

## INTRODUCTION

Nontuberculous mycobacteria (NTMs) were initially recognized as pathogens in the mid-1950's [1]. More ~~and more~~ species were discovered ~~with recognize~~ ~~of~~by medical workers and improvement of ~~inspection-detection~~ techniques. Some ~~NTM~~ species ~~of them~~ may cause diseases in humans [1]. ~~NTM has been reported in m~~More than 170 species ~~of NTMs have been reported~~ worldwide, including ~~two types:~~ rapidly growing mycobacteria (RGMs) and slow growing mycobacteria (~~SGM~~) [2]. *Mycobacterium mucogenicum* (*M. mucogenicum*) belongs to the ~~RGM group~~ ~~of RGM~~, ~~which and~~ is considered ~~to be an~~ environmental microorganism with a worldwide distribution. It is associated with a wide spectrum of clinical diseases, including osteomyelitis, respiratory tract, bloodstream, and disseminated infections in both immunocompetent and immunosuppressed individuals [3]. However, time-consuming techniques and low detection rates ~~s~~ for identifying this pathogen usually leads to delayed or missed diagnosis. So, we need a rapid and ~~board~~broad-spectrum method to identify some rare pathogens. Next-generation sequencing (NGS) is an unbiased approach whose RefSeq contains 4,152 ~~whole~~ ~~whole~~-genome sequences ~~s~~ of viral taxa, 3,446 ~~bacterial~~bacterial genomes or scaffolds, 206 human pathogenic fungi ~~related to human infection~~, and 140 human parasites ~~associated with human diseases~~. ~~It~~ NGS identifies the nucleotides in the targeted ~~ed~~ samples and compares the detected nucleotides against the catalog~~ue~~ library of pathogens, thereby listing the possible causative agents in the clinical samples [4-7]. However, there are little few reports ~~in of~~ formalin-fixed, paraffin-embedded (FFPE) specimens. ~~And and~~ whether ~~the clinical~~ ~~significance of~~ detection ~~results~~ using FFPE specimens is useful or not is unclear.

Here, we report a case ~~with of~~ disseminated infection ~~with the pathogen~~ ~~identified~~ ~~as caused by~~ *M. mucogenicum* diagnosed by NGS using FFPE specimens.

## DISCUSSION

*M. mucogenicum*, a ubiquitous RGM, has been recognized as a species since 1995. The organism was first called *M. ycobacterium chelonae*-like organism (~~M.CLO~~) ~~as causing diseases in humans~~ in 1982, during two outbreaks of peritonitis associated with peritoneal dialysis in the ~~United States~~ USA in 1976 and 1978<sup>[10]</sup>. *M. mucogenicum* is more closely related to the *M. fortuitum* group by 16S rDNA sequencing than other ~~M.~~ *Mycobacterium* groups<sup>[8]</sup>. This mucoid surface provides the ability to form a protective biofilm, which contributes to infection of central venous catheters (~~CVC~~) and skin and soft-tissue, usually ~~post-traumatically, clinically the more common sites for M. mucogenicum infections~~<sup>[11]</sup>. Moreover, it can cause respiratory infections, bloodstream infections, ~~osteomyelitis~~ osteomyelitis, and disseminated infections, which is common ~~happened~~ in immunocompromised hosts<sup>[3, 12]</sup>. Some equipments exposed to ~~contaminated~~ water contaminated by *M. mucogenicum* can lead to nosocomial infection, including ~~central-central-venous-venous~~ catheter-related and hemodialysis-related infections<sup>[1, 13]</sup>.

There was no evidence of immunodeficiency in ~~this~~ our patient. ~~Undoubtedly~~ Undoubtedly, ~~it's~~ it is very necessary to determine the source of infection. ~~But~~ However, regrettably, we did ~~n't~~ not have ~~the~~ evidence of NTM pneumonia ~~with~~ NTM-infected. We ~~can't~~ could not perform fiber ~~bronchoscope~~ bronchoscopy on her because ~~that~~ she ~~underwent~~ had cardiac insufficiency at the initial examination, and ~~her~~ pneumonia ~~was~~ improved rapidly after anti-NTM treatment. ~~Apparently, it~~ It is unlikely that the infection was nosocomial. Due to the pulmonary lesions and multiple skin lesions and the good therapeutic ~~effect~~ response, it is reasonable to presume that the ~~source~~ route of infection ~~with this microorganism is~~ was ~~via~~ from the upper to ~~the~~ lower respiratory tract, then ~~go~~ through the bloodstream ~~infection, finally~~ to skin and soft-tissue, ~~that cause the whole infectious process~~.

~~As to~~ For identification of this microorganism, there are several major

methods ~~as follows~~. The first is conventional culture ~~methodology~~. ~~It Culture~~ is still difficult and delayed ~~to culture~~ ~~can lead leading~~ to missed diagnosis ~~by conventional culture methodology~~<sup>[14]</sup>. The second is DNA sequencing, with 16S rRNA gene, *rpoB*, and *hsp65* being recognized as useful targets<sup>[15-17]</sup>. ~~But~~ ~~However~~, due to its targeting, limitations ~~is unavoidable~~ in identification ~~for of~~ uncertain pathogens ~~are unavoidable~~. The third is ~~Matrixmatrix~~-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Several investigators have demonstrated that MALDI-TOF mass spectrometry ~~could~~ ~~can~~ accurately identify mycobacteria<sup>[18-19]</sup>. ~~But~~ ~~However~~, these methods are not available in many laboratories.

Moreover, ~~the result of~~ biopsy ~~on of~~ the right psoas was not ~~satisfied~~ ~~satisfactory~~. So, we ~~took a try~~ ~~tried~~ to perform NGS using FFPE ~~specimens~~. NGS works well with short nucleic acid sequences, thus, it can ~~in theory~~ be used ~~easily~~ to analyze fragmented DNA and RNA extracted from standard FFPE clinical specimens ~~in theory~~. In ~~the~~ previous researches, it ~~can~~ ~~has been~~ used for ~~whole-whole~~-genome sequencing, mainly in cancer patients <sup>[20-21]</sup>. Using FFPE tissues for pathogen identification ~~was~~ ~~has~~ also ~~been~~ reported in recent years, such as 16S ~~PCR~~ ~~polymerase chain reaction~~ from brain tissue <sup>[22]</sup>, RNA sequencing of 1918 influenza samples <sup>[23]</sup> and ~~diagnosis of~~ corneal infections ~~diagnosis~~<sup>[24]</sup>.

To our knowledge, this is the first report ~~on of~~ *M. mucogenicum* confirmed by ~~the~~-NGS using FFPE ~~specimens~~. ~~In this study, it is~~ ~~It was~~ feasible to use metagenomic NGS of DNA extracted from FFPE clinical specimens to identify ~~the~~ causative microorganism. This method holds ~~great~~-promise for the ~~relatively~~ rapid detection of microorganisms, including rare pathogens ~~and in~~ cases in which conditional cultures ~~were~~ ~~have~~ not ~~been~~ attempted or ~~have~~ failed to yield positive results.

After ~~a~~-combination therapy ~~of with~~ clarithromycin, doxycycline and TMP-~~SMX~~, the patient was treated for ~~over~~ ~~>~~ 12 months, and ~~the~~ improvement was observed. ~~But~~ ~~However~~, the optimal duration of

antimicrobial therapy for disseminated infection with *M. mucogenicum*-related is unknown. It is certain that prolonged therapy for ~~one to two~~1-2 years ~~even more~~ contributes to ~~eradicate~~eradication of infection and reduced ~~the~~ chance of ~~the~~ recurrence <sup>[1]</sup>.