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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) samples 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 average counts of *Nocardia* spp. were 1.3×10^4 , 2.6×10^4 and 7.4×10^3 from cow's milk (dairy farms, dairy shops and street vendors, respectively), 10×10^3 , 1.2×10^4 and 5.9×10^3 from buffalo's milk (dairy farms, dairy shops and street vendors, respectively), 1.4×10^4 and 7.6×10^3 CFU/ml. from sheep's and goat's milk, respectively on Nocardia medium.

While on Bushnell-Hass medium, the average counts were 2×10^4 , 3.3×10^4 and 1.5×10^4 from cow's milk (dairy farms, dairy shops and street vendors, respectively), 2.4×10^4 , 2.5×10^4 and 5.9×10^3 from buffalo's milk (dairy farms, dairy shops and street vendors, respectively), 7×10^3 and 1.9×10^3 CFU/ml. from sheep's and goat's milk, respectively.

Of 118 *Nocardia* isolates obtained from the examined milk samples on Nocardia medium, 30 were *N.asteroides*, 13 *N.farcinica*, 25 *N.brasiliensis*, 5 *N.carnea*, 7 *N.brevicatena*, 9 *N.otitidiscavarium*, 13 *N.transvalensis*, 15 *N.amarae* and 1 *N.vaccinii*.

On other hand, of 106 *Nocardia* isolates from the examined cow's, buffalo's, sheep's and goat's milk on Bushnell-Hass medium, 32 were *N.asteroides*, 13 *N.farcinica*, 19 *N.brasiliensis*, 6 *N.carnea*, 6 *N.brevicatena*, 5 *N.otitidiscavarium*, 12 *N.transvalensis*, 13 *N.amarae*, while, *N.vaccinii* couldn't be detected.

By comparing the efficiency of the two media used for the enumeration and isolation of *Nocardia* spp., it was found that, there is no significant difference between the results obtained using Nocardia and Bushnell-Hass media.

Response of rat's inoculation to various isolates of *Nocardia* species injected intraperitoneally proved that 7 out of 9 species of *Nocardia* were virulent depending on the lesions which occurred on rat model, the lesions were severe inflammation in all organs specially in GIT, brain, testis and in sub-cutaneous arteries with enlargement of testis and sudden death in some rats. These lesions occurred by *N.asteroides*, *N.brasiliensis*, *N.brevicatena*, *N.otitidiscavarium*, *N.transvalensis*, *N.amarae* and *N.vaccinii*. While only 2 species of *Nocardiae* (*N.farcinica* and *N.carnea*) were non-pathogenic.

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 discusse

Conclusion and Recommendation

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. 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 in farm and transportation. Moreover, absence of the cool system may enhance the multiplication of most pathogenic microorganisms.

The results of this investigation emphasized that *Nocardia* spp. including *N.asteroides*, *N.farcinica*, *N.brasiliensis*, *N.carnea*, *N.brevicatena*, *N.otitidiscavarium*, *N.transvalensis*, *N.amarae* and *N.vaccinii* were detected in cow's, buffalo's, sheep's, and goat's milk in different percentages. This reflects the lack of hygienic supervision, poorly cleaned and sanitized dairy farm equipments which constitute the major sources of contamination of different types of milk.

From the aforementioned data it is evident that *Nocardia* spp. could be isolated from sheep's and goat's milk with higher incidence than which isolated from cow's and buffalo's milk and that clarify the bad environmental condition surrounding sheep's and goat's milk production and that is important to recognize their presence which may result in potential health hazards.

Also, from the results of the present study, there are differences in the distribution of species and the proportion of strains within a given species that may possess virulence factors. So that raw milk under the influence of it's nutritive value provides and constitutes from the epidemiological point of view a dangerous source of infection to human beings.

The results obtained suggested that the use of *Nocardia* medium was enough for isolation of different species of *Nocardia* but also, using Bushnell-Hass medium was selective to avoid the contamination

by other microorganisms. So that both Nocardia and Bushnell-Hass media are recommended for isolation of *Nocardia* spp.

Nocardia are opportunistic pathogens, causing disease primarily among the young, the elderly, and those who are immunocompromised. It can be present with various clinical syndromes, range from chronic skin lesions to a progressive pulmonary diseases with documented haematogenous dissemination to virtually any organ in the body. These bacteria secrete various proteins (virulence factors) that increase their pathogenicity and this study indicated that 7 out of 9 species isolated from milk samples (cow's, buffalo's, sheep's and goat's milk) tested were pathogenic to rats.

Therefore, the aforementioned data proved that 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. So, many steps should be taken in consideration as follows:-

- Production of high quality milk regarding the most possible hygienic conditions should be in consideration.
- Proper cleaning and sanitizing of all dairy utensils and equipments used in milk production.
- Avoid the extensive use of antibiotics in the treatment of mastitis as this may increase the incidence of *Nocardial* infection.
- Avoid consuming raw milk, the public should be made aware of the hazard of *Nocardia* and other pathogens in raw milk and

should be advised to consume pasteurized milk and milk products as *Nocardia* will not survive in the efficient pasteurization process for milk and milk products.

- The use of refrigeration during processing, storage and handling of the milk and dairy products is also essential.
- Handling and distribution of milk should be done under strict hygienic measures.
- Health examination of persons who handle milk to prevent transmission of *Nocardia* spp. by food handlers into the food chain.
- Educational programs for farmers, consumers and plant employees are also indispensable. There are two mains by which attempts have been made to obtain a safe food supply: a-Inspection and regulation, b-End product testing.
- The HACCP should be designed to establish environment-monitoring programs to minimize the potential for contamination of finished products and to identify contaminated foods before distribution.
- Application of the Good Manufacturing Practices (GMPs) in milk manufacturing units should be regarded by the regulatory authorities as basic or compulsory requirements.
- Strict application of the "General Principles of Food Hygiene" issued by the "Codex Alimentarius Commission" and should be practiced in all chain of milk and milk products manufacturing.