

Diagnosis of Rare Bone Infection Caused by *Nocardia* by 16S rRNA Gene Sequencing

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Abstract: *Nocardia* is an aerobic actinomycete that causes serious opportunistic infections in immunocompromised individuals. Gene sequencing is the gold standard for pathogenic bacteria diagnosis. This study uses 16S rRNA gene sequencing to diagnose three cases of bone infections caused by *Nocardia*, including one rare case (*N. cyriacigeorgica*), and the clinical features, etiological characteristics, treatment, and prognosis of the patients.

Keywords: *Nocardia*, 16S rRNA gene sequencing, bone infection, clinical features

Introduction

A *Nocardia* infection is an acute, sub-acute or chronic rare disease caused by gram-positive, modified positive-staining acid-fast bacteria that can occur in skin, lung, and disseminated (eg. brain and meninges) infections.¹ Bone infections caused by *Nocardia* are relatively rare, and their clinical features are atypical, which should attract the attention of clinicians and docimasters. This study analyses the clinical manifestations and pathogenic characteristics of three patients with *Nocardia* bone infections who were admitted to the Fourth Medical Center of PLA General Hospital from September 2019 to November 2021.

Case Information

Case 1 was a 62-year-old female with a duration of 33 years who was admitted to our hospital for amputation to eliminate pain due to a crush injury on the right leg caused by a car accident, which had resulted in massive avulsion and infection of the soft tissue and protracted wound healing of the affected limb. After multiple transfers and surgeries, the bacterial cultures were all negative. A physical examination showed several sinus tracts of approximately 0.3 cm in diameter on the anterior and medial sides of the right thigh, a small amount of yellowish exudation, and local tenderness, as shown in Figure 1A. Laboratory tests showed the following: erythrocyte sedimentation rate: 2 mL/h; high-sensitivity C-reactive protein: 0.5 mg/L; blood routine white blood cells: $6.1 \times 10^9/L$; neutrophil: 62.6%; pro-B-type natriuretic peptide: 436.4 pg/mL; and urine routine white blood cells: 11.88/ μL . The magnetic resonance imaging (MRI) results showed discontinuous signal intensity in the right femoral bone, local cortical thickening, a less uniform signal intensity in the medullary cavity, a dot-like slightly long T2 signal, and an abnormal shape and signal intensity in the right femur, which is consistent with the chronic osteomyelitis findings shown in Figure 1B.

On July 5th, 2019, vascular nerve exploration for the debridement of the right-thigh lesion was performed under general anaesthesia. Antibacterial bone cement was used to fill the bone defect site, with resection of the right-iliac mass and external fixation vacuum aspiration. During the operation, tissues and pus were removed and sent for pathological examination and microbial culture. The pathology results showed acute and chronic inflammatory cell infiltration in the fibroadipose tissue of the mass, with small vessel hyperplasia, which is consistent with suppurative inflammatory

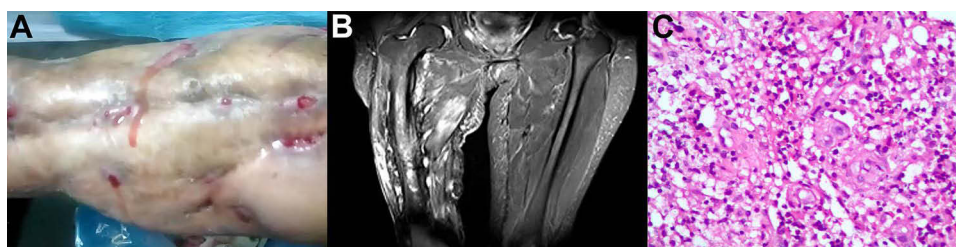


Figure 1 Three images of the affected area in case 1. (A), Features of osteomyelitis caused by *Nocardia otitidiscaviarum*; (B), Magnetic resonance imaging manifestation of chronic osteomyelitis; (C), Pathological examination of septic changes under a 10×20x microscope.

changes, as shown in Figure 1C. The submitted tissue was identified as *N. otitidiscaviarum* by 16S rRNA gene sequencing after 9 days' microbial culture. The patient was discharged after their inflammatory parameters and symptoms had improved with a change in empirical medication gentamicin injection (480,000 units/day), moxifloxacin hydrochloride injection (0.4 g/day) and oral trimethoprim–sulfamethoxazole (0.96 g/day) for symptomatic treatment. Sequential oral trimethoprim–sulfamethoxazole (0.96 g/day) was administered orally, and the patient recovered 12 months later.

Case 2 was a 2-year-old male who presented with ulceration and exudation of the left index finger for more than 1 month caused by a knife cut. The physical examination showed that the wound of the index finger was approximately 1 cm long, with a small amount of bleeding and pain. Laboratory tests revealed the following: erythrocyte sedimentation rate: 15 mL/h; alkaline phosphatase: 275 U/L; high-sensitivity C-reactive protein: 0.7 mg/L; white blood cells $11.9 \times 10^9/L$; neutrophil: 36.7%; and platelets: $403 \times 10^9/L$. The MRI results showed soft tissue swelling around the left index finger with slightly long T1 and T2 signal shadows. Additionally, the middle phalanx lost its normal shape in T1WI, the normal signal intensity of the middle and distal bone marrow disappeared, and the middle phalanx of the left index finger was consistent with osteomyelitic changes. On Aug. 30th 2019, the patient underwent debridement of the lesion in the left index finger and Kirschner wire fixation with vacuum aspiration under general anaesthesia. Intraoperative secretions were removed and sent for pathology, which showed inflammatory cell infiltration in the second bone block, fibrous connective tissue, and immature bone tissue of the left index finger. The secretion was cultured in a Columbia blood plate for 9 days, and the colony was identified as *N. cyriacigeorgica* by 16S rRNA gene sequencing. The patient was given symptomatic treatment with ceftriaxone sodium (0.5 g/day) by injection and discharged 7 days later. On September 23, the erythrocyte sedimentation rate, C-reactive protein, and white blood cell count returned to normal. The patient was discharged and followed up for 12 months and recovered.

Case 3 was a 56-year-old female who underwent endoscope-assisted L2-5 interbody release and interbody fusion cage implantation using a lateral anterior lumbar approach under general anaesthesia on Sep. 8th 2020 due to scoliosis deformity and lumbar spinal stenosis. A posterior route percutaneous minimally invasive pedicle screw system internal fixation was performed. Three months after lumbar surgery, the incision ruptured intermittently during the preceding 1 month and showed significant granulation tissue proliferation, a small amount of yellowish exudation, no redness or swelling around the incision, and normal local skin temperature. Laboratory tests revealed the following: leukocytes: $7.6 \times 10^9/L$; procalcitonin: 0.020 ng/mL; high-sensitivity C-reactive protein: 3.3 mg/L; erythrocyte sedimentation rate: 13 mL/h; and D-dimer: 223 ng/mL. The MRI results showed metal fixation shadows in the L2-5 vertebral body and corresponding intervertebral space, a heterogeneous T2WI signal of the intervertebral disc, and an uneven soft tissue signal in the lower back, suggestive of postoperative changes.

On Jan. 5th 2021, the patient underwent lumbar debridement, sinus tract resection, and closed lavage under general anaesthesia. Sinus secretions were cultured for 9 days and were identified as *N. farcinica* by 16S rRNA sequencing. The patient had a history of a sulphonamide allergy and was discharged with anti-infective treatment with the sensitive

antibacterial drug linezolid (600 mg/day) + imipenem and cilastatin sodium (1000 mg/day) until the wound healed well and the inflammatory parameters were normal.

Pathogenesis-Related Tests

Identification

All three strains of *Nocardia* were sent to the microbiology laboratory for bacterial culture from the samples taken during surgery and were grown on blood culture dishes for 9 days before 16S rRNA gene sequencing. Each *Nocardia* strain was identified by 16S rRNA gene sequencing (1465 bp), primers were the bacterial 16S rRNA universal primer pairs, 27F and 1492R (5'AGAGTTTGATCATGGCTC3', 5'TAGGGTTACCTTGTTACGACTT3'). NCBI GenBank BLAST analysis was used in this study.

(<http://www.ncbi.nlm.nih.gov/blast>, identification accuracy >99.5%), which detected *N. otitidiscaviarum*, as shown in Figure 2A. The colony growth was characterised by dryness, an asteroid appearance and a hard texture, as shown in Figure 2B. The colony growth of *N. cyriacigeorgica*, as shown in Figure 2C, was characterized by white limestone (in colours and shapes), as shown in Figure 2D. The colony growth of the case of *N. farcinica*, as shown in Figure 2E, was characterised by yellowish soft and central convex colonies, as shown in Figure 2F. The three strains tested positive for modified acid-fast staining (the staining solution was purchased from BASO DIAGNOSTICS INC. ZHUHAI. Address: No.286, Tongchang Road, Xiangzhou District, Zhuhai City 519,000, Guangdong Province, China).

Results of Antimicrobial Susceptibility Testing

Eleven antimicrobial agents, including trimethoprim–sulfamethoxazole, linezolid, ciprofloxacin, imipenem, moxifloxacin, cefepime, amikacin, ceftriaxone, minocycline, tobramycin, and clarithromycin, were tested for antimicrobial susceptibility, using broth microdilution. The clinical breakpoints were interpreted with reference to the Clinical and Laboratory Standards Institute M24-2A standards for susceptibility. The antimicrobial susceptibility testing results are shown in Table 1.

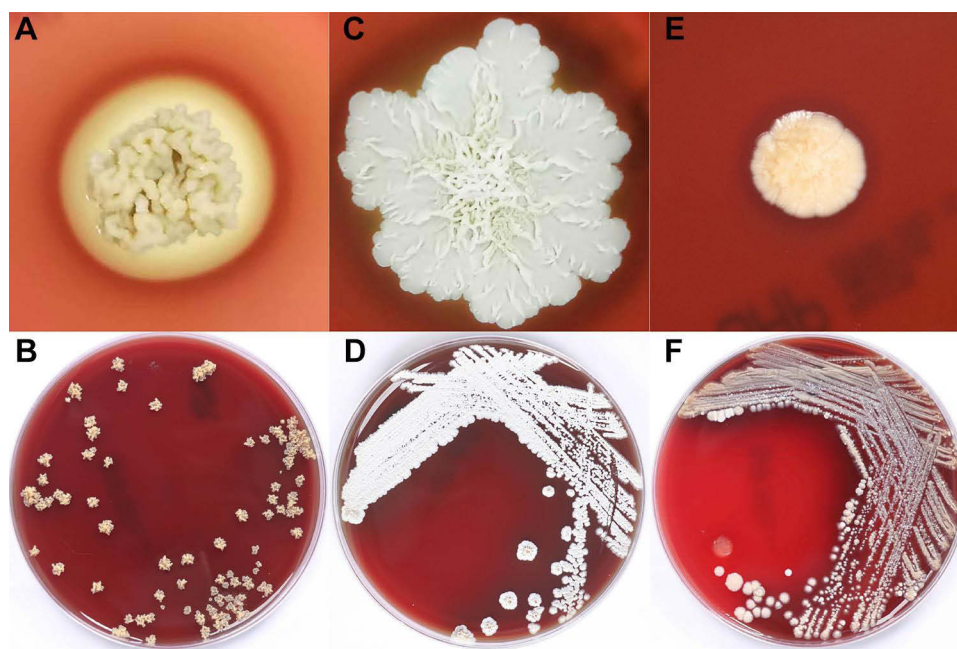


Figure 2 Colony morphology of three *Nocardia* species after 9 days of Columbia blood agar culture. (A and B), *Nocardia otitidiscaviarum*; (C and D), *Nocardia cyriacigeorgica*; (E and F), *Nocardia farcinica*.

Table 1 Antimicrobial Susceptibility Testing Results of Nocardia in Three Patients with Bone Infection

Pathogenic Bacteria	SXT		LZD		CIP		IMI		MXF		FEP		AMI		AXO		MIN		TOB		CLA	
	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R	MIC	R
		I		I		I		I		I		I		I		I		I		I		I
		S		S		S		S		S		S		S		S		S		S		S
Nocardia otitidiscaviarum	1/19	S	≤1	S	I	S	≥16	R	0.5	S	4	S	≤1	S	≥64	R	2	I	8	I	2	S
Nocardia farcinca	1/19	S	2	S	0.5	S	≤2	S	≤0.25	S	32	R	≤1	S	≥64	R	4	I	16	R	4	I
Nocardia cyriacigeorgica	0.25/4.75	S	≤1	S	4	R	≤2	S	I	S	8	S	≤1	S	≤4	S	4	I	≤1	S	≥16	R

Note: Explanation of drug sensitivity:
Abbreviations: SXT, trimethoprim-sulfamethoxazole; LZD, linezolid; CIP, ciprofloxacin; IMI, imipenem; MXF, moxifloxacin; FEP, cefepime; AMI, amikacin; AXO ceftriaxone; MIN, minocycline; TOB, tobramycin; CLA, clarithromycin; S, susceptible; I, intermediate; R, resistant; MIC, minimum inhibitory concentration (μg/mL).

Discussion

In orthopaedic patients, wound infections are a common and difficult problem encountered in clinical practice. A wound infection can easily lead to difficult wound healing, which may cause problems, such as osteomyelitis, and in severe cases may result in disability or partial loss of function. The most common pathogenic bacteria in orthopaedic patients are *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.¹ *Nocardia* infections are rare, atypical, and easily confused with tuberculosis, fungal or non-tuberculous mycobacterial infections, which can lead to misdiagnoses. This is a growing concern.²

Nocardia is an aerobic actinomycete that is commonly found in decaying plants, water, and soil. It causes severe opportunistic infections in immunocompromised individuals, such as patients with acquired immunodeficiency syndrome, chronic granulomas, transplants, and long-term high-dose hormone use.³ A *Nocardia* infection is not thought to be transmitted from person to person and manifests clinically as acute or chronic pyogenic granulomas, often with sinus tract formation, usually occurring in the lungs and skin. According to the three patients in this study who suffered from car accident injury, knife wound, and post-operative orthopaedic infection, respectively, trauma or an invasive operation is a suspected risk factor in the diagnosis of *Nocardia* in immunocompetent people and should not be ignored. The published literature reports approximately 500–1000 cases of *Nocardia* infections in the United States every year, most of which are in the lungs, with only 18.7% in the muscles and bones.⁴ French expert D. Lebeaux et al⁵ showed in their study that from a total of 793 strains of *Nocardia* in 2010 and 2015, joint fluid and bone tissue accounted for 20 cases (2.5%). Yashik Bansal et al⁶ revealed that pulmonary infections accounted for approximately 70% of *Nocardia* infections, and orthopaedic *Nocardia* infections accounted for 4% of patients in northern India. Hao Wang et al's study⁷ analysed a total of 441 strains of *Nocardia* collected from 21 provinces, cities, and autonomous regions in China from 2009 to 2021, of which three strains were present in bone-associated infections, accounting for 0.68% of the study.

Nocardia grows slowly and generally requires approximately 1 week of culture to form more typical colonies, with some slow-growing colonies requiring at least 2 weeks of culture.⁸ In contrast, a negative report can be sent in 2–3 days for common bacteria. For rare *Nocardia* colonies, microbiological testing personnel need to have relatively extensive professional knowledge, and insufficient culture time may be one of the reasons for the missed detection of *Nocardia*. If there is a mixture of these slow-growing bacteria and common fast-growing bacteria when an infection occurs, the fast-growing bacteria will often completely mask the presence of *Nocardia*, thus causing misdiagnoses and missed detection. In clinical practice, pathogenic results are often negative in patients with chronic osteomyelitis. To increase the rate of positive culture detection, it is recommended to discontinue antimicrobial drugs for at least 1 to 2 weeks prior to sampling the infected bone tissue.⁹ When sampling at the site of an implant-associated chronic bone infection, specimens should be taken from at least five sites around the implant to ensure accurate sampling.

From clinical manifestations, the patient in Case 1 in this study, who had a disease duration of up to 33 years, had not been diagnosed after several transfers and surgeries. This patient had the longest duration of *Nocardia* infection osteomyelitis in the world to be reported in detail. This indicates that the clinical characteristics of patients with this bacterial infection are atypical, the anti-infective treatment is not effective, and the disease is persistent. Case 2 was the youngest patient with *Nocardia* osteomyelitis reported globally, indicating that *Nocardia* infections caused in children should not be ignored. Case 3 was postoperative incisional rupture, which suggests the possibility of surgical infection.

Laboratory examinations of patients with a *Nocardia* bone infection showed that the degree of increase in inflammatory parameters, such as white blood cells, C-reactive protein, procalcitonin, and the erythrocyte sedimentation rate, was not specific to other bacterial infections. Alkaline phosphatase was elevated in one patient with osteomyelitis, which may be associated with osteolytic changes. The pathological examination showed inflammatory cell infiltration and suppurative changes, and MRI has great advantages in indicating abdominal and soft tissue lesions and joint and tendon ligament injuries of the extremities. Moreover, the prognosis and recurrence of the patients could be monitored by follow-up examinations, such as computed tomography or MRI¹⁰ scanning.

Nocardia infections remain difficult to diagnose due to the slow growth of the bacteria, and there is no serological method for the clinical diagnosis of this type of infection. Traditional biochemical methods for identifying *Nocardia* are

very time-consuming and can easily lead to a delay in diagnosis.¹¹ Appropriate antimicrobial therapy during the early stage of the disease depends on the early diagnosis of a *Nocardia* infection and is essential in reducing the mortality rate. Gene sequencing is the gold standard for pathogenic diagnosis, and 16S rRNA has high sensitivity and specificity for the molecular diagnosis and strain identification of *Nocardia*.¹² In this study, this method detected three cases of *Nocardia* causing a bone infection: *N. otitidiscaviarum*, *N. cyriacigeorgica*, and *N. farcinica*. In addition, antimicrobial susceptibility varies widely between species based on CLSI M24-A¹³ breakpoints.⁴ Masahiro Toyokawa et al¹⁴ showed that imipenem was 100% resistant to *N. otitidiscaviarum* and that linezolid had the highest sensitivity in in vitro susceptibility testing. According to the research data of scholars in the United States,¹⁵ Japan,¹⁶ and France,¹⁷ trimethoprim-sulfamethoxazole, linezolid, and amikacin have shown good antibacterial activity against *Nocardia*. The results of the present study suggest that the use of compound sulphonamide, linezolid, and imipenem also achieves good therapeutic effects.

In summary, it is rare for *Nocardia* to cause a bone infection. However, to address the poor specificity of clinical manifestations, gene sequencing as the gold standard for pathogenic diagnosis and strong communication between clinicians and laboratory personnel should be improved to facilitate accurate diagnosis and treatment.

Patient Consent and Ethics Statement

Patients have provided informed consent for the case details and images to be published. This study was approved by the Ethics Committee of the Fourth Medical Center of PLA General Hospital, Beijing, China (No. 2022KY130-KS001).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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