

## Incidence of *Nocardia* species in raw milk collected from different localities of Assiut City of Egypt

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Published online at www.veterinaryworld.org on 25-03-2011

### Abstract

This study aimed to isolate and enumerate *Nocardiae* from the examined raw milk samples. 240 random milk samples were collected from cows, buffaloes, sheep and goats from different localities in Assiut city- Egypt. The incidences of *Nocardia* spp. were 47.8, 43.3, 53.3 and 66.7% with average counts of  $3.8 \times 10^4$ ,  $4.5 \times 10^4$ ,  $1.4 \times 10^4$  and  $7.6 \times 10^3$ /CFU/mL of the examined samples, respectively. Pathogenicity of the isolates was also studied. *N. otitidiscavarium* and *N. brasiliensis* caused sudden death of rats while, *N. farcinica* and *N. carneae* strains were non pathogenic. Other species caused several lesions. It was concluded from the study that, *Nocardia* species are existed in retailed and fresh milk of different farm animals. Most of the isolated strains were highly pathogenic to rats. Consequently, preventive measures should be taken to protect consumers from being infected.

Keywords: *Nocardia* species, Raw milk, Incidence, Pathogenicity, Zoonosis.

### Introduction

*Nocardia* species are one of the worldwide bacterial infections and has been implicated in the pathogenesis of pulmonary nocardiosis, brain abscesses, enteritis, arthritis, and endocarditis in all infected humans, including children and immune suppressed patients (Maria Bernadete et al., 2007). Infections due to *Nocardia* spp. are generally acquired through inhalation, trauma and ingestion (Dwight et al., 2004 and Martínez et al., 2008). The seriousness of the disease returns to the fact that clinical diagnosis is difficult due to non-specific manifestations (Saubolle and Sussland, 2003) and contamination of soil from the infected patient who plays an important role in wide spreading of the pathogens (Pisoni et al., 2008).

Economically, the most important host is the dairy cow. High incidence of bovine mastitis (due to *nocardia* infection) has been reported all over the world (Pisoni, 2008). Presence of *Nocardia* spp. in raw milk is not surprising as they are soil-borne aerobic actinomycetes (Saubolle and Sussland, 2003), and may contaminate milk during milking, handling, storage and transportation (Schinger, 1994). The present work investigated the existence of *Nocardia* species in different raw milk (cows, buffaloes, sheep and goats) as well as their pathogenicity.

### Material and Methods

Milk samples: A total of 240 random samples of raw

milk were collected during year 2009 from different localities in Assiut city, Egypt. The samples included cow's milk (90), buffalo's milk (90), sheep's milk (30) and goat's milk (30). Cow and buffalo's milk samples were collected from dairy farms, dairy shops and street vendors (30 samples of each).

Isolation of *Nocardia* species: One mL of each milk sample was aseptically inoculated into Bushnell-Haas broth (Difco) (Schaal, 1972). The inoculated tubes were incubated at 37 °C for 2-7 days and examined every 2 days. A loopfull from the incubated broth cultures was streaked onto plates of *nocardia* media (Difco) and incubated at 37 °C for 2-7 days (Gordon et al., 1974).

Identification of the isolates: Suspected colonies were identified morphologically and microscopically (Brown and McNeil, 2003). Biochemical tests and confirmation of species were applied according to Cowan and Steel, (1974).

Enumeration of *Nocardia* species (APHA, 1992): 10 mL of each prepared sample were added to 90 mL of sterile saline solution, mixed to make a dilution of 1/10, from which ten fold serial dilutions were prepared. 0.1 mL from each dilution was transferred and spread over a dry surface of *nocardia* agar plates (Difco). The plates were incubated at 37 °C for 2-7 days and the numbers of *Nocardia* spp./mL were calculated.

Pathogenicity of the isolated *Nocardia* strains:

Table-1: Incidence of the isolated *Nocardia* spp. recovered from milk samples

<i>Nocardia</i> 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.	%	No.	%	No.	%
<i>N.asteroides</i>	7	23.3	3	10	6	20	6	20	2	6.7	3	10	2	6.7	1	3.3
<i>N.farcinica</i>	4	13.3	1	3.3	0	0	2	6.7	0	0	1	3.3	1	3.3	4	13.3
<i>N.brasiliensis</i>	1	3.3	2	6.7	3	10	4	13.3	0	0	4	13.3	4	13.3	7	23.3
<i>N.carnea</i>	0	0	1	3.3	0	0	1	3.3	0	0	1	3.3	2	6.7	0	0
<i>N.brevicatena</i>	0	0	1	3.3	1	3.3	0	0	0	0	1	3.3	2	6.7	2	6.7
<i>N.otitidis scavarium</i>	1	3.3	0	0	1	3.3	1	3.3	3	10	0	0	1	3.3	2	6.7
<i>N.transvalensis</i>	4	13.3	0	0	0	0	3	10	0	0	0	0	2	6.7	4	13.3
<i>N.amarae</i>	2	6.7	2	6.7	3	10	1	3.3	5	16.7	0	0	2	6.7	0	0
<i>N.vaccinii</i>	0	0	0	0	0	0	0	0	1	3.3	0	0	0	0	0	0
<b>Total</b>	<b>19</b>	<b>63.3</b>	<b>10</b>	<b>33.3</b>	<b>14</b>	<b>46.6</b>	<b>18</b>	<b>60</b>	<b>11</b>	<b>36.7</b>	<b>10</b>	<b>33.3</b>	<b>16</b>	<b>53.3</b>	<b>20</b>	<b>66.7</b>

Animals: 30 male Spargue Dawley Albino rats (from 9 to 12 weeks old, weighing about 250 gm) were obtained from the Animal House, Fac. Medicine, Assiut Univ.

Strain culture: Isolated *Nocardia* species (9 strains) were sub cultured into nutrient broth and incubated at 37°C for 48h. Ten fold serial dilutions up to 1/10<sup>10</sup> were prepared; plate counts revealed that such broth cultures contain approximately 10<sup>9</sup>CFU/mL.

The intraperitoneal inoculation of rats: 27 rats were I/P inoculated with nine different *Nocardia* species (3 rats for each strain). Each rat was injected I/P with one mL of the broth culture. Another 3 rats were kept as a control (Pier et al., 1961). Two weeks later, all animals that survived were sacrificed and subjected to PM inspection and histopathology according to Bancroft et al., (1996). The representative lesions were cultured for reisolation of the inoculated *Nocardia* species.

## Results

*Nocardia* spp. were isolated from cow's, buffalo's, sheep and goat's milk samples in percentages of 47.8, 43.3, 53.3 and 66.7% and average counts of 3.8 x10<sup>4</sup>, 4.5x10<sup>4</sup>, 1.4x10<sup>4</sup> and 7.6x10<sup>3</sup> CFU/mL, respectively. 19 (63.3%) out of 30 cow's milk samples from dairy farms, were found to be contaminated with *Nocardia* spp. with average count of 1.3x10<sup>4</sup> CFU/mL. Lower percentages (46.7, 33.3 %) were found in milk samples from street vendors and dairy shops with average counts of 7.4x10<sup>3</sup> and 2.6x10<sup>4</sup> CFU/mL, respectively. In buffalo's milk samples, the highest incidence (60%) was obtained from milk samples from dairy farms. However, the incidences in buffalo's milk samples from dairy shops and street vendors were 36.7% and 33.3% with average counts of 1.2x10<sup>4</sup> and 5.9x10<sup>3</sup> CFU /mL, respectively. *Nocardia* spp. could be isolated from sheep and goat's milk samples in

percentages of 53.3 and 66.7% and average values of 1.4x10<sup>4</sup> and 7.6x10<sup>3</sup> CFU/mL, respectively. *N. asteroides* was the highest frequency distributed species in all examined milk samples; the distribution of the species was illustrated in Fig. 1. PM inspection showed that *N. asteroides* caused severe congestion in intestine, brain, parynchymatous and genital organs with clear enlargement of testis. *N. brasiliensis*, *N. brevicatena*, *N. otitidis-cavarium*, *N. transvalensis*, and *N. vaccinii* were also virulent causing similar lesions. *N. otitidiscavarium* and *N. brasiliensis* caused sudden death. On the other hand, *N. farcinica* and *N. carnea* were non pathogenic. Histopathological studies showed inflammatory edema between the villi of the intestine and increase of the goblet cells (Fig. 2A). Liver showed degeneration of the hepatic cells especially in the portal area (Fig. 2B and C). Lung showed degeneration of the bronchial epithelium, congestion of the bronchial blood vessels and thickening in the interalveolar septa with inflammatory cells (Fig. 3A). Vaccular degeneration in the tubular epithelium of the kidneys (Fig 3B), pertubular edema with tubular degeneration of the testis (Fig. 3C) and congestion in the cerebral blood vessels were noticed (Fig. 3D).

## Discussion

In the current study the incidence of *Nocardia* spp. in milk samples was considered higher than other previous studies (Abdel-Fattah, 1996 and Seddek, 2001). This higher incidence may be attributed to the fact that they are wide spread in nature. They may be introduced into the udder by insertion of cannula, contaminated preparations, drug mixture and tips of injectors (Seddek, 2001). The extensive use of antibiotics in the treatment of mastitis increased the incidence of *Nocardia* infection (Pisoni et al. 2008). Furthermore, the lack of hygienic measures and poorly sanitized dairy farm equipments constitute the major

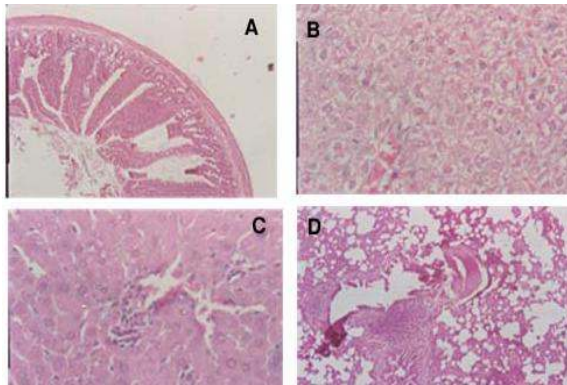


Figure-1. Intestine (A), liver (B and C) and lung (D) of *Nocardia* infected rats (H&Ex10).

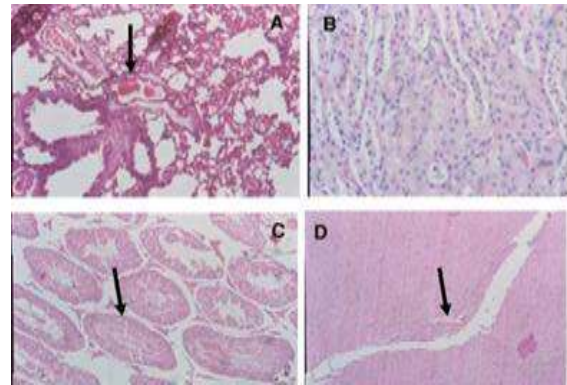


Figure-2. Lung (A), kidney (B), testis (C) and brain (D) of *Nocardia* infected rats (H&Ex10).

sources of contamination of different types of milk. The highest incidence of *Nocardia* spp. in goat's milk associated with poor hygienic nomadic rearing conditions in housing and in the milking parlours (Contreras et al., 2002). Some environmental factors facilitate multiplication of these organisms in milk such as hot and wet weathers. Contamination of soil from the infected patient plays an important role in wide spreading of the pathogens (Pisoni et al., 2008). Moreover, low income people use raw milk in preparation of some products increasing the potential risk of infections spread among community elements.

Pathogenic *Nocardiae* are saprophytes in many climates, soils and water, either as endogenous microbiota or as contaminants (Dwight et al., 2004) so, it is very important to study the severity and pathogenicity of these species that have been isolated from milk samples. Intraperitoneal inoculation of the isolated strains revealed that *N. otitidiscaviarum* and *N. brasiliensis* caused sudden death of the inoculated rats. Other species were also virulent causing several lesions. In other experimental animal models, Malik and Bakshi, (2002) found that *N. asteroides*, *N. farcinica*, *N. brasiliensis* and *N. otitidiscaviarum* were the most pathogenic species that caused infections in most laboratory animals. Despite *N. farcinica* was avirulent in this study, it is claimed to be the etiology of human nocardiosis (Rivero et al., 2008) because of its tendency to dissemination and its resistance to antibiotics (Torres et al., 2000 and Hitti and Wolff, 2005). So, further investigations should be conducted to study the virulence of different *nocardia* strains. It was concluded that the prevalence of *Nocardia* spp. in milk samples was high. All recovered *Nocardiae* were pathogenic strains except *N.farcinica* and *N. carnea*. So, proper cleaning and sanitizing of all dairy utensils

and equipment used in milk production was essential. The extensive use of antibiotics in the treatment of mastitis should be avoided as this may increase the incidence of *Nocardial* infection. Moreover, persons who handle milk should be periodically examined to prevent transmission of *Nocardia* spp. by food handlers into the food chain.

#### Acknowledgements

Authors are sincerely grateful for the great help provided by members of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Assiut University.

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