Human Wound Infection with *Mannheimia glucosida* following Lamb Bite

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*Mannheimia* spp. are veterinary pathogens that can cause mastitis and pneumonia in domestic cattle and sheep. While *Mannheimia* species are difficult to distinguish from one another, typically being identified as *M. haemolytica*, there have been no reported cases of human infection with this organism.

**CASE REPORT**

A 64-year-old man sustained a bite on the right thumb from a 10-month-old lamb. The lamb’s teeth punctured his thumbnail while he was holding the lamb’s mouth open during anthelmintic treatment with a malfunctioning dosing device. He applied epoxy resin to the nail to prevent the shattered edges from catching. Following this application, he developed pain around the wound, and 10 days after the injury, he presented to the hospital. On examination, the nail was discolored, with localized wound tenderness but no discharge, and there was erythema tracking from the right thumb up to the axilla. The wound was explored surgically, the nail plate was removed, and pus was washed out and sent to the laboratory for microscopy and culture. The patient was treated with intravenous flucloxacillin for 24 h, followed by oral cephalexin for 7 days. On review 1 week later, the erythema had resolved and the wound had healed well.

Microscopy revealed large numbers of polymorphonuclear cells, but no organisms were seen on Gram staining. After 1 day of incubation on horse blood agar, there was heavy growth of a Gram-negative bacillus that was catalase positive, oxidase positive, and indole negative. It was hemolytic on 5% horse blood agar and Mueller-Hinton agar containing 5% sheep blood. After a second day of incubation, a lighter growth of *Escherichia coli*, *Enterococcus faecalis*, coagulase-negative staphylococci, and mixed anaerobes was apparent.

With the use of the Vitrek 2 GN card (bioMérieux, Marcy l’Etoile, France), the Gram-negative bacillus was identified as *Sphingomonas paucimobilis* (97% match). Notably, the trehalase reaction was negative, the beta-glucosidase reaction was positive, and the ornithine decarboxylase reaction was negative on the Vitrek card. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based identification was performed using the Microflex LT mass spectrometer (Bruker Daltonik), and the results were analyzed using the MALDI Biotyper software program (version 4.0.0.1). The top score for this Gram-negative organism was *Mannheimia haemolytica*, with a score of 2.177 and the accompanying comment “species of this genus have very similar patterns, therefore distinguishing their species is difficult.” Other *Mannheimia* species appeared in the top 10 identifications listed, including *M. haemolytica* and *M. glucosida*, the highest score for which was 1.305.

Partial 16S rRNA gene sequencing was performed using the MicroSeq 500 bacterial identification kit (Perkin-Elmer/Applied Biosystems, Foster City, CA), with sequence analysis performed on 500 nucleotides using MicroSeq 500 (version 2.2). A result for *M. haemolytica* was reported, with a specimen score of 43 and a 98.4% match (consensus length of 488 bp out of the 489-bp library length). Sequence analysis using GenBank BLAST version 2.0 demonstrated 93% homology with *M. glucosida* (accession no. DQ301921.1) as the top match. The MicroSeq database includes *M. haemolytica* but not *M. glucosida*.

Given the uncertainty surrounding the identity of the isolate, amplification and sequencing were performed on two housekeeping genes, 16S rRNA (1,464 bp; GenBank accession no. KT222023) and partial *rpoB* (534 bp; accession no. KT222022), as well as one virulence gene, *lktA* (2,862 bp; accession no. KT222021), using primers and protocols described previously (1–3). The sequences were then compared and aligned with those obtained previously from ovine mastitis isolates (4), using ClustalW in Geneious version 8.0 (Biomatters Ltd., Auckland, New Zealand). The analysis showed that the 16S rRNA gene from the isolate was 99.91% identical to that of *M. glucosida* isolates F1 and H2, obtained previously from cases of mastitis in sheep (4). A lower level of identity (98.47%) was found with *M. haemolytica* ATCC 33396T. Moreover, the isolate was 100% identical to the two *Mannheimia glucosida* isolates in their partial *rpoB* gene sequences (4) and shared 98.96% identity with the *M. haemolytica* type strain ATCC 33396. The *lktA* gene was 100% identical to that of *M. glucosida* isolate H2, obtained previously from a case of mastitis in a sheep (3), and contained only one nonsynonymous


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substitution compared with M. glucosida isolate PH498 (GenBank accession no. AF314518), an isolate originally obtained from a sheep in the United Kingdom (5). Pairwise nucleotide differences of 6.6 and 16.0% between the leukotoxins of M. glucosida and those of the different alleles of M. haemolytica have previously been reported (5). On the basis of the similarity of the 16S rRNA, from Animals microb Disk and Dilution Susceptibility Tests for Bacteria Isolated and nia.n17, and aids in bacterial survival by interacting with the rpoB genes alone can be inconclusive for identification of this species and needs to be combined with phenotypic tests. Identification of M. haemolytica, however, can be achieved by amplification and sequencing of the rpoB gene, as M. haemolytica isolates from sheep mastitis have been found to be 100% identical in their partial rpoB gene sequences (4).

This is the first case of M. glucosida infection in humans identified using modern molecular methods. The older literature does report human infections with Pasteurella haemolytica, including an aortic graft infection (18), infective endocarditis (19, 20), respiratory infections (21), and superficial wound infections (22). The actual identification of the causative agent in this older literature is clouded by the taxonomic rearrangements that have occurred with Pasteurella haemolytica and the lack of molecular tests at that time. Trehalose-negative strains of the Pasteurella haemolytica complex were transferred to the genus Mannheimia in 1999 (11). In detail, P. haemolytica biogroup 1 became M. haemolytica, containing reference strains of serovars 1, 2, 5 to 9, 12 to 14, and 16 of the former P. haemolytica. Biotypes 3A to 3H and 9 and also serovar 11 of the former P. haemolytica were reclassified as M. glucosida (13). Biotypes and serotypes were not clearly reported in these previously published cases, making any retrospective conversion to the modern taxonomy impossible.

In the current case, a number of phenotypic (Vitek and MALDI-TOF MS) and genotypic (MicroSeq 500) commercial identification systems failed to confidently identify the M. glucosida isolate. It is well recognized that commercial identification systems can have databases that focus on common medical pathogens and have deficiencies for bacteria encountered more rarely in medical cases, such as the Pasteurellaceae (23). While MALDI-TOF MS has been shown to confidently identify some species within the genus Mannheimia, this prior work was limited to just three species, M. granulomatis, M. haemolytica, and M. varigena (24). Misidentification by MALDI-TOF MS has been associated with insufficient numbers of reference strains within the database (25). The difficulties of confident species identification by MALDI-TOF MS reported in the current case are similar to those reported for another genus of the family Pasteurellaceae, Avibacterium (26). The failure of the MicroSeq system to identify the isolate was essentially due to the absence of M. glucosida from the database. Diagnostic laboratories need to be aware that animal bite-based infections have a high likelihood of yielding members of the family Pasteurellaceae (especially Pasteurella multocida) and need to be prepared to use conventional (non-kit-based) sequencing of key genes such as 16S rRNA and rpoB and extensive databases such as GenBank or a more focused, specialized database such as the EzTaxon database (27).

Antimicrobial susceptibility data for Mannheimia spp. are limited, and the available studies have focused on M. haemolytica due to its significance as a veterinary pathogen. Tetracyclines are widely used in veterinary medicine (1) and are often used as a first-line treatment for cases of severe ovine mastitis (28). However, tetracycline resistance has been described for M. haemolytica, M. glucosida, and M. varigena isolates from cases of pneumonia in cattle (29). This resistance is mediated by tetracycline resistance (tet) genes found on plasmids and chromosomes in these isolates and has been demonstrated to be able to transfer horizontally between members of different Mannheimia species (29, 30). In vitro susceptibility surveys have been reported using different methods, including broth microdilution, disk diffusion, and cali-
brated dichotomous sensitivity (CDS) testing. Various rates of resistance to ampicillin, penicillin, and trimethoprim-sulfamethoxazole have been described (29, 31, 32).

This patient did not receive appropriate empirical antibiotics for a polymicrobial animal bite infection including *Pasteurella* or *Mannheimia* species. However, the clinical outcome was favorable, highlighting the importance of appropriate surgical management in these cases.

**Nucleotide sequence accession numbers.** Sequences have been deposited in GenBank under accession no. KT222021 to KT222023.

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**REFERENCES**


