Dear editor,

We thank the editors and reviewers for the constructive comments on our manuscript. The manuscript has been carefully revised according to these comments and suggestions. In the revised manuscript, all the changes made in response to these comments are clearly indicated using yellow highlight. We also corrected language issues in the entire manuscript with the assistance of a professional medical editing service (Medjaden, Inc.)

Reviewer 1

1. The abstract introduces the topic concisely but could benefit from a clearer articulation of the study's importance, highlighting the uniqueness of the case and its implications for diagnostic and treatment approaches.

Authors' reply: Thank you for your valuable suggestion. We described the case more clearly in the Background of the revised **Abstract** as follows:

> BACKGROUND
>
> Infections by nontuberculous mycobacteria (NTM) have become more common in recent years. *Mycobacterium canariasense* was first reported as an opportunistic pathogen in 2004, but there have been very few case reports since then. *Nocardia* is a genus of aerobic and Gram-positive bacilli, and these species are also opportunistic pathogens in the Mycobacteriales order. Conventional methods for diagnosis of NTM are inefficient. Metagenomic next-generation sequencing (mNGS) can rapidly detect many pathogenic microorganisms, even rare species. Most NTM and *Nocardia* infections occur in immunocompromised patients with atypical clinical symptoms. There are no previous reports of infection by *M. canariasense* and *N. farcinica*, especially in immunocompetent patients. This case report describes an immunocompetent 52-year-old woman who had overlapping infections of *M. canariasense*, *N. farcinica*, and *Candida parapsilosis* based on mNGS.

2. The introduction would be strengthened by providing a broader context on

non-tuberculous mycobacteria (NTM) and Nocardia infections, emphasizing their rarity and the clinical challenges they pose.

Authors' reply: We added additional information about non-tuberculous mycobacteria (NTM) and *Nocardia* infections in the revised **Introduction** as follows:

INTRODUCTION

The non-tuberculous mycobacteria (NTM), also referred to as environmental mycobacteria, atypical mycobacteria, or anonymous mycobacteria, are ubiquitous species and potential causes of infectious diseases. Based on their growth characteristics determined from subcultures, the NTM are classified as rapidly growing mycobacteria (RGM; mature colonies in less than 7 days) or slowly growing mycobacteria (SGM; mature colonies in more than 7 days)[(1)]. Several RGM species have been identified as etiologic agents of bacteremia, especially in patients with low immunity, such as those with HIV infections or malignant tumors. *M. canariasense* is a rare species of RGM that is closely related to *M. diernhofer*[(2, 3)], but has never been described separately in immunocompetent persons.

The genus *Nocardia* is also in the order Mycobacteriales, and includes at least 50 species that are aerobic Gram-positive bacilli which can invade the lungs, skin, or central nervous system, especially in immunocompromised persons. The symptoms of patients with *Nocardia* infections are often nonspecific, and include chronic cough, low-grade fever, fatigue and weight loss[(4)].

It can be difficult to diagnose infections by *Mycobacterium* and *Nocardia* from the conventional culture tests used in clinical practice, and delayed diagnosis may have serious adverse consequences. The rapid and efficient identification of the pathogen responsible for an infectious disease is a prerequisite for the effective treatment of these patients. Macro-genomics with next-generation sequencing (mNGS) does not require traditional microbial culture, and instead directly provides high-throughput sequencing of nucleic acids in clinical samples, which are then compared with a database. This method can rapidly and objectively detect many pathogenic microorganisms (including viruses, bacteria, fungi, and parasites) in clinical samples

without the need for specific amplification, and is especially suitable for the diagnosis of acute and critical diseases and difficult infections. In this paper, we describe the use of mNGS to detect the rare co-occurrence of *M. canariasense* and *N. farcinica* in an immunocompetent adult. This identification allowed administration of an effective treatment and led to patient cure.

3. The case presentation could be enriched by integrating a more detailed patient history and diagnostic reasoning.

Authors' reply: We provided more details about the patient's history and diagnostic reasoning as follows:

History of present illness

The patient experienced a relapse of productive cough, persistent chest pain, and moderate-grade fever that was accompanied by chills and shivering, but there was no hemoptysis. She was diagnosed with pneumonia and treated with cefuroxime sodium (0.75 g per 8 h) and levofloxacin (0.5 g per day) at a local hospital for 1 week and experienced defervescence. However, the cough and chest pain persisted, and this affected her ability to work and study. The patient did not visit another outpatient clinic for persistent cough and chest pain, so a malignant tumor could not be excluded. She presented at our hospital for further diagnosis and treatment.

4. The discussion could benefit from integrating alternative diagnostic methods or treatments that were considered.

Authors' reply: The patient had a history of cough and chest pain, and these symptoms remained after receipt of anti-infection treatment at a local hospital. An inpatient CT examination at our hospital showed nodular lesions of the lung, but we could not exclude a malignancy. However, the puncture biopsy results of the injured part of the lung were not consistent with malignancy. Combined with the mNGS results, the diagnosis in this patient was pneumonia due to overlapping infections of *M. canariasense*, *N. farcinica*, and *C. parapsilosis*. Thank you for your comments.

5. In the Graphics, Tables, and Figures section, it is recommended to ensure that all visual elements are of high quality and clearly labeled. In Table 1, the label is incorrect: "Laboratary test results of blood," the correct one is: "Laboratory test results of blood."

Authors' reply: We apologize for these errors. We have checked the **Tables** and **Figures** and made corrections as requested. Thank you for your comments.

6. Finally, the conclusion should succinctly encapsulate the main findings and implications, suggesting directions for future research. Implementing these suggestions could significantly improve the clarity, depth, and impact of the article, making it a valuable contribution to the field.

Authors' reply: Thank you for your comments. We revised the **Conclusion** section as follows:

Conclusion

In summary, this is the first report of a patient who had overlapping infections of *M. canariasense* and *N. farcinica*. Infections by either of these pathogens are generally rare in immunocompetent hosts. The presence of lung nodules that are partially leafy and have burrs may lead to a misdiagnosis of lung cancer. We highlight the importance of identifying the causative pathogen by use of mNGS, a powerful tool for the detection of mixed infections, especially in patients with atypical symptoms who are infected by rare pathogens. The results from mNGS, combined with analysis of a patient's underlying clinical status and laboratory indicators, provide clinicians with a more complete characterization of disease and the causative pathogen. This case report also confirmed the potential role of *M. canariasense* and *N. farcinica* as opportunistic pathogens. It is important to note that due to the rarity of overlapping infections by *M. canariasense* and another pathogen, empirical antibiotic treatment without mNGS results may not be adequate.