**Nocardia farcinica** – a significant cause of mastitis in goats in Sudan

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**ABSTRACT**

Fifteen of 100 mastitic milk samples from goats suffering from mastitis were tentatively identified as members of the genus *Nocardia* on the basis of selected phenotypic and chemotaxonomic characteristics. Six of the 15 strains were confirmed as *Nocardia farcinica* by 16S rDNA gene sequencing and subsequent aligning with relevant actinomycetes found in electronic databases and 2 by other identification criteria. *N. farcinica* is a serious cause of mastitis with a significant prevalence (15%) among the examined goats. Efforts are needed to optimise and simplify isolation and identification methods.

**Key words**: goats, mastitis, Nocardia farcinica, Sudan.


**INTRODUCTION**

Many infective agents have been implicated as causes of mastitis in cattle, but the disease is most commonly caused by *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli*. Miscellaneous causes of mastitis in bovines, such as *nocardiae*, have been described\(^{6,20}\). Although mastitis in goats has been reported from some parts of the world, including Africa\(^{1,6,20,22}\), it has received little attention in Sudan.

An early study reported the isolation of *Mycoplasma* spp., *Nocardia* sp. and *Corynebacterium pseudotuberculosis* from mastitic goats in Sudan\(^6\), and another study confirmed *Mycoplasma agalactiae* as one of the causes of goat mastitis in Sudan\(^1\). To our knowledge, no general survey of the causes of mastitis among goats in Sudan has been undertaken.

Various microbial agents have been isolated from African goats with clinical or subclinical symptoms or from normal milk samples. These include *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Acinetobacter* spp., *Actinomyces* spp., *Pseudomonas* spp. and *colliforms*\(^{1,8,10}\). Various bacteria, including *Actinomyces* spp., have been isolated from clinically normal milk samples from a mixed dairy goat flock in Kenya\(^8\). In Sudan there were two reports of bovine mastitis caused by *nocardiae*\(^{11,26}\) and a single report of mastitis in a goat caused by *Nocardia asteroides*\(^4\).

*Nocardiosis* is known to cause a variety of suppurative infections in humans and animals\(^{12–15}\). The most commonly reported pathogenic species are *Nocardia africana*, *N. asteroides*, *N. farcinica* and *N. nova*, followed in order of importance by *N. brasiliensis*, *N. otidiscaviarum*, *N. pseudobrasiliensis* and *N. transvalensis*. The incidence of such infections in humans and animals in tropical countries is unknown although nocardiosis has been reported from most regions of the world. However, it is well established that *nocardiae* can be easily overlooked during routine culture and smear examinations.

The aim of this study was to identify to species level a number of actinomycetes-like strains that had been isolated from mastitic goats in Sudan, using 16S rDNA sequence analysis of representatives of major phenotypic clusters identified recently\(^4\).

**MATERIALS AND METHODS**

**Animals and area of investigation**

The goats examined during the course of this report belonged to the Nubian type and 1 was a Saanen type. All goats were from Khartoum State, central Sudan. Thorough clinical examination with special attention to udders was conducted. Milk samples from 100 mastitic goats were collected in sterile containers and immediately transported to the laboratory for bacteriological investigations.

**Bacterial isolations**

Primary isolation of the causal agents was carried out using tryptic soya agar (TSA; Difco). TSA plates were incubated aerobically at 37°C for up to 5 days. Subsequent subcultures of the primary cultures were made using glucose yeast extract agar (GYEA: 10 g glucose, 10 g yeast extract, 14 g agar, 100 ml distilled water; pH 6.8).

**Mycolic acid analysis**

The 15 nocardia-like strains and other actinomycete-like strains were examined for the presence of nocardomycobolates by thin-layer chromatography (TLC). Extraction of nocardomycolic acids and TLC analysis of extracted mycolates were performed as previously described\(^5\). The presence of single-spot co-chromatograms, compared with *N. farcinica* (ATCC 3888), confirmed the presence of nocardomycobolates.

**Phenotypic identification**

Cultures were subjected to an identification scheme using selected morphological and cultural characteristics\(^{12,13}\). One of the diagnostic features of actinomycetes, such as *nocardiae*, is the presence of acid-fast branching filaments\(^1\). To detect strains of *Nocardia farcinica*, rapid opacification of Middlebrook 7H10 agar was also included as a test in the identification scheme\(^{10}\).

**Sequencing of 16S rDNA**

Isolation of chromosomal DNA and 16S rDNA sequencing were carried out according to the method of Chun and Goodfellow\(^1\). The resulting PCR amplicons were separated by gel electrophoresis and purified using Nucleospin Extraction Kits (Macherey-Nagel, Dueren, Germany) according to the manufacturer’s instructions. Sequencing of the almost complete 16S rDNA gene was performed as previously described\(^1\).

**Phylogenetic analyses**

The resulting sequences were manually aligned against other nocardiae and representative sequences from members of the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Rhodococcus*, *Skenmania*, *Tsakamurella* and *Williamsia*.

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retrieved from the DDBJ/EMBL/GenBank databases using PHYDIT (http://plaza.snu.ac.kr/~jchun/phydit/). Evolutionary trees were inferred by using 4 treeing algorithms, namely, the least-squares, maximum-likelihood, maximum-parsimony and neighbour-joining using the PHYLIP suite of programs. Bootstrap analyses were used to evaluate the treeing topologies of the neighbour-joining data according to Jukes and Cantor based on 1000 resamplings.

RESULTS
The presence of branching filamentous organisms was taken as the cause of mastitis as they are not considered to be part of the normal udder flora; other organisms were considered contaminants. Based on phenotypic properties, 15 of the 100 isolated strains were recognised members of the genus Nocardia. Most of the strains were isolated in pure form from the milk samples. The 1st isolate (SD1800) was isolated in pure form from a nodular granulomatous mastitic case. The procedure was repeated 3 times, and this case was then used a benchmark for the isolation procedure. TSA is not a selective medium and therefore our procedure did not target particular organisms but rather revealed actinomycetes as they were encountered.

The 15 strains showed: a) rapid growth with rough, wrinkled colonies that varied in colour from orange (Fig. 1), to grey to cream-yellow; (b) colonies were firmly attached to the medium, were difficult to emulsify, and had sparse or non-aerial hyphae. Microscopically, the organisms were Gram-positive, weakly to non-acid fast and contained branched filaments that fragmented into short chains and, occasionally, rods.

In TLC analysis, the 15 strains were found to contain mycolic acids. These mycolates were considered nocardomycolates as they co-migrated with those typical of Nocardia sp.

Eight of the fifteen strains were identified phenotypically as Nocardia farcinica as all opacified the Middlebrook agar 7H10 whereas none of the other recognized species of Nocardia do. It should be noted, however, that neither Nocardia asiatica nor N. puris were included for comparison because type strains are still unavailable.

Six of the 8 strains showed a 16S rDNA gene similarity of 100 % with that of Nocardia farcinica ATCC 33188 (Fig. 2). A bootstrap value of 100 % was attained in the neighbor-joining tree. Based on these results, the eight strains could be confidently assigned to N. farcinica.

DISCUSSION
The number of cases of mastitis due to nocardiae reported in this study is relatively high (8/100 milk samples from mastitic goats). This is surprising in view of the lack of previous reports of mastitis in goats caused by N. farcinica. There are, however, reports of nocardiae causing mastitis in cattle and other diseases in animals and a single report of mastitis in a goat caused by Nocardia asteroides.

Little is known about the incidence of...
infections in goats, not only with regard to mastitis, but other diseases as well. In Sudan there are about 20 million goats, and as our pilot study suggests, a surprisingly large proportion of the population might be suffering from infections due to Nocardia farcinica and possibly other actinomycetes as well.

The initial identification criteria used in the present study, namely colony morphology, opacification of Middlebrook 7H10 agar and the detection of nocardomycobacterales are highly diagnostic of Nocardia farcinica, as confirmed by 16S rDNA gene sequencing of 6 of the 15 isolated strains. Our results on opacification of agar media is in agreement with those of previous studies, in which this test was recommended as a useful adjunct to routine methods when identifying strains of Nocardia farcinica.

To our knowledge, this is the 1st report of mastitis in goats due to Nocardia farcinica and it is evident from the present study that better and more accurate methods for the quick identification of pathogenic nocardiae should be urgently sought and evaluated.

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