From postmortem lung tissue, metagenomic detection of pathogenic microorganisms for pneumonia

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ABSTRACT

The validity of bacterial cultures from lung tissue for postmortem identification of pneumonia-associated pathogens has been questioned by pathologists. As a result, we investigated whether pathogenic bacteria that causes pneumonia could be detected using metagenomics analysis of lung tissue from 11 pneumonia patients and nine non-pneumonia cases collected at autopsy. We showed that metagenomics analysis of the postmortem lung microbiota could identify a bacterial genus as a pneumonia pathogen even when the genus was common and well-known. The presence of Enterobacteriaceae or anaerobic bacteria in the lung microbiota of non-pneumonia patients suggests postmortem transfer and replacement. Furthermore, based on postmortem anaerobic alterations and artificial culture conditions, we verified that postmortem bacterial culture from lung tissue might be deceptive for identifying the pneumonia pathogen. To our knowledge, this is the first study to show that metagenomics analysis of postmortem lung tissue may identify bacterial species that cause pneumonia under certain conditions.

Key Words: *Pneumonia; Postmortem diagnosis; 16S rRNA metagenomic analysis; Bacterial culture*

INTRODUCTION

Pneumonia is a prevalent cause of mortality, according to clinical practice as well as postmortem examinations. Pathologists use bacterial culture to identify the bacterial culprit when pneumonia is suspected based on fever, respiratory symptoms, and postmortem Computed Tomography (CT) findings. Because they are typically unexpected out-of-hospital deaths, health problems, a medical history, the clinical course, and the postmortem interval are all unknown in most instances that necessitate postmortem inquiry. Pathologists also can't depend on postmortem bacterial culture due to contamination during the procurement process and postmortem bacterial translocation. Furthermore, they are unable to replicate the in vivo circumstances of deadly bacteria proliferation, which are unknown in terms of air and nutrition needs and are influenced by unknown bacterial species, non-bacterial microorganisms, and host immunity. As a result, a new method is needed to replace bacterial culture in forensic autopsies for the identification of pneumonia pathogens. The introduction of next-generation sequencing (NGS) has made amplicon capture simple, quick, thorough, and cost-effective. Amplicons are DNA fragments that have been amplified from a region of a marker gene, such as bacterial 16S DNA, in a tiny quantity of a microbiota-containing sample. A distinct bacterial population at a certain anatomical region is referred to as the microbiota.

Despite the inconsistency of bacterial culture detection of local microorganisms, healthy people's lungs and lower airways were thought to be sterile. However, precise identification of resident bacteria in the oral cavity or upper airways in samples from the lower airway or lung has been mistaken as contamination during sampling on occasion. Even in healthy humans, the lung microbiota has been shown to contain not only normal lung microbiota that overlaps with the upper airway microbiota but also microbes aspirated from the upper airway and oral cavity into the lower airway, thanks to the development of bacterial metagenomics analysis. To use 16S rRNA metagenomics analysis of postmortem samples to identify pneumonia-causing bacteria, we must define not only the normal microbiota but also postmortem alterations in the lung microbiome. A metagenomics investigation of postmortem bacterial microbiota has been conducted in two studies. Within 24 hours-48 hours after death, the microbiomes in the eyes, nose, mouth, and rectum of 188 persons resembled ante mortem health status, according to Pechal. Javan found bacteria in the liver, spleen, heart, brain, blood, and oral cavity of 27 cadavers that were common to postmortem and ante mortem human tissues, showing a relationship between the ante mortem and postmortem microbiota (24 h). The postmortem microbiota in numerous organs has been defined in this research, but not in the lungs. Pneumonia is caused by bacterial invasion from outside the lungs, which occurs in the setting of weakened host immunity, which is impacted by age, illnesses, and the environment.

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Furthermore, bacteria undertake postmortem transfer from the colon or upper airway to the lungs, as well as postmortem replenishment in anaerobic environments. To use 16S rRNA metagenomics analysis for the postmortem diagnosis of pneumonia, the latter possibility must be explored by examining the lung microbiota in non-pneumonia patients. To use 16S rRNA analysis to identify a bacterial genus as a pneumonia pathogen, the genus must be abundant in the lungs of patients diagnosed with pneumonia, but not in cases without a history or symptoms of pneumonia.

CONCLUSION

Finally, we showed for the first time that 16S rRNA metagenomics analysis of postmortem lung tissue may be utilized to identify the bacterial genus that caused pneumonia in the deceased. The presence of only known pneumonia-related bacterial species might help to identify the aetiology of pneumonia in this case. However, in instances when these bacteria are abundant, the possibility of postmo--rtam transfer and growth of Enterobacteriaceae or anaerobic bacteria must be ruled out. Furthermore, because metagenomics analysis cannot distinguish pathogenic from non-pathogenic species within the same bacterial genera, such as Streptococcus, we were unable to identify several bacterial genera as pneumonia pathogens. Based on postmortem alterations and challenges in selecting culture conditions that mimic the diseased tissue, bacterial culture from lung tissue has been proven to be deceptive for the identification of pneumonia-causing microorganisms. Finally, bigger metagenomics investigations with more pneumonia and non-pneumonia cases are needed to determine the normal lung microbiota and postmortem alterations in the microbiome before making a metagenomics diagnosis of pneumonia from postmortem lung tissue. With the use of the postmortem CT equipment established after this study, we will widen our examination of the concerns unaddressed in this study following the COVID-19 pandemic.