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Case Report

Severe chronic osteomyelitis caused by *Morganella morganii* with high population diversity

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SUMMARY

A case of chronic osteomyelitis probably caused by *Morganella morganii*, occurring over a period of 30 years, is reported. The organism was identified through a combination of sample culture, direct sequencing, and 16S RNA gene amplicon sequencing. Further whole-genome sequencing and population structure analysis of the isolates from the patient showed the bacterial population to be highly diverse. This case provides a valuable example of a long-term infection caused by an opportunistic pathogen, *M. morganii*, with high diversity, which might evolve during replication within the host.

1. Introduction

Morganella morganii is typically prevalent in the environment and in the human intestinal tract as a commensal organism. On rare occasions, it can act as an important opportunistic pathogen, causing infection among postoperative, immunocompromised, and intensive care unit patients. *M. morganii* can be isolated from patients with several types of infection; however, only a few cases with bone involvement have been reported in the literature.[1–4]

A case of severe chronic osteomyelitis caused by *M. morganii* and lasting for approximately 30 years in the patient is reported here. After radical debridement followed by skeletal fixation and antibiotic therapy, the osteomyelitis was cured without recurrence. Further analysis of the bacterial diversity by genomic sequencing of multiple colonies revealed that the bacterial population was highly diverse, supporting the diverse-community model of bacteria during chronic infection suggested in recent studies.[5–7] This case provides a valuable example of a long-term infection caused by an opportunistic pathogen, *M. morganii*, with high diversity, which might evolve during replication within the host.[8]

2. Case report

A 44-year-old man was admitted with a high fever and wound pain, with ulceration and odorous liquid exudation from his left distal thigh. He reported a trauma to the left leg caused by falling from a car approximately 30 years ago during childhood. He had not received treatment, and the affected area had become swollen a few days later. He had developed pain, a high fever, and coma, and nearly died. After receiving treatment at a local hospital involving simple surgery (the details were unknown), his symptoms improved, but a sinus tract near the surgical site emerged with pus effusion, which occasionally self-closed. Over the past 30 years, the patient experienced spasticity in the left knee, and the left lower limb was gradually shortening, but weight-bearing was unaffected. During this period, the patient occasionally experienced pain and ulceration with pale yellow pus oozing

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around the affected area accompanied by a high fever. After antibiotic treatment (data unavailable), his body temperature returned to normal and the skin ulceration area healed. This situation recurred two to three times annually. Thirteen years ago, the patient had gradually developed a limp and the left thigh showed various deformities.

Laboratory tests of the patient's blood showed a white blood cell count of 6.1×10^9 cells/l (normal range 4.0–10.0), erythrocyte sedimentation rate (ESR) of 15 mm/h (normal range 0–30), and C-reactive protein (CRP) level of 6.5 mg/l (normal range 0.3–8). A physical examination revealed that the left limb was 5 cm shorter than the right limb. The femur in the upper left knee joint showed inward angulation and a sinus tract was observed inside the upper left knee with odorous pus effusion. An additional closed sinus tract outside of the upper left knee was observed. Bacterial cultures of the purulent liquid were positive for *M. morganii*. Plain radiographic examination revealed a deformity in the left distal femur and the potential existence of dead bone. Sinus angiography showed that this sinus tract was connected with a bony sequestrum and a very large cavity with a volume of approximately 56.8 mm^3 around the sequestrum (Figure 1A). Three-dimensional reconstruction of computed tomography images revealed disappearance of the left knee joint space, pronation of the left distal femur, and the presence of a large section of dead bone in the medullary cavity (data not shown).

The patient underwent excision of the sinus tract and debridement of the medullary masses (Figure 1B, C). Intraoperative findings, pathology, and culture revealed an abscess caused by *M. morganii*. After skeletal fixation of the left knee joint and left femur, he was given therapy with closed lavage and negative pressure drainage for 3 days and was treated with 1 g intravenous imipenem every 12 h for 5 weeks. Although his markers increased upon completing the operation, the wound gradually closed, and inflammation markers such as the white blood cell count, CRP, and ESR decreased to normal ranges 1 month later. The external fixation was removed 3 months later and the osteomyelitis has not recurred.

3. Methods

To preclude contamination during culture and comprehensively explore the pathogen responsible for this 30-year chronic infection, clinical samples and isolates were further investigated by meta-genome sequencing, 16S RNA amplicon sequencing, and whole-genome sequencing. To identify the pathogen causing this infection with an unbiased comprehensive approach, next-generation DNA sequencing (NGS) was used to directly determine all DNA sequences present in the clinical samples obtained from this patient.

16S RNA gene amplicon combined with deep sequencing is less expensive and time-consuming than NGS. Therefore, 16S RNA gene amplification with primers (forward: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAYTGGGYDTAAAGNG; reverse: CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCACGCCTACNVGGGTATCTAATCC) and subsequent deep sequencing was used to determine whether similar results could be achieved.

To further characterize this patient's clinical isolates, 10 colonies of *M. morganii* were selected randomly and subjected to whole-genome sequencing. Briefly, genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Genomic libraries were

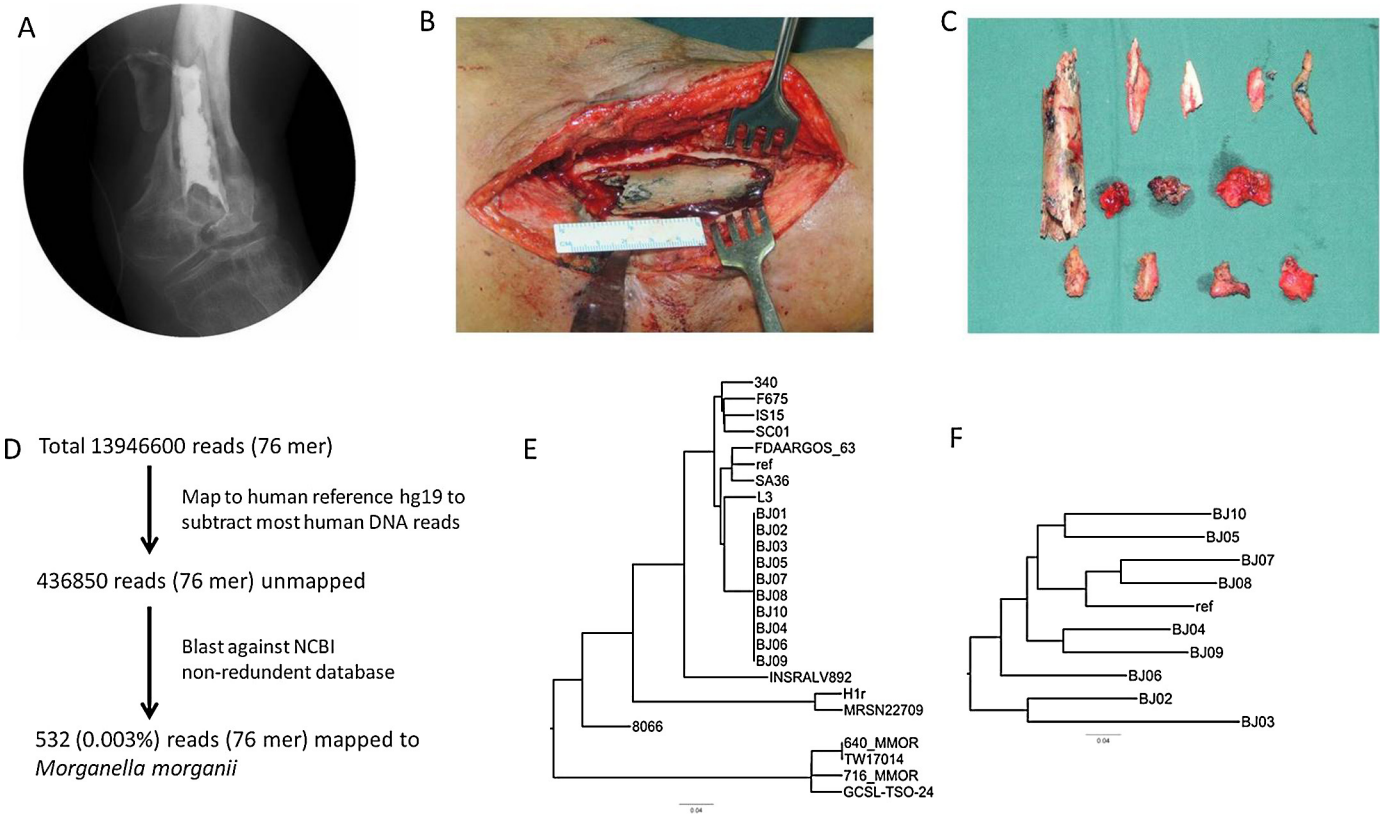

Figure 1. (A) Sinus angiography showing the appearance of a bony sequestrum. (B) (C) Views of dead bones surrounded by normal bones and soft tissues: a total of 12 dead bones were excised from this site, with the longest being approximately 8.35 cm. (D) Detection of potential pathogens by whole-genome sequencing: schematic representation of the direct sequencing of clinical samples. (E) (F) Maximum likelihood dendrogram for 10 clinical isolates together with 16 publically available *Morganella morganii* genomes or 10 clinical isolates, supporting the diverse-community model of the bacterial population during chronic infection.