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Isolation of *Moraxella canis* from an Ulcerated Metastatic Lymph Node

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***Moraxella canis* was isolated in large numbers from an ulcerated supraclavicular lymph node of a terminal patient, who died a few days later. Although the patient presented with septic symptoms and with a heavy growth of gram-negative diplococci in the lymph node, blood cultures remained negative. *M. canis* is an upper-airway commensal from dogs and cats and is considered nonpathogenic for humans, although this is the third reported human isolate of this species.**

Case report. A 51-year-old patient who was a 55-pack-per-year smoker and who had a long history of serious, chronic obstructive pulmonary disease was sent to the hospital in October 1998 because of dyspnea. A primary bronchial carcinoma was diagnosed. Biopsy of the left supraclavicular lymph node revealed a moderately differentiated adenocarcinoma. Palliative radiotherapy was started but was stopped in December 1998, and the patient was sent home during the first week of January.

The patient was in a cachectic, immunodepressed condition, presented with fever and chills, and had a large ulcer at the left supraclavicular lymph node. A differential diagnosis of sepsis and tumor was made. Computed tomography of the thorax showed a large necrotic nodus at the left supraclavicular lymph node and a tumor at the right lower lobe in association with pleural fluid effusion and a diffuse metastasized bronchial carcinoma. *Staphylococcus aureus* in small numbers and *Moraxella canis* in large numbers were isolated from the ulcer wound. Blood cultures remained negative. The patient was discharged 2 days later and died shortly thereafter at home.

The oxidase-positive gram-negative diplococci were given a preliminary identification as *M. canis* after observation of brown pigmentation of the Mueller-Hinton II agar (MHAII; BBL Becton Dickinson, Cockeysville, Md.), used routinely for disk diffusion susceptibility testing. This characteristic was present in 15 of the 16 previously described isolates of *M. canis*, while absent in all other *Moraxella* species (2). Further differentiation from other *Moraxella* species was possible on the basis of a positive DNase reaction, acetate assimilation positivity, and a positive gamma glutamyl aminopeptidase reaction (2).

The isolate (LBV436) was resistant to ampicillin and susceptible to co-trimoxazole, doxycycline, fucidic acid, vancomycin, rifampin, gentamicin, and quinolones. Production of β-lactamase has been demonstrated in some strains of *M. canis* (2), and this strain was also β-lactamase positive.

M. canis, together with *Moraxella catarrhalis*, *Moraxella cuniculi*, *Moraxella caviae*, *Moraxella ovis*, and *Moraxella* sp. incertae sedis strain NCTC 4103 belong to the coccal moraxellae, which, in contrast to the bacillary moraxellae, all exhibit DNase activity. *M. canis* and strain NCTC 4103 differ from all other coccoid moraxellae by the production of gamma glutamyl aminopeptidase and the ability to grow on MHAII at room temperature. Moreover, *M. canis* and, to a weaker extent, strain NCTC 4103 produce a brownish pigment on this growth medium, which is a feature not shown by any other moraxellae.

To our knowledge, this is the third isolation of *M. canis* from humans. The first isolate ($N7^T$, CCUG $8415A^T$) was from a dog bite wound in a Swedish female in 1979, for which no further clinical data are available (2). Wüst et al. (7) described a blood culture isolate (U33, BLU8387) obtained in 1988 from a 61-year-old alcoholic Swiss male with bleeding esophageal varices who was hospitalized for symptoms suggesting pneumonia. The cachectic immunodepressed condition of our patient is comparable to that of the Swiss patient (7). All other known isolates of this species are commensals isolated from dog saliva (P37, Paris) (2) or swabs from dog muzzles (one was from a cat muzzle) (2), from which *M. canis* could be cultured only after suppression of other commensal bacteria with a selective medium (4).

Sequence determination of the first 450 to 700 bp of the PCR-amplified 16S rRNA gene was carried out for isolate LBV436, for four of the previously collected *M. canis* strains (O18, P37, U33, and W4), for strain NCTC 4103, for an unidentified gram-negative diplococcus isolated from a dog bite (MOR32), and for a *Moraxella* strain producing yellowish pigment on MHAII (LBV438). The complete sequence was determined for the *M. canis* type strain N7. Amplification was done by PCR with the primers 5′-AGT TTG ATC CTG GCT CAG and 5′-TAC CTT GTT ACG ACT TCG TCC CA. The reactions were performed in a final reaction mixture of 50 μl containing 25 μl of Master Mix (Qiagen, Hilden, Germany), a 0.2-μM concentration of each primer, and 5 μl of a DNA suspension obtained by alkaline lysis. Alkaline lysis was done by suspending one colony in 20 μl of 0.25% sodium dodecyl sulfate–0.05 N NaOH and heating at 95°C for 15 min, followed by a final dilution with 180 μl of distilled water. The amplification reactions were performed in a GeneAmp PCR System 9600 (Applied Biosystems, Foster City, Calif.) with the following cycling parameters: 94°C for 5 min, followed by 3 cycles of 45 s at 94°C, 2 min at 50°C, 1 min at 72°C, and 30 cycles of 20 s at 94°C, 1 min at 50°C, 1 min at 72°C, with a final extension at 72°C for 7 min. The presence of amplification products was checked by electrophoresis on 2% agarose gels stained with ethidium bromide. The amplification products were then pu-

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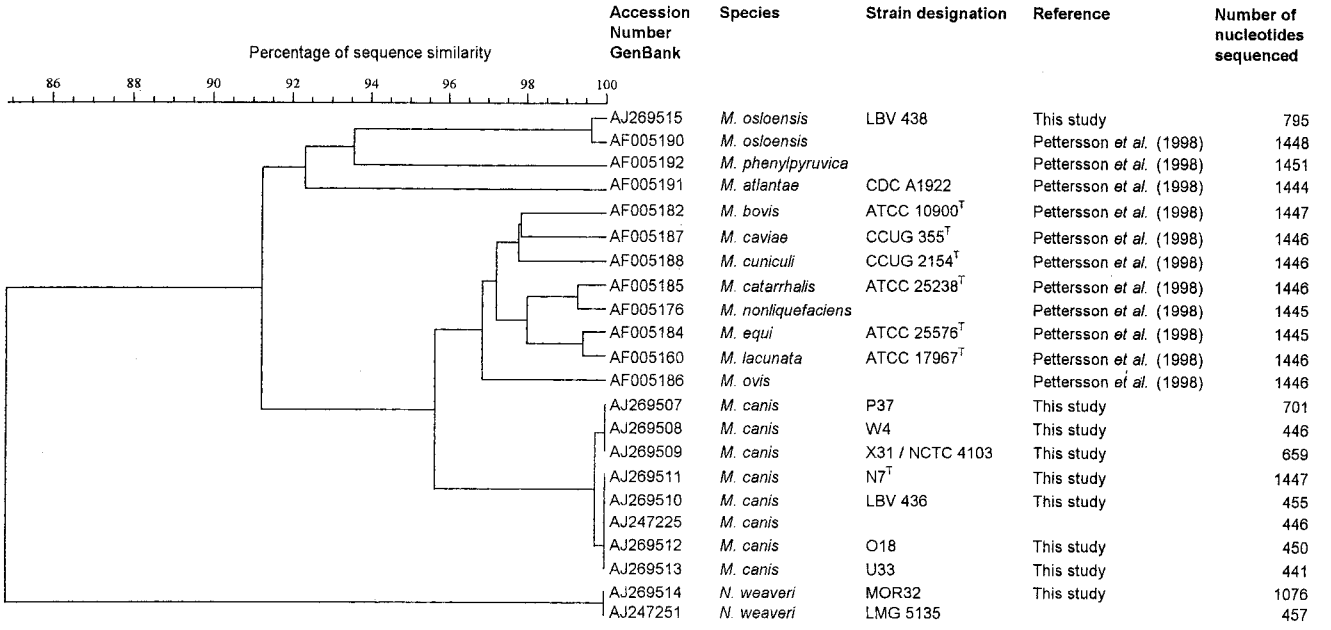

FIG. 1. Dendrogram based on clustering by means of the unweighted pair groups method using arithmetic averages (open gap penalty, 100%; unit gap penalty, 0%) of 16S rDNA sequences published for the genus *Moraxella* and obtained in this study.

rified with the Concert PCR purification kit (Gibco BRL Life Technologies, Merelbeke, Belgium), used according to the manufacturer's instructions. Sequencing was done using the ABI Big Dye cycle sequencing reaction kit with Ampli*Taq* FS DNA polymerase (Applied Biosystems) with the following primers: 5′ AGT TTG ATC CTG GCT CAG (*Escherichia coli* 16S rRNA gene sequence position 8 to 27), 5′CTCCTACGG GAGGCAGCAGT (339 to 358 bp), 5′CAGCAGCCGCGG TAATAC (519 to 536), 5′AACTCAAAGGAATTGACGG (908 to 926), 5′AGTCCCGCAACGAGCGCAAC (1093 to 1112), and 5′GCTACACACGTGCTACAATG (1222 to 1241) (1). Electrophoresis was performed on an ABI 310 analyzer. Analysis of the sequences and clustering was done by GeneCompar, version 2.0 (Applied Maths, Kortrijk, Belgium).

The clinical isolate reported here revealed 100% 16S rDNA sequence identity with U33 (the clinical isolate described by Wüst et al. [7]) and above 99% identity with the other four *M. canis* strains sequenced and with strain NCTC 4103. Less than 96% identity was observed with the 16S rDNA sequences of other *Moraxella* species (3). The dog bite isolate MOR32 was identified as *Neisseria weaveri*, and the *Moraxella* isolate which produced yellow pigmentation on MHAII (LBV438) was identified as *M. osloensis* (Fig. 1).

M. canis can also be differentiated from all other *Moraxella* species by amplification of the tRNA intergenic spacers (6) and determination of the tRNA intergenic-spacer lengths by means of capillary electrophoresis (5). *M. canis* and *M. catarrhalis* had tRNA spacer fragments with lengths of 66, 81, and 215 bp in common, which were absent in *M. caviae*, *M. cuniculi*, *Moraxella lacunata*, *Moraxella nonliquefaciens*, *M. osloensis*, and *M. ovis* and could be differentiated from each other by the presence of a fragment of 193 bp in *M. canis* tDNA PCR fingerprints and the presence of fragments of 167 and 179 bp in *M. catarrhalis* fingerprints.

Despite the fact that *M. canis* was isolated in large numbers from the clinical site, the organism has to be considered an opportunistic pathogen, since this strain was isolated from a heavily debilitated patient, as was the case for the Swiss patient (7). Whether our patient lived in close contact with dogs or cats could not be investigated.

In conclusion, gram-negative oxidase-positive diplococci which produce brownish pigment on MHAII are most probably *M. canis*, a *Moraxella* species that is a commensal of dogs and cats and that exceptionally can be isolated from clinical samples in humans.

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