Animal Health Research Institute
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ISOLATION OF *PSEUDOMONAS AERUGINOSA* FROM RAW AND PASTEURIZED CREAM

(With 3 Tables)

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عزل ميكروب السيدوموناس ايروجينوزا من القشدة الطازجة والمبسترة

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يعتبر ميكروب السيدوموناس ايروجينوزا من الميكروبات المحبة للبرودة التي تلعب دورا خطيرا في فساد الأغذية بالإضافة إلى خطورته على الصحة العامة لما يسببه من تسمم غذائي نتيجة تناول أغذية ملوثة به, لذلك أجريت هذه الدراسة على مائة عينة من القشدة (خمسين من الطازجة , خمسين من المبسترة) جمعت عشوائيا من بعض الأماكن المتفرقة بمحافظة أسيوط لاستبيان مدى تواجد هذا الميكروب فيها وذلك باستخدام مستنبتى Pseudomonas Agar F base , CNA وقد تبين من الفحص أن معدل وجود السيدوموناس ايروجينوزا فى القشدة الطازجة كان بنسبة ٤% , ٤٢% وذلك على مستنبتى Pseudomonas Agar F base , CNA على التوالي, بينما أمكن عزلها من القشدة المبسترة بنسبة ٢١% وذلك على مستنبت Pseudomonas Agar F base فقط. وقد دلت النتائج على أن مستنبت Pseudomonas Agar F base افضل من مستنبت CNA لعزل هذا الميكروب وقد تم اختبار حساسية عترات السيدوموناس ايروجينوزا (٢٩ عترة) المعزولة من القشدة لبعض المضادات الحيوية وقد وجد أن العترات حساسة للبوليمكسينB والكاربنيسلين ولم يلاحظ لباقي المضادات الحيوية أي تأثير على العترات المعزولة. هذا وقد ناقش البحث أهمية الميكروب من الناحية الاقتصادية وتأثيره على الصحة العامة والشروط الواجب اتخاذها لمنع تلوث الألبان ومنتجاتها بهذا الميكروب للحد من خطورته

SUMMARY

One hundred random samples of raw and pasteurized cream (50 each) were collected from different localities in Assiut Governorate to be examined for the presence of *Pseudomonas aeruginosa*. The organism could be isolated from 4% and 42% of the examined raw cream samples using CNA and Pseudomonas Agar F base, respectively, while in case of

pasteurized cream *Pseudomonas aeruginosa* could be isolated from 12% of the examined samples on pseudomonas Agar F base and it failed detection on CNA. These results indicate that Pseudomonas Agar F base is more reliable than CNA for recovery of *Pseudomonas aeruginosa*. Isolated *Pseudomonas aeruginosa* strains from cream were tested for some antibiotics. Most of the isolated *Pseudomonas aeruginosa* strains were sensitive to Polymyxin B and Carbenicillin but were resistant to other antibiotics. The public health hazard and suggestive measures were discussed.

INTRODUCTION

Pseudomonas aeuroginosa is a Gram negative bacteria belongs to the psychrotrophic microflora which are associated with spoilage as they grow rapidly at refrigeration temperatures and dominate the microbial population.

Ps. aeruginosa is ubiquitous in nature in both winter and summer and is frequently present in hospital environment (air and dust) especially moist places such as skin, bowls, drains, cleaning buckets and inadequately treated water supplies (Lantos et al. 1969 and Ross 1979). Also the organism is present in the intestinal tract of both man and animals, and its presence in food could be taken as an index of faecal contamination (Hoddly and McCoy, 1968 and Bergan, 1975).

Now *Ps.aeruginosa* is recognized by public health authorities as it plays an important role in causing many infections as otitis media, 11% of all nosocomial infections mainly of the lower respiratory tract, urinary tract and surgical wounds (Botzenhart and Ruden 1987). Moreover, the organism is responsible for a number of cases of mastitis and remains in the udder for a number of years (Howell, 1972). Also, *Ps. aeruginosa* was reported as a causative agent of severe forms of acute gastroenteritis (Sutter et al., 1966 and Pereird et al., 1977). Several cases of food poisoning due to Ps. aeruginosa have been reported in Tanta and Assiut Cities due to consumption of dairy products (Abd El-Aziz, 1979 and Ahmed, 1980). Ensing et al (1946) reported an outbreak of food poisoning affecting 24 infants, 9 of them died due to *Ps.aeruginosa*. Also, Abd El- aziz (1979) reported that one out of 101 outbreaks of food poisoning recorded through a year was due to *Ps.aeruginosa*.

Milk and milk products are liable to contamination with *Ps. aeruginosa* during handling, production and processing, also, milk from animals suffering from mastitis due to such organism. This organism

could grow and multiply to numbers sufficient to induce food poisoning and spoilage of contaminated milk (Cheung and Westhoff, 1983).

Ps.aeruginosa could be isolated in variable percentages from milk and milk products by Ahmed (1980), Ergullu (1982), Saad (1983), while Grover and Srinivasan (1988) found high incidence (90%) of *Ps.aeruginosa* in the examined raw cream samples.

Because of the public health significance of Ps.aeruginosa as well as the economic losses caused by it, the present study was undertaken to study the prevalence of it in raw and pasteurized cream and to evaluate two selective plating media for its isolation.

MATERIAL and METHODS

A total of 100 random samples of cream (50) raw obtained from different farmer's houses in Assiut Governorate and (50 pasteurized) obtained from the milk technology laboratory of Faculty of agriculture Assiut University and from different supermarkets. All samples were collected under sterile conditions in sterile containers and dispatched to the laboratory with a minimum of delay and held in refrigerator untill examination for the occurrence of *Ps.aeruginosa*.

A- Preparation of samples:

All cream samples (raw and pasteurized) were thoroughly mixed before being examined.

B- Enrichment procedure: (Lowbury, 1951)

One milliliter of each prepared mixed sample was inoculated into Cetrimide broth and incubated at 42°C for 48 h.

C- Isolation and Identification: (Shriniwas, 1975)

Loopfuls from enrichment broth were streaked on Cetrimide nutrient agar (Oxoide. M3) and Pseudomonas Agar F base (King et al., 1954)

The inoculated plates were incubated at 42°C for 48h. After incubation, the presence of *Ps.aeruginosa* was detected by blue green pigment production (Soluble in both water and chloroform) and the cultures have a distinctive smell (fruity smell) on the media used due to 2 amino acetophenone production.

From the suspected colonies, agar slants were made and incubated for further identification according to King et al., (1954) and Finegold and Martin (1982) and incubated at 42°C for 48 h.

Antibiotic sensitivity test of *Ps.aeruginosa* strains isolated from cream samples was carried out according to the recommended manufacture's instructions using the following antibiotics: Ampicillin 10µg, Erythromycin 15µg, Neomycin 30µg, Streptomycin 10µg, Tetracyclin 30µg, Chloramphenicol 30 µg, Polymyxin B 300 units and Carbenicillin 100µg (Difico Laboratories, Deteriot Michigan, USA)

RESULTS

The results obtained from the examined samples are recorded in tables 1-3.

DISCUSSION

It is evident from the data recorded in Table 1 that *Ps.aeruginosa* could be detected in 4% (2/50) and 42% (21/50) of the examined raw cream samples using Cetrimide Nutrient agar and Pseudomonas Agar F base, respectively. The obtained results are lower than those recorded by Grover and Srinivasan (1988) and mild to those obtained by Ahmed (1980), Ergullu (1982) and Saad (1983) and agree to a certain extent with those of Grun (1969) who recorded high incidence of *Pseudomonas aeruginosa* (87%).

Table 2 emphsizes that no isolates could be recovered from pasteurized cream samples using Cetrimide Nutrient agar, while using Pseudomonas Agar F base, 12% (6/50) of the examined samples found to be positive for *Ps.aeruginosa* and this result is considered high if compared with those recorded by Grover and Srinivasan (1988) and agree with those of Haiadova and Iacova (1982).

The presence of Ps.aeruginosa in raw cream may be attributed to the bad hygienic measures applied during production and handling, using of polluted water in dairy farm, milking equipments as well as milker's hands (Otte et al., 1978), while its presence in pasteurized cream may be due to post pasteurization contamination.

Table 1 and 2 show that using of Pseudomonas Agar F base gave a good recovery for Ps.aeruginosa than Cetrimide Nutrient agar in all of the examined samples and these results are in accordance with those obtained by Grover and Srinivasan (1988), Saad (1983) and korashy (1992).

Table 3 shows that all the isolates of Ps. aeruginosa were found to be resistant to all antibiotic discs and sensitive only to Carbencillin and polymyxin B and this agree with Rita R. Colwell (1964), while Haagsma and Pereboom (1965) found Tetracyclines, Polymyxin and Chloramphenicol to be ineffective in the control of an outbreak of haemorrhagic pneumonia in mink caused by Pseudomonas aeruginosa whereas, Collins and Donnelly (1966) reported that Sulphathiazole was of some benefit in the control of this disease, and in general, most workers agree that antibiotics are of limited value in the treatment of pseudomonas infections in man and animals.

In conclusion, milk and milk products are liable to contaminate by *Ps.aeruginosa* during production, handling or processing and the organism could grow to numbers sufficient to induce food poisoning.

Therefore, governmental regulations should be imposed for those who are concerned with dairy industry. Also, stringent hygienic measures must be followed during all steps of milk production and its manufacture to dairy products.

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Table 1: Incidence of *Ps.aeruginosa* in the examined raw cream samples.

Media used	No. of examined samples	No. of positive samples	%
1-Cetrimide Nutrient agar	50	2	4
2-Pseudomonas Agar F. base	50	21	42

Table 2: Incidence of *Ps.aeruginosa* in the examined pasteurized cream samples.

Media used	No. of examined samples	No. of positive samples	%
1-Cetrimide Nutrient agar	50	-	-
2-Pseudomonas Agar F. base	50	6	12

Table 3: Antibiotic sensitivity of isolated *Ps.aeruginosa* from raw and pasteurized cream

Antibiotic disc	No. of samples	Reaction
Ampicillin (10µg)	29	-
Erythromycin (15µg)		-
Neomycin (30µg)		-
Streptomycin (10µg)		-
Tetracyclin (30µg)		-
Chloramphenicol (30 µg)		-
Polymyxin B (300 units)		+ + +
Carbenicillin (100µg)		+ +

- = resistant + = low sensitive ++ = moderate sensitive +++ = high sensitive