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CASE REPORT
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Bovine Mastitis Caused by Multidrug-Resistant *Nocardia farcinica*

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ABSTRACT

***Background*:** Mastitis caused by *Nocardia* is characterized by pyogranulomatous inflammation related to inadequate hygiene conditions and is difficult to treat. Prompted by the absence of documentation of *Nocardia farcinica* associated to bovine mastitis in the Northeast region of Brazil, this is the first report to describe bovine mastitis caused by multidrug-resistant *N. farcinica*.

***Case*:** Four milk samples (one from each teat) obtained from a 3-year-old Jersey cow raised on a property located in the metropolitan region of Recife, Pernambuco state, Brazil, were submitted to the Laboratory of Infectious-Contagious Diseases of the Veterinary Hospital at Universidade Federal Rural de Pernambuco [UFRPE]. At the laboratory, samples were cultured in base agar enriched with 7% sheep blood (blood agar) in a microbiological incubator at 37°C under aerobic conditions for 72 h. After only 48 h, however, pure bacterial colony growth was observed in all samples. Macroscopic analysis revealed small colonies, with an irregular shape, dry aspect, and greyish in color. Gram-positive rods forming filaments and/or ramifications were observed using a Gram staining method. *Nocardia* spp. were identified according to morphotinctorial characteristics. Susceptibility testing using the disc-diffusion method in agar (antibiogram) was performed using the following antibiotics: penicillin (10 IU), tetracycline (30 µg), amoxicillin (10 µg), gentamicin (10 µg), cephalexin (30 µg), erythromycin (15 µg), cephalothin (30 µg) and ampicillin (30 µg). However, the organism exhibited resistance to all drugs; as such, a new milk sample was obtained at the same location the initial samples were collected. Samples (approximately 5 mL) were collected aseptically and separately from all four teats in sterile bottles, during which the presence of granular material was noted. Bacterial culture was performed as previously described and, after 48 h, colony growth with the same characteristics as the first isolation were observed, and with same morphotinctorial characteristics in the Gram stain. A resistance profile was observed for 14 of the antimicrobial drugs tested; sensitivity was verified only for ciprofloxacin and amoxicillin with clavulanic acid. One bacterial colony was selected and sent to the Center of Strategic Technology of Northeast (CETENE-PE) for species identification using a matrix-associated laser desorption ionization-time of flight (MALDI-TOF/MS) technique, which confirmed the species to be *N. farcinica.* Molecular characterization of 16s ribosomal DNA was performed using polymerase chain reaction with universal prokaryotic primers 516F-13R. Subsequently, the amplified product was subjected to sequencing, and the result was analyzed for quality using Phred base calling software; bases with a Phred value > 20 were kept. The sequence was evaluated using GenBank, in which the isolate exhibited 99% similarity to *N. farcinica.*

***Discussion*:** Clinical findings and animal history associated with microbiological culture and bacterial identification using the MALDI-TOF technique, as well as DNA sequencing, confirmed the case of clinical mastitis to be caused by *N. farcinica.* These bacteria are considered saprophytes, and their occurrence is associated with deficiencies in hygienic-sanitary management, such as not using pre- and post-dipping, which may favor mammary gland infection. Treatment of *N. farcinica* mastitis is effective only when properly performed, with agent identification and antibiotic sensitivity tests in vitro associated with the adoption of hygienic-sanitary measures. This is the first description of bovine mastitis caused by *Nocardia farcinica* in the northeast of Brazil. Multidrug resistance should raise awareness of producers searching for laboratory aids in agent identification as well as antibiotic sensitivity tests, and to develop a proper therapeutic protocol based on results obtained in laboratory examinations.

Keywords: dairy cattle, nocardiosis, mastitis, multidrug resistance.

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INTRODUCTION

Mastitis is considered to be an infectious disease with a strong negative impact on dairy cattle production. It is characterized by an inflammatory reaction in the mammary glands caused by diverse microorganisms that culminates in reduced milk quality and production, in addition to treatment expenses and early animal disposal [1,2].

It has a pluri-etiological and multifactorial etiology, including bacteria and fungi. *Nocardia* is one of the less documented genera of bacteria involved in environmental mastitis, generally occurring as isolated cases or epizooties. A variety of species in this genus can cause mastitis, including *Nocardia asteroides, Nocardia nova,* and *Nocardia farcinica* [3-5].

Mastitis caused by *Nocardia* is characterized by a pyogranulomatous inflammatory reaction, often related to inadequate environmental hygiene and is difficult to treat given its multidrug resistance to various antimicrobial classes, causing a reserved or unfavorable prognosis [3,4].

N. farcinica has previously been described to cause bovine mastitis in an epizooty in Canada [3], in bovines from São Paulo state [4], isolated from a milk tank in the State of São Paulo [6], and in cases of bovine abortion in Kansas, United States [7].

Prompted by the absence of documentation of *N. farcinica*-associated bovine mastitis in the Northeast region of Brazil, the present report provides the first description of bovine mastitis caused by multidrug-resistant *N. farcinica.*

CASE

Four milk samples (one from each teat) obtained from a 3-year-old Jersey cow raised on a property located in the metropolitan region of Recife, Pernambuco state, Brazil, were submitted to the Laboratory of Infectious-Contagious Diseases of the Veterinary Hospital at Universidade Federal Rural de Pernambuco (UFRPE). According to the owner, the cow presented clinical signs compatible with mastitis, such as granular material, reduced milk production, and painful reaction to touch. It was also reported that pre- and post-dipping and antimicrobial treatment were previously performed using gentamicin by intramammary infusion. Due to treatment failure and, after two months of gentamicin use, there was a change in protocol to a penicillin-based antibiotic, with gland inflammation recurring five months after the first treatment. Considering the recurrence of mastitis, the owner forwarded milk samples to the laboratory with the objective of establishing a definitive diagnosis.

At the laboratory, samples were cultured in base agar enriched with 7% sheep blood (blood agar) in a microbiological incubator at 37°C under aerobic conditions for 72 h. After only 48 h, however, pure bacterial colony growth was observed in all samples. Macroscopic analysis revealed small colonies, with irregular shape, dry aspect, and greyish in color. Gram-positive rods forming filaments and/or ramifications were observed using the Gram staining method. *Nocardia* spp. were identified according to morphotinctorial characteristics. Susceptibility testing using a disc-diffusion method in agar (antibiogram) was performed using penicillin[1] (10 UI), tetracycline[2] (30 µg), amoxicillin[1] (10 µg), gentamicin[2] (10 µg), cephalexin[1] (30 µg), erythromycin[1] (15 µg), cephalothin[1] (30 µg), and ampicillin[1] (30 µg). However, the organism exhibited resistance to all drugs. Considering the bacterial resistance to multiple antibiotics, it was decided to visit the property for an environmental evaluation and new sample collection one week after the first examination, in which peccary hygiene conditions were noted in the presence of feces and trash.

Samples (approximately 5 mL) were collected aseptically and separately from all four teats in sterile bottles; during milk collection, the presence of granular material was noted. Samples were identified and aconditioned in isothermal boxes containing recyclable ice and forwarded to the laboratory for processing. Bacterial culture was performed as previously described and, after 48 h, colony growth with the same characteristics as the first isolation were observed, and with the same morphotinctorial characteristics in Gram stain (Figure 1). In addition to the antimicrobials previously tested, amoxicillin with clavulanic acid[1] (30 µg), kanamycin[1] (30 µg), oxacillin[1] (1 µg), florfenicol[2] (30 µg), azithromycin[1] (15 µg), ciprofloxacin[1] (5 µg), trimethoprim[1] (5 µg), and cefoxitin[1] (30 µg) were also evaluated (Figure 2). A resistance profile was observed for 14 of the antimicrobial drugs tested, and sensitivity was verified only to ciprofloxacin and amoxicillin with clavulanic acid.

A bacterial colony was selected and sent to Center of Strategic Technology of Northeast (CETENE-PE) for species identification using a matrix-associated laser

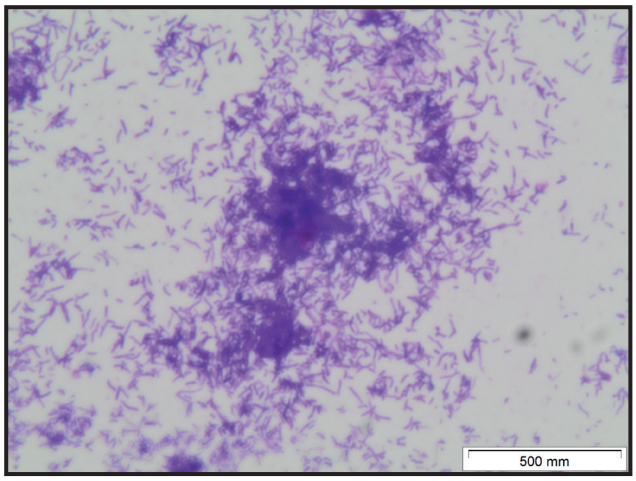

Figure 1. Microphotography revealing a Gram-positive tangle isolated from milk samples forming filamentous structures and/or ramified, non-septate entities. [Gram stain; original magnification ×100].

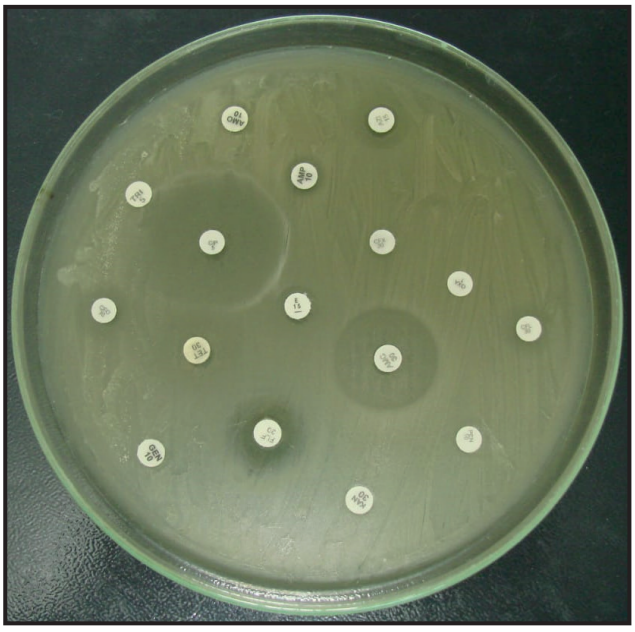

Figure 2. Antibiogram of the mastitis isolate exhibiting the sensitivity profile of *Nocardia farcinica*. Of 16 antibiotics tested, *N. farcinica* was sensitive only to ciprofloxacin and amoxicillin with clavulanic acid.

desorption-ionization-time of flight (MALDI-TOF/MS) technique, confirming the species as *N. farcinica.*

Molecular characterization of 16s ribosomal DNA was performed using polymerase chain reaction (PCR) with universal prokaryote primers 516F-13R [8]. Subsequently, the amplified product was subjected to sequencing, and the result was analyzed for quality using Phred base calling software [9]; bases with a Phred value > 20 were kept. The sequence was evaluated using GenBank® (www.ncbi.nlm.nih.gov/genbank/), with the isolate exhibiting 99% similarity to *N. farcinica.*

DISCUSSION

Clinical findings and animal history associated with microbiological culture and bacterial identification using the MALDI-TOF/MS technique, as well as DNA sequencing, confirmed the case of clinical mastitis to be caused by *N. farcinica.* These bacteria are considered saprophytes, and their occurrence is associated with deficiencies in hygienic-sanitary management, such as not using pre- and post-dipping, which may favor mammary gland infection [4].

Regarding diagnosis, molecular characterization techniques, such as DNA and protein profile evaluation, are of significant importance for classification of pathogens with rare occurrence in mastitis etiology, especially in which biochemical testing may yield inaccurate results [10]. Combining culture methods with antibiogram is important for proper diagnosis of bovine mastitis and to avoid unnecessary antimicrobial drug use; for these reasons, its application should be encouraged by veterinarians [11].

At the property, the presence of organic material in the milking place and the lack of teat disinfection before and after milking were observed, this being one of the main risk factors observed during the visit. The presence of granular material in milk indicates a pyogranulomatous reaction. This characteristic of mastitis caused by *Nocardia* has been described in previous studies [3,4,12,14].

Aside from the clinical-epidemiological characteristics of mastitis, it was noted that it was difficult to treat multidrug-resistant *N. farcinica*, which was confirmed *in vitro* and, thus, a limiting factor in treatment [3,4,13]. Another limiting factor to *N. farcinica* mastitis treatment is its intracellular nature, which impairs antibiotic action, and virulence factors that stimulate a pyogranulomatous reaction process [14].

Knowing the etiological agent of mastitis may be fundamental in clinical decision-making regarding therapy, establishing prophylactic measures, as well as decisions regarding animal maintenance in the flock [10]. Treatment based on gentamicin and penicillin - both of which were unsuccessful - indicates bacterial resistance *in vivo,* which was confirmed by antibiogram (*in vitro*). We also observed that the bacteria presented resistance to all ß-lactam antibiotics tested in the antibiogram, except for amoxicillin with clavulanic acid, which demonstrated *in vitro* sensitivity. ß-lactam resistance may occur due to the capacity of *N. farcinica* to

produce beta-lactamase enzyme [15], and its sensitivity to amoxicillin with clavulanic acid may be explained by clavulanic acid inhibition of beta-lactamase enzyme action [16].

Regarding recurrent mastitis cases, aside from bacterial resistance, other causes may be associated, including the presence of the pathological agent in the environment favoring reinfection, and the lack of teat disinfection before and after milking [3,4,14]. Treatment of *N. farcinica* mastitis is effective only when properly performed, with agent identification and antibiotic sensitivity testing *in vitro* and the adoption of hygienic-sanitary measures.

To our knowledge, this is the first report of bovine mastitis caused by multidrug resistant *N. farcinica* in the northeast of Brazil. Multidrug resistance should raise awareness to producers searching for laboratory aids in identifying pathological agents as well as antibiotic sensibility tests, and to develop a proper therapeutic protocol based on results obtained in laboratory examinations.

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