



Sudan University



College of Graduate Studies and Scientific Research

**Isolation and Molecular Identification and Sequencings of
Nocardia and Nocardia- like spp Isolated from Soil and Milk of
Goats, Sheep, Cattle and with Mastitis in Khartoum Stats**

(العزل والتعريف التسلسلي لبكتريا النوكارديا وأشباه النوكارديا من عينات التربة ولبن الماعز
والأغنام والأبقار المصابات بالتهاب الضرع في ولاية الخرطوم)

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Dedication

This work is dedicated to my

WonderfulMother

The symbol of love and giving

To my Uncles

Who Help and Stand Beside me

To my Husbandand To my Sons

Who always with me

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ABSTRACT

This work was intended to investigate the role of *Nocardia* and *Nocardia*-like organisms as causative agents of mastitis in soil sample and milk sample from goats, cow and sheep. It was carried out at different farms in Khartoum Stat.

A total of three hundred milk samples of cow, sheep and goat and twenty soil samples were collected from the same farms in different sites for isolation and identification of *Nocardia* and *Nocardia* — like organisms.

All milk samples were cultivated onto Tryptic Soya Agar (TSA), and soil cultured on to Tryptic Soya Agar medium (TSA) supplemented with a combination of tetracycline (5mg/ml) and nystatin (50 mg/ml).

All isolates 11(11%) from milk of goats, 13(13%) from milk of cow and 7(35%) from soil sample were identified phenotypically as *Nocardia* spp by cultural, morphological, and biochemical tests (urease test, catalase test and degradation of xanthine, tyrosine, casein, sugar fermentation, growth at 45°C), mycolic acid content and antimicrobial susceptibility test.

The isolates were tentatively identified as member of the genus *Nocardia* on the morphological, biochemical and mycolic acid pattern. Comparative analysis of the 16S RNA gene sequencing confirm that the isolates fall within the phylogenetic branch which accommodates member genus *Mycobacteria*, *Dietzia* spp and *Rhodococcus* spp.

In this study we consider the recognition of *Nocardia* and *Nocardia*-like based on phenotypic tests was strenuous, but definitive identification was attainable by molecular methods.

المستخلص

أجريت هذه الدراسة بهدف التقصي عن دور النوكارديا وشبه النوكارديا كمسبب لالتهاب الضرع في الأغنام والماعز والأبقار. نفذت الدراسة في مزارع مختلفة بولاية الخرطوم ، السودان .

جمعت ثلاثمئة عينة من لبن الأغنام والماعز والأبقار وعشرون عينة من التربة من نفس المزارع التي جمعت منها عينات اللبن وذلك بغرض عزل وتصنيف بكتريا النوكارديا واشباه النوكارديا .

زرعت كل عينات اللبن في اجار التربتيك صويا والعينات التي عزلت من التربة في اجار التربتيك صويا مضاف اليه 5 مايكروجرام/ مل من مضاد التتراسايكلين و50 مايكروجرام/ مل من النيستاتين.

عزلت إحدى عشر(11%) عينة من لبن الاغنام وثلاثة عشر(13%) عينة من لبن الابقار وسبعة عينات(35%) من التربة تم تصنيفها لانواع النوكارديا (باستعمال الشكل المورفولوجي والاختبارات الاستيبانية والاختبارات الكيمو حيوية) مثل إختبار الكاتليز واختبار اليوريا واختبار تحلل الزنثين الكازيين والتايروسين والنشأ واختبار تخمر السكريات والقدرة على النمو في درجة حرارة 45 مئوية) واختبار حامض المايكولك (*mycolic acid*) واختبار الحساسية للمضادات الحيوية .

تم تصنيف كل العينات مؤقتا الى نوكارديا بناءا عي الشكل المورفرولوجي والاختبارات الكيموحيويه واختبار حمض المايكولك وبقارنة نتائج 16S rRNA اثبت ان العينات تستوعب مايكو بكتريا وبكتريا الدتسيا وبكتريا الرودوكوكس.

لخص الدراسة الى ان التعرف على النوحارديا واسبابه النوحارديا بالطرق المصهريه
غالبا مايكون مضني ولايحدد الانواع بشكل قاطع لكن التمييز الفاعل يتم بواسطة
الطرق الجزئية.

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LIST OF ABBREVIATION

SCC	Somatic Cells count
DMCC	Direct Microscopic Somatic Cells count
MACA	Mycolic Acid Containing Actinomycetes
TLC	Thin Layer Chromatography
MZN	Modified Ziehl – Neelsen
LJ	Lowen stein - Jensen
DST	Diagnostic Sensitivity Test
HPLC	High Performance Liquid Chromatography
GYEA	Glucose Yeast Extract Agar
TSAM	Tryptic Soya Agar Media
I/v	Intravenous
I/M	Intramuscular
No	Number
U. of K.	University of Khartoum
PCR	Polymerase Chain Reaction

