



Case report

A case of chronic wounds caused by *Sporothrix schenckii* infection was rapidly detected by metagenomic next generation sequencing

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ABSTRACT

The dimorphic fungus *Sporothrix schenckii* is widely distributed in soil, vegetation, and decaying organic matter, and can cause sporotrichosis when the patient's skin trauma was exposed to contaminated material with *Sporothrix* spp. The cases of *Sporothrix schenckii* infection in chronic wounds are rarely reported. Here we reported a 53-year-old male construction worker who was admitted to our hospital on July 9, 2022, without underlying disease presented with a painless subcutaneous hard nodule on his right calf, which later ulcerated and oozed, with an enlarged wound and no fever during the course of the disease. His procalcitonin, C-reactive protein, erythrocyte sedimentation rate increased, and necrotic histopathology suggested chronic granulomatous inflammation. Then his necrotic tissue and pus were sent for metagenomic next generation sequencing (mNGS), the result reported *Sporothrix schenckii* after 43 hours, which was consistent with the result of culture after 18 days. mNGS might be more useful and valuable in diseases such as sporotrichosis where it is difficult to see the yeast cells in the tissues.

1. Introduction

Chronic wounds can occur in the patients of all ages with a long course, resulting in not only poor quality of life but increase of the medical burden on families and society. The causes of wounds include nutritional and metabolic diseases, genetic diseases, vascular diseases, autoimmune diseases, tumors, hematologic diseases, drug-related factors, dermatologic diseases, infectious diseases, mental

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disorders, etc. Chronic infectious wounds caused by pathogens are relatively rare [1]. Sporotrichosis is a disease caused by *Sporothrix* spp. which is a dimorphic fungus, characterized by chronic nodules or ulcers in the skin and subcutis. The pathogenic clade mainly include four phylogenetically closely related species: *Sporothrix schenckii sensu stricto*, *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix luriei*. These species differ in geographic distribution, host range, virulence, and antifungal susceptibility [2]. *Sporothrix globosa* was considered as the highest prevalence and clinical importance Chinese species, particularly in northeastern China [3]. The clinical symptoms of sporotrichosis are difficult to distinguish from infections caused by other pathogens such as *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, *Francisella tularensis* and etc [4]. Methods for diagnosing sporotrichosis include culture, histopathological examination, serology testing, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), and polymerase chain reaction (PCR). *Sporothrix* spp. grow at 28 °C on medium with Sabouraud dextrose agar or at 37 °C in a brain-heart infusion medium (BHI). The colony microscopy reveals branched septate hyaline hyphae and hyaline conidia at room temperature, but it can revert the mycelium-to-yeast form in BHI, after incubation at 37 °C [5]. Serological ELISA test using the *S. schenckii* concanavalin A-binding (SsCBF) may be useful in the diagnosis of sporotrichosis [6]. MALDI-TOF MS has been used for species identification of the *S. schenckii* species complex based on measurement of proteins [7]. It can be distinguished and identified at the species level by gene sequencing based on PCR using genetic loci such as translation elongation factor, ITS, CAL, and etc [8]. Delayed diagnosis may lead to the appearance of more nodules, abscesses, and even the spread of infection to other organ system throughout the body, making the disease worse. In this study, we reported a case of chronic wounds caused by *Sporothrix schenckii* infection that was rapidly detected by metagenomic next-generation sequencing in South China.

1.1. The study

A 53-year-old male construction site worker was admitted to our hospital with chronic wounds but no underlying disease. Four months before admission, there was no obvious cause of depression edema in the middle or lower parts of the right thigh, with several subcutaneous indurations of the skin on the inner side of the right leg, gradually breaking the exudate. Although there was a history of external application of traditional Chinese medicine, the drainage wound was cleaned in the local hospital and the patient was administered cefuroxime for one week, but despite this the wound became further enlarged. No chills, fever, joint pain, joint swelling or pain, limb dysfunction or sensory abnormalities were detected. Dermatological examination revealed an ulcer of approximately 16 × 11 cm on the inner side of the right calf, deep into the muscle layer, with exudation. The wound base was red and covered with a small amount of necrotic tissue. The skin pigmentation around the wound was changed, and the skin temperature was slightly higher in this area. Several dark red hard nodules of different sizes were observed around the wound, without tenderness. The skin temperature, blood flow, sensation of the right lower extremity and the dorsalis pedis artery pulsation were normal. Laboratory tests showed that the white blood cell count, neutrophil percentage, hemoglobin level, CD4 cell count, 1,3-beta-D-glucan assay, random blood glucose level,

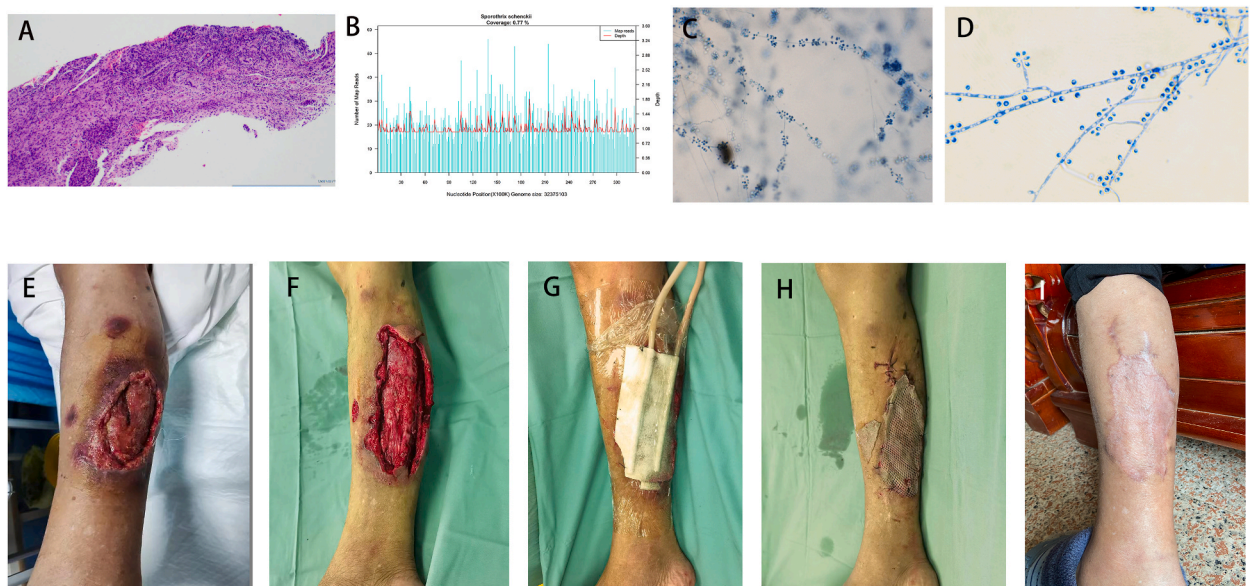


Fig. 1. Pathogenic testing of chronic wounds caused by *Sporothrix schenckii* infection of the patient and Patient's chronic wound before and after treatment. (A) Pathology suggested chronic granulomatous inflammation, PAS (–). (B) mNGS revealed *Sporothrix schenckii* with reads of 2894. (C) Culture results. Mycelial growth at room temperature. Direct microscopic examination showed that the branches had septate hyphae, ovoid conidia grew along the hyphae, short stalk tips in clusters, daisy flower microspores in growth. (D) A high-power field of *S. schenckii* (100× oil immersion lens). (E) Chronic wounds on the medial right calf of the patient, with several dark red nodules of various sizes seen around the wounds. (at admission) (F) Ulcer surface debridement (day 9 after diagnosis) followed by (G) vacuum sealing drainage (VSD) of the wound (lasting 7 days) (H) mesh implant (day 16 after diagnosis) (I) wound healing (follow-up 6 months later).

antinuclear antibodies (ANAs), serum antistreptolysin O (ASO) and rheumatoid factor (RF) level were normal. The C-reactive protein was 19.76 mg/L (reference <10), the erythrocyte sedimentation rate was 27mm/h (reference 1–10), the procalcitonin was 1.100ng/ml, and the serum galactomannan (GM) testing result was 0.575. No abnormalities were seen on ultrasound of the lower extremities. Excision of the nodular lesion showed a small amount of dark yellow pus, and necrotic histopathology suggested chronic granulomatous inflammation with PAS staining (–) (Fig. 1A). The patient's sample of skin lesion tissue was detected by mNGS. Firstly, the total nucleic acid in the sample was extracted and constructed to library with Nextera XT DNA Library Prep Kit. Library pools were then loaded onto an Illumina Nextseq CN500 sequencer for 75 cycles of single-end sequencing of 20 million reads for each library. After the sequencing was completed, low quality reads, adapter contamination, duplicated reads and human sequence data were removed. The remaining sequence data were aligned to the current bacterial, virus, fungal, and protozoan databases (NCBI; <ftp://ftp.ncbi.nlm.nih.gov/genomes>). Finally, it reported 2894 mapped reads of *Sporothrix schenckii* after 43 hours (Fig. 1B). Culture of a tissue sample presented colonies after 18 days and their micromorphological characteristics, such as separation of branch hyphae and ovoid conidia were characteristic of *Sporothrix* spp. (Fig. 1C and D). The wound on admission to hospital (Fig. 1E) was debrided for the ulcerated surface (Fig. 1F), then subjected to vacuum sealed drainage (Fig. 1G), followed by skin grafting (Fig. 1H). Meanwhile, amphotericin B (45 mg daily) was administered intravenously for 3 weeks followed by oral itraconazole (200 mg twice daily) for three months. The wound healed (Fig. 1I) and no recurrence by six months of follow-up.

2. Discussion

There are many reasons for chronic wound formation, such as nutritional metabolic and genetic diseases, vascular diseases, autoimmune diseases, tumors, blood diseases, drug-related factors, infectious diseases and etc. In this case, we reasonably hypothesized that it was caused by pathogen infection based on C-reactive protein, erythrocyte sedimentation rate, procalcitonin, serum galactomannan and the pathological results, then it was diagnosed as *Sporothrix schenckii* infection by mNGS and culture. The patient improved after treatment. In the process of diagnosis of infectious diseases, it was very important to determine whether it was infection according to the infectious biomarkers and pathological results. For rare infections and emergency cases, mNGS detection may be a better way with its wide detection range and short detection time.

Culture is the reference standard for the diagnosis of sporotrichosis. It has been widely used in clinical practice. However, it is less sensitive, time-consuming, and could not accurately identify pathogens to the species level. Although with cross-reactive, serological methods have good sensitivity and specificity, produce rapid results, therefore it can be applied for diagnostic screening and therapeutic monitoring in highly endemic areas. There are species-specific primers for *Sporothrix* spp. using PCR and Sanger sequencing, but it needs to preset pathogens. Unfortunately, these techniques are only at the research level and not widely available in health service. Since rapid diagnosis could have a positive impact on patient clinical outcomes, it is also important to consider the financial burden on patients and the accessibility of technology.

Few cases have been reported of the etiology of infection using mNGS to detect chronic wound. There has been reported a case of wound infection detected by mNGS, the etiologic agent is *Coxiella burnetii* which is very difficult to isolate using conventional culture methods [9]. In addition to sporotrichosis, the diagnosis of diseases caused by dimorphic fungi like histoplasmosis or blastomycosis depended on cultures and/or morphological identification of the yeast cells. Further diagnostic methods are therefore increasingly needed to meet the clinical demand.

The genome sequences of *Sporothrix schenckii*, *Sporothrix globosa*, *Sporothrix brasiliensis* and several other species of the genus *Sporothrix* have been published in NCBI database. By mNGS analysis, there were 2894 reads that specifically matched to the *Sporothrix schenckii* genome and no reads that specifically matched to other species such as *Sporothrix globosa*, so we believed that the species detected was *Sporothrix schenckii*, not *S. globosa*. Therefore, we infer that *S. schenckii* and *S. globosa* co-occur in China, which is similar to some current research reports [10].

Metagenomic next-generation sequencing (mNGS) is a culture-independent and random sequencing method that can simultaneously and rapidly detect all potential pathogens in the same sample and avoid present detection ranges [11]. At present, the value of mNGS in the diagnosis of infectious diseases is gradually recognized. In a study on the diagnosis of pneumonia in children, it was found that the positive detection rate of mNGS and the coincidence rate with the final diagnosis were significantly higher than those of conventional methods such as culture, smear and serology [12]. Comparing the performance of mNGS and conventional urine culture in the diagnosis of infection and identification of pathogenic microorganisms, it was found that the detection rate and sensitivity of mNGS were significantly higher than that of culture. In addition, mNGS detected a wider range of pathogens [13]. In a study on 641 patients with infectious diseases, by comparing the detection efficiency of mNGS and traditional culture method for different pathogens, the positive rate of mNGS was 69.89 %, significantly higher than that of traditional culture 22.31 % [14]. However, there were few reports of mNGS targeting chronic wound infection, especially co-infection with multiple pathogens. The study of this case indicated that mNGS was feasible in detecting the infection of *Sporothrix* spp. . Although the cost of mNGS is relatively high compared with culture, its detection time is much shorter, and it can be identified to the species level. With the gradual reduction of sequencing cost and the improvement of technology maturity, mNGS is very likely applied in the future to diagnose the case.

3. Conclusion

This case presented the rapid detection of chronic wounds infected with *Sporothrix schenckii* by mNGS, suggesting that mNGS could be able to rapidly and accurately detect pathogens in the process of diagnosing the etiology of chronic wounds, helping to clarify the diagnosis and precise treatment as early as possible, and also play an important role in excluding infectious etiology of chronic wounds.

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Ethics approval and consent to participate

This study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University [2023-E038-01].

Consent for publication

The patient's written consent has been obtained regarding publishing his data and photographs.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Wenxiang Jin: Writing – original draft. **Yong Liu:** Supervision. **Qiuyue Ning:** Data curation. **Shuwen Wu:** Formal analysis. **Sibiao Su:** Project administration. **Dongyan Zheng:** Validation. **Shasha Ma:** Software. **Jun Zou:** Validation, Conceptualization. **Min Yang:** Conceptualization. **Diefei Hu:** Methodology. **Huarong Ding:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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