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# OCCURRENCE OF NOCARDIA SPECIES IN RAW MILK

(With 5 Tables and 3 Figures)

By

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## تواجد أجناس النوكارديا فى الألبان الخام

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تلعب ميكروبات النوكارديا دوراً خطيراً فى فساد الألبان ومنتجاتها. حيث إنها تسبب نزول اللبن مدمم ومتجلط من الضرع الملتهب بسبب النوكارديا . و أيضا تسبب إنخفاض إنتاج اللبن بنسب واضحه مما يؤدى إلى خساره إقتصاديه كبيره. علاوة على الأثر الكبير على الصحة العامة، لما قد تسببه من إلتهابات بالجهاز الهضمى كله والأمراض الرئوية والإلتهابات الجلديه الشديده وأمراض المخ والجهاز العصبى هذا غير ما قد تسببه من أعراض أخرى مختلفه تؤثر على الإنسان وقد تودى إلى الوفاه وخاصة إن لم يتم معالجتها سريعا. ومن هنا تم عمل هذه الدراسه لمعرفة مدى تواجد هذا الميكروب فى الأنواع المختلفه من الألبان وقد إشتملت هذه الدراسه على التالى : جمع 240 عينه عشوائيه من اللبن الخام والتى جمعت من أماكن مختلفه فى مدينة أسيوط من لبن بقرى وجاموسى ونعاج وماعز بواقع 90عينة لكل من اللبن البقرى والجاموسى مقسمه إلى 30 عينه لكل من لبن المزارع والمحلات والباعه الجائلين و 30 عينه لكل من لبن النعاج والماعز. ولقد تبين بالفحص البكتريولوجى أن 43 (47, 8%) ،39 (43, 3 %) ، 16 ( 53, 3) ، 20 ( 66, 7 %) من العينات المفحوصة كانت ايجابية للميكروب بأستخدام مستنبت النوكارديا بينما كانت النتائج 37 (41, 1 %) ، 39 (43, 3) ، 15 ( 50%) ، 15 ( 50%) من عينات نفس اللبن السابق ذكرها كانت ملوثه بهذه الميكروبات على التوالى بأستخدام مستنبت البوشنل هاس . وقد بلغ متوسط عدد النوكارديا بأستخدام مستنبت النوكارديا $3,1 \times 10^4$ ، $2,6 \times 10^4$ ، $7,4 \times 10^3$ ميكروب/مللى من عينات اللبن البقرى المجمعة من المزارع والمحلات والباعه الجائلين على التوالى ومن عينات اللبن الجاموسى $10 \times 10^3$ ، $1,2 \times 10^4$ ، $5,9 \times 10^3$ المجمعه أيضا من مزارع الألبان ومحلات الألبان والباعه الجائلين وأما عينات لبن النعاج والماعز فقد كان متوسط عدد هذا الميكروب $1,4 \times 10^4$ ، $7,6 \times 10^3$ ميكروب/مللى على الترتيب. وبالنسبه لمستنبت البوشنل هاس كان متوسط العد الكلى لهذا الميكروب كالآتى: $2 \times 10^4$ ، $3,3 \times 10^4$ ، $5,1 \times 10^4$ من عينات اللبن البقرى المجمعه من المزارع والمحلات والباعة الجائلين على التوالى وأيضا $2,4 \times 10^4$ ، $2,5 \times 10^4$ ، $5,9 \times 10^3$ من عينات اللبن الجاموسى

المجمعه أيضا من مزارع الألبان ومحلات الألبان والباعة الجائلين $7 \times 10^3$ ، $9,1 \times 10^3$ ميكروب/مللى من عينات لبن النعاج والماعز على الترتيب. هذا وقد تم تصنيف ميكروبات النوكارديا المعزوله من جميع العينات المفحوصه ووجد ان اعلى نسبة كانت للنوكارديا أستيرويدز تليها النوكارديا برازيلينسس. هذا وقد تم مناقشة النتائج مع ذكر الشروط الصحية الواجب إتباعها لمنع تلوث الأغذيه وخاصة الألبان بميكروب النوكارديا وذلك لحماية صحة المستهلك وحفاظا على المنتج من الفساد.

## SUMMARY

Two hundred and forty random samples of raw milk including cow's (90), buffalo's (90), sheep's (30) and goat's milk (30) were collected from dairy farms, dairy shops and street vendors in Assiut city. These samples were examined for the prevalence of *Nocardia* spp. using two selective media: *Nocardia* and Bushnell-Hass media. The recorded data revealed that 43 (47.8%), 39 (43.3%), 16 (53.3%) and 20 (66.7%) of the examined cow's, buffalo's, sheep's and goat's milk samples, respectively were contaminated with *Nocardia* spp. on *Nocardia* medium. However, the incidence of *Nocardia* spp. on Bushnell-Hass medium was 37 (41.1%), 39 (43.3%), 15 (50%), and 15 (50%) in the same samples, respectively. The highest *Nocardia* positive samples were from goat's and sheep's milk. Different counts of *Nocardia* spp. from milk samples on both media were recorded. N.asteroides was the predominant species, it could be isolated in percentages of 25.4 and 30.2% on *Nocardia* and Bushnell-Hass medium respectively, other *nocardia* species were isolated in different percentages. The public health significance of the organisms and the precautions which should be taken to control this organism in dairy industry as well as the recommended sanitary measures, were also discussed.

***Key words:*** *Nocardia spp., cow, buffalo, sheep, goat, raw milk*

## INTRODUCTION

*Nocardia* species continue to be highly dangerous human pathogens. There is an increase interest of *Nocardia* species particularly N.asteroides as health risk affecting both human and animal. *Nocardiae* are found extensively world wide (Brown and McNeil, 2003). They are aerobic, Gram positive, non-motile, non-spore forming rods that usually show branching and aerial hyphae and are usually partially acid-fast and

there is considerable confusion in the taxonomy of *Nocardia* (Carter and Darla, 2004).

About sixteen species of Nocardiae have been implicated in human infection (Saubolle and Sussland, 2003).The most important pathogenic species are "*Nocardia* asteroides complex" which infect domestic animals and human (Carter and Darla, 2004). The term "N.asteroides complex" is used to include N.asteroides, N.caviae, N.brasiliensis and N.otitidiscaviarum (Saubolle, 2002 and Brown and McNeil, 2003).

Nocardiosis is a bacterial disease of man and animal caused by *Nocardia* species. Concomitant pulmonary and cerebral nocardiosis have been reported in many cases and there is evidence of hematogenous spread (Emmons *et al.,* 1977 and Patrick *et al.,* 1998). In human, it may be manifested as bacteremia, empyema, brain abscess, pericarditis, synovitis, soft tissue infection, peritonitis and corneal ulcers; Nocardiosis is characterized by an acute inflammatory response terminating in necrosis and abscess formation (Burgert, 1999; Saubolle, 2002 and Brown and McNeil, 2003).

The fatality rate in human from Nocardiosis is high (Carter and Darla, 2004).The medical importance of Nocardiae may cause significant morbidity and mortality, particularly in immunocompromised patients, including transplant recipients, patients infected with human immunodeficiency virus and in those receiving long-term corticosteroid therapies (McNeil and Brown, 1994).

Economically, the most important host is the dairy cow. Mastitis caused by N.asteroides was reported in Hawaii (USA) in a herd from which 100 cows had to be culled and an additional 80 cows died of the infection. The problem arises there not only belongs to the economic coasts to control nocardial mastitis, but mainly due to the potential risk to human health from contamination of milk and the cross reaction to tuberculin test in case of pulmonary nocardiosis (Willett *et al.,* 1982; Costa *et al.,* 1987 and Schoonderwoerd *et al.,* 1990). Moreover Nocardial species were considered as environmental thrive organisms. Pathogenic Nocardiae are saprophytes found in many climates in soils and water, either as indigenous flora or as contaminants. The main routes of infection are inhalation, trauma and ingestion (Dwight *et al.* 2004).

As, the ability of *Nocardia* species to grow and multiply in raw milk as well as organisms are potential causative agents of food borne diseases, it is essential to estimate the prevalence of *Nocardia* species in

different types of milk including cow's, buffalo's, sheep's and goat's milk.

## MATERIALS and METHODS

**1-Collection of samples:**

A total of 240 random samples of milk including cow's and buffalo's milk were collected from dairy farms, dairy shops and street vendors (30 samples of each). Sheep's milk (30) and goat's milk (30) were collected from different localities in Assiut city. Each milk sample was mixed and tested for heat treatment using Storch test (Lampert, 1975).

**2-Preparation of samples:-**

Ten fold serial dilutions were prepared according to A.P.H.A (1992).

**3- Isolation and enumeration of *Nocardia* spp.:-**

Enrichment of *Nocardia* spp. was adopted using Bushnell-Haas broth and incubated at 37ºC for 2-7 days (Schaal, 1972). Isolation and enumeration was done using surface spreading technique on *Nocardia* and Bushnell-Haas agar incubated at 37ºC for 2-7 days but cultures should be examined every 2 days (Waksman, 1967 and Gordon *et al.,* 1974). Suspected colonies should be optimized by seeing filamentous, rosette, white to yellow to orange colonies, may be with aerial and delicate mycelia (Schaal, 1984; Saubolle, 2002 and Brown and McNeil, 2003).

**4- Identification of isolates:-**

Initial visualization of phenotypic colony coloration and morphology, together with the presence of aerial hyphae, rosette shape often provides initial clues to the genus of the isolates (Carter, 1984). Specific tests include Gram stain, modified Ziehl-Neelsen stain, catalase test, hemolysis on blood agar and other biochemical reactions.

Differentiation between the suspected colonies was done according to Maldonado *et al.,* 2000 and Hamid *et al.,* 2001.Confirmation of the different three *Nocardia* spp. (N.asteroides, N.brasiliensis and N.caviae) was done according to Larone, 1995 and Brown *et al.,* 1999.

# RESULTS

**Table 1:** Incidence of *Nocardia* spp. in the examined milk samples.

| Examined samples | No. | *Positive samples on media used* | | | |
|---|---|---|---|---|---|
| | | *Nocardia* medium | | Bushnell-Hass medium | |
| | | No. | % | No. | % |
| Cow's milk | 90 | 43 | 47.8 | 37 | 41.1 |
| Buffalo's milk | 90 | 39 | 43.3 | 39 | 43.3 |
| Sheep's milk | 30 | 16 | 53.3 | 15 | 50 |
| Goat's milk | 30 | 20 | 66.7 | 15 | 50 |

**Table 2:** Statistical analytical results of *Nocardia* spp. in the examined milk samples on *Nocardia* medium.

| Samples | No. | Positive samples | | Count/ml. | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| 1. Cow's milk Dairy farms | 30 | 19 | 63.3 | $2x10^3$ | $7x10^4$ | $1.3x10^4$ |
| Dairy shops | 30 | 10 | 33.3 | $2x10^3$ | $7x10^4$ | $2.6x10^4$ |
| Street vendors | 30 | 14 | 46.7 | $1x10^3$ | $3x10^4$ | $7.4x10^3$ |
| 2. Buffalo's milk Dairy farms | 30 | 18 | 60 | 9x10 | $3.2x10^4$ | $10x10^3$ |
| Dairy shops | 30 | 11 | 36.7 | $1x10^3$ | $3x10^4$ | $1.2x10^4$ |
| Street vendors | 30 | 10 | 33.3 | $1x10^2$ | $2.7x10^4$ | $5.9x10^3$ |
| 3. Sheep's milk | 30 | 16 | 53.3 | $7x10^2$ | $7.9x10^4$ | $1.4x10^4$ |
| 4. Goat's Milk | 30 | 20 | 66.7 | 5x10 | $2.4x10^4$ | $7.6x10^3$ |

**Table 3:** Statistical analytical results of *Nocardia spp.* in the examined milk samples on Bushnell-Hass medium.

| Samples | No. | Positive samples | | Count/ml. | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Average |
| 1. Cow's milk<br>Dairy farms | 30 | 17 | 56.7 | $2x10^3$ | $8.6x10^4$ | $2x10^4$ |
| Dairy shops | 30 | 9 | 30 | $14x10^3$ | $8.3x10^4$ | $3.3x10^4$ |
| Street vendors | 30 | 11 | 36.7 | $4x10^2$ | $4.9x10^4$ | $1.5x10^4$ |
| 2. Buffalo's milk<br>Dairy farms | 30 | 18 | 60 | $3x10^2$ | $9.2x10^4$ | $2.4x10^4$ |
| Dairy shops | 30 | 11 | 36.7 | $1.3x10^3$ | $6x10^4$ | $2.5x10^4$ |
| Street vendors | 30 | 10 | 33.3 | $2x10^2$ | $4x10^4$ | $9.5x10^3$ |
| 3. Sheep's milk | 30 | 15 | 50 | 1x10 | $5.8x10^4$ | $7x10^3$ |
| 4. Goat's Milk | 30 | 15 | 50 | 1x10 | $6x10^3$ | $1.9x10^3$ |

**Table 4:** Incidence of the isolated *Nocardia* spp. recovered from milk samples using *Nocardia* medium.

| *Nocardia Spp.* | cow's milk samples | | | | | | buffalo's milk samples | | | | | | Sheep's milk | | Goat's milk | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dairy farms | | Dairy shops | | Street vendors | | Dairy farms | | Dairy shops | | Street vendors | | | | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No | % | No. | % |
| N.asteroides | 7 | 23.3 | 3 | 10 | 6 | 20 | 6 | 20 | 2 | 6.7 | 3 | 10 | 2 | 6.7 | 1 | 3.3 |
| N.farcinica | 4 | 13.3 | 1 | 3.3 | 0 | 0 | 2 | 6.7 | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 4 | 13.3 |
| N.brasiliensis | 1 | 3.3 | 2 | 6.7 | 3 | 10 | 4 | 13.3 | 0 | 0 | 4 | 13.3 | 4 | 13.3 | 7 | 23.3 |
| N.carnea | 0 | 0 | 1 | 3.3 | 0 | 0 | 1 | 3.3 | 0 | 0 | 1 | 3.3 | 2 | 6.7 | 0 | 0 |
| N.brevicatena | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 0 | 0 | 0 | 0 | 1 | 3.3 | 2 | 6.7 | 2 | 6.7 |
| N.otitidi scavarium | 1 | 3.3 | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 3 | 10 | 0 | 0 | 1 | 3.3 | 2 | 6.7 |
| N.transvalensis | 4 | 13.3 | 0 | 0 | 0 | 0 | 3 | 10 | 0 | 0 | 0 | 0 | 2 | 6.7 | 4 | 13.3 |
| N.amarae | 2 | 6.7 | 2 | 6.7 | 3 | 10 | 1 | 3.3 | 5 | 16.7 | 0 | 0 | 2 | 6.7 | 0 | 0 |
| N.vaccinii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 19 | 63.3 | 10 | 33.3 | 14 | 46.6 | 18 | 60 | 11 | 36.7 | 10 | 33.3 | 16 | 53.3 | 20 | 66.7 |

**Table 5:** Incidence of the isolated *Nocardia* spp. recovered from milk samples using Bushnell-Hass medium

| *Nocardia Spp.* | cow's milk samples | | | | | | buffalo's milk samples | | | | | | Sheep's milk | | Goat's milk | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dairy farms | | Dairy shops | | Street vendors | | Dairy farms | | Dairy shops | | Street vendors | | | | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No | % | No. | % |
| N.asteroides | 4 | 13.3 | 3 | 10 | 6 | 20 | 6 | 20 | 2 | 6.7 | 4 | 13.3 | 3 | 10 | 4 | 13.3 |
| N.farcinica | 4 | 13.3 | 1 | 3.3 | 0 | 0 | 2 | 6.7 | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 4 | 13.3 |
| N.brasiliensis | 2 | 6.7 | 1 | 3.3 | 1 | 3.3 | 4 | 13.3 | 2 | 6.7 | 3 | 10 | 4 | 13.3 | 2 | 6.7 |
| N.carnea | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 1 | 3.3 | 1 | 3.3 | 1 | 3.3 | 3 | 10 | 0 | 0 |
| N.brevicatena | 0 | 0 | 0 | 0 | 1 | 3.3 | 0 | 0 | 0 | 0 | 1 | 3.3 | 1 | 3.3 | 2 | 6.7 |
| N.otitidi scavarium | 5 | 16.7 | 1 | 3.3 | 0 | 0 | 1 | 3.3 | 2 | 6.7 | 0 | 0 | 0 | 0 | 1 | 3.3 |
| N.transvalensis | 2 | 6.7 | 2 | 6.7 | 2 | 6.7 | 3 | 10 | 0 | 0 | 0 | 0 | 1 | 3.3 | 2 | 6.7 |
| N.amarae | 4 | 13.3 | 3 | 10 | 6 | 20 | 1 | 3.3 | 4 | 13.3 | 0 | 0 | 2 | 6.7 | 0 | 0 |
| N.vaccinii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 17 | 56.7 | 9 | 30 | 11 | 36.7 | 18 | 60 | 11 | 36.7 | 10 | 33.3 | 15 | 50 | 15 | 50 |

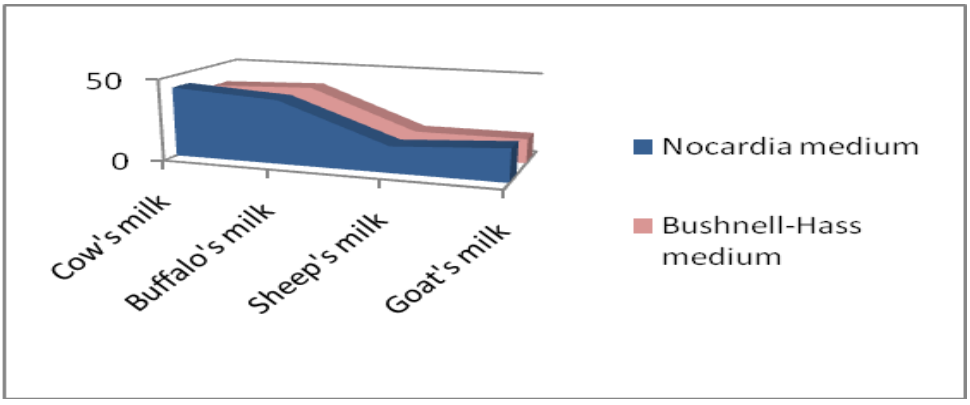


**Fig. 1:** Differentiation between media used for isolation of *Nocardia* spp.

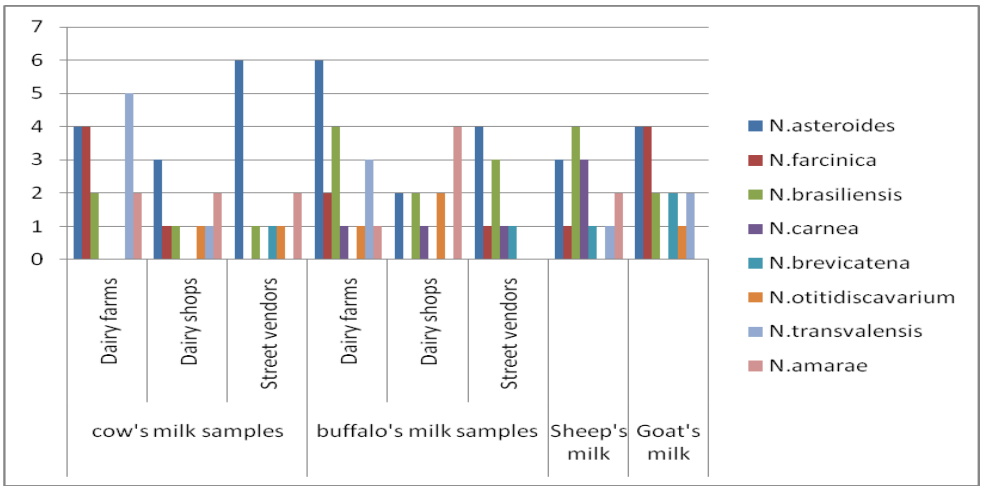


**Fig. 2:** Incidence of the isolated *Nocardia* spp. recovered from milk samples using *Nocardia* medium.

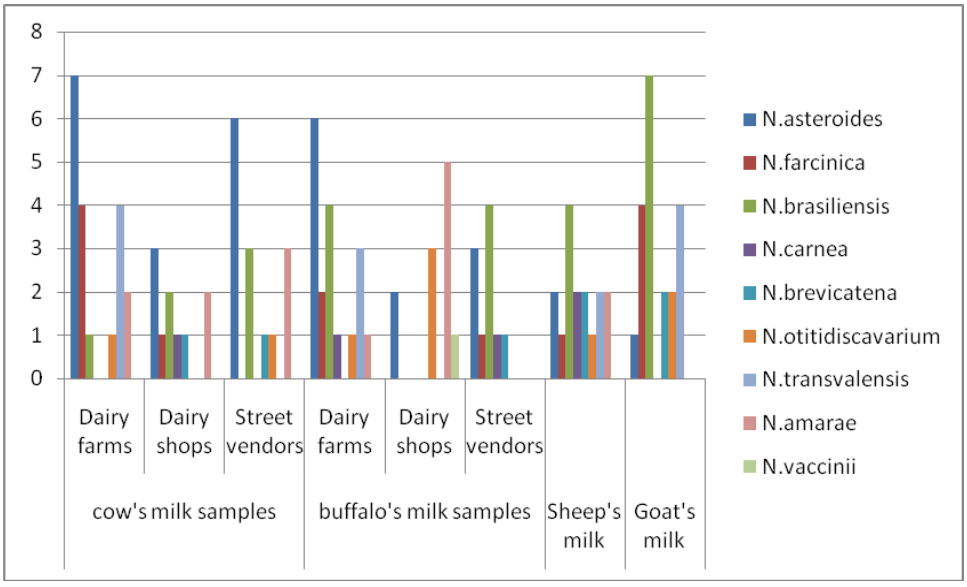


**Fig. 3:** Incidence of the isolated *Nocardia* spp. recovered from milk samples using Bushnell-Hass medium.

# DISCUSSION

Different types of milk are unique and ideal foods for human. However, they are highly nutritious media in which microorganisms will thrive and lead to deterioration and spoilage. *Nocardia* spp. is one of the world wide bacterial infections and has been implicated in the pathogenesis of pulmonary nocardiosis, enteritis, arthritis, multiple skin abscesses, central nervous system nocardiosis and endocarditis in all infected humans, including children, immunosuppressed and rare in non-immunocompromised patients (Maria Bernadete *et al.*, 2007).

Nocardia spp. was isolated from 47.8, 43.3, 53.3 and 66.7% of cow's, buffalo's, sheep's and goat's milk samples on *Nocardia* medium and 41.1, 43.3, 50 and 50 % of the same samples on Bushnell-Hass medium, respectively (Table 1).

Presence of *Nocardia* spp. in milk is not surprising in view of fact that it is widely spread in nature and contaminates the milk during milking, handling, storage and transportation. Moreover, dust, soil and plant materials serve as sources of contamination; the problem was complicated by the absence of the cool system that may enhance the multiplication of most pathogenic microorganisms.

Nocardia medium is preferable than Bushnell-Hass medium for isolation of *Nocardia* spp. Also N.vaccinii can be isolated on Nocardia medium and not on Bushnell-Hass medium (Fig. 1 and 2). So, for successful detection of different species of *Nocardia*, using of *Nocardia* medium is necessary.

Out of the 30 examined raw dairy farms milk samples, 19 (63.3%) and 17 (56.7%) were found to be contaminated with *Nocardia* spp. on *Nocardia* and Bushnell-Hass media, respectively. The average count/ml. was $1.3x10^4$ on *Nocardia* medium while on Bushnell-Hass medium the average value was $2x10^4$ CFU/ml. (Tables 2 and 3). Nearly similar findings were reported by Vaissaire *et al.* (1984) while, lower values were recorded by Seddek (2001) and Friedman *et al.* (2004). *Nocardia* spp. is an uncommon cause of mastitis, but the outbreaks have typically been reported in dairy farms due to poor hygienic and management conditions (Pisoni *et al.* 2008).

*Nocardia* spp. were isolated from samples collected from dairy shops and street vendors in lower percentages but nearly similar counts (33.3and 30% for dairy shops and 46.7 and 36.7% for street vendors samples) on *Nocardia* and Bushnell-Hass media respectively, (Tables 2 and 3). That may be due to supply using illegal preservatives

which inhibit the growth of most organisms including *Nocardia* spp. present in milk and prolong the shelf life of raw milk in dairy shops.

*Nocardia* spp. were isolated from teat tips in fore milk stripping (Schinger, 1994). Furthermore, the *Nocardia* introduced into the udder by insertion of cannula, contaminated preparations, drug mixture and tips of injectors (Battig *et al.* 1990 and Seddek 2001) this recorded information may support the high incidence of *Nocardia* spp. in this investigation. The environmental factors facilities for all activities of *Nocardia* spp. involving the multiplication of this organism such as hot and wet weathers, bad hygienic measure as contamination of soil from the infected human patient which plays an important role in wide spreading of the pathogens (Pisoni *et al.*, 2008).

Nearly the same result was obtained from buffalo milk samples, The highest incidence (60%) of *Nocardia* spp. was recorded from the examined dairy farm samples followed by dairy shops (36.7%) and street vendors ( 33.3%) on both media (Tables 2 and 3).

16 (53.3%) and 15(50%) of the examined sheep milk samples proved to harbor *Nocardia* spp. on both *Nocardia* and Bushnell-Hass media, respectively. The minimum and maximum counts on *Nocardia* medium were $7x10^2$ and $7.9x10^4$, respectively with an average value of $1.4x10^4$ CFU/ml. On Bushnell-Hass medium the contamination level ranged from 1x10 to $5.8x10^4$ and the average was $7x10^3$ CFU/ml. (Tables 2 and 3). *Nocardia* spp. cause several lesions in ovines as ovine abortion, mastitis and milk infections (Watson, 1977 and John *et al.*, 1980).

Regarding goat's milk, 20 (66.7%) and 15 (50%) of samples were found to be contaminated with *Nocardia* spp. The average count was $7.6x10^3$ and $1.9x10^3$ CFU/ml. on both *Nocardia* and Bushnell-Hass media, respectively. The high incidence of Nocardia spp. in goat's milk was clarified that the major intramammary pathogens in goats are associated with poor hygienic conditions in housing and in the milking parlous (Contreras *et al.* 2002). This present study showed that the highest *Nocardia* positive samples were from goat's and sheep's milk. This emphasized the role of primitive way of producing, bad handling and unhygienic measure of these particular types of milk as an important source of contamination with *Nocardia* spp. Furthermore, Nocradiae are ubiquitous in the environment and can be found in fresh- and salt water, soil, dust, decaying vegetation and decaying fecal deposits from animals (Diskensoy *et al.* 2004).

The highest frequency distributed species in all milk samples in both media was N.asteroides followed by N.brasiliensis which considered the most common species prevalent in more temperate climates (Tables 4 and 5, Fig.2 and 3). The same results were demonstrated by Patrick *et al.* (1998), Petersen *et al.* (2007) and Yin *et al.* (2007) who found that the most common species of *Nocardia* associated with human disease are N.asteroids, N.brasiliensis, N.otitidiscaviarum, and N.farcinica. Also, N.asteroids was the causative agent of an outbreak of mastitis in different animals and in different countries (Al-Bassam *et al.* 1989 and Seddek 2001).

N.asteroides is the species of *Nocardia* that is most commonly associated with human disease, which is primarily opportunistic; occurring in immune-compromised patients (Ryan and Ray, 2004). N.brasiliensis is one of the causes of nocardiosis in humans (Carter and Darla, 2004). Although it is associated with tropical environments, it is the second most common isolate after N.asteroides in the United States and has a higher prevalence in the south western and south eastern regions (McNeil and Brown, 1994; Saubolle, 2002 and Brown and McNeil, 2003,). While in Japan, about 303 cases of nocardiosis between 1992 and 2001 were reported, about 72% of the strains belonged to the" N.asteroides complex", including 81 strains of N.farcinica , which was the most frequent isolate, 66 strains were N.brasiliensis (Kageyama *et al.*, 2004).

Finally, the presence of any *Nocardia* spp. should be a warning to dairy producers that conditions exist for the growth of pathogenic species of *Nocardia* and the corrective measures needed to ensure pathogenic-free products. So, great attention must be paid to the problems of these pathogens in our foods. Consequently, more restriction and preventive measures should be taken to improve the quality of raw milk to protect consumers from being infected by this and other organisms.

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