm raw milk	25	25	100	3.83 (6.7×10^3)	5.28 (1.9×10^5)	4.45* ($2.8\times10^{4*}$)	0.09 (1.23)
Market raw	25	25	100	6.11 (1.2×10^6)	8.40 (2.5×10^8)	7.25 ($1.7\times10^{7*}$)	0.12 (1.31)

Analysis based on logarithms to base 10 of counts (log10 cfu/ml). Anti log values (cfu/ml) are shown in brackets. Means bearing different superscripts differ significantly (P<0.05).

Table (3): Incidence of *Pseudomonas Spp.* Isolated from raw milk (farm raw milk, market raw milk) plated on *Pseudomonas* Agar base.

No. of samples	Raw milk			
	Farm raw milk		Market raw milk	
	Positive samples	%	Positive samples	%
25	14	56	23	92

Table (4): No. of *Pseudomonas Spp.* Isolated from raw milk (farm raw milk, market raw milk).

Pseudomonas Spp.	Raw milk			
	Farm raw milk		Market raw milk	
	No.	%	No.	%
Ps. Fluorescens	11	52.4	24	63
Ps. cepacia	8	38.1	6	16
Ps. Putida	2	9.5	8	21
Total	21	100	38	100

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الملخص العربى

تقييم البكتريا المحبة للبرودة وخصوصاً ميكروبات الزوائف فى اللبن الخام

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قسم الرقابة الصحية على الأغذية-كلية الطب البيطري-جامعة المنصورة *

قسم الرقابة الصحية على الأغذية-كلية الطب البيطري-جامعة كفر الشيخ **

اللبن الخام غذاء أساسى للأطفال ومكمل غذائى للكبار ولهذا تكمن أهمية الدراسة. وقد أجرينا تلك الدراسة لتحديد العد للميكروبات المحبة للبرودة وكذلك ميكروبات السيدوموناس فى اللبن الخام المبرد والذى تم جمعه من المزارع والمحلات الصغيرة الخاصة ببيع الألبان ومنتجاتها من محافظة دمياط. ووجد أن متوسط العد للميكروبات المحبة للبرودة وكذلك ميكروبات الزوائف وكان (3,3x710/ ، 2,7x410مل) و (7,1x710/ ، 8,2x410مل) من عينات اللبن الخام المبرد والذى تم جمعه من المزارع والمحلات الصغيرة الخاصة ببيع الألبان على الترتيب. ومن ناحية أخرى تم تصنيف ميكروبات السيدوموناس التى تم عزلها باستخدام طريقة API20NE. وقد عزلت ميكروبات Ps. putida ، Ps. cepacia ، Ps. fluorescens بنسب قدرها 5,9% ، 1,38 ، 4,52على التوالى من عينات اللبن الخام المبرد والذى تم جمعه من المزارع. بينما تم عزل هذه الميكروبات بنسب قدرها 21% ،16 ،63 على التوالى من عينات اللبن الخام المسوق بالمحلات الصغيرة الخاصة ببيع الألبان. وقد وجد أن ميكروب Ps. fluorescens هو الأكثر شيوعا بين ميكروبات السيدوموناس. ومن المعروف أن هذا الميكروب يؤدى الى فساد اللبن ومنتجاته نظرا لافرازه الانزيمات المقاومة للحرارة التى تؤدى الى تحلل البروتين و الدهن فى اللبن.