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SANITARY CONDITIONS OF MILKING ENVIRONMENT IN ASSIUT DAIRY FARMS AND THE QUALITY OF THEIR PRODUCED MILK

(With 7 Tables)

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الحالة الصحية لبيئة مزارع الألبان في أسيوط ونوعية الحليب المنتج

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تم فى هذا البحث دراسة التلوث الميكروبي لعينات عشوائية من هواء وأسطح جدران وحدات إنتاج اللبن ومسحات من الحلمات والضرع ومسحات من أيدي الحلابين بالإضافة إلى مسحات من السطح الداخلي لأواني الحليب وذلك لمعرفة مدى حالة التلوث بالميكروبات بالإضافة إلى تحديد أنواعها المختلفة وعلاقة ذلك أيضا بمدى حالة التلوث للألبان المنتجة منها. وإستيفاءاً لهذا الغرض تم اختيار ثلاثة مزارع مختلفة في محافظة أسيوط شملت مزرعة كلية الزراعة ومزرعة مستشفى كلية الطب البيطري ومزرعة مدرسة الزراعة الثانوية. تم تجميع وفحص إجمالي عدد ٤٣٢ عينة (٧٢ عينة هواء ، ٧٢ مسحة من أسطح جدران المحلب الداخلي ،٧٢ مسحة من أسطح الضرع والحلمات ،٥٤ مسحة من أيدي الحلابين قبل الحلب مباشرة ، ٧٢ مسحة من السطح الداخلي لأواني الحليب ، ١٨ مسحة من السطح الداخلي لحلمات ماكينات الحلب الآلي ، ٧٢ عينه لبن مأخوذة أثناء الحلب مباشرة). وقد تركزت هذه الدراسة على إجراء العد الطبقي الكلى القياسي للميكروبات عند درجة حرارة ٣٧° م ، ٢٢° م وكذلك تم إجراء العد الكلى لميكروب الكوليفورم وميكروب الإشرشيا القولوني البرازي النموذجي وميكروب الكلوستريديوم ولش. وقد أظهرت النتائج وجود متوسطات أعداد متباينة من الميكروبات لكل من العينات المختبرة من الألبان والبيئة المحيطة بها ، كما تم عزل وتصنيف العديد للميكروبات من البيئة المحيطة بالحيوانات أثناء إجراء عملية الحلب وكذلك من اللبن المنتج منها مباشرة والتي تمثلت في عزل إجمالي ٩٠٦ عترة ميكروبية شملت ٤٨٣ ، ١٤١ ، ١٩٩عترة من البيئة المحيطة بالحيوانات وأيضا ٤١ ، ١٥، ٢٧ عترة من عينات اللبن المأخوذة منها وذلك لكل من مزرعة كلية الزراعة ومزرعة مستشفى كلية الطب البيطري ومزرعة مدرسة الزراعة الثانوية بأسيوط على التوالي . وكان من أهم العترات المعزولة ميكروب مكور العنقود الذهبي ، الميكروب السبحي البرازي ، ميكروب الإشرشيا القولوني النموذجي، وميكروب الكلوستريديوم ولش وخلافة من الميكروبات المرضية والملوثة لمنتج اللبن و بنسب عزل مختلفة . وقد تم

الإشارة للأهمية الصحية لبعض عترات الميكروبات المعزولة. كما اتضح من خلال هذه الدراسة بأن البيئة المحيطة داخل وحدات إنتاج اللبن تلعب دوراً كبيراً في تلوث ونقل الكثير من الميكروبات إلى منتج اللبن. وقد تم ذكر بعض أهم التوصيات والاحتياطات الوقائية بالإضافة إلى بعض الإجراءات الصحية والتي يجب وضعها في الاعتبار داخل مزارع إنتاج الألبان للحصول على منتج لبن يخلو من الملوثات الميكروبية حفاظاً على صحة كل من الإنسان والحيوان على السواء.

SUMMERY

Special attention must be paid to the increased health risks resulting during milk production in dairy farms which are of growing in number and size allover the world , thus monitorial programs and methods of identifying and controlling these risks must be offered periodically. A total number of 432 random samples (represented by 72 samples of air; 72 wall surfaces swabs; 72 udder and teat surfaces swabs ; 54 milker's hands swabs; 18 teat cups swabs; 72 milk equipments swabs and 72 milk samples) were collected from the milking units of three experimented dairy farms included Fac. of Agriculture; Fac. of Vet. Medicine and Secondary School of Agriculture in Assiut Province and examined bacteriologically to evaluate the distribution of pathogenic and potentially pathogenic bacteria and their role in milk contamination. Variable loads of total bacterial counts/unit were estimated. The maximum of total bacterial mean count of $7.2 \times 10^5 \pm 0.49/m^3$ was detected in examined air samples of Fac. of Vet. Medicine, while the minimum mean count of $1.74 \times 10^2 \pm 0.56$/ml was achieved in milk samples of the milking unit of Fac. of Agriculture. The maximum of total coliforms mean count was $4.38 \times 10^4 \pm 1.55/m^2$ from inner surfaces of milk utensils of Fac. of Agriculture and minimum of total coliforms mean count was $0.0/m^3$ in examined air samples collected from dairy farms of Fac. of Agriculture and Secondary School of Agriculture. The maximum mean count of *Escherichia coli* was estimated by $3.92 \times 10^3 \pm 1.98/m^2$ from wall surfaces in Fac. of Vet. Med. Farm, while the minimum of mean count of *Escherichia coli* was $0.0/m^3$ in examined air samples in both dairy farms of Fac. of Agriculture and Secondary School of Agriculture. The maximum mean count of *Clostridium perfringens* was $6.0 \times 10^3 \pm 0.21/m^2$ from wall surfaces in Fac. of Vet. Med. Farm, while the minimum mean count was estimated by $0.0/m^3$ in all examined air samples of the experimented three dairy farms and also from milk samples of both dairy farms of Fac. of Agriculture and Secondary School of Agriculture. It had been found that the mean values of total bacterial counts, total coliforms counts, *Escherichia coli* counts and

Clostridium perfringens counts were greatly variable from one experimented dairy farm to another. Moreover, it could be noticed that there was a direct relation between the different bacterial counts obtained from the surrounding environment and that of corresponding counts in milk produced under these circumstances. Wide varieties of total 906 of pathogenic and potentially pathogenic bacterial isolates could be detected and identified from all examined samples with variable incidence and frequency percentages. The most common bacterial isolates included *Staphylococcus aureus; Staphylococcus epidermidis; Streptococcus faecalis; Streptococcus bovis; Streptococcus agalactia; Pseudomonas aeruginosa; Escherichia coli; Klebsiella pnemoniae; Klebsiella mastitis; Arizona species; Salmonella species; Clostridium perfringens* and others. The total bacterial isolates were represented by 483, 141 and 199 from milking units and 41, 15 and 27 of produced milk from the three examined dairy farms in Fac. of Agriculture; Fac. of Vet. Medicine and Secondary School of Agriculture respectively. Animal and public health significance of the most common bacterial pathogens and contaminants from all examined samples from the experimented dairy units and milk, as well as the precautionary measures and hygienic recommendations were briefly discussed.

Key words: *Hygiene of dairy or milking environment; sanitary conditions in dairy farms; dairy herds and milk quality.*

INTRODUCTION

The extent of bacterial contamination in the environment of milking units are of particular importance in milk industry. Environment surrounding animals constitutes the main source of pathogenic and potentially pathogenic microorganisms. Such organisms involved the causative agents of milk deterioration. Environmental conditions can markedly influence the diseases that may be higher if large number of animals are allowed to house in unhygienic conditions (Johens, 1980 and Quigley *et al.*, 1995). The occurrence and persistence of the microorganisms in air, wall surfaces, udder ant teat surfaces, milk utensils and equipments have been largely overlooked as a problem in the hygienic condition of milk (Ahmed, 1975; Anderson *et al.*, 1999; Fox *et al.*, 1990; Kloos & Musselwhite, 1975; Matos *et al.*, 1991; Mowafi *et al.*, 1980; Roberson *et al.*, 1994; Roberson *et al.*, 1998 and Schacken *et al.*, 1996). *Clostridium perfringens*, enterococci and

coliforms constitute the major bacterial groups that commonly detected from contaminated dairy environment (Parrakova & Fratic, 1980).

Enterohemorrhgic *Escherichia coli* was first recognized as a pathogen in the last decade, several outbreaks that induce hemorrhagic colitis and hemolytic syndrome caused by the pathogens via consumption of contaminated raw milk (Duncan *et al.*, 1987). Presence of enterobacteriaceae in milk is always taken as a definite index of faecal contamination (Synage, 2000 and Zakaria *et al.*, 1980). The initial microflora of milk has a marked influence on the keeping quality of raw milk and once the milk comes outside the udder contamination of various degrees occur mainly from milk handling and from the environmental contamination (Anderson *et al.*, 1999 and Rendose *et al.*, 1975). Moreover, air, walls, milker's hands, milk equipments as well as soiled teats and udder with dung or mud from bedding materials are washed into milk during milking (Nazem *et al.*, 1995). The load of number and type of microorganisms vary according to the type and amount of smeared soil on the teats and udder (Gierl and Putz, 1992). The milk production of good keeping quality requires healthy cows, good management, thorough cleaning and disinfection of the dairy units, premilking teats and udder disinfection beside reduction of air dust particles (Bodman *et al.*, 1988; Ingawa *et al.*, 1992; McKinnon *et al.*, 1990; Nickerson , 1989 and Rasmussen *et al.*, 1991) .

The present work was carried out in order to screen the microflora contamination of the milking environment as well as those present in the produced milk of three different dairy farms in Assiut Province with particular concern for evaluation the hygienic condition of milk quality and discuss the hazard effects of animal and public health significance of the major bacterial isolates.

MATERIALS and METHODS

Experimental dairy farms:

The present investigation was conducted in three different dairy farms at Assiut Province. The construction of the experimented dairy farms included:

Faculty of Agriculture Farm:

The farm accommodated for total 676 animals of which 121 were lactating and milked. Animals were housed in open yard system in dirty floor and supplied with milking unit. The lactating caws were milked manually.

Faculty of Vet. Medicine Farm:

The farm accommodated for total 20 animals of which 11 were lactating and milked. Animals were housed in stalls of concrete floor (cow-house system). The stalls mainly used for milking and housing. The lactating caws were milked manually.

Secondary School of Agriculture:

The farm accommodated for total 40 animals of which 18 were lactating and milked. Animals were housed in open yard system of dirty floor and supplied with milking parlour which holding a pipeline milking machine with teat cups (Alfa–Laval System). The lactating caws were milked automatically.

Sampling and bacteriological examination of specimens:

A total number of 432 random samples (represented by 72 samples of air; 72 wall surfaces swabs; 72 udder surfaces swabs; 54 milker's hands swabs; 18 teat cups swabs; 72 milk equipments swabs and 72 milk samples) were collected under complete aseptic condition from milking units of the three experimented dairy farms in Assiut Province. The obtained specimens were kept separately cooled and carried to the laboratory with a minimum of delay for the further bacteriological examination.

The bacteriological examination of environmental specimens and milk samples:

Air samples: Seventy-two air samples were collected from the tested milking units using sterile liquid impingers supplied with electric counter vacuum pump. The technique used by Cown *et al.* (1956) and Brachman *et al.* (1964) was adopted.

Wall surfaces: Seventy-two swabs were collected from the inner surfaces of the building of the examined milking units aseptically, according to Rendos *et al.* (1975). The swabs were inoculated into sterile test tubes, each containing sterile 10 ml of nutrient broth.

Udder swabs: Seventy-two udder and teat swabs were collected from dairy cows just before milking time, according to Rendos *et al.* (1975). The swabs were inoculated into sterile test tubes containing sterile nutrient broth.

Milker's hands: Fifty-four swabs were collected from the milker's hands. Each sterile swab moisten with 10 ml. of sterile broth was rubbed on the skin surface of palm.

Teat cups: Eighteen swabs were collected from the inner surfaces of teat cups of the milking machine, just before milking time.

Milk utensils (equipments): Seventy-two swabs were collected from the inner surfaces of milk utensils just before milking time.

Milk samples: Seventy-two milk samples were collected in sterile screw bottles under complete aseptic condition.

The bacteriological counts/units of environmental specimens and milk samples included:

The total bacterial counts/unit; total coliforms; *Escherichia coli* count and *Clostridium perfringens* count were done according to the technique described by Beerens *et al.* (1980); Carter (1979); Cruickshank *et al.* (1980); Johnson & Curl (1972) and Oblinger & Koburger (1975).

The bacteriological cultivation, isolation and identification of the examined samples included:

Bacterial culture, isolation and identification of the isolated strains on different liquid and solid media were carried out according to Baily & Scott (1974); Carter (1979); Cruickshank *et al.* (1980); Edward & Ewing (1972); Koneman *et al.* (1988); Krieg & Holt (1984); Murry *et al.* (1984); Topley & Wilson (1975) and Treagan & Pulliam (1982).

RESULTS

The obtained results were illustrated in Tables (1 – 7).

DISCUSSION

The primary entry points of pathogens in milk are dairy animals , milk handlers , equipments and other contact environmental sources. Cross-contamination of milk can occur from poor hygiene of milking environment in animal enclosures (Bringe, 1989 and McKinnon *et al.*, 1983).

Microbial quality of air samples:

The obtained results of the examined air samples in Tables (1,2 & 3) revealed that, the total colony counts/m^3 were widely differed in the three experimented dairy farms. They were as high as 1.8 x $10^6/m^3$ at 37°c and 6.3 x 10^4 / m^3 at 22°c in dairy farm of Fac. of Vet. Medicine, while they were 3.2 x $10^4/m^3$ at 37°c and 4.0 x $10^3/m^3$ at 22°c in Secondary school farm, and 8.0 x 10^3 /m^3 at 37°c and 4.8 x $10^4/m^3$ at 22°c in case of the dairy farm of Fac. of Agriculture. The high count in dairy farm of Fac. of Vet. Med. may be attributed to the long time spend by animals inside the farm, which was supported by Benham and Egdell

(1970) who reported that, the high extent of microbial contamination of air samples was likely pollute the atmosphere of the milking environment and would create a hazard in the production of clean milk. Neither coliforms, *Escherichia coli* nor *Clostridium perfringens* were detected from examined air samples in both farms of Fac. of Agriculture and Secondary School of Agriculture, while air samples of Fac. of Vet. Medicine Farm contained 2.38 x 10 ±0.83 and 1.64 x 10 ±0.58 as mean counts/m^3 of total *Coliforms* and *Escherichia coli* respectively (Tables, 1-3). Biological air contamination is considered as one of the major sources of milk contamination with microbial pathogens in dairy confinements (Benham & Egdell, 1970; Dykstra, 1961; El-Agrab, 1977; Perry *et al.* 1958; Torre, 1955 and Wilson & Miles, 1957).

Microbial quality of wall surfaces:

The obtained results concerning inner wall surfaces (Tables, 1-4) showed that, the highest total bacterial mean counts/m^2 (4.77 x 10^4 ±0.32); total coliforms count/m^2 (2.48 x 10^4 ±0.69); *E. coli* count / m^2 (3.92 x 10^3 ±1.98) and *Cl. perfringens* count / m^2 (6.0 x 10^3 ±1.21) were observed in case of Fac. of Vet. Med. Farm, while the lower total bacterial mean counts/m^2 (1.11 x 10^4 ±0.30); total coliforms count/m^2 (4.09 x 10^3 ±1.22); *E. coli* count/m^2 (3.04 x 10^3 ±0.90) and *Cl. perfringens* count/m^2 (4.98 x 10^2 ±0.61) were detected in the farm of Secondary School of Agriculture. The sanitary control measures should be incorporated in any building design and the whole building should be cleaned and disinfected periodically (Galton & Merrill, 1987 & 1988).

Microbial quality of teat and udder surfaces:

Illustrated microbial data of teats and udder surfaces (Tables, 1-4) revealed a high total bacterial mean count/m^2 of 1.44 x 10^5 ±0.40 and 3.60 x 10^4 ±1.01 in case of Secondary School of Agriculture farm and Fac. of Vet. Med. Farm respectively, while the lower total bacterial mean count/m^2 of 1.20 x 10^4 ±0.37 was observed in case of Fac. of Agriculture dairy farm. *E. coli* and *Cl. perfringens* mean counts/m^2 were (5.02 x 10^2 ±1.89 & 3.20 x 10^2 ±1.42); (2.36 x 10^2 ±0.63 & 7.62 x 10 ±1.10) and (1.12 x 10^2 ±0.26 & 6.04 x 10^2 ±2.78) in case of Fac. of Agriculture; Secondary School of Agriculture and Fac. of Vet. Medicine dairy farms respectively. Udder and teats surfaces represent important sources of milk contamination as was confirmed in a bacteriological survey of six parlours of milked herds carried out by Underwood *et al.* (1974).

Microbial quality of milker's hands:

Results concerning the microbial contamination of the milker's hands (Tables, 1-4) showed a higher total bacterial mean count/palm of $3.24 \times 10^3 \pm 0.91$ in case of Fac. of Vet. Medicine farm while, the lower total mean count/palm of $1.61 \times 10^3 \pm 0.44$ was detected in case of Fac. of Agriculture dairy farm. Moreover, the *E. coli* and *Cl. perfringens* mean counts/palm were $7.28 \times 10 \pm 2.91$ and $3.00 \times 10 \pm 0.17$ in case of Fac. of Agriculture dairy farm , while these mean counts were $1.36 \times 10 \pm 0.32$ and $4.96 \times 10 \pm 2.31$ in case of Fac. of Vet. Medicine dairy farm. Complete hand sanitation is nearly impossible under practical conditions so milkers must wear sterile smooth rubber gloves and dip them in a sanitizing solution to reduce contamination during milking operation (Eberhart, 1987).

Microbial quality of machine teat cups:

The obtained results concerning microbial contamination of the milking machine teat cups (Tables, 1-4) revealed mean counts/m^2 of ($1.45 \times 10^4 \pm 0.50$); ($1.25 \times 10^4 \pm 0.34$); ($0.93 \times 10^3 \pm 0.24$) and ($6.77 \times 10 \pm 1.20$) for the total bacterial count; total coliforms; *E. coli* and *Cl. perfringens* in case of Secondary School of Agriculture farm. All containers including teat cups, rubber parts that come into contact with milk and vacuum hoses of milking machine must be thoroughly cleaned and soaked in an effective sanitizer for 2 to 3 hours before each use (John, 2001).

Microbial quality of milk utensils (equipments):

The obtained results of milk equipments (Tables, 1-4) showed total bacterial mean counts/m^2 of $5.08 \times 10^4 \pm 1.44$; $4.34 \times 10^4 \pm 1.23$ and $1.46 \times 10^4 \pm 0.39$ in dairy farms of Fac. of Vet. Medicine; Fac. of Agriculture and Secondary School of Agriculture. *E. coli* and *Cl. perfringens* mean counts/m^2 were ($2.08 \times 10^3 \pm 0.65$ & $1.50 \times 10^2 \pm 0.63$); ($1.46 \times 10^3 \pm 0.48$ & $1.12 \times 10^2 \pm 0.33$) and ($6.18 \times 10^2 \pm 1.74$ & 0.0) in case of Fac. of Agriculture; Secondary School of Agriculture and Fac. of Vet. Medicine dairy farms respectively. So the contact surfaces of all equipments and utensils used in handling, storage or transportation of milk should be cleaned and treated with an effective sanitizer before and after each usage (Galton & Merrill, 1987 & 1988).

Microbial quality of milk:

Results concerning the microbial contamination of examined milk samples (Tables, 1-4) revealed total bacterial mean counts/ml of ($1.39 \times 10^3 \pm 6.97$ at 37°c & $7.99 \times 10^4 \pm 1.53$ at 22°c); ($6.10 \times 10^2 \pm$

0.36 at 37°c & $4.56 \times 10^2 \pm 1.23$ at 22°c) and ($1.74 \times 10^2 \pm 0.56$ at 37°c & $3.76 \times 10^2 \pm 1.46$ at 22°c) in case of Fac. of Vet. Medicine; Secondary School of Agriculture and Fac. of Agriculture dairy farms respectively. *E. coli* and *Cl. perfringens* mean counts /m^2 were (4.2 ± 0.13 & 0.0); (6.4 ± 3.03 & 0.0) and (4.0 ± 0.19 & 2.8 ± 1.52) in case of Fac. of Agriculture; Secondary School of Agriculture and Fac. of Vet. Medicine dairy farms respectively. *Cl. Perfringens* was only detected in examined milk samples obtained from Fac. of Vet. Medicine dairy farm which indicated poor hygiene within its milking stalls. The obtained results were more or less coincided with those recorded by (Ahmed, 1975; Brander, 1973; El-Masry, 1989; Johens, 1980 and Mnatsakanov *et al.*, 1991). Milk is good medium for growth of different microorganisms which may contaminate it during milking , handling and processing (Bodman *et al.*, 1988). Some of bacterial strains isolated from milk are classified as pathogens which induce health hazards, but other organisms act as milk spoilers that cause deterioration of its keeping quality in several ways like milk spoilage and curdling or undesirable flavors like rancidity or milk sourness, the production of good quality milk required healthy dairy animals, good management and hygienic environment (Duncan *et al.*, 1987 and Galton & Merrill, 1987 and Tybor & Gilson, 1989).

The obtained results (Tables, 5-7) revealed that a wide variety of total 906 of pathogenic and potentially pathogenic bacterial isolates could be detected from all examined specimens (air, wall surfaces, teats and udder, milker's hand, teat cups and milk samples) with variable incidence and frequency percentages of major animal and public health significance included *Staphylococcus aureus; Staphylococcus epidermidis; Streptococcus faecalis; Streptococcus bovis ; Streptococcus agalactia; Pseudomonas aeruginosa; Escherichia coli; Klebsiella pnemoniae; Klebsiella mastitis; Arizona species; Salmonella species; Clostridium perfringens* and others. The wide differences of percentages of bacterial isolation may be attributed to variation of environmental management and hygienic conditions within the experimented dairy farms (Acres, 1985 and Hinton *et al.*, 1994). The total bacterial isolates were represented by 483, 141 and 199 from milking units and 41, 15 and 27 from produced milk that obtained from the three examined dairy farms in Fac. of Agriculture; Fac. of Vet. Medicine and Secondary School of Agriculture in Assiut Province respectively. *Staphylococcus aureus* was the most prominent pathogen which isolated with overall incidence percentages of (13.5, 9.93 and 9.55 from milking

environment) and (9.76, 6.67 and 3.70 from milk samples) in case of examined dairy farms from Fac. of Agriculture; Fac. of Vet. Medicine and Secondary School of Agriculture respectively (Tables, 5-7), the obtained results indicate that *Staphylococcus aureus* is considered as one of the main bacterial strains isolated from the dairy farm environment. *Staphylococci* may be present in milk as a result of contamination from udder or other sources and may grow in milk and milk products and produce potent enterotoxins which are thermostable as not destroyed by pasteurization and causing food poisoning among consumers (Minor & Marth, 1972 and Hekneby & Gondrosen, 1982). *Streptococcus faecalis* (Tables, 5-7) was isolated with overall incidence percentages of (5.80, 5.67 and 5.53 from milking environment) and (2.44, 0.0 and 0.0 from milk samples); *Streptococcus bovis* (Tables, 5-7) was isolated with overall incidence percentages of (1.45, 1.42 and 3.52 from milking environment) and the organism failed to be detected from examined milk samples); *Streptococcus agalactia* (Tables, 5-7) was isolated with overall incidence percentages of (1.24, 1.42 and 3.52 from milking environment) and (2.44, 0.0 and 7.41 from milk samples); *Pseudomonas aeruginosa* (Tables, 5-7) was isolated with overall incidence percentages of (5.59, 3.55 and 4.02 from milking environment) and (7.32, 0.0 and 3.70 from milk samples); *Klebsiella pnemoniae* (Tables, 5-7) was isolated with overall incidence percentages of (0.62, 1.42 and 0.50 from milking environment) and the organism failed to be detected from milk samples) ; *Klebsiella mastitis* (Tables, 5-7) was isolated with overall incidence percentages of (1.03, 1.42 and 2.01 from milking environment) and (2.44, 0.0 and 3.70 from milk samples); *Escherichia coli* (Tables, 5-7) was isolated with overall incidence percentages of (10.9, 9.22 and 6.53 from milking environment) and (9.76, 6.67 and 4.44 from milk samples); *Arizona species* (Tables, 5-7) was isolated with overall incidence percentages of (0.83, 0.0 and 0.0 from milking environment) and (0.0, 0.0 and 0.0 from milk samples); *Salmonella species* (Tables, 5-7) was isolated with overall incidence percentages of (0.41, 1.42 and 6.53 from milking environment) and the organism failed to be detected from milk samples) and *Clostridium perfringens* (Tables, 5-7) was isolated with overall incidence percentages of (4.97, 5.67 and 6.53 from milking environment) and (0.0, 6.67 and 0.0 from milk samples) in all examined samples of the dairy farms from Fac. of Agriculture; Fac. of Vet. Medicine and Secondary School of Agriculture respectively. These results were more or less inagreement with those recorded by Johansen (1972); Fiser & Svitasvsky (1973); Rendose *et al.*

(1975); Eberhart (1977); Mowafi *et al.* (1980); Zakaria *et al.* (1980); Mostafa (1984); Saad (1993) and El-Masry (1996). Occurrence of enteric pathogens of enterobacteriaceae in food and milk may constitute a public health hazard, sever gastroenteritis, summer diarrhea in children and may lead to food poisoning outbreaks, also presence of *Escherichia coli* in milk act as a primary route for human and animal illness either through physical contact or by contamination from food chain (Ahmed *et al.*, 1988; Edward & Ewing, 1972; Fantasia *et al.*, 1975; Finegold & Martin, 1982; Hobbs & Gilbert, 1978 and Irodanov *et al.* 1970). *Escherichia coli* is considered as a commensal in the alimentary tracts of animals and man, so its presence in milking environment and milk is used as an indicator of its faecal contamination (Synage, 2000 and Zakaria *et al.*, 1980). Moreover, many strain of *Escherichia coli* have the ability to produce verotoxins which can induce variable illness and diseases in animals and man (Law, 2000). Occasionally cow's udders become infected with hemolytic streptococci of human origin, which may result in milk-borne epidemics of scarlet fever or septic sore throat, and isolation of environmental pathogens including *staphylococci, streptococci, coliforms* and *E. coli* indicate poor hygiene either during equipment cleaning and sanitation, during milking, or between milkings (Gerald & Jones, 2001). The obtained results (Tables, 1-4) showed a positive correlation between the load of microbial contamination of milk and that of its surrounding environment which represented by the high counts of total colony counts, total coliforms and *Escherichia coli* in examined milk samples and that of the inner wall surfaces; udder and teats surfaces; milk utensils; teat cups and milker's hands. In general hygienic production of milk usually requires dairy environment of cleanable atmosphere, drainage facilities as well as restricted hygiene and disinfection of all animal surroundings. The sanitary instructions should be strictly imposed together with educational programs in order to improve hygiene condition of the milking environment as well as improve the hygienic quality of milk.

Conclusion:

It is epidemiologically significant that the abundance of pathogenic and potentially pathogenic microorganisms demonstrate the unhygienic conditions existing in milking environment of the experimented dairy farms which have a direct relation to animal and public health. From the obtained results of the present investigation, it could be concluded that there was a direct relationship between the contamination load of milking environment with microflora and the

corresponding contaminants in milk that produced under these circumstances. The conditions inside the milking confinements play a great role in contamination of milk. To avoid milk contamination by environmental pathogens, some of the sanitary recommendations must be followed to overcome contamination of milk and milking environment in dairy farms as good hygiene and restricted measures including adequate ventilation, hygienic disposal of sewage, frequent and through cleaning and disinfection of dairy confinements and milk equipments as well as teats and udder before milking.

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Table (1) : Statistical analytical counts of microflora in milking environment samples
(Fac. of Agriculture Dairy Farm , Assiut University)

Sampling specimens	Count / unit	Total forming units (T.C.C.)		Total coliforms count	*E. coli* count	*Cl. perfrigens* count
		22°c	37°c			
Air /m^3	Minimum :	1.2×10^2	2.3 x 10	0.0	0.0	0.0
	Maximum :	4.8×10^4	8.0×10^3	0.0	0.0	0.0
	Mean :	$2.36 \times 10^4 \pm 0.85$	$4.38 \times 10^3 \pm 1.79$	0.0	0.0	0.0
Wall surfaces / m^2	Minimum :	1.6×10^2	6.3 x 10	1.5 x 10	0.8x 10	2.1 x 10
	Maximum :	5.7×10^4	3.2×10^4	5×10^4	4.1×10^3	2.8×10^3
	Mean :	$2.61 \times 10^4 \pm 0.58$	$2.36 \times 10^4 \pm 0.66$	$1.48 \times 10^4 \pm 1.01$	$2.45 \times 10^3 \pm 1.04$	$1.44 \times 10^3 \pm 0.49$
Udder surface / m^2	Minimum :	6.8×10^2	4.3×10^2	3.7x 10	2.8x 10	0.8x 10
	Maximum :	2.8×10^4	2×10^4	5.6×10^3	1.0×10^3	7.0×10^2
	Mean :	$1.69 \times 10^4 \pm 0.52$	$1.20 \times 10^4 \pm 0.37$	$3.33 \times 10^3 \pm 1.30$	$5.02 \times 10^2 \pm 1.89$	$3.20 \times 10^2 \pm 1.42$
Milker' hands / palm	Minimum :	7.2x 10	5.8x 10	0.8x 10	0.3x 10	0.2x 10
	Maximum :	3.1×10^3	2.2×10^3	2.5×10^3	1.4×10^2	0.8×10^2
	Mean :	$2.01 \times 10^3 \pm 0.57$	$1.61 \times 10^3 \pm 0.44$	$0.92 \times 10^3 \pm 0.50$	$7.28 \times 10 \pm 2.91$	$0.30 \times 10^2 \pm 0.17$
Milk utensils / m^2 (Equipments)	Minimum :	1.3×10^2	1.2×10^2	8.2x 10	2.1x 10	0.9x 10
	Maximum :	7.9×10^4	6.0×10^4	7.4×10^4	3.2×10^3	3.4×10^2
	Mean :	$5.26 \times 10^4 \pm 1.53$	$4.34 \times 10^4 \pm 1.23$	$4.38 \times 10^4 \pm 1.55$	$2.08 \times 10^3 \pm 0.65$	$1.50 \times 10^2 \pm 0.63$
Milk / ml	Minimum :	1.3x 10	1.1x 10	0.4x 10	0.0	0.0
	Maximum :	8.0×10^2	3.3×10^2	0.2×10^2	0.7x 10	0.0
	Mean :	$3.76 \times 10^2 \pm 1.46$	$1.74 \times 10^2 \pm 0.56$	8.2 ± 0.34	4.2 ± 0.13	0.0

Table (2) : Statistical analytical counts of microflora in milking environment samples
(Fac. of Veterinary Medicine Hospital Dairy Farm , Assiut University)

Sampling specimens	Count / unit	Total forming units (T.C.C.) 22°c	Total forming units (T.C.C.) 37°c	Total coliforms count	*E. coli* count	*Cl. perfrigens* count
Air /m^3	Minimum :	2.1×10^2	3.4×10^2	0.0	0.0	0.0
	Maximum :	6.3×10^4	1.8×10^6	2.3×10^2	1.3×10^2	0.0
	Mean :	$5.50 \times 10^4 \pm 2.17$	$7.2 \times 10^5 \pm 0.49$	$2.38 \times 10 \pm 0.83$	$1.64 \times 10 \pm 0.58$	0.0
Wall surfaces / m^2	Minimum :	1.2×10^3	6.3×10^2	1.8×10^2	2.5×10	1.2×10
	Maximum :	1.1×10^5	6.0×10^4	3.2×10^4	8.2×10^3	1.0×10^4
	Mean :	$0.84 \times 10^5 \pm 0.23$	$4.77 \times 10^4 \pm 1.32$	$2.48 \times 10^4 \pm 0.69$	$3.92 \times 10^3 \pm 1.98$	$6.0 \times 10^3 \pm 0.21$
Udder surface / m^2	Minimum :	1.4×10^2	1.3×10^2	2.1×10	1.8×10	2.1×10
	Maximum :	1.1×10^5	4.8×10^4	3.0×10^3	1.4×10^2	1.2×10^3
	Mean :	$8.24 \times 10^4 \pm 2.33$	$3.60 \times 10^4 \pm 1.01$	$2.04 \times 10^3 \pm 0.61$	$1.12 \times 10^2 \pm 0.26$	$6.04 \times 10^2 \pm 2.78$
Milker' hands / palm	Minimum :	1.3×10	1.5×10	0.8×10	0.2×10	0.8×10
	Maximum :	6.0×10^3	4.3×10^3	5.0×10^2	1.7×10	1.0×10^2
	Mean :	$4.32 \times 10^3 \pm 1.24$	$3.24 \times 10^3 \pm 0.91$	$3.22 \times 10^2 \pm 1.01$	$1.36 \times 10 \pm 0.32$	$4.96 \times 10 \pm 2.31$
Milk utensils / m^2 (Equipments)	Minimum :	2.3×10^2	1.9×10^2	2.1×10^2	0.9×10	0.0
	Maximum :	1.6×10^5	6.9×10^4	1.6×10^4	8.4×10^2	0.0
	Mean :	$8.32 \times 10^4 \pm 3.64$	$5.08 \times 10^4 \pm 1.44$	$7.64 \times 10^3 \pm 3.86$	$6.18 \times 10^2 \pm 1.74$	0.0
Milk / ml	Minimum :	1.4×10	1.2×10	0.1×10	0.0	0.0
	Maximum :	1.7×10^3	2.9×10^3	0.6×10	0.8×10	0.6×10
	Mean :	$7.99 \times 10^2 \pm 4.15$	$1.39 \times 10^3 \pm 6.97$	4.2 ± 0.10	4.0 ± 0.19	2.80 ± 1.52

Table (3) : Statistical analytical counts of microflora in milking environment samples
(Secondary School of Agriculture Dairy Farm , Assiut City)

Sampling specimens	Count / unit	Total forming units (T.C.C.)		Total coliforms count	*E. coli* count	*Cl. perfrigens* count
		22°c	37°c			
Air /m^3	Minimum :	1.8×10^2	1.2×10^2	0.0	0.0	0.0
	Maximum :	4.0×10^3	3.2×10^4	0.0	0.0	0.0
	Mean :	$2.60 \times 10^3 \pm 0.78$	$1.30 \times 10^3 \pm 0.88$	0.0	0.0	0.0
Wall surfaces / m^2	Minimum :	2.3×10^2	1.8×10^2	3.6×10	1.7×10	2.1×10
	Maximum :	1.4×10^5	1.4×10^5	6.0×10^3	4.4×10^3	3.2×10^3
	Mean :	$1.04 \times 10^5 \pm 0.29$	$1.11 \times 10^4 \pm 0.30$	$4.09 \times 10^3 \pm 1.22$	$3.04 \times 10^3 \pm 0.90$	$4.98 \times 10^2 \pm 0.61$
Udder surface / m^2	Minimum :	3.4×10^2	4.5×10^2	5.1×10^2	1.8×10	0.0
	Maximum :	1.6×10^5	1.9×10^5	4.8×10^4	3.2×10^2	1.1×10^2
	Mean :	$1.16 \times 10^5 \pm 0.33$	$1.44 \times 10^5 \pm 0.40$	$2.12 \times 10^4 \pm 1.23$	$2.36 \times 10^2 \pm 0.63$	$7.62 \times 10 \pm 1.10$
Milk cups / m^2	Minimum :	4.6×10^3	3.2×10^3	5.3×10^3	6.2×10	0.0
	Maximum :	4.2×10^5	2.4×10^5	1.7×10^5	1.2×10^3	4.4×10^2
	Mean :	$3.17 \times 10^5 \pm 0.88$	$1.45 \times 10^4 \pm 0.50$	$1.25 \times 10^4 \pm 0.34$	$9.3 \times 10^2 \pm 0.24$	$6.77 \times 10 \pm 1.20$
Milk utensils / m^2 (Equipments)	Minimum :	2.7×10^2	8.2×10^3	2.8×10^2	1.1×10^2	1.2×10
	Maximum :	1.1×10^5	1.9×10^5	6.4×10^4	2.4×10^3	1.6×10^3
	Mean :	$0.82 \times 10^5 \pm 0.23$	$1.46 \times 10^4 \pm 0.39$	$3.28 \times 10^4 \pm 1.47$	$1.46 \times 10^3 \pm 0.48$	$1.12 \times 10^2 \pm 0.33$
Milk / ml	Minimum :	4.2×10	3.2×10	1.2×10	0.0	0.0
	Maximum :	6.4×10^2	1.4×10^3	2.1×10^2	1.2×10	0.0
	Mean :	$4.56 \times 10^2 \pm 1.23$	$6.1 \times 10^2 \pm 0.36$	$9.4 \times 10 \pm 0.53$	6.40 ± 3.03	0.0

Table (4) : Statistical mean counts of microflora in dairy environment of examined 3 dairy farms in Assiut Province

Sampling specimens	Total No. of examined samples	Mean count / unit	Fac. of Agriculture milking unit	Fac. of Vet. Med. milking stalls	School of Agriculture milking parlour
Air /m^3	72	T.F.U/ unit at 37°c	$4.38 \times 10^3 \pm 1.79$	$7.2 \times 10^5 \pm 0.49$	$1.30 \times 10^3 \pm 0.88$
		T.F.U/ unit at 22°c	$2.36 \times 10^4 \pm 0.85$	$5.50 \times 10^4 \pm 2.17$	$2.60 \times 10^3 \pm 0.78$
		Total coliforms count	0.0	$2.38 \times 10 \pm 0.83$	0.0
		E. coli count	0.0	$1.64 \times 10 \pm 0.58$	0.0
		Cl. perfringens count	0.0	0.0	0.0
Wall surfaces / m^2	72	T.F.U/ unit at 37°c	$2.36 \times 10^4 \pm 0.66$	$4.77 \times 10^4 \pm 1.32$	$1.11 \times 10^4 \pm 0.30$
		T.F.U/ unit at 22°c	$2.61 \times 10^4 \pm 0.58$	$0.84 \times 10^5 \pm 0.23$	$1.04 \times 10^5 \pm 0.29$
		Total coliforms count	$1.48 \times 10^4 \pm 1.01$	$2.48 \times 10^4 \pm 0.69$	$4.09 \times 10^3 \pm 1.22$
		E. coli count	$2.45 \times 10^3 \pm 1.04$	$3.92 \times 10^3 \pm 1.98$	$3.04 \times 10^3 \pm 0.90$
		Cl. perfringens count	$1.44 \times 10^3 \pm 0.49$	$6.0 \times 10^3 \pm 0.21$	$4.98 \times 10^2 \pm 0.61$
Udder surface / m^2	72	T.F.U/ unit at 37°c	$1.20 \times 10^4 \pm 0.37$	$3.60 \times 10^4 \pm 1.01$	$1.44 \times 10^5 \pm 0.40$
		T.F.U/ unit at 22°c	$1.69 \times 10^4 \pm 0.52$	$8.24 \times 10^4 \pm 2.33$	$1.16 \times 10^5 \pm 0.33$
		Total coliforms count	$3.33 \times 10^3 \pm 1.30$	$2.04 \times 10^3 \pm 0.61$	$2.12 \times 10^4 \pm 1.23$
		E. coli count	$5.02 \times 10^2 \pm 1.89$	$1.12 \times 10^2 \pm 0.26$	$2.36 \times 10^2 \pm 0.63$
		Cl. perfringens count	$3.20 \times 10^2 \pm 1.42$	$6.04 \times 10^2 \pm 2.78$	$7.62 \times 10 \pm 1.10$
Milker' hands / palm	54	T.F.U/ unit at 37°c	$1.61 \times 10^3 \pm 0.44$	$3.24 \times 10^3 \pm 0.91$	0.0
		T.F.U/ unit at 22°c	$2.01 \times 10^3 \pm 0.57$	$4.32 \times 10^3 \pm 1.24$	0.0
		Total coliforms count	$0.92 \times 10^3 \pm 0.50$	$3.22 \times 10^2 \pm 1.01$	0.0
		E. coli count	$7.28 \times 10 \pm 2.91$	$1.36 \times 10 \pm 0.32$	0.0
		Cl. perfringens count	$3.00 \times 10 \pm 0.17$	$4.96 \times 10 \pm 2.31$	0.0

Continue of Table (4) : Statistical mean counts of microflora in dairy environment of examined 3 dairy farms in Assiut Province

Sampling specimens	Total No. of examined samples	Mean count / unit	Fac. of Agriculture milking unit	Fac. of Vet. Med. milking stalls	School of Agriculture milking parlour
Milk cups / m^2	18	T.F.U/ unit at 37°c	0.0	0.0	$1.45 \times 10^4 \pm 0.50$
		T.F.U/ unit at 22°c	0.0	0.0	$3.17 \times 10^5 \pm 0.88$
		Total coliforms count	0.0	0.0	$1.25 \times 10^4 \pm 0.34$
		E. coli count	0.0	0.0	$9.3 \times 10^2 \pm 0.24$
		Cl. perfringens count	0.0	0.0	$6.77 \times 10 \pm 1.20$
Milk utensils / m^2 (Equipments)	72	T.F.U/ unit at 37°c	$4.34 \times 10^4 \pm 1.23$	$5.08 \times 10^4 \pm 1.44$	$1.46 \times 10^4 \pm 0.39$
		T.F.U/ unit at 22°c	$5.26 \times 10^4 \pm 1.53$	$8.32 \times 10^4 \pm 3.64$	$0.82 \times 10^5 \pm 0.23$
		Total coliforms count	$4.38 \times 10^4 \pm 1.55$	$7.64 \times 10^3 \pm 3.86$	$3.28 \times 10^4 \pm 1.47$
		E. coli count	$2.08 \times 10^3 \pm 0.65$	$6.18 \times 10^2 \pm 1.74$	$1.46 \times 10^3 \pm 0.48$
		Cl. perfringens count	$1.50 \times 10^2 \pm 0.63$	0.0	$1.12 \times 10^2 \pm 0.33$
Milk / ml	72	T.F.U/ unit at 37°c	$1.74 \times 10^2 \pm 0.56$	$1.39 \times 10^3 \pm 6.97$	$6.1 \times 10^2 \pm 0.36$
		T.F.U/ unit at 22°c	$3.76 \times 10^2 \pm 1.46$	$7.99 \times 10^2 \pm 4.15$	$4.56 \times 10^2 \pm 1.23$
		Total coliforms count	8.2 ± 0.34	4.2 ± 0.10	$9.4 \times 10 \pm 0.53$
		E. coli count	4.2 ± 0.13	4.00 ± 0.19	6.40 ± 3.03
		Cl. perfringens count	0.0	2.80 ± 1.52	0.0
Total	432				

Table (5) : Incidence percentages and frequency distribution of bacterial isolates in milking environment samples (Faculty of Agriculture dairy farm , Assiut University).

Bacterial Isolates	Examined samples of milking environment																					
	Air (45 samples)			Walls (45 samples)			Udder (45 samples)			Milker's hands (45 samples)			Equipments (45 samples)			Overall percentages (225 samples)			Milk (45 samples)		
	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	Total Number	T.I %	T.F %	No. of Isolates	Inc %	Frq %
Staphylococcus aureus	17	27.4	37.8	23	12.9	51.1	7	6.19	15.6	6	9.68	13.3	12	17.4	26.7	65	13.5	28.9	4	9.76	8.89
Staphylococcus epidermidis	32	51.6	71.1	31	17.5	68.9	12	10.6	26.7	13	20.9	28.9	17	24.6	37.8	105	21.7	46.7	11	26.8	24.4
Micrococcus species	13	20.9	28.9	18	10.2	40.0	9	7.96	20.0	10	16.1	22.2	11	15.9	24.4	61	12.6	27.1	8	19.5	17.8
Streptococcus faecalis	0	0.0	0.0	14	7.91	31.1	8	7.08	17.8	2	3.22	4.44	4	5.80	8.89	28	5.80	12.4	1	2.44	2.22
Streptococcus bovis	0	0.0	0.0	5	2.82	11.1	2	1.77	4.44	0	0.00	0.00	0	0.00	0.00	7	1.45	3.11	0	0.00	0.00
Streptococcus agalactia	0	0.0	0.0	3	1.69	6.67	1	0.88	2.22	0	0.00	0.00	2	2.90	4.44	6	1.24	2.67	1	2.44	2.22
Pseudomonas aeraginosa	0	0.0	0.0	11	6.21	24.4	8	7.96	17.8	5	8.06	11.1	3	4.35	6.67	27	5.59	12.0	3	7.32	6.67
Alcaligenes faecalis	0	0.0	0.0	9	5.08	20.0	10	8.85	22.2	6	9.68	13.3	3	4.35	6.67	28	5.80	12.4	2	4.88	4.44
Escherichia coli	0	0.0	0.0	14	7.91	31.1	27	23.9	60.00	4	6.45	8.89	8	11.6	17.8	53	10.9	23.6	4	9.76	8.89
Klebsilla pneumoniae	0	0.0	0.0	2	1.13	4.44	0	0.0	0.0	1	1.61	2.22	0	0.0	0.0	3	0.62	1.33	0	0.00	0.00
Klebsilla mastitis	0	0.0	0.0	2	1.13	4.44	2	1.77	4.44	0	0.00	0.00	1	1.45	2.22	5	1.03	2.22	1	2.44	2.22
Enterobacter aerogenes	0	0.0	0.0	5	2.82	11.1	12	10.6	26.7	7	11.3	15.6	2	2.90	4.44	26	5.38	116	1	2.44	2.22
Arizona species	0	0.0	0.0	2	1.13	4.44	2	1.77	4.44	0	0.00	0.00	0	0.00	0.00	4	0.83	1.78	0	0.00	0.00
Providencia species	0	0.0	0.0	4	2.26	8.89	1	0.88	2.22	1	1.61	2.22	0	0.00	0.00	6	1.24	2.67	0	0.00	0.00
Serratia marcescens	0	0.0	0.0	5	2.82	11.1	2	1.77	4.44	1	1.61	2.22	1	1.45	2.22	9	1.86	4.00	1	2.44	2.22
Proteus species	0	0.0	0.0	11	6.21	24.4	6	5.31	13.3	3	4.84	6.67	4	5.80	8.89	24	4.97	10.7	4	9.76	8.89
Salmonella species	0	0.0	0.0	2	1.13	4.44	0	0.0	0.0	0	0.00	0.00	0	0.00	0.00	2	0.41	0.89	0	0.00	0.00
Clostridium perfringens	0	0.0	0.0	16	9.04	35.5	4	3.54	8.89	3	4.84	6.67	1	1.45	2.22	24	4.97	1.07	0	0.00	0.00
Total	62			177			113			62			69			483			41		

Table (6) : Incidence percentages and frequency distribution of bacterial isolates in milking environment samples (Faculty of Vet. Medicine Hospital dairy farm , Assiut University).

Bacterial Isolates	Examined samples of milking environment																					
	Air (9 samples)			Walls (9samples)			Udder (9 samples)			Milker's hands (9 samples)			Equipments (9 samples)			Overall percentages (45 samples)			Milk (9 samples)		
	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	Total Number	T.I %	T.F %	No. of Isolates	Inc %	Frq %
Staphylococcus aureus	4	21.1	44.4	5	11.1	55.6	2	5.26	22.2	1	7.14	11.1	2	8.00	22.2	14	9.93	31.1	1	6.67	11.1
Staphylococcus epidermidis	6	31.6	66.7	7	15.6	77.8	3	7.89	33.3	2	14.3	22.2	4	16.0	44.4	22	15.6	48.9	3	20.0	33.3
Micrococcus species	5	26.3	55.6	4	8.89	44.4	3	7.89	33.3	2	14.3	22.2	3	12.0	33.3	17	12.1	37.8	2	13.3	22.2
Streptococcus faecalis	0	0.00	0.00	3	6.67	33.3	4	10.5	44.4	0	0.0	0.0	1	4.00	11.1	8	5.67	17.8	0	0.0	0.0
Streptococcus bovis	0	0.00	0.00	0	0.00	0.00	1	2.63	11.1	0	0.0	0.0	1	4.00	11.1	2	1.42	4.44	0	0.0	0.0
Streptococcus agalactia	0	0.00	0.00	0	0.00	0.00	1	2.63	11.1	0	0.0	0.0	1	4.00	11.1	2	1.42	4.44	0	0.0	0.0
Pseudomonas aeruginosa	0	0.00	0.00	1	2.22	11.1	1	2.63	11.1	2	14.3	22.2	1	4.00	11.1	5	3.55	11.1	0	0.0	0.0
Alcaligenes faecalis	0	0.00	0.00	3	6.67	33.3	4	10.5	44.4	2	14.3	22.2	2	8.00	22.2	11	7.80	24.4	2	13.3	22.2
Eschirachia coli	1	5.26	11.1	4	8.89	44.4	5	13.2	55.6	2	14.3	22.2	1	4.00	11.1	13	9.22	28.9	1	6.67	11.1
Klebsilla pneumoniae	0	0.00	0.00	1	2.22	11.1	1	2.63	11.1	0	0.0	0.0	0	0.0	0.0	2	1.42	4.44	0	0.0	0.0
Klebsilla mastitis	0	0.00	0.00	1	2.22	11.1	1	2.63	11.1	0	0.0	0.0	0	0.0	0.0	2	1.42	4.44	0	0.0	0.0
Enterobacter aerogenes	0	0.00	0.00	1	2.22	11.1	2	5.26	22.2	0	0.0	0.0	1	4.00	11.1	4	2.84	8.89	1	6.67	11.1
Arizona species	0	0.00	0.00	1	2.22	11.1	1	2.63	11.1	0	0.0	0.0	0	0.0	0.0	2	1.42	4.44	0	0.0	0.0
Providencia species	0	0.00	0.00	2	4.44	22.2	1	2.63	11.1	0	0.0	0.0	1	4.00	11.1	4	2.84	8.89	1	6.67	11.1
Serratia marcescens	0	0.00	0.00	2	4.44	22.2	1	2.63	11.1	1	7.14	11.1	2	8.00	22.2	6	4.25	13.3	1	6.67	11.1
Proteus species	3	15.8	33.3	6	13.3	66.7	4	10.5	44.4	1	7.14	11.1	5	20.0	55.6	19	13.5	42.2	2	13.3	22.2
Salmonella species	0	0.00	0.00	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
Clostridium perfringens	0	0.00	0.00	4	8.89	44.4	3	7.89	33.3	1	7.14	11.1	0	0.0	0.0	8	5.67	17.8	1	6.67	11.1
Total	19			45			38			14			25			141			15		

Table (7) : Incidence percentages and frequency distribution of bacterial isolates in milking environment samples (Secondary school of Agriculture dairy farm , Assiut City).

Bacterial Isolates	Examined samples of milking environment																							
	Air (18 samples)			Walls (18 samples)			Udder (18 samples)			Teat cups (18 samples)			Equipments (18 samples)			Overall percentages (90 samples)			Milk (18 samples)					
	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	No. of Isolates	Inc %	Frq %	Total Number	T.I %	T.F %	No. of Isolates	Inc %	Frq %			
Staphylococcus aureus	3	25.0	16.67	8	13.1	44.4	3	6.00	16.7	3	7.32	167	2	5.71	11.1	19	9.55	21.1	1	3.70	5.55			
Staphylococcus epidermida	5	41.7	27.8	11	18.0	61.1	7	14.0	38.9	5	12.2	27.3	4	11.4	22.2	32	16.1	35.6	4	14.8	22.20			
Micrococcus species	4	33.3	22.2	7	11.5	38.9	8	16.0	44.4	3	7.32	167	4	11.4	22.2	26	13.1	28.9	5	18.5	27.3			
Streptococcus faecalis	0	0.0	0.0	4	6.56	22.2	5	10.0	27.3	1	2.44	5.55	1	2.86	5.55	11	5.53	12.22	0	0.0	0.0			
Streptococcus bovis	0	0.0	0.0	2	3.28	11.1	2	4.00	11.11	2	4.88	11.1	1	2.86	5.55	7	3.52	7.78	0	0.0	0.0			
Streptococcus agalactia	0	0.0	0.0	0	0.0	0.0	3	6.00	16.7	2	4.88	11.1	2	5.71	11.1	7	3.52	7.78	2	7.41	11.1			
Pseudomonas aeruginosa	0	0.0	0.0	2	3.28	11.1	1	2.00	5.55	2	4.88	11.1	3	8.57	16.7	8	4.02	8.89	1	3.70	5.55			
Alcaligenes faecalis	0	0.0	0.0	3	4.92	16.7	3	6.00	16.7	3	7.32	167	3	8.57	16.7	12	6.03	13.33	2	7.41	11.1			
Escherichia coli	0	0.0	0.0	4	6.56	22.2	4	8.00	22.22	3	7.32	167	2	5.71	11.1	13	6.53	14.40	2	7.41	11.1			
Klebsilla pneumoniae	0	0.0	0.0	1	1.64	5.55	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	1	0.50	1.11	0	0.0	0.0			
Klebsilla mastitis	0	0.0	0.0	1	1.64	5.55	1	2.00	5.55	1	2.44	5.55	1	2.86	5.55	4	2.01	4.44	1	3.70	5.55			
Enterobacter aerogenes	0	0.0	0.0	1	1.64	5.55	2	4.00	11.11	2	4.88	11.1	3	8.57	16.7	8	4.02	8.89	1	3.70	5.55			
Arizona species	0	0.0	0.0	2	3.28	11.1	1	2.00	5.55	1	2.44	5.55	0	0.0	0.0	4	2.01	4.44	1	3.70	5.55			
Providencia species	0	0.0	0.0	2	3.28	11.1	2	4.00	11.11	1	2.44	5.55	1	2.86	5.55	6	3.01	6.67	2	7.41	11.1			
Serratia marcescens	0	0.0	0.0	3	4.92	16.7	2	4.00	11.11	1	2.44	5.55	1	2.86	5.55	7	3.52	7.78	2	7.41	11.1			
Proteus species	0	0.0	0.0	6	9.84	33.3	2	4.00	11.11	8	19.5	44.4	5	14.3	27.3	21	10.6	23.3	3	11.1	16.7			
Salmonella species	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0			
Clostridium perfringens	0	0.0	0.0	4	6.56	22.2	4	8.00	22.22	3	7.32	167	2	5.71	11.1	13	6.53	14.40	0	0.0	0.0			
Total	12			61			50			41			35			199			27					