Arthritis caused by *Legionella micdadei* and *Staphylococcus aureus*: metagenomic next-generation sequencing provides a rapid and accurate access to diagnosis and surveillance

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Abstract: *Legionella* spp. is an important pulmonary pathogen but rarely causes extra-pulmonary infections. We report a case of joint infection caused by *Legionella micdadei* and *Staphylococcus aureus* in a 54-year-old male with medication history of oral steroid for systemic lupus erythematosus (SLE). He developed arthritis in his right metacarpophalangeal (MCP) joints without precursor pneumonia. In the joint aspirate, *S. aureus* was detected through culture. The existence of *L. micdadei* and *S. aureus* were indicated by metagenomic next-generation sequencing (mNGS) and confirmed by 16S rRNA sequence analysis. After oral levofloxacin treatment for 54 days, the patient's symptoms ameliorated and blood test results improved, which were consistent with the dynamic trend of reads numbers in mNGS data. Our case included, arthritis caused by *Legionella* spp. have been reported in 11 patients. However, our case is the first to report septic arthritis caused by *L. micdadei* in native joints and monitored by mNGS. This case demonstrated an application of mNGS for etiological diagnosis and semi-quantification in joint aspirate. mNGS may serve as a promising tool for rapid and accurate etiological diagnosis and surveillance, contributing to appropriate antimicrobial drug applications and timely medication adjustments when necessary.

Keywords: Arthritis; legionella; high-throughput nucleotide sequencing

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Introduction

Legionella spp. is widely known as the causative pathogen of community-acquired pneumonia, the common symptoms of which include fever, cough, expectoration, dyspnea and radiological signs. Extra-pulmonary infections due to *Legionella spp*. are rare. The difficulties in culture remains to be a challenge in clinical diagnosis.

Metagenomics Next-generation sequencing (mNGS) is a new technique which is increasingly used for infectious diagnosis. It allows for culture-independent identification of various pathogens in complex microbial samples and semi-quantification of pathogen loads. Now we report a case of septic arthritis caused by *L. micdadei* and *Staphylococcus aureus* unexpectedly diagnosed by mNGS.

Case presentation

A 54-year-old male visit the clinic of the Infectious Diseases of Zhongshan Hospital because of new-onset swelling with progressive pain in the 1st and 3rd metacarpophalangeal (MCP) joints of his right hand for 16 days. He denied any symptom of local trauma, fever, respiratory disorder or febrile illness before the onset. He had medication history of oral corticosteroid for systemic lupus erythematosus (SLE). After the onset, he increased the steroid doses without doctor's advice. In fact, he developed progressive pain and swelling in the right elbow joint one month earlier, which was ended up with spontaneous rupture of the joint mass and discharge of dark red pus.

He had a visit to the rheumatologic clinic on the 9th day

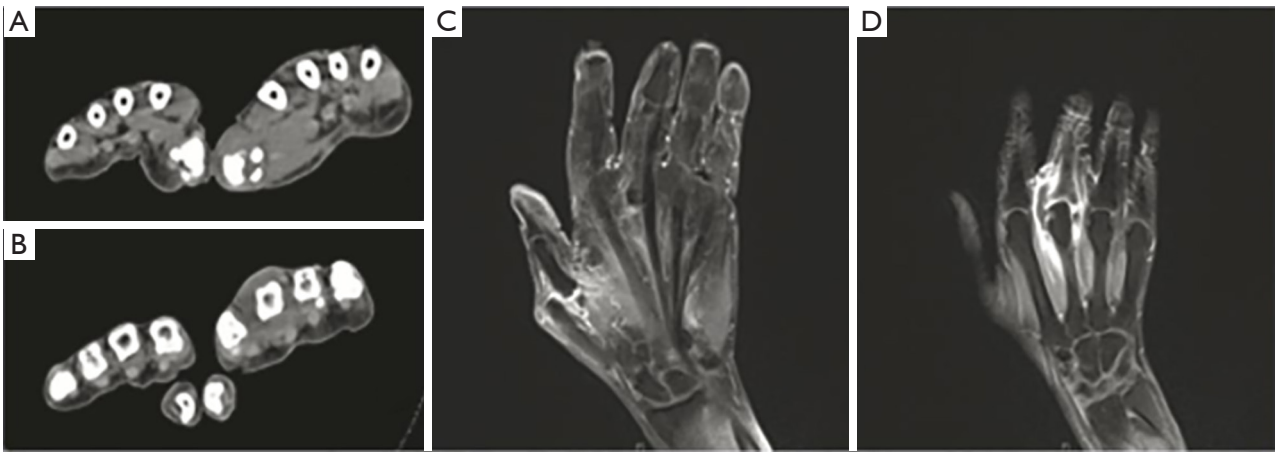

Figure 1 CT and MRI of the patient's 1st and 3rd MCP joints on admission. (A,C) Swelling and subluxation of the patient's 1st MCP joints shown in CT on day 0 and MRI-T2 on day 6, respectively. (B,D) Swelling and subluxation of the patient's 3rd MCP joints shown in CT on day 0 and MRI-T2 on day 6, respectively. MCP, metacarpophalangeal.

of the onset. The initial analysis detected a leukocytosis count of 13.39×10^9/L, neutrophil proportion of 93.1%, a slight increase in erythrocyte sedimentation rate (ESR, 52 mm/h) , significant increase in C-reactive protein (CRP, 117.4 mg/L), undetected pro-calcitonin (PCT) and a similar level of autoantibodies as before (ANA 1:100, dsDNA 100 IU/mL). CT of hands showed some swelling of the 1st and 3rd MCP joints with subluxation (*Figure 1*). His SLE was evaluated as stable, with a possibility of infection. He was not admitted to the rheumatologic ward. The rheumatologic clinician ordered a decreased dose of corticosteroid and a normal dose of oral levofloxacin, which slightly relieved the symptoms in the joints.

The patient was admitted into the ward of Infectious Diseases on the day of the clinic. He was afebrile and normotensive. His MCP joints were swollen, tender and warmer than surrounding tissues, especially in the 3rd MCP joint. Blood tests indicated increased levels (leukocytosis count 19.79×10^9/L, neutrophil proportion 90.9%, ESR 78 mm/h, CRP 242.1 mg/L, PCT 0.33 ng/mL). A purulent aspirate (about 3ml in all) obtained by arthrocentesis on admission was sent to microbiological lab for conventional microbial culture and to a tertiary sequencing lab for mNGS test simultaneously (*Figure 2*).

Brief methods of mNGS were according to the standard protocol of the BGISEQ-100 sequencing platform (BGI, China). (I) DNA Extraction. (II) Library Construction and Sequencing. The DNA extracted was sonicated to generate 200–300 bp fragments. The fragments were then end-repaired, added specific barcodes, amplified by PCR and purified. Quality control was performed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Reads of short (<35 bp) and low-quality were filtered out. Human host sequences (mapped to the human reference genomes hg19) were eliminated. (III) Data processing and analysis. High-quality sequencing data were aligned to the Microbial Genome Database. The mapping sequences were then advanced analyzed. The reference genomes were downloaded from the National Center for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes/) including 3,297 bacteria, 4,152 viruses, 206 fungi, 140 parasites, 104 mycobacteria and 45 mycoplasma/chlamydia, all associated to human diseases (1).

Twenty-four hours later, we received the mNGS report. A large number of standardized strict mapping reads (SDSMR) of *L. micdadei* and small numbers of SDSMR of *S. aureus*, *L. dumoffii* and *L. pneumophila* were detected. The SDSMR numbers of *L. micdadei*, *S. aureus*, *L. dumoffii* and *L. pneumophila* were 63,169, 361, 300 and 31; with genomic coverage rate of 76.05%, 2%, 0.4143% and 0.1958%, respectively (*Figure 3*). In consideration of microorganism genome's rupture into short fragments and the sequence homology among different Legionella species, the *non-micdadei Legionella* reads were assumed as micdadei reads that have mapped to non-micdadei reference sequences. In addition, several skin colonizers such as *Propionibacterium acnes* and coagulase negative staphylococcus were also present in the results (4 and 1, respectively), yet were considered as contamination due to the small numbers of reads and mis-mapping due to the sequence homology among different

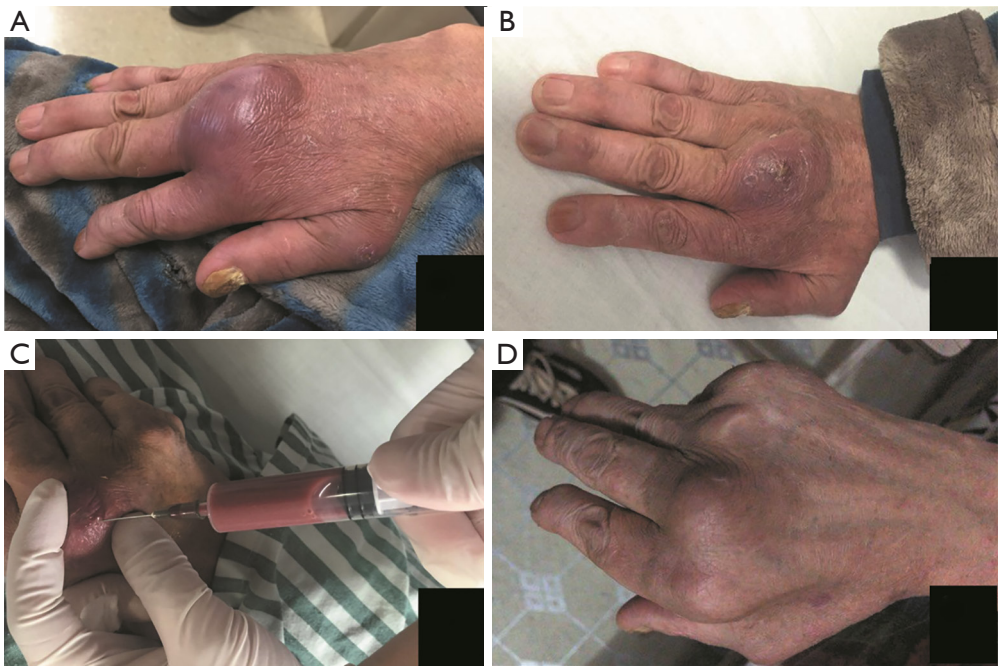

Figure 2 The patient's 3rd MCP joint before, during and after treatment. (A) Day 0. The obviously swollen joint on admission. (B) Day 6. The swelling alleviated significantly after first arthrocentesis and treatment of oral levofloxacin. (C) Day 6. About 10 mL dark red aspirate obtained in the 2nd arthrocentesis. (D) Day 56. The joint was almost recovered. MCP, metacarpophalangeal.

staphylococcus. The following 16s rRNA sequence analysis confirmed the presence of *L. micdadei*. Oral levofloxacin treatment was continued after mNGS test.

Sixty hours later, a growth of methicillin-sensitive *S. aureus* (MSSA) in standard culture medium was reported. The *S. aureus* was sensitive to levofloxacin. In consideration of the patient's economic situation, we did not add new antibiotics although levofloxacin is not a first-choice for S. aureus. Oral levofloxacin treatment was continued.

On day 6, the patient's pain and swelling in the joint were alleviated significantly. Blood tests showed decreases in routine blood test indexes and inflammatory biomarkers (leukocytosis count 14.14×10^9/L, neutrophil proportion 89%, ESR 62 mm/h, CRP 43.8 mg/L, PCT 0.09 ng/mL). MRI still showed swelling and subluxation of the 1st and 3rd MCP joints (*Figure 1*). Purulent aspirate (about 10 mL) obtained by a complete arthrocentesis was sent to the two labs again (*Figure 2*).

Twenty-four hours later, mNGS reported *Legionella* species and *S. aureus* again. Compared to the previous report, most of the bacteria had decreased significantly in sequencing reads (*Figure 3*), which were consistent with the alleviation of symptoms and improvement of laboratory test results. *Propionibacterium acnes* and coagulase negative staphylococcus were negative this time. 54 h later, the conventional culture reported a single MSSA again. The patient was prescribed with another 60 days' oral levofloxacin. Eight weeks later, the patient revisited the outpatient clinic and was found almost fully recovered.

Discussion

This report describes a case of septic arthritis caused by *L. micdadei* and *S. aureus* in an immunocompromised patient. The incidence of legionellosis has been increasing in the United States and in Europe (2-4), with a nearly 3.5-fold increase between 2000 and 2011 in the United States (3). The typical mode of transmission for *Legionella* is inhalation of aerosols which leads to pneumonia. Although rare in frequency, extra-pulmonary infections caused by *Legionella* species includes prosthetic valve endocarditis (5), pyelonephritis (6), sinusitis (7) and cellulitis (8). *Legionella* species may also cause wound infections due to direct inoculation and skin contamination (9,10). The majority of extra-pulmonary infections caused by *Legionella* species occur in immunocompromised individuals (11).

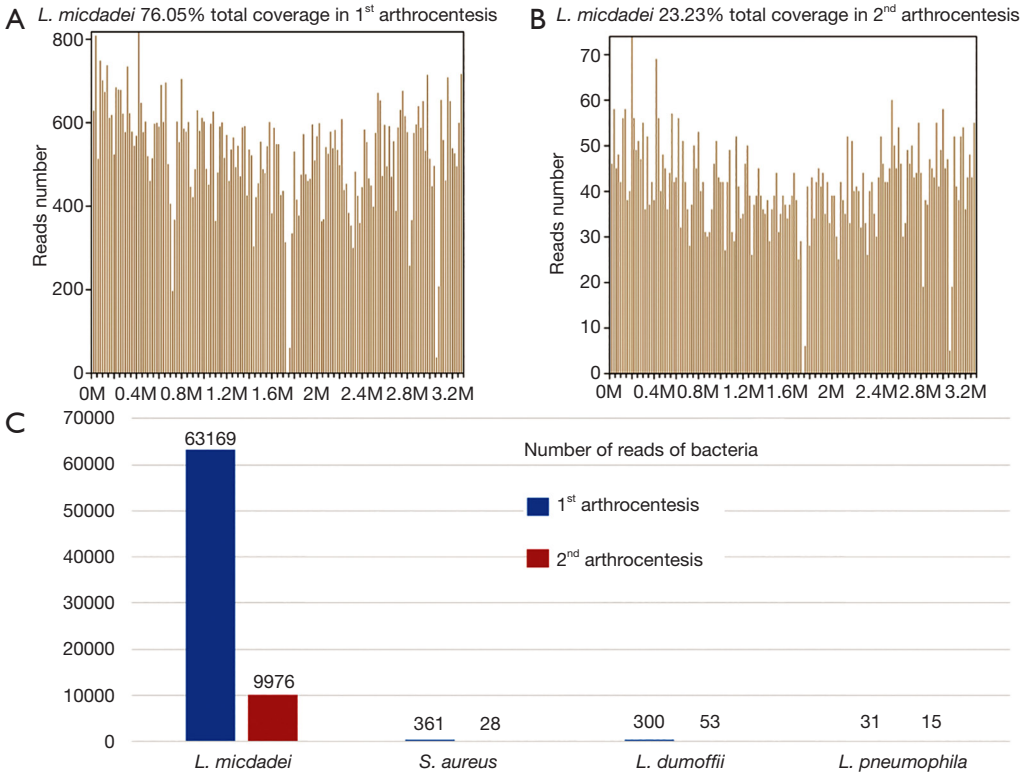

Figure 3 mNGS results in 1st arthrocentesis and 2nd arthrocentesis. (A) In 1st arthrocentesis, 63,169 of bacterial reads corresponded to *L. micdadei*, with a coverage of 76.05%. (B) In 2nd arthrocentesis, 9,976 of bacterial reads corresponded to *L. micdadei*, with a coverage of 76.05%. (C) The most abundant four bacteria in the two arthrocentesis remained the same. The number of reads in 2nd arthrocentesis decreased significantly, which was consistent with the alleviation of patients' symptoms and improvement of laboratory test results. mNGS, metagenomics next-generation sequencing.

To date, ten cases of *Legionella* arthritis have been reported, including eight septic (12-19) and two reactive (20,21) arthritis (*Table 1*). However, we present the first case of septic arthritis in native joint caused by *L. micdadei*, accompanied with *S. aureus*. Our case included, 8/11 patients were immunocompromised. Of the other three cases, two were diagnosed as reactive arthritis and had pneumonia history about 2 weeks before the onset. *L. pneumophila* is the leading pathogen (5/11). The others are *L. longbeachae* (one case) (14), *L. Dumoffii* (one case) (15), *L. micdadei* (two cases, one in prosthetic joint (19) and our case of septic arthritis in native joint), *L. bozemanae* (one case) (16) and *L. cincinnatiensis* (one case) (18). Both small and large joints could be affected. Except for one 32-year-old patient, who had dived using a compressed air breathing apparatus 2 weeks before a CT detected atypical pneumonia and clinical examination detected arthritis (20), all patients were more than 50 years old. Intra-articular injection and aerosolized water therapy may be the potential risk factors. Besides SLE, we did not find other risk factors for *Legionella micdadei* infection.

The urinary antigen test (UAT) and serum antibody examination are common methods for *Legionella* detection. UAT is designed for *L. pneumophila* serogroup 1. To a great extent, it is the availability of this test that *L. pneumophila* serogroup 1 had most confirmed cases. While other species do not have their specific tests and could not achieve such accurate etiological diagnosis. Serum antibody examination requires several weeks for seroconversion. Most microbiological diagnosis was established upon joint specimen culture and 16s rRNA sequence analysis. *Legionella* species grow on special culture media (buffered charcoal yeast extract, BCYE) instead of standard ones, impeding the detection of this pathogen in routine culture. Hence the true frequency of infection may be significantly underestimated. Interestingly, *Legionella*

Table 1 Case reports of arthritis caused by *Legionella* species including the present case

No.	Age	Gender	Medical history and possible risk factors	Species	Diagnostic methods
1 (12)	*51*	Male	Late recurrence of thymoma	*L. pneumophila serogroup 1*	Serology (IgG, IgM)+; UAT+
			Chemotherapy		Culture (aspirate and blood, medium specific for mycobacteria +BCYE. medium)+; Culture (sputum)–
2 (13)	80	Female	Osteoarthritis	*L. pneumophila serogroup 4*	Serology (IgG)+; Culture (Joint tissue, chocolate agar)+
					Immunofluorescence revealed *L. pneumophila serogroup 4* (insufficient material for culture in BCYE medium)
					Culture (blood)-; UAT-
3 (20)	*32*	Male	None	*L. pneumophila*	Serology (*L. pneumophila* antibody *serogroup 1*)+
			Diving using a compressed air breathing apparatus 2w before a CT detected atypical pneumonia and clinical examination detected arthritis		UAT+
					Culture (aspirate， medium not mentioned)–
4 (21)	*80*	*Female*	Chronic renal failure	*L. pneumophila serogroup 1*	UAT+; Gram staining (aspirate)–
			Pneumonia 16d before onset		Culture (aspirate, standard and BCYE medium)–
					16S rRNA PCR+, sequence analysis revealed *L. pneumophila serogroup 1*
5 (14)	56	Male	RA + type 2 diabetes mellitus	*L. longbeachae*	Gram staining(aspirate)+
			Methotrexate + prednisolone		Culture (aspirate, BCYE medium)+, 16S rRNA PCR+, sequence analysis revealed *L. longbeachae*
			No environmental risk factors or precedent pneumonia		Culture (blood)–
6 (15)	58	Female	SLE-like disease	*L. dumoffii*	Culture (aspirate, BCYE medium)+
			Methotrexate + prednisolone		Culture (blood)-
			Intra-articular injection of triamcinolone acetonide in the week prior to admission		16S rRNA PCR+, sequence analysis revealed *L. dumoffii*
7 (19)	83	Female	RA	*L. micdadei*	Gram staining (aspirate)–
			Methotrexate + deflazacort		Culture (aspirate, standard medium)–
			Replacement of the old prosthesis 32m before, progressive pain followed by swelling in her right knee 20m before, pneumonia 8m before admission; aerosolized water therapy at a spa in the year of onset and 2 arthrocentesis at the onset; no large air-conditioning units near the residence		Culture (aspirate, BCYE medium)+
					16S rRNA PCR+, sequence analysis revealed *L. micdadei*
					Culture (sputum)–
					UAT–

Table 1 *(continued)*

Table 1 *(continued)*

No.	Age	Gender	Medical history and possible risk factors	Species	Diagnostic methods
8 (16)	71	Female	Amyopathic dermatomyositis	*L. bozemanae*	Serology (IgG, IgM)+
			Methotrexate+ prednisolone		16S rRNA PCR (aspirate)+, sequence analysis revealed *L. bozemanae*
			A rehabilitation stay at a rheumatologic hospital. *L. bozemanae* not detected from water samples from the patient's home, the rehabilitation hospital or the department		Culture (aspirate, MWY medium)+, MALDI-TOF MS revealed *L. bozemanae* by
					Culture (blood + urine, standard medium)-
9 (17)	70	Female	Thymoma, lymphocytes, low IgG and IgA	*L. pneumophila serogroup 1*	Culture (blood + aspirate, standard medium)-
			No detectable CD19 and CD20		Culture (aspirate, BCYE medium)+
					16S rRNA PCR (aspirate)+, sequence analysis revealed *L. pneumophila serogroup 1*
			Pneumonia 2y before the first onset, native valve endocarditis at the recurrence		UAT-
10 (18)	90	Female	Chronic kidney disease + presumed chondrocalcinosis	*L. cincinnatiensis*	Gram staining (intraoperative pus specimen)+
			Prednisone + etoricoxib		Culture (intraoperative pus specimen, standard medium)-
			Intra-articular corticosteroid before onset. No contact with soil, potting mix or fountains, no respiratory symptoms or febrile illness before onset		16S rRNA PCR (intraoperative pus specimen)+, sequence analysis revealed *L. cincinnatiensis/longbeachae*
					Culture (intraoperative pus specimen, BCYE medium)+, MALDI-TOF MS and MIP gene sequencing revealed *L. cincinnatiensis*
11 (current case)	54	Male	SLE	*L. micdadei*	Culture (aspirate, standard medium) revealed *S. aureus*
			Corticosteroid		mNGS revealed *L. micdadei and aureus, L. micdadei was confirmed by* 16S rRNA sequence analysis

L., Legionella; UAT, urinary antigen test; BCYE medium, buffered charcoal yeast extract medium; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MWY medium, modified Wadowsky Yee medium; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; MIP gene, macrophage infectivity potentiator gene.

species also grew unexpectedly on other culture media, such as chocolate agar (13) and mycobacteria growth medium (12). For culture-negative infections, 16s rRNA sequence analysis may help. Since cases of Legionella infections are still few, it is hard to tell the differences on epidemiology, toxicity, pathogenicity or other biological characteristics between *Legionella micdadei* and other Legionella species. As mNGS gaining its popularity, it would be possible to summarize the characteristics of different Legionella species in future.

Given the single positive result in conventional culture, Legionella infections would not be taken into consideration for the patient. mNGS detected the mixed infection and identified the species, making a great supplement to conventional culture. Besides, mNGS is time-saving because it's culture-independent. In immunocompromised patients, complex mixed infections are very common, where conventional culture-dependent methods might not be enough for etiological diagnosis. mNGS is a new technique that is increasingly used for the clinical diagnosis of infectious diseases. Theoretically, with sufficiently long reads, multiple hits in the microbial genome, and a complete reference database, most microorganisms can be uniquely identified through mNGS (22). Althrough PCR based testing other than next generation, full-genome sequencing can be used to seek viral and fungal pathogens, mNGS has its superiority in diagnosis: (I) that a single test is able to identify bacterial, viral, fungal and parasitic pathogens, (II) that it is able to identify the presence of multiple organisms, (III) that it is able to provide a relative quantification of the multiple organisms present in a single sample and (IV) that it greatly saves time, especially for the mycobacteria infection. Several case reports and clinical studies have demonstrated the use of mNGS as a promising diagnostic tool for infectious diseases (22-24). Our case is the first one in which septic arthritis was diagnosed through mNGS of the aspirate sample.

Currently, clinical surveillance of infection progression is mainly guided by clinical manifestations, routine laboratory tests and culture. However, we still need a tool to directly monitor the dynamics of the pathogen. Apart from etiological diagnosis, mNGS offers a quick way to semi-quantitively monitor the changes in pathogen loads. Thus we consider mNGS a rapid method for disease surveillance (25). Our case implies the promising potential of mNGS as an effective tool for rapid and accurate infection diagnosis and surveillance.

Sample contamination is a common problem in mNGS. There were several possible contaminating microorganisms in this study. In consideration of the high sensitivity of mNGS, the relatively small reads numbers and the sequence homology among different staphylococcus, *P. acnes* and coagulase negative staphylococcus were considered as contamination. This contamination mainly caused by commensal microbes on the skin surface and are also very common in culture (especially coagulase negative staphylococcus). Similarly, in sputum sample, commensal oral flora such as S*treptococci* and anaerobe are frequently reported. Clinicians should be familiar with the background microorganisms in various parts of human body where specimens are collected. However, sometimes commensal microorganisms and pathogens can perform mutual transformations.

In conclusion, we present the first case of septic arthritis in native joint caused by *L. micdadei*, accompanied with *S. aureus*, diagnosed through mNGS. Conventional culture may not be able to detect all the pathogens. mNGS is a good supplement for rapid and accurate etiological diagnosis and surveillance. mNGS may play an important role in complicated infections, especially in immune-compromised patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

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