

Application of PCR Sequencing and Next-Generation Sequencing in the Diagnosis of Sporotrichosis

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Abstract: Sporotrichosis is a common chronic fungal infection and the clinical manifestations are often untypical. Diagnosis of sporotrichosis relies conventionally on fungal culture, histopathological examination, and species identification by molecular test. We reported that a 70-year-old man presented with a cutaneous lesion on the back of his right hand (present for 6 months). The cutaneous bacterial infection was diagnosed at a local hospital and the lesion had not improved. Physical examination revealed an infiltrative reddish plaque with purulent secretion and crusts. Histopathological examination revealed scattered round yeast cells in the dermis. Fungal culture revealed multiple, velvety, brown colonies on Sabouraud dextrose agar (SDA). *Sporothrix globosa* was identified by PCR-sequencing and next generation sequencing (NGS) method. Finally, a case of sporotrichosis caused by *Sporothrix globosa* was diagnosed by histopathological examination, mycological examination, and molecular identification. The patient was treated with oral itraconazole 400 mg/day for 2 months. The lesion was dramatically ameliorated.

Keywords: sporotrichosis, PCR sequencing, next generation sequencing

Introduction

Sporotrichosis is a common chronic fungal infection usually caused by trauma through which the spores or yeast cells enter the host.¹ The clinical manifestation can range from fixed-cutaneous form to lymphocutaneous sporotrichosis and even disseminated sporotrichosis.² Diagnosis of sporotrichosis relies mainly on pathogen isolation and culture, histopathological examination, and species identification by molecular tests.² Currently, there is a critical need to rapidly identify infectious organisms in clinical samples. Next generation sequencing (NGS), as a novel and promising approach, has been used in clinical aspects and has the ability to identify pathogens and detect microorganisms.³ Here, we reported a case of sporotrichosis confirmed by a combination of fungal culture, histopathological examination, PCR-sequencing, and NGS.

Case Report

A 70-year-old Chinese man presented with a painful cutaneous lesion on the back of his right hand which had been present for 6 months and was admitted to our department. Six months ago, a few small pustules appeared on the back of his right hand. The patient denied a history of surgery or trauma and did not receive any treatment. No fever, chills, headache, and weight loss had occurred. The rashes gradually expanded into an infiltrative reddish plaque accompanied by ulceration and purulent secretion. One month ago, the patient presented to a local hospital and a skin biopsy was performed. The bacterial culture of the cutaneous tissue was positive, indicating that the cutaneous infection was caused by *Cronobacter* spp, whereas the fungal culture was negative. Hence, he was diagnosed with bacterial cutaneous infection and treated with intravenous piperacillin, however, the lesions did not significantly improve. He had a 10-year history of poorly controlled hypertension.

Physical examination revealed a 3.5×2 cm infiltrative reddish plaque with unclear boundaries. Purulent secretion and crusts were observed on the uneven surface of plaque (Figure 1A). Histopathological examination of biopsy tissue revealed pseudoepitheliomatous hyperplasia in the epidermis and the infiltration of lymphocytes, neutrophils, histiocytes and multinucleated giant cells in the dermis, using hematoxylin eosin (HE) staining (Figure 1B), suggestive of an infective granuloma. Fortunately, scattered round yeast cells were observed in the dermis by periodic acid-Schiff (PAS) staining (Figure 1C). Fungal

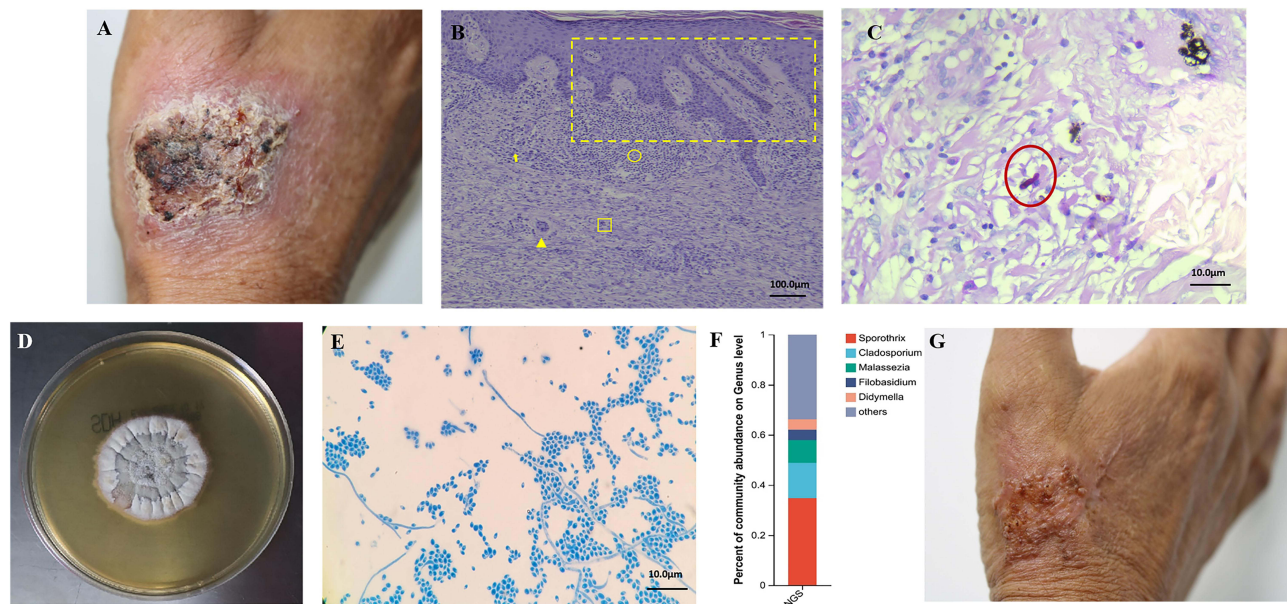


Figure 1 Clinical presentation of the patient and laboratory findings of the excisional specimen. **(A)** A 3.5×2 cm infiltrative reddish plaque with unclear boundaries and yellow-white crusts was observed on the back of right hand. **(B)** HE staining of biopsy tissue showed pseudoepitheliomatous hyperplasia in the epidermis and the infiltration of lymphocytes, neutrophils, histiocytes and multinucleated giant cells in the dermis (magnification×100). The pseudoepitheliomatous hyperplasia in the epidermis was annotated by dashed boxes. The infiltration of lymphocytes, neutrophils, plasma cell and multinucleated giant cells in the dermis was annotated by yellow circle, solid box, arrow and triangle, respectively. **(C)** PAS staining of biopsy tissue showed the horizontal section of hyphae (red oval, magnification ×400). **(D)** Fungal culture of biopsy specimen and purulent secretion revealed multiple, velvety, brown colonies on Sabouraud dextrose agar (SDA) for 14 days. **(E)** The microscopic characteristics of the isolate showed thin, branched, hyaline septate hyphae (magnification ×400). **(F)** The total *Sporothrix* read number detected in biopsy tissue accounted for 34.7% of the total amount of reads. **(G)** The skin lesion was dramatically ameliorated after treatment with oral itraconazole 400mg/day for 2 months.

culture of biopsy specimen and purulent secretion from the infection site both revealed multiple, velvety, white colonies on Sabouraud dextrose agar (SDA) for 7 days at 28°C. (Figure 1D). The microscopic characteristics of the isolate showed thin, branched, hyaline septate hyphae (Figure 1E). The genomic DNA of the isolate was extracted and used to amplify the sequence of internal transcribed spacer (ITS) by PCR. The PCR product showed 100% similarity with *Sporothrix globosa* sequences in the CBS database (<http://www.cbs.knaw.nl/Collections>). Additionally, the pathogen in biopsy tissue was also identified by using 16S rRNA and ITS genes amplicon NGS approach. The total *Sporothrix* read number detected in biopsy tissue was 20005 reads, accounting for 34.7% of the total reads (Figure 1F). Findings from complete blood cell count, liver function and renal function were unremarkable. The bacterial culture of the secretion and biopsy specimen was negative. Therefore, the patient was diagnosed with fixed sporotrichosis caused by *Sporothrix globosa* by histopathological examination, and PCR-sequencing combined with NGS. Since itraconazole is the first drug of choice for the treatment of sporotrichosis, the in vitro susceptibility to itraconazole of the isolate was tested and the MIC was 0.5 µg/mL. The patient was empirically treated with oral itraconazole 400mg/day for 2 months. The cutaneous lesions were dramatically ameliorated (Figure 1G). The patient was still taking the medicine and being followed-up.

Discussion

Sporotrichosis is a prevalent subacute-to-chronic fungal infection caused by the dimorphic *Sporothrix* spp., which is found worldwide and distributed in soil and plants.^{1–3} Sporotrichosis typically presents as papules or pustules that form ulcerated nodules involving local lymphatics, which needs to be distinguished from some diseases such as nontuberculous mycobacterial infection, chronic eczema, or Majocchi granuloma.^{1–3} In this study, our patient presented with a chronic unilateral, deep infiltrating plaque covered with crusts on the back of his right hand, which was initially diagnosed as bacterial cutaneous infection at a local hospital, which led to delayed treatment and aggravated the condition.

Fungal culture is the gold standard for diagnosing sporotrichosis.^{3,4} Species identification is usually achieved by conventional molecular techniques such as PCR-sequencing and nest PCR, which are also based on the positive result of tissue or secretion fungal culture.^{5,6} However, fungal culture commonly takes 2–4 weeks to obtain the result, which makes rapid clinical diagnosis more challenging.^{4,5} Additionally, fungal culture requires

professional mycological expertise, which is not widely available and may be absent in some health care settings. Recently, NGS has gradually become a hot-spot since NGS can directly and quickly detect the pathogens in biopsy samples.⁵ Previous studies reported that the rapid development of NGS provided accurate and timely diagnostic tools for some rare and complex pathogens such as *Mycobacterium marinum* and *Nocardia huaxiensis*.^{6,7}

In this study, the cutaneous tissue biopsy was initially sent for NGS to detect particular or unexpected pathogens. The findings of NGS were suggestive of *Sporothrix* spp., which is consistent with the histopathological examination, fungal culture, and PCR-sequencing. Fortunately, the histopathological examination of biopsy tissue also showed scattered round yeast cells which are often difficult to observe in clinic. To the best of our knowledge, we reported a valuable case of typical fixed sporotrichosis diagnosed by a combination of histopathological examination, fungal culture, PCR-sequencing, and NGS. Additionally, this study also helped us to better understand the advantages of NGS in identifying the causative pathogens in clinical samples, which provided timely diagnosis and appropriate treatment.

Ethics Approval and Informed Consent

We have obtained all appropriate patient consent forms. The patient gave written informed consent for the publication of clinical information and photographs. Institutional approval was not required for the case details.

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Disclosure

The authors have no competing interest to declare in this work.

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