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PSEUDOMONAS AERUGINOSA AND *STAPHYLOCOCCUS AUREUS* PROFILE IN SOME DAIRY FARMS

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ABSTRACT

The purpose of the current study was to evaluate the degree of contamination of dairy farms with both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in which, a total of 150 swab samples were collected from 3 different dairy farms (50 each) distributed in Assiut governorate (farm A) and El-Minia governorate (farms B & C), Egypt. The swabs were taken from different locations in the dairy farms such as teats of dairy animals, milking machines, workers' hands and stanchions, etc. All the samples were examined for detection of *Ps. aeruginosa* (conventionally biochemical followed by MICROBACT™ identification kits) and for *Staph. aureus* (coagulase +ve). The obtained results revealed the overall incidence of *Ps. aeruginosa* as 14%, 2% and 4% in farms A, B & C, respectively; while, the incidence of *Staph. aureus* was 4%, 30% and 4%, respectively. In conclusion, the profile of both *Ps. aeruginosa* and *Staph. aureus* among the examined dairy farms is highlighting the hygienic state of each farm separately.

Key words: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Dairy farms.

INTRODUCTION

Unlike some livestock systems, the dairy farm does not have the luxury of an 'all in, all out' stocking policy. The reduction of bacteria in the immediate surroundings must reduce the opportunity for bacteria to gain access to the animal and cause diseases. Therefore, maintaining proper hygiene on a dairy farm presents challenges. These challenges can be met with an array of products designed to keep your dairy farm clean and safe.

Cleaning is the process of removing unwanted substances, such as dirt, infectious agents, and other impurities, from an object or environment. Cleaning is often performed for aesthetic, hygienic, functional, environmental, or safety purposes. Cleaning occurs in many different contexts and uses many different methods. Poor cleaning methods can cause the spread of disease and pathogens from one milking to the next.

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Several bacteria genera have been described as psychrotolerant microorganisms and *Pseudomonas* is the genus of most technological relevance (Hantsis-Zacharov and Halpern 2007). *Pseudomonas* spp. was isolated from all milk samples and from all sampling points, so the dissemination of *Pseudomonas* spp. in dairy farm environment was demonstrated (Vidal *et al*., 2017a). Milkers' hands, surface of cows' teats, teat cups and cooling tanks were associated with raw milk contamination with *Pseudomonas* spp. on farms with manual and mechanical milking systems, showing that regardless of the type of milking system and season, proper hygiene procedures of equipment, utensils and workers' hands are essential to avoid contamination of raw milk (Vidal *et al*., 2017a).

Staphylococcus aureus is responsible for all kinds of mastitis in dairy animals. The unhygienic conditions at farms along with other risk factors may result in infection to animals irrespective of age. Economic damages that are outcomes of clinical and subclinical mastitis are entitled as reduced milk yield, spoiled milk, lower milk quality, unstable taste, reduced milk processing, lower shelf life and decreased yield of milk products.

Therefore, the current investigation aimed to explore the profile of both *Pseudomonas aeruginosa* and *Staphylococcus aureus* in some dairy farms related to Assiut and El-Minia governorates.

MATERIALS AND METHODS

Samples collection:

A total of 150 swab samples were collected from 3 different dairy farms (50 each) located in Assiut governorate (farm A) and El-Minia governorate (farms B & C), Egypt. The swabs were taken from different locations in the dairy farms such as teats of dairy animals, milking machines, workers' hands and stanchions, etc.

After rubbing the wetted cotton swab with the surface, it was immediately immersed in a sterile tryptic soya broth (TSB) and placed in an ice box and transported to the laboratory for direct analysis. All the swab samples were incubated at 37° C for 24 - 48 h followed by further isolation of both of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Isolation of *Pseudomonas aeruginosa*:

A loopful from each of the incubated swabs was streaked onto the surface of Pseudomonas selective agar (Oxoid, CM 0559) plates supplemented with Pseudomonas CN supplement (Oxoid, SR0102). The plates were incubated at 25° C for 24 - 48 h. The colonal and cellular morphology of *Ps. aeruginosa* (Quinn *et al*., 1994) were identified, in which, the suspected colonies (large flat spread green bluish, brown or florescence pigmentation with a characteristic grape-like odor of O-aminoacetophenone which *Ps. aeruginosa* was unique of its production) were taken on TSA agar slants for maintenance and further identification by conventional biochemical reactions (like oxidase test), followed by **MICROBACT™** identification kits which is Gram-negative identification system.

Isolation of *Staphylococcus aureus* (APHA, 2004)**:**

A loopful from each of the incubated swabs was streaked onto the surface of Baird-Parker's agar (Oxoid, CM 0275) plates supplemented with egg yolk tellurite (Oxoid, SR0054). The plates were incubated at 37° C for 24 h. The suspected colonies were taken on TSA agar slants for maintenance and further identification. The coagulase test (Mackie and McCartney, 1996) was done by tube coagulase (free coagulase).

RESULTS

Table 1: Incidence of the examined bacteria in the farm A

Farm A		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Samples type	**Samples (no.)**	**Positive samples (no.)**	**Incidence (%)**	**Positive samples (no.)**	**Incidence (%)**
Animal teat	24	2	8.33	-	-
Milking machine	10	4	40	1	10
Milk cooler tank	2	-	-	-	-
Milker's hand	2	-	-	-	-
Milking stanchion	2	-	-	-	-
Milk utensil	7	1	14.29	1	14.29
Milk strainer	1	-	-	-	-
Farm stanchion	2	-	-	-	-
Total	50	7	14	2	4

Table 2: Incidence of the examined bacteria in the farm B

Farm B		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Samples type	**Samples (no.)**	**Positive samples (no.)**	**Incidence (%)**	**Positive samples (no.)**	**Incidence (%)**
Animal teat	32	-	-	12	37.5
Milker's hand	2	1	50	1	50
Milking stanchion	2	-	-	-	-
Milk utensil	8	-	-	1	12.5
Milk strainer	4	-	-	1	25
Farm stanchion	2	-	-	-	-
Total	50	1	2	15	30

Table 3: Incidence of the examined bacteria in the farm C

Farm C		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Samples type	**Samples (no.)**	**Positive samples (no.)**	**Incidence (%)**	**Positive samples (no.)**	**Incidence (%)**
Animal teat	12	-	-	-	-
Milking machine	20	-	-	-	-
Milker's hand	2	1	50	-	-
Milking stanchion	6	1	16.67	2	33.33
Milk utensil	7	-	-	-	-
Farm stanchion	3	-	-	-	-
Total	50	2	4	2	4

DISCUSSION

The present study threw light on the profile of *Ps. aeruginosa* in the current examined dairy farms. The summarized results in Table 1 revealed that the incidence of *Ps. aeroginosa* in farm A was 14% distributed among animal teats, milking machines and milk utensils in percentages of 8.33%, 40% and 14.29%, respectively. While, in farm B, *Ps.*

aeruginosa could be detected in only one sample (2%) contaminating milker's hands (Table 2).

For farm C, Table 3 cleared that 2 out of the 50 swab samples (4%) were contaminated with *Ps. aeruginosa*, which was isolated from milker's hands and milking stanchions in percentages of 50 and 16.67%, respectively. Aziz *et al*. (2022) reported a lower result as *Ps. aeruginosa* was isolated in a percentage of 28% from hand swabs. While Vidal *et al*. (2017b) revealed a higher percentage as *Pseudomonas* spp. were isolated from milkers' hands in 80%. *Ps. aeruginosa* was isolated from different sources; reflecting its survival in different conditions.

The farm A results (Table 1) showed that 4% of the examined swab samples were contaminated with *Staph. aureus* which was found in milking machines and milking utensils. Nearly higher findings of 20% swabs of milking buckets were reported by Regasa *et al*. (2019).

The highest incidence of isolated *Staph. aureus* in the current investigation by 30% was obtained from farm B (Table 2). The distribution of *Staph. aureus* among teat samples, milker's hands, milk utensils and milk strainer are shown in Table 2. It was remarkable that *Staph. aureus* contaminated 12 out of 32 of the animal teats (37.5%). For farm C, *Staph*. *aureus* was isolated from the milking stanchions only (Table 3).

The rates of isolation of *Staph*. *aureus* from the present 3 dairy farms (A, B and C) were 4%, 30% and 4%, respectively (Tables 1, 2 & 3). These variations might be due to the difference in the management system used by the farms, in addition to, the farm size, the number of animals per farm, the hygienic conditions, the milking system, the geographical location and the season.

One of the main differences among the examined dairy farms was the milking method, in which farm B (highest *Staph*. *aureus* isolation) was hand milked system, while farms A and C were machine milked. Hand milking is prone to high risks of distributing bacteria from the milker to the milk itself (Robinson, 2005).

The observations in the current investigation showed the bad practices in milk production and handling, especially the farm B; for example, unsatisfactory hand washing of the workers, no clothes change before milking, no head cover during milking, drinking and smoking during milking, and motorcycle milk transportation. All in turn, enhance the chances of milk contamination.

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صورة تواجد السيدوموناس إيريجينوزا والمكور العنقودي الذهبي في بعض المزارع الحلابة

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الغرض من الدراسة الحالية هو تقييم درجة تلوث المزارع الحلابة بكل من السيدوموناس إيريجينوزا والمكور العنقودي الذهبي، حيث تم جمع عدد ١٥٠ مسحة من ٣ مزارع حلابة مختلفة (٥٠ مسحة لكل مزرعة) موزعة في محافظة أسيوط (مزرعة أ) ومحافظة المنيا (مزرعة ب و ج)، مصر. وقد تم أخذ المسحات من أماكن مختلفة بالمزارع مثل حلمات الحيوانات الحلابة وماكينات الحلب وأيدي العمال والزناقات إلخ. وقد تم فحص كل العينات لتحديد وجود السيدوموناس إيريجينوزا (الطرق البيوكيميائية ثم التعريف بواسطة MICROBACT™ kits) وكذلك لتحديد نسب المكور العنقودي الذهبي (إيجابي التجلط)، وقد أظهرت النتائج أن وجود السيدوموناس إيريجينوزا كان بالنسب ١٤% و ٢% و ٤% في المزارع أ و ب و ج، على التوالي، بينما ظهر المكور العنقودي الذهبي بالنسب ٤% و ٣٠% و ٤%، على التوالي. ونستخلص من الدراسة الحالية أن صورة تواجد كل من السيدوموناس إيريجينوزا والمكور العنقودي الذهبي في المزارع الحلابة هي إنعكاس للحالة الصحية لكل مزرعة على حدة.

الكلمات المفتاحية: السيدوموناس إيريجينوزا، المكور العنقودي الذهبي، مزارع الألبان.