

Animal Health Research Institute
Assiut Regional Laboratory

**ISOLATION OF NOCARDIA ORGANISMS FROM
SOIL AND MASTITIC MILK OF DAIRY COWS
(FRIEZIAN AND HOLESTEIN)
ON DIFFERENT MEDIA ON SOME
GOVERNOMENTAL FARMS
IN ASSIUT GOVERNORATE**

(With 3 Tables)

By

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**عزل ميكروب النيوكارديا من التربة ولبن الأبقار ذات الضرع الملتهب
في الأبقار الفرزيان والهولشتين على أوساط غذائية مختلفة
في بعض المزارع الحكومية في أسيوط**

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لقد أجريت هذه الدراسة على عدد ٣٦ عينة من لبن الأبقار المصابة بالتهاب الضرع الإكلينيكي وكذلك ٧٢ عينة من التربة من بعض مزارع الأبقار الحكومية بمحافظة أسيوط. وقد أظهرت نتائج الفحص البكتريولوجي لعينات اللبن من أبقار مصابة بالتهاب الضرع أن ٩ عينات بنسبة (٢٥%) كانت إيجابية الزرع للنيوكارديا اسبيشرز، ١٢ عينة إيجابية الزرع بنسبة (١٦,٦٦%) من عينات التربة، كذلك أوضحت النتائج أن أهم أنواع النيوكارديا المعزولة هي النيوكارديا استيرويرز حيث أنه قد تم عزل ٧ عزلات بنسبة (١٩,٤٤%) من عينات اللبن و ٨ عزلات من النيوكارديا استيرويرز بنسبة (١١,١١%) من عينات التربة. ولقد وجد أقصى نمو للنيوكارديا اسبيشرز المعزولة من عينات اللبن قد تم على الاجاز المحتوى على (٥%) من دم الإنسان حيث كانت نسبة العزل ٩ عزلات بنسبة (٢٥%) وكانت نسبة العزل على الاجاز الترتيتوزي ٨ عزلات (٢٢,٣٠%) وعلى السبروت وكستروز اجاز ٦ عزلات (١٦,٧٠%) وفي حالة عينات التربة المأخوذة من نفس المزارع كانت نسبة العزل على نفس الأوساط الغذائية وهي الاجاز والأجاز الترتيتوزي المحتوى (٥%) من دم الإنسان والسبروت دكستروز أجاز هي على الترتيب كالتالي (١٦,٦٦%) (١٢%) و (١٣,٩٠%). أيضا تم عزل النيوكارديا برازيلنسيس في النيوكارديا المعزولة باستخدام ١١ نوع من المضادات الحيوية المختلفة وقد أوضحت النتائج أن معظم عزلات نيوكارديا شديدة الحساسية للنيوميسين ومتوسطة الحساسية لكل من الأمبسلين والأستربتومايسين وضعيفة الحساسية للبريثوميسين ومقاومة للمضادات الحيوية الأخرى.

SUMMARY

This study was carried out on 36 milk samples that were taken from dairy cows clinically affected with mastitis and 72 soil samples were taken from soil of some Governmental dairy farms in Assiut Governorate. Bacteriological examination of the mastitic milk samples revealed that 9(25%), and 12(18.66%) culturally positive for *Nocardia* species. The main bacterial isolates were *Nocardia asteroides* where it was isolated with an incidence 7(19.44%) from mastitic milk samples and 8(11.11%) from soil samples. The maximum growth of *Nocardial* species on 3 different utilized media trypticase soya agar, blood agar and Sabourad dextrose agar were found superior on blood agar, where the number of isolated strains on blood agar from milk samples were 9 isolates (25%), on trypticase agar 8 (22.30%) and SDA 6 (16.70%). In case of soil samples the isolated strains of *Nocardia* on the same 3 utilized media were 12 (16.66%), 12 (16.66%) and 10(13.90%) respectively. Also *N. brasiliensis* was isolated with percentage 2 (2.77%) from soil only. The antibiotic sensitivity test for isolated *Nocardia* spp using 11 types of antibiotics were discussed. The results showed that the most isolated strains were highly sensitive to Neomycin, moderately sensitive to Streptomycin, and weak sensitive to Erythromycin and complete resistant to the other antibiotics.

Key words: *Nocardia* isolation from soil and milk.

INTRODUCTION

Nocardia infections (*Nocardiosis*) are observed in men and animals in spite of their occurrence as a soil contaminant (Fey *et al.*, 1954; Fuchs and Boretius, 1972; Lindt *et al.*, 1961; Wendt *et al.* (1969a); Trabia *et al.*, 1993). According to Lechevaller (1986) *nocardia* belong to the family *Nocardiaceae* with 9 different species and they are pleomorph and aerobic growing, acid-resistant, Gram positive and required 1-5 day for culturing (Bisping and Mtsberg, 1988). These organisms are account for many pyogenic diseases of animals (Harvey *et al.*, 1993 and Lobetti, *et al.* 1993). These *Nocardial* mastitis in cattle is very important and it is obvious that soil plays the main role in the epidemiology of the disease as it is the source of infection (HIBBS *et al.*; 1980; Willett *et al.*, 1982; Valssaire *et al.*, 1984; Pellerin *et al.*, 1987 and Dohoo, 1989).

High incidence of bovine mastitis due to *Nocardia* has been reported all over the world (Sears, 1983; Argente; *et al.*, 1983; Nicolas *et al.*, 1984 and Stark and Anderson 1990). Though some epidemics of *Nocardia* mastitis were also recorded (Olubummi and Ayeni, 1983 and Rivard & Turgeon, 1984).

The problem arises here not only belongs to the economic costs to control nocardial mastitis (Willett; *et al.*, 1982) but mention is made of the potential risk to human health from contamination of milk, in addition to the cross reaction to tuberculin test in cases of pulmonary Nocardiasis (Costa, *et al.*, 1987 and Schoonderwoerd, *et al.*, 1990).

Pier *et al.* (1961) gave an account of *Nocardia asteroides* as a causative agent of an out break of bovine mastitis.

Many cases of Nocardial mastitis were described every where, in Europe (Battig *et al.*, 1990), USA (Dohoo, 1989 and Trabia *et al.*, 1993), Asia (Al-Bassam, *et al.*; 1989 and Ootani *et al.*, 1991) and Africa (Shigidi and Mamoun, 1981).

Udder nocardiasis is occurring sporadically but also enzootically and the clinical course acute or chronic. The course of disease is dependent on the virulence strain and the resistance of animal (Battig *et al.*, 1990).

MATERIAL and METHODS

Collection of Samples:

A sum of 72 soil samples representing an area of 3 farms at Assiut Governorate of milk production farm, were collected by scraping about 15 gms of the most upper layer of the soil with sterile spatula and transferred to a sterile bag.

A total of 36 milk samples were collected under aseptic condition in evacuated sterile test tubes from clinical cases of bovine mastitis on the dairy farms in Assiut Governorate.

Examination of Specimens:

3 sets of solid media were used, Sabouraud dextrose agar (SDA), Tryptose soya agar and blood agar containing 5-10% of human's R.BCs (Cruickshank *et al.*, 1975). Gentamycin 25 mg/l was added to the media in order to reduce background bacteria and allow *Nocardia* species to be detected more easily, 1-2 gms of soil sample were transferred to a test tube containing 5 ml sterile saline. The tube was thoroughly, mixed and left to stand for about 10 min. 3 Loopfuls of supernatant fluid of saline – soil suspension were streaked onto the surface of the 3 media. The same culture technique was performed on the sediment of mastitic milk samples after centrifugation.

The inoculated plates were incubated at 37°C for up to 1-5 days and inspected daily for any microbial growth. Suspected cultures were identified according to its colonial features and staining reaction using Gram and Modified Ziehl-Neelson (MZN) stains (Cheesbrough, 1984). Positive cultures of *Nocardia* were identified as non-haemolytic which powdery coloured colonies on TSA, and blood agar but light orange colour or brown to pink on SDA. They are microscopically identified as G +ve and acid fast small rods, or branched filaments.

Identification of Isolated Strains:

N. species are identified mainly by colonial and biochemical characteristics. The colonial feature of nocardial growth is evident in 1-5 days of inoculated plates and colonies are irregularly folded, raised and smooth or granular. The color varies from yellow, deep orange brown to pink on SDA and powdery chalk adhered not easily detached on blood agar. Gram-positive partially acid-fast branched mycelial filaments, which break up into bacillary forms, are evident under oil immersion. The presence of mycelial element distinguishes *Nocardia* from saprophytic and atypical mycobacteria. The mycelial forms of the *Nocardia* can be readily seen in slide cultures of SDA. The spores grow well at 45°C (Carter, 1984)

This species can be distinguished from less common *Nocardia* species according to the methods outlined by casein hydrolysis on milk agar. Urease activity (Cheesbrough (1984) and Carter (1984).

Antibiotic sensitivity tests for the isolated *Nocardia* species was carried out by agar diffusion method using 11 different types of antibiotics discs, Gentamycin (30 ug) Chloramphenicol (30 ug), Kanamycin (30 ug), Neomycin (30 ug), Pencillin (10 IU), Nalidixic acid (30 ug), Ampicillin (30 ug), Erythromycin (30ug), Streptomycin (30 ug), Cloxacillin (30 ug), and sulphamexathole plus trimethoprim (30 ug).

This study is designed in order to isolate *Nocardia* microorganisms from soil and mastitic milk of dairy cows animals on different media. Also, to evaluate different media, for isolation, identification of this pathogenic *Nocardia* species. Antibiotic test was carried out using different types of antibiotics by mean of agar diffusion-test.

RESULTS

The results were illustrated in Tables 1,2 and 3.

DISCUSSION

The results revealed that the main clinical finding of Nocardial mastitis were depression, anorexia, general status weak, increase the body temperature (39-42°C), the diseased quarter of the udder was swollen, firm in consistency, reddened and highly painful, enlargement of udder lymphnode, reduced milk yields. The milk secretion is similar to milk serum or bloody and contained sometimes grayish clots of pus with very offensive odour which contain milk particles. These recorded signs agree to that mentioned by Schuh *et al.*, (1994) and Weissentbock *et al.*, (1995). Nocardial species were considered as environmental pathogens and the environmental factors favour the spreading of infections in a community. Soil is one of the most important reservoir of many pathogenic Nocardiosis (Kishimoto and Baer, 1969). So isolation of N-species from soil with an incidence (16.66%) indicates the habitual characters of this species as soil inhabitants. This finding agrees with that previously recorded by many authors who predicted nocardia as one the major pathogens which are naturally occurring in the soil in many regions and responsible for serious diseases as Nocardial bovine mastitis (Kishimoto & Baer, 1969, Atia, 1976; Pier & Fichtner, 1981; Philpott-Howard, 1993 and Lopez-Martinez, *et al.*, 1993). Also the isolation of N-species with an incidence (16.66%) from soil of some Governomental dairy farms at Assiut Governorate was lower than that obtained by Abdel-Fattah (1996) who isolated N. species with higher incidence (21.45%) from soil of some dairy farms at Assiut Governorate. This different variation of isolated percentage may be due to environmental factors such a hot and wet weather, bad hygienic measure as contamination of soil with infected human patient which play an important role in wide spreading of infected pathogens (Pier and Fichtner, 1981).

Isolation of *Nocardia asteroides* in this study (11.11%) from soil of some dairy farms were nearly recorded by many authors who predicted N-asteroides as the major pathogens which naturally occurring in the soil of many regions and incriminated in occurrence many serious diseases (Kishimoto & Baer, 1989 and Pier & Fichtner, 1981). Also isolation of *N. brasilinses* from some dairy farms in this investigation (2.77%) indicate that this Nocardial types were uncommon pathogens and its frequency or its presence in soil are few and rare. Other than the two species of *N-brasilinses* and *N-asteroides* are uncommon

opportunistic pathogens but have been recovered on occasion as untappable Nocardial species in this investigation (Cheesbrough, 1984).

N-species was detected from the milk of mastitic cows with an incidence percentage (22-30 %). This tendency was reported previously by many authors as Sears (1983); Rivar & Turgeon (1984); Pellerin *et al.* (1987); Dohoo (1989); Manninen *et al.* (1993) and Weissenbock *et al.* (1995) who could isolate Nocardia species with an incidence (23%) from acute and chronic mastitis of dairy cows. On other hand in bacteriological investigation of acute and chronic mastitis Costa *et al.* (1987) and Battig *et al.* (1990) isolated Nocardia species with a lower incidence (6.6%).

In epidemiological properties studies of pus forming bacteria Abdel-Fattah (1996) isolated Nocardia species from mastitic milk with high incidence (23.33) similar to that revealed in our investigative study. Also, during a survey on the role of Nocardia in the aetiology of bovine mastitis, Vaissire *et al.* (1984) recorded a high incidence of morbidity rate ranged to about (50%), while a lower incidence of Nocardial mastitis (4.2%) was observed in Canada (1989) and (4.9%) in Siwtzerland from (1987-1989) was recorded by Dohoo (1989).

To clarify the cause of the increase of Nocardial mastitis it was found that Nocardia species isolated from teat tips in fore milk strippings (SCHINGER, 1994). According to Schoonderwoerd and Lynch (1989) and Battig *et al.* (1990), the Nocardia introduced into the udder by insertion of infusion cannula, contaminated preparations, drug mixture and tips of injectors, these recorded information supported the high incidence of Nocardia (25%) in their investigation. From previously mentioned information and because Nocardia species are optimal udder pathogens and predisposing factors are necessary to cause Nocardial mastitis, in addition to influence of environmental factors, immunosuppressed state and general weakness, resulting in differences of isolation percentage of Nocardia species.

In our study the results cleared that the N-asteroides isolated percentage from mastitic cows milk were (19.44%) and soil of dairy cows farm (12.45%) that indicate the higher incidence of the disease and this micro-organisms represent the main cause of the disease than other types of Nocardia species. These recorded results are accordance with that obtained by (Sears, 1983; Argente *et al.*, 1983 and Vaissaire *et al.*, 1984), who recorded a higher incidence of N-asteroides presenting about 50% of Nocardial bovine mastitis. In another study N-asteroides were isolated from infusion cannules medicine vials, milk samples positive in

californian mastitis test, teat tips, teat canal, subclinical mastitic milk and fore milk (Schinger, 1994). Also (Pier *et al.*, 1958, Kielwein, 1959, Pier *et al.*, 1961b, Wendt *et al.*, 1969a, Lynch, 1988; Battic *et al.*, 1990, Stark and Anderson, 1990 and Ollis *et al.*, 1991) mentioned that Nocardial *asteroides* mastitis are in connection with antibiotic application with dry-off and there is a host-parasite relationship between Nocardial mastitis and alter application of antibiotics, this explains that N-*asteroides* are the most common and wide spreads pathogens among Nocardial species in acute and chronic mastitis of dairy cows.

Concerning the evaluation of 3 different utilized media, TSA, SDA and blood agar, it was found that the high enrichment constituents of blood agar media containing 5% human blood give maximum growth of the Nocardial organisms than other media (TSA and SDA), these recorded results are in agreement to that reported by (Nsbit and Gunaskaran, 1993). Also Dolan (1972) found that the growth of 5 strains of N-*asteroides* on TSA containing 5% sheep or human blood was inferior to other media, this finding similar to our results.

In an epidemiological properties of pus-forming bacteria Abdel-Fattah (1996) found that the growth and percentage rate of N-species isolated from soil and mastitic milk more efficient and increase on blood agar than the other media (SDA), such this recorded results agree our explained finding in this investigation.

In concerning to antibiotic sensitivity tests the isolated Nocardial strains were tested against 11 different antibiotic types, the results cleared that the most isolated Nocardial strains (100%) were sensitive to Neomycin and moderate sensitive to Streptomycin and Ampicillin and less sensitive to Erythromycine and resistant to Kanamycin, Pencillin, Chloramphenicol, Naldixic Acid, Gentamycin, Bacterin, (sulphamexathole and trimethoprim) and Cloxacillin, these obtained results are in agreement to that obtained by Schuh *et al.* (1994). Many authors found good sensitivity of nocardia to antibiotics chloramphenicol and Trimethoprim (Battig *et al.*, 1990 and Tarabia *et al.*, 1993) which disagreed to our finding.

In referring to the resistance of isolated strains to most antibiotics types may be attributed to that isolated Nocardial strains produce beta-Lactamase against Pencillin G, Ampicillin and some other penicillins and Cephalosporins (Cheesbrough, 1984).

CONCLUSION

The argument of soil pathogens as an environmental problem indicate that it is difficult if not impossible to superimpose *Nocardia* infections from other infections. Therefore, *Nocardia mastitis* is not highly contagious but is more likely to occur from environmental contamination. The development methods of controlling the environmental pathogens would have significant impact in reducing the economic importance of the disease infection among human and dairy farm animals. Also medical instruments used in treatment of mastitis must be sterilized to overcome the infection through instruments.

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Table 1: Incidence of Nocardia spp. In soil and milk samples on different Governmental dairy farms.

Locality	Types of Samples					
	Soil Samples			Mastitic Milk Samples		
	Total No	Ve+	%	Total No	Ve+	%
Friesian Bari-Mor Farm	30	5	16.70	17	3	17.65
Manshiet Kashaba (Holstein darry cows farm)	35	6	17.14	15	5	33.33
Secondary agriculture school farm	7	1	14.29	4	1	25
Total	72	12	16.66	36	9	25

Table 2: Distribution of the isolated Nocardial species from soil and mastitic milk samples of infected cattle on utilized media.

Types of Nocardia Species	Total Samples of Mastitic milk (36)						Total Samples of Soil (72)					
	TSA		Blood agar		SDA		TSA		Blood agar		SDA	
	No. of Isolates	%	NO.	%	NO.	%	No.	%	No.	%	No	%
Nocardia asteroides	7	19.4	7	19.4	5	13.88	8	11.11	8	11.11	7	9.72
Nocardia brasiliensis	0	0	0	0	0	0	2	2.77	2	2.77	2	2.77
Non Typable Nocardia	1	2.77	2	5.56	1	2.77	2	2.77	2	2.77	1	1.39
Total number of isolates	8	22.30	9	25	6	16.70	12	16.66	12	16.66	10	13.90

The percentage of isolates calculated according to total number of samples.

Table 3: The antibiogram of isolated strains recovered from mastitic milk and soil.

Antibiotic type	GM (30ug)	S (30ug)	K (30ug)	C (30ug)	B (30ug)	P (10 IU)	N (30ug)	Amp (30ug)	E (30ug)	Cox (30ug)	NA (30ug)
Nocardia asteroides	-	++	-	-	-	-	+++	++	+	-	-
Nocardia brasiliensis	-	++	-	-	-	-	+++	++	+	-	-
Non Typable Nocardia species	-	++	-	-	-	-	+++	++	+	-	-

Abbreviations:

GM: Gentamycin S: Streptomycin K: Kanamycin C: Chloramphenicol B: Bacterin P: Pencillin
 N: Neomycin Amp: Ampicillin E: Erythromycin Cox: Coloxocillin NA: Nalidixic acid.