

CASE REPORT

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Community-acquired pneumonia caused by *Micrococcus antarcticus*: a rare case report

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Abstract

Background *Micrococcus antarcticus* (*M. antarcticus*) is an aerobic Gram-positive spherical actinobacterium that was initially isolated from Chinese Great-Wall station in Antarctica in 2000. *M. antarcticus* was considered to be of low pathogenicity, no previous cases of human infection by this organism have been reported. Here we describe the first report with community-acquired pneumonia (CAP) caused by *M. antarcticus*.

Case presentation An 87-year-old female was presented to the Central Hospital of Wuhan in November 2023 with a chief complaint of cough, sputum production, and chest tightness for 2 weeks. Microbial culture of the patient's bronchoalveolar lavage fluid (BALF) and identification of the isolates using Matrix-assisted laser desorption ionization/time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing revealed *M. antarcticus* infection. Combined with clinical symptoms, laboratory and imaging examination, the patient was diagnosed with CAP. Then cefoperazone/sulbactam and levofloxacin was administrated, the patient's condition was improved and she was discharged after a week after admission, no abnormalities were detected during a 5-month follow-up.

Conclusions This case highlights that *M. antarcticus*, first identified from a patient with CAP, is an extremely rare pathogenic microorganism. Clinicians should be aware of its potential as a pathogen in the diagnosis and treatment of CAP.

Keywords *Micrococcus antarcticus*, 16S rRNA gene sequencing, MALDI-TOF MS, Community-acquired pneumonia, Case report

Background

The genus *Micrococcus* is a Gram-positive, aerobic, non-motile, and non-sporeforming actinomycetes that are generally isolated from various habitats, such as activated sludge [1], feces of black pig [2], aquatic plant [3–6], oreochromis niloticus in Egypt [7], human skin [8], soil [9], air [10], and dairy industry waste [11]. The typical perception of *Micrococcus* species is that they are non-pathogenic, yet there have been occasional instances of *Micrococcus* infections among individuals with compromised immune systems [12]. *M. antarcticus*, a member of the genus *Micrococcus*, was originally isolated from Chinese Great-Wall station in Antarctica in 2000 [13]. Literature review revealed a series of researches on the

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discovery and identification of β -glucosidase BglU, which is related to the cold-adapted mechanism of *M. antarcticus*, and its relevant molecular structure basis [14, 15]. However, the role and pathogenicity of this bacterium in the field of medicine remains unknown. Currently, no reports of human infection caused by *M. antarcticus* have been found. Hence, we present the first case of community-acquired pneumonia case caused by *M. antarcticus* in China.

Case presentation

On November 21, 2023, an 87-year-old female patient, known for a medical history comprising coronary atherosclerotic heart disease, hypertension, renal insufficiency, colon polyps, and previous treatment for endometrial cancer, was admitted to our hospital with a chief complaint of “cough, sputum production, and chest tightness for 2 weeks”. The patient presented with symptoms including a runny nose, nasal congestion, mild cough, fatigue, poor appetite, without fever, headache, or palpitations, but accompanied by nausea after catching a cold 2 weeks ago. Despite self-administering of “Chinese herbal medicine” in an attempt to alleviate symptoms, the cough exhibited a progressive aggravation with no relief. The physical examination revealed the following: body temperature of 36.5 °C, pulse rate of 87 beats per minute, blood pressure of 113/81mmHg, blood oxygen saturation of 97%, coarse breath sounds in both lungs, and moist rales audible in the lower left lung. The laboratory test results are as follows: white blood cell count of $5.51 \times 10^9/L$ (75.2% neutrophils), platelet count of $100 \times 10^9/L$ (normal 125–350 $\times 10^9/L$), erythrocyte sedimentation rate of 51 mm/h (normal 0–20 mm/h), hyper-sensitive C-reactive protein (whole blood) of 8.82 mg/dL (normal <7 pg/mL), interleukin-6 of 8.54 pg/mL (normal <7 pg/mL), creatinine of 108.9 $\mu\text{mol/L}$ (normal 41–81 $\mu\text{mol/L}$), uric acid of 437 $\mu\text{mol/L}$ (normal 155–357 $\mu\text{mol/L}$), and estimated glomerular filtration rate of 39.46 mL/min/1.73m² (normal >90 mL/min/1.73m²). In addition, no significant abnormalities were identified in the tests for cardiac troponin, SARS-CoV-2, *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, influenza A, and influenza B. Furthermore, the chest computed tomography scan showed enhanced texture in both lungs, and a patchy clouding opacity can be seen in the left lower lung, which suggested a newly developed lung infection in the patient (Fig. 1A). Based on the results mentioned above, the patient was diagnosed with CAP and was prescribed empirical antimicrobial treatment with intravenous (i.v.) cefoperazone/sulbactam 2 \times 1 g/day (q 12 h) and levofloxacin 1 g/day (qd). This empirical treatment was selected to provide broad-spectrum coverage against potential Gram-positive and Gram-negative pathogens.

Following that, a bronchoscopy was performed on the patient under general anesthesia, which showed inflammation of the bronchial mucosa bilaterally (Fig. 1B). BALF was then collected from the affected area, gram stained and inoculated onto Columbia blood agar plates, MacConkey agar plates, chocolate agar plates, and Sabouraud agar plates (Guangzhou Dijing Microbial Technology Co., Ltd., Guangzhou, China). The plates were incubated at 35 °C with 5% CO₂ for 18–24 h.

Actually, a large number of gram-positive cocci were observed under the microscope by Gram staining of BALF, some of which were distributed in and around the leukocytes. Moreover, in the BALF culture of the patient, three bacterial species have been isolated and classified by MALDI-TOF MS (Bruker Daltonik GmbH, Germany), including *Micrococcus sp.*, *Streptococcus oralis*, and *Streptococcus parasanguinis*, among which *Micrococcus sp.* was the dominant bacterium. Since *Streptococcus oralis* and *Streptococcus parasanguinis* are part of common normal flora, and their colony counts were significantly lower than that of *Micrococcus sp.*, the isolated *Micrococcus sp.* named CAP44B11 was considered to be the causative agent in this case. The strain CAP44B11 is yellow, mucoid, smooth-edged and circular colony on the Columbia blood agar plate which was confirmed to be Gram-positive cocci through Gram staining (Fig. 1C).

Subsequently, 16S rRNA gene sequencing was employed for further identification. Genomic DNA was extracted from purified bacteria using the TIANamp Bacteria DNA Kit (TIANGEN Biotech, Co, Ltd, Beijing, China) in accordance with the instructions provided. The 16S rRNA gene was amplified using universal primers (forward, 5'-AGTTTGATCCTGGCTCAG-3'; reverse, 5'-GTATTGCCGCGGCTGCTG-3') and sequenced. A total of 1,413 contiguous nucleotides were obtained. The complete 16S rRNA sequence of the strain CAP44B11 was analyzed with the EzBioCloud Database [16]. The strain CAP44B11 exhibited the highest (99.58%) 16S rRNA gene sequence similarity with the type strain of *M. antarcticus* T2^T (accession no. AJ005932). Among the 1,413 bases, there was only a six-base difference between the two strains. The CLUSTALW algorithm was used to calculate the multiple alignments and sequence similarity levels of the most closely related *Micrococcus* species [17]. The phylogenetic tree was constructed using the neighbor-joining method in MEGA software version 11 [18]. Bootstrap resampling method of Felsenstein (1985) was employed to repetitively evaluate the topology of the phylogenetic tree through 1000 replicates [19]. The above results demonstrated that strain CAP44B11 was clustered with the type strain *M. antarcticus* T2^T, with a bootstrap value of 99% (Fig. 2), which indicated that the isolated strain CAP44B11 belongs to the *M. antarcticus* species. The 16S rRNA sequence of *M. antarcticus*

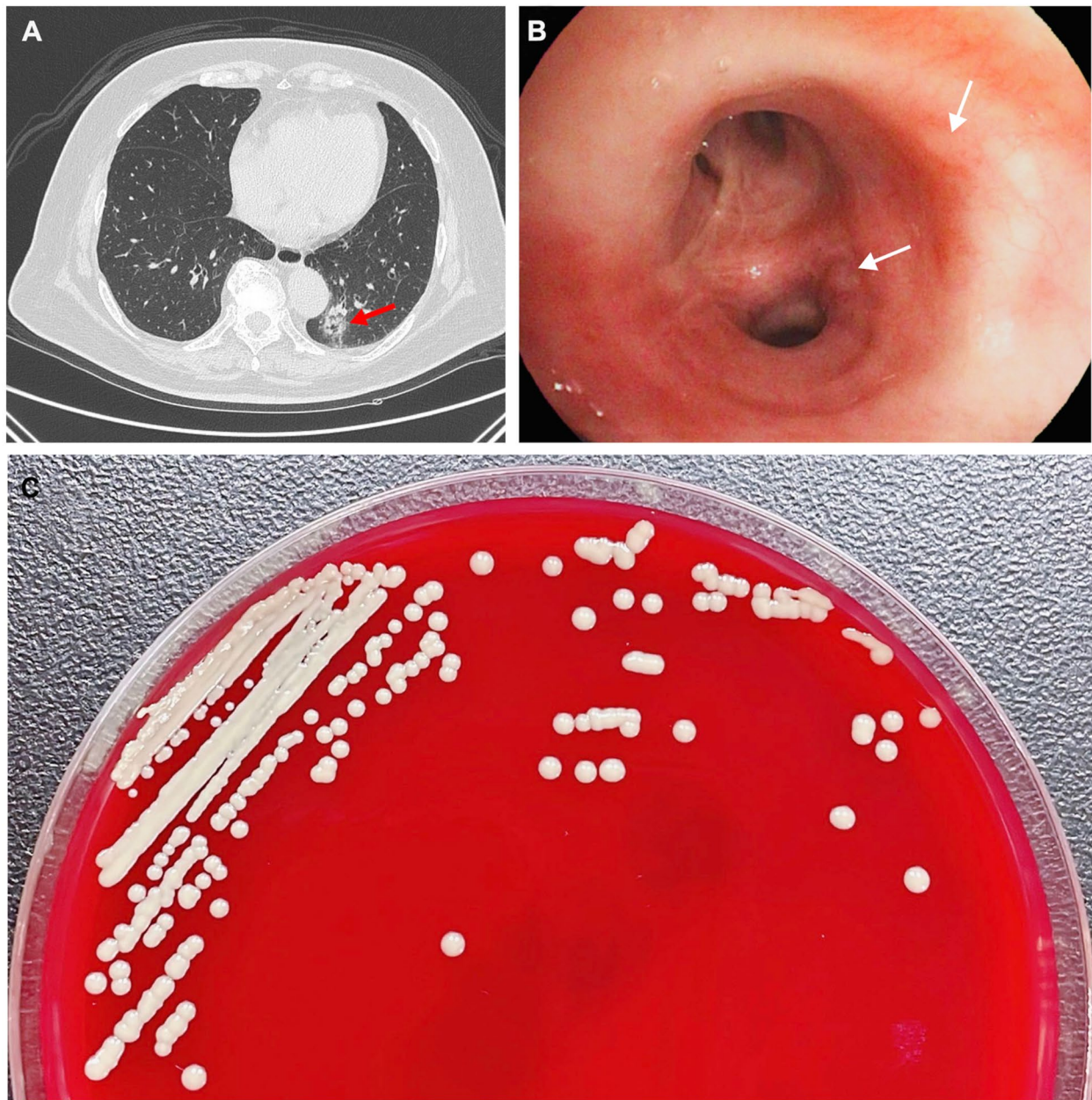


Fig. 1 Results of computed tomography (CT) and electronic bronchoscope, and the morphology of the *Micrococcus antarcticus* strain CAP44B11. **(A)** Chest CT demonstrating infectious lesions in the lower left lung (red arrow). **(B)** The electronic bronchoscope shows bronchial mucosa inflammation with redness and purulent secretion as indicated by white arrows. **(C)** Bacterial colonies on Columbia blood agar after being cultured at 37 °C in the presence of 5% CO₂ for 24 h.

CAP44B11 has been deposited into GenBank under accession number of PP535566.

AutoMic-i600 platform (Autobio Co., Ltd., Zhenzhou, China) was used to test the antimicrobial susceptibility of the strain *M. antarcticus* CAP44B11, and *Staphylococcus aureus* ATCC 25,923 and *Escherichia coli* ATCC 25,922 were used as the quality control strains. The drug susceptibility was executed in accordance with the EUCAST

2024 standard V 14.0 [20]. According to the results, the *M. antarcticus* demonstrates sensitivity to a majority of antibiotics, including ceflorin, ciprofloxacin, and moxifloxacin, etc. (Table 1). Although the *M. antarcticus* was sensitive to ceflorin and ceftiofur, cefoperazone/sulbactam offers a broader antimicrobial spectrum. The use of levofloxacin was based on the patient’s sensitivity to ciprofloxacin and moxifloxacin, indicating a good response

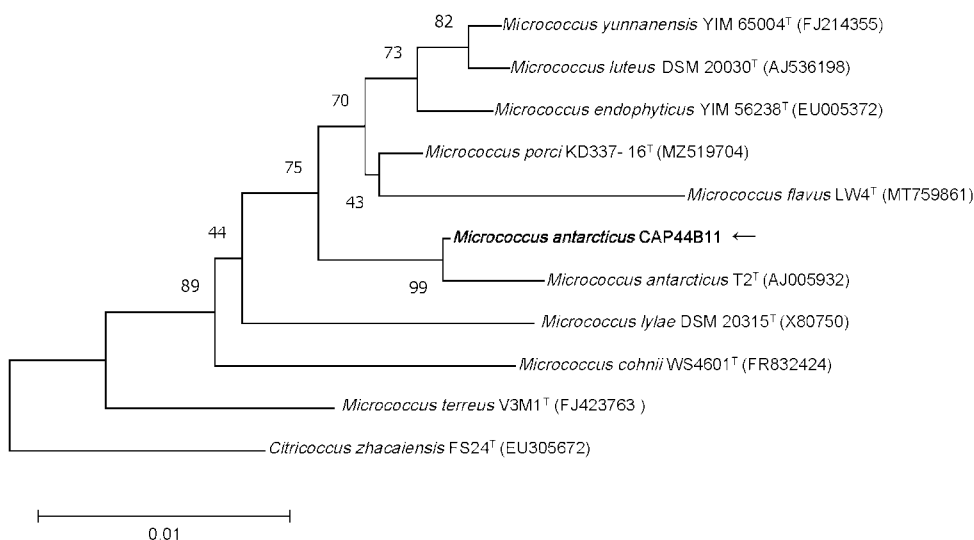


Fig. 2 The phylogenetic tree based on the 16S rRNA gene sequences showing the relationship of isolated strain CAP44B11 (black arrow) and members within genus *Micrococcus*. The tree was reconstructed by the neighbor-joining method, and *Citricoccus zhacaiensis* FS24^T (EU305672) was used as an outgroup. Bootstrap values (> 50%) based on 1,000 replicates are shown at branch nodes. T, type strain.

Table 1 Drug susceptibility results of *M. Antarcticus* CAP44B11

Antibiotics	MIC (ng/μL)*	Antibiotics	MIC (ng/μL)
Penicillin	≤ 0.12(S)	Ampicillin	≤ 0.5(S)
Ceflorin	≤ 0.12(S)	Cefoxitin	≤ 1(S)
Oritavancin	≤ 0.015(S)	Teicoplanin	≤ 1(S)
Ciprofloxacin	≤ 0.12(S)	Vancomycin	≤ 0.5(S)
Tetracycline	≤ 1(S)	Moxifloxacin	≤ 0.12(S)
Gentamicin	≤ 2(S)	Tigecycline	≤ 0.03(S)
Co-trimoxazole	≤ 0.5/9.5(S)	Linezolid	≤ 1(S)
Rifampicin	≤ 0.015(S)	Daptomycin	≤ 0.12(S)
Clindamycin	≤ 0.25(S)	Erythromycin	≤ 0.25(S)

* Drug sensitivity was judged according to EUCAST 2024 standard V 14.0. S: sensitive; I: intermediate; R: resistant

to fluoroquinolones. Combining cefoperazone/sulbactam with levofloxacin can provide a synergistic effect, enhancing overall treatment efficacy and reducing the risk of resistance to a single drug. After a comprehensive evaluation of the patient, blood cultures were not performed before initial antibiotics. However, after 5 days of treatment with cefoperazone/sulbactam combined with levofloxacin, the patient’s symptoms showed significant improvement, indicating that the treatment is feasible and effective. Then the patient was discharged after a week, no abnormalities were detected during a 5-month follow-up.

Discussion and conclusions

The genus *Micrococcus*, classified within the family *Micrococcaceae*, order *Micrococcales*, and phylum *Actinomycetota*, was originally proposed by Cohn (1872) and later revised by Stackebrandt et al. [21] and Wieser

et al. [22] based on the utilization of glucose, analysis of the G+C content of the genomic DNA and phylogenetic analysis of the 16S rRNA gene. Up to date, eight species have been identified with validly published and correct names under the List of Prokaryotic Names with Standing in Nomenclature (LPSN) [23]: *Micrococcus antarcticus*, *Micrococcus cohnii*, *Micrococcus endophyticus*, *Micrococcus flavus*, *Micrococcus luteus*, *Micrococcus lylae*, *Micrococcus porci*, and *Micrococcus terreus*. Members of the genus *Micrococcus* are Gram-positive, aerobic, non-motile, and non-sporeforming actinomycetes. Among *Micrococcus spp.*, only a few species have been reported to be associated with human infectious diseases. Notably, *Micrococcus luteus* have been implicated in various conditions, such as brain abscess [24], bloodstream infection [25], infecting endocarditis [26], bacteremia in immunocompromised patients [27]. Additionally, peritoneal dialysis-associated peritonitis has been attributed to

Micrococcus aloeverae [28], while community-acquired pneumonia has been linked to *Micrococcus yunnanensis* [12]. Nevertheless, there are no accounts of human infection with *M. antarcticus*, so its pathogenicity remains largely unexplored. In this instance, we reported the debut case of CAP caused by *M. antarcticus*, aspiring to offer further insights into this rare infection.

CAP presents as a significant public health challenge worldwide, characterized by high mortality and morbidity rates along with economic burdens. Despite its impact, research on CAP in China has been relatively limited, with increasing attention being drawn to this issue only in recent years. The research conducted by Academician Xu's team at the Chinese Center for Disease Control and Prevention has revealed that the incidence of CAP in China is higher than previously understood [29]. Moreover, studies indicate that bacteria still hold a dominant position in adult pneumonia cases [30]. Therefore, the prompt and accurate identification of causative pathogens is of paramount importance for the early diagnosis and management of CAP. There have been previous reports about CAP caused by *Micrococcus yunnanensis* [12], and in this case, we have documented a case of CAP caused by *M. antarcticus*, highlighting the importance of considering the potential for *Micrococcus spp.* infections in the diagnosis of CAP in the future.

M. antarcticus was initially discovered from Chinese Great-Wall station in Antarctica, with studies indicating its optimal temperature to be 15–17 °C [13]. However, it is noteworthy that *M. antarcticus* was detected under the conditions of overnight cultivation in a 5% CO₂, 35 °C incubator, which diverges from its typical cold adapted habitat. This finding in a clinical patient raises intriguing questions about its environmental adaptability and potential implications. As demonstrated in Chen's study [31], *Aeromonas salmonicida* is a commonly known cold-water pathogenic bacterium, and its most notable and characteristic virulence factors are aerolysin and hemolysin. Interestingly, the genes *aerA* and *hlyA*, which encode these toxins, were found to be significantly upregulated at both 28 °C and 37 °C. This indicates that the virulence and environmental adaptation-related genes of certain bacteria are significantly influenced by the environmental temperature. Although the optimal growth temperature for *M. antarcticus* is relatively low, it may have acquired the ability to survive and grow under non-optimal conditions through genetic mutations or other evolutionary mechanisms. This could enable it to briefly survive within the human body and potentially cause infection. Nevertheless, further investigations are required to substantiate this hypothesis.

Previous research has revealed that individuals with immunocompromised function are more susceptible to be infected by *Micrococcus spp.* [24, 25, 32], and age

is a well-known risk factor for CAP, especially among the older population [33]. In this case, the patient is an 87-year-old elderly woman with a previous medical history of surgical intervention for endometrial cancer in 2022 as well as diagnoses of coronary artery atherosclerosis heart disease, hypertension, and renal dysfunction. Hence, she is considered to be part of the immunocompromised group, which could be the underlying reason for her CAP caused by the infection of *M. antarcticus*. However, the patient has denied having been to Antarctica recently, so the exact source of the isolated bacteria remains unknown. Nonetheless, the elevated inflammatory markers in the patient and the identification of the pathogenic bacteria isolated from the BALF have confirmed the presence of an infection.

In recent years, MALDI-TOF MS has emerged as a widely utilized method in microbiology laboratories worldwide, offering a rapid and reliable identification of pathogenic bacteria based on phenotype and specific proteomic characteristics. This technique is valued for its simplicity, high accuracy, and cost-effectiveness. Recent studies have also highlights its significant importance in the classification and identification of specific microbiota [34]. However, despite its numerous advantages, MALDI-TOF MS does have its limitations, particularly in the identification of novel or rare bacterial species [35, 36]. Take our case for example, MALDI-TOF MS failed to accurately identify the isolated bacteria at the species level. Thus, we employed 16S rRNA gene sequencing, a technique widely used for identifying isolates that cannot be determined by conventional methods [37]. Through this approach, the causative organism was finally confirmed as *M. antarcticus*, highlighting the utility of 16S rRNA gene sequencing as an alternative method in situations where MALDI-TOF MS fails to yield satisfactory results, particularly in the identification of uncommon or rare bacteria. Thus, 16S rRNA gene sequencing emerges as a valuable and effective option for overcoming challenges encountered in microbial identification.

This case report presents the first documented instance of CAP caused by *M. antarcticus*, with the patient exhibiting satisfactory recovery after receiving appropriate symptomatic treatment. Our findings suggest that the potential emergence of *M. antarcticus* as a pathogenic agent capable of causing human infections. 16S rRNA gene sequencing facilitated this rare pathogen identification in our case and should be recommended for the identification of unknown bacteria. However, the underlying mechanism of *M. antarcticus* induced human infections remains unclear. Further research is therefore required to explore the virulence, pathogenicity, and other characteristics of this bacterium in order to gain a better understanding of *M. antarcticus*.

Abbreviations

CAP	Community-acquired pneumonia
BALF	Bronchoalveolar lavage fluid
MALDI-TOF MS	Matrix-assisted laser desorption ionization/time of flight mass spectrometry
AST	Antimicrobial susceptibility test

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Not applicable.

Author contributions

JX, YZ, and ZL conceived and designed the research. LS and HW collected the clinical data. JX and YZ performed the experiments and analyzed the data. MZ performed quality control. JX drafted the primary manuscript. ZL reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analyzed during the current study are available in the GenBank under accession number of PP535566.

Declarations

Ethics approval and consent to participate

The study was approved by Medical Ethics Committee of the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology (Project no. WHZXKYL2023-094).

Consent for publication

Written informed consent was obtained from the patient for publication of case report and accompanying images.

Competing interests

The authors declare no competing interests.

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