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Microbial Aerosols: New Diagnostic Specimens for Pulmonary Infections

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Abstract

Pulmonary infections are important causes of global morbidity and mortality, but diagnostics are often limited by the ability to collect specimens easily, safely and in a cost-effective manner. We review recent advances in the collection of infectious aerosols from patients with tuberculosis and with influenza. Although this research has been focused on assessing the infectious potential of such patients, we propose that these methods have the potential to lead to the use of patient-generated microbial aerosols as non-invasive diagnostic tests of disease as well as tests of infectiousness.

Introduction

Pulmonary infections are major public health problems globally, with tuberculosis (TB) the most lethal infectious disease and lower respiratory infections the 4th leading cause of death. In the United States, pneumonia and influenza together are the 8th leading cause of death. Despite considerable technological advances in molecular diagnostics and in clinical

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microbiology, the diagnosis of pulmonary infections is limited by the ability to easily obtain adequate specimens in a safe and cost-effective manner. There have been advances in the collection of infectious aerosols over the past two decades, especially in the research of tuberculosis and influenza. We will briefly review these advances, and we propose that patientgenerated aerosols have the potential to become clinically useful specimens for the diagnosis of pulmonary infections in the near future.

The most commonly used specimen to diagnose pulmonary infections currently is expectorated sputum. However, sputum production varies immensely across diseases. It is usually available either spontaneously or by induction with inhaled saline in HIV-negative adults presenting with pulmonary TB or with exacerbations of bronchiectasis due to cystic fibrosis. In contrast, about 50% of patients with nontuberculous mycobacterial infections associated with nodular bronchiectasis are unable to produce sputum, and about one-third of patients with bacterial pneumonia may not produce sputum.

The utility of sputa specimens for the diagnosis of community-acquired pneumonia remains controversial.[1] Although outcomes are best when treatment is directed at specific pathogens, over 95% of cases of ambulatory pneumonia are successfully treated with empiric antibiotic therapy. A critical issue is the quality of the sputum specimen, as many that are collected are saliva from the upper respiratory tract and are not representative of the lower respiratory tract. Children usually do not produce good quality sputum specimens, so the etiologic agent is often inferred by the clinical presentation, epidemiology, and laboratory or radiographic patterns. When good quality sputum specimens cannot be obtained, bronchoscopy is often indicated, especially if the patient is very ill.

Brief History of Pulmonary Specimens

In 1882, Robert Koch identified what became known as *Mycobacterium tuberculosis* in autopsy tissue and in the sputum from patients suffering from ‘phthisis’, what we know now as tuberculosis (TB).[2] Sputum has continued to be the most commonly ordered specimen to diagnose TB and other respiratory infections globally. Several years after Koch’s discovery, face masks were used to collect the aerosols generated by coughing from TB patients in a research study, using microscopy to detect the bacilli.[3] However, this method was never adapted to the collection of clinical specimens.

Although flexible bronchoscopes were developed in the 1960s, it was not until the 1980s that they were used routinely to obtain bronchoalveolar lavage (BAL) to diagnose respiratory infections, driven largely by the AIDS epidemic.[4] The use of sputum induction by inhalation of hypertonic saline also increased around this time, especially in attempts to non-invasively diagnose *Pneumocystis* pneumonia and tuberculosis among HIV-infected patients. Unfortunately, bronchoscopy is an invasive and relatively expensive procedure that is not available in all settings; although typically well-tolerated, it is not without risk. Sputum induction is more readily available and less risky, although it can be uncomfortable and is not universally available.

Tuberculosis

Shortly after Koch's demonstration that the 'tubercle bacillus' was the etiologic agent of TB, the discovery of the acid-fast characteristics of the bacillus led to the clinical use of sputum microscopy for acid-fast bacilli (AFB), still the method of diagnosing TB in much of the world today, 137 years later. The mode of transmission of TB remained controversial for the next 80 years until the elegant studies of Riley and colleagues that proved airborne transmission by demonstrating that guinea pigs developed TB upon breathing air exhausted from a remote experimental ward housing TB patients.[5] Thus, *Mycobacterium tuberculosis* bacilli must be in or on particles in the air, i.e., in an 'aerosol', a system of particles dispersed in the air. In spite of this knowledge that TB is transmitted by airborne bacilli and not by sputum, the infectiousness of patients with pulmonary TB (from here on written only as 'TB') has continued to rely upon the demonstration of AFB in sputum due to the ease of sputum testing and the lack of technology to detect airborne bacilli, i.e., infectious aerosols.

In an initial proof of concept study of a novel cough aerosol sampling system (CASS) (Figure 1), only 4 of 12 (25%) hospitalized patients with TB produced culturable *M. tuberculosis* from the aerosols generated by voluntary coughing.[6] Subsequent cohorts of smear-positive culture-proven TB patients from Uganda and Brazil found that 100/233 (43%) patients produced culturable aerosols and of these 57 (24%) were high aerosol producers defined as production of ≥ 10 colony forming units (CFU) of *M. tuberculosis* from aerosol sampling.[7-9] Two other studies from South Africa have been done, one using a minor modification of the CASS method[10] and the other a novel stationary respiratory air sampling chamber in which the whole patient sits.[11] Infectious aerosol production has been found to be only partially related to bacterial burden measured as AFB smear grade or time to positivity in liquid culture media, and it appears to decrease rapidly after commencement of TB therapy. In studies from Brazil and Uganda, aerosol production predicted the risk of TB infection among household contacts (HHCs) better than the sputum AFB smear. Furthermore, microbiologically-proven secondary TB disease in HHCs clustered among contacts of aerosol positive TB cases.

The finding of cough aerosol cultures collected from patients in South Africa with extensively drug-resistant refractory to treatment suggests the worrisome potential for ongoing transmission.[10] The results of cough aerosol cultures from two different groups of investigators in South Africa have been similar to those found in Uganda and Brazil by another team. The use of polymerase chain reaction (PCR) assays that have identified molecular signals in exhaled breath at higher concentrations than the colony-forming units in solid cultures.[11] A group from the UK has developed a novel method of mask aerosol collection of *M. tuberculosis* using a modified face mask (Figure 2).[12] They have also found high concentrations of *M. tuberculosis* DNA in the exhaled breath of TB patients using molecular methods. The discordance between the results of the molecular assays versus culture-based methods is likely due to differences in viability of the bacilli in the aerosols. It is possible that bacilli are in a viable but nonculturable state after exposure to the stresses of aerosolization, desiccation and temperature change, as can occur after exposure to hypoxia or antibiotics, but this warrants further research.

Influenza

Influenza is thought to be spread at least in part by contact and droplets. However, increasing evidence suggests that airborne transmission by droplet nuclei also plays an important role, especially at short ranges. Viral respiratory tract infections are now commonly diagnosed by collecting a sample of respiratory mucus and analyzing it with an antigen or PCR-based system.[13] Currently, the usual methods for obtaining clinical specimens from the respiratory tract are nasopharyngeal or oropharyngeal swabs, nasopharyngeal aspirates and nasal washes, tracheal aspirates, bronchoalveolar lavage, or the collection of sputum. Each of these techniques has drawbacks: Nasopharyngeal and oropharyngeal swabs, aspirates, and washes provide mucus from the upper respiratory tract, which does not always contain the same viral load or the same species of viruses as the lower respiratory tract.[13] The collection of nasopharyngeal swabs is uncomfortable for patients, especially pediatric patients, and nasal washes and nasopharyngeal aspirates are often preferred in pediatric practice. Bronchoalveolar lavage provides a more definitive sample of lower respiratory tract infections, but the procedure is considerably more invasive and unpleasant, and therefore is rarely used. Sputum often contains upper respiratory mucus; producing sputum can be difficult and distasteful for patients and may require induction by nebulization, which can lead to bronchospasm.

The collection of aerosol particles produced by patients during coughing and tidal breathing potentially provides a non-invasive method for the collection of diagnostic specimens of respiratory viruses. Respiratory viruses have been detected in the exhaled breath and cough aerosols from infected patients, especially influenza virus.[14,15] Milton and colleagues collected the aerosol particles in the exhaled breath and cough of 37 subjects for 30 minutes and found influenza virus RNA in 35 samples (95%)(Figure 3).[16] Lindsley and colleagues collected the particles in three coughs from 47 influenza-positive subjects and detected influenza RNA in 38 samples (81%)(Figure 4).[15] Milton and colleagues[16] and Yan and colleagues[17] also found that the influenza viral copy numbers in nasopharyngeal swabs were at most weakly correlated with the viral copy numbers in aerosol particles, suggesting that viral aerosols stem from lung infections, not infections in the head airways. Respiratory aerosols therefore might provide a more representative source of diagnostic specimens for lower respiratory tract infections than nasopharyngeal swabs.

The biggest challenge to the use of respiratory aerosol analysis as a diagnostic tool is the small amount of viral material contained in the aerosols and the subsequent difficulty in the detection of viruses. This is especially true for analysis by multiplex PCR assay or genomic sequencing, which require larger amounts of material than single-species PCR analysis. In their experiments, Yan and colleagues[17] found that the influenza viral content collected from respiratory aerosols was 10^4 lower than found in nasopharyngeal swabs. The small amounts of viral material in exhaled aerosols leads to a requirement for extended collection times, which can present practical difficulties in the clinical setting. The system used by Yan et al. collected samples for 30 minutes,[17] while the system used by Lindsley et al. required about 20 minutes.[18] Mitchell et al. analyzed filters from patient ventilators, which allowed non-invasive sample collection for 24 hours.[19]

Analysis of exhaled breath aerosols for respiratory viruses also requires a collection method suitable for bioaerosols.[20] For example, systems that collect exhaled breath condensate do not necessarily collect exhaled breath aerosols efficiently,[21] and two analyses of exhaled breath condensate for respiratory viruses reported poor results.[22, 23] Simple filter-based bioaerosol collection systems have been used in several respiratory virus studies[14, 22, 23] and for many applications a filter system would be sufficient when culture is not required. Newer systems of exhaled breath aerosol collection allowing normalization to respiratory lining fluid volume (by monitoring exhaled droplet volume), size fractionation, and highly efficient collection and recovery of aerosol particles may provide clinicians and researchers with new tools in the near future.[24]

Tests of Infectiousness versus Disease

The studies reviewed above were all designed to assess infectiousness, i.e., the potential of patients with TB or influenza to transmit to others. However, they were not intended to assess the ability of these methods to diagnose disease. In our studies of TB (KF), we have observed that there were occasionally positive cough aerosol collections when patients did not produce a sputum specimen (unpublished data). Similarly, with influenza patients, we have sometimes observed patients with negative nasopharyngeal swabs who had positive cough aerosols.[15] These observations and the development of increasingly sensitive molecular markers suggest the potential of cough or breath aerosols to be used as specimens to diagnose disease. Microbial aerosols as diagnostic tests of disease may be especially useful for populations where sputum collection is particularly challenging, such as children, patients infected with the human immunodeficiency virus (HIV), other immunosuppressed patients, and the elderly. As suggested above, microbial aerosols may also be more representative of lower respiratory tract disease in viral illnesses in which sputum production is not common. Because exhaled aerosol collection is non-invasive, repeated sample collection should be more acceptable to patients than traditional methods. If the limitations can be overcome, exhaled aerosol analysis could become a useful tool for the diagnosis of respiratory infections and for monitoring the course of illness and response to treatment.

Microbial aerosol measurements may prove to be very useful as test of infectiousness for infection control or public health officers. For example, the identification of an aerosolpositive phenotype of TB patient who could be highly infectious, also known as ‘superspreaders’, opens the possibility for interventions aimed to prioritize case finding and targeted preventive therapy for contacts of high aerosol producers. The development of novel point of care tests incorporating polymerase chain reaction (PCR) or other amplification methods would enable aerosol sampling to be used to prioritize infection control resources within hospitals and other health care facilities, e.g., airborne isolation rooms in resource-limited settings.

Limitations and Challenges

The major advantages of patient-generated microbial aerosols are that they are collected non-invasively and that they likely provide more specific data on infectiousness. However, an inherent limitation of the collection of microbial aerosols from either coughs or tidal

breathing is the dilution of the pathogens in a large volume of air, compared to the relatively concentrated nature of some sputum specimens. This technical limitation was overcome in these early studies by collecting large volumes of air and by minimizing additional dilution by the entrainment of ambient room air. However, some of those studies were also focused on providing data on the particle size distribution of the aerosols to understand the potential for deposition within the respiratory tract and for remaining airborne. This goal resulted in technologically complex systems that are not appropriate for clinical settings. As the particle size data is no longer needed for clinical purposes, future collections can be much simpler. A disposable point-of-care device has been developed that collects infectious aerosols onto a filter that can be washed,[25] but it has only been studied in patients with cystic fibrosis (Figure 5). Further development of mask sampling or simple point-of-care devices paired with more sensitive diagnostic tests, e.g. molecular assays, will provide the opportunity for patientgenerated aerosols to become routine diagnostic specimens.

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Disclaimer

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Figure 1.

Cough Aerosol Sampling System initially used for detection of aerosols of culturable *M. tuberculosis*.. View inside of chamber with two Andersen cascade impactors and settle plate (left) and set up in procedure room ready for use (right). (Figure 1. Cough Aerosol Sampling System. View inside of chamber with two Andersen cascade impactors and settle plate (left) and set up in procedure room ready for use (right).

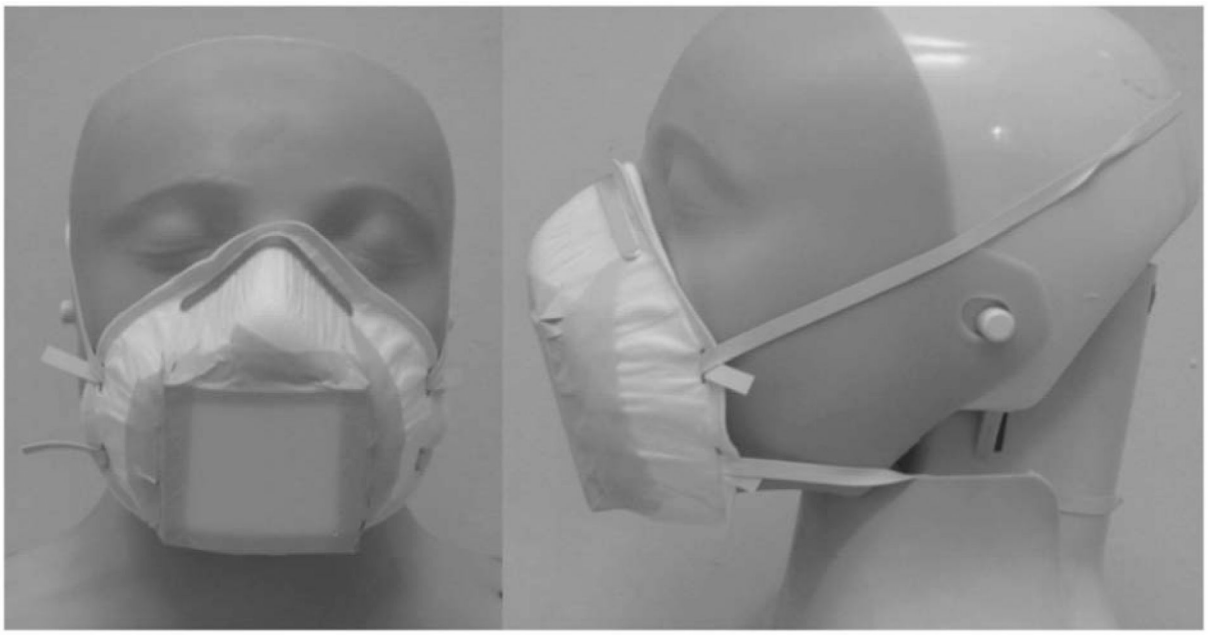

Figure 2.

Mask with filter for sampling of aerosols. (from Williams CML, Cheah ESG, Malkin J, et al. Face mask sampling for the detection of *Mycobacterium tuberculosis* in expelled aerosols. PLOS One 2014 9(8): e104921. doi:10.1371/journal.pone.0104921. Although this method has been developed for detection of *M. tuberculosis,* further developments may render it useful for detection of other pathogens.

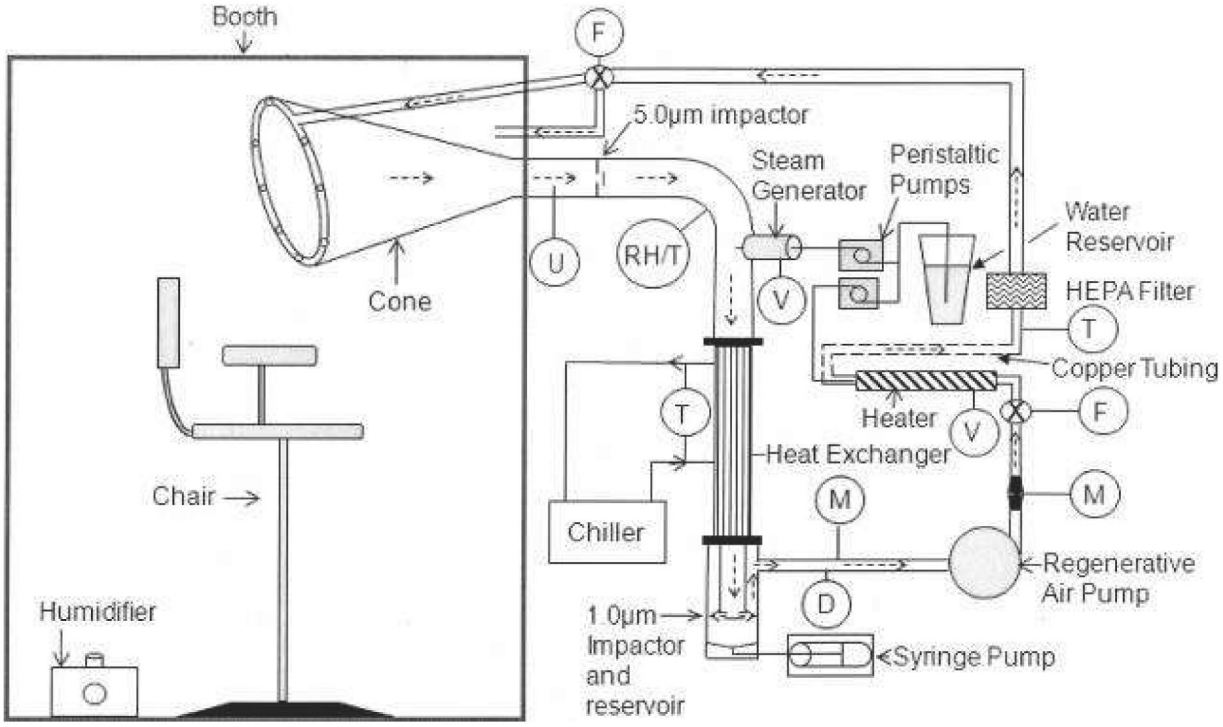

Figure 3.

System for collection of exhaled breath for sampling of influenza aerosols. (Courtesy of Donald Milton.) The patient or research participant sits in the chair and breaths into the cone which draws air into the sampling system.

Figure 4.

Cough aerosol sampling system for collection of influenza aerosols. (Courtesy of William Lindsley.) With this system, the patient or research participant breaths and coughs into a mouthpiece connected to a spirometer. The aerosol particles expelled by the patient are then collected by an aerosol sampler (yellow and black) attached to the spirometer. The brown instrument on the outside is a vacuum pump that pulls air through the aerosol sampler.

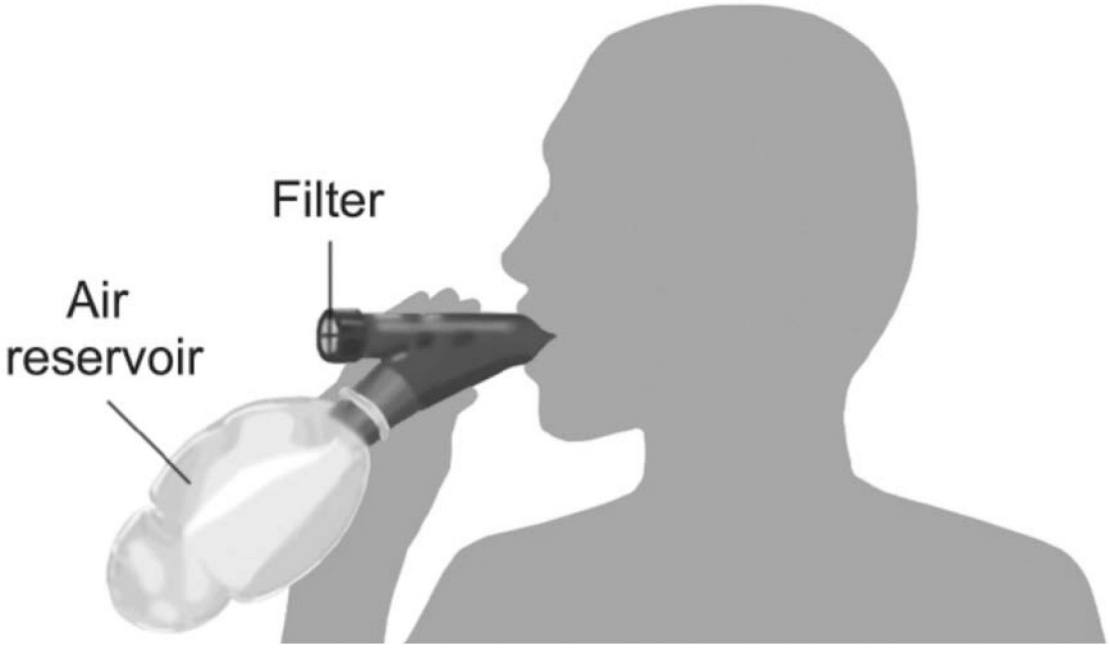

Figure 5.

PneumoniaCheck™ device for point-of-care sampling of cough aerosols. (from Ku DN, Ku SK, Helfman B et al. Ability of device to collect bacteria from cough aerosols generated by adults with cystic fibrosis. *F1000Research* 2016, **5**:1920 (doi: 0.12688/f1000research.9251.1) When a patient coughs into the device, the air reservoir collects air and aerosol particles from the mouth and upper airways. When the air reservoir is full, the air and particles from the lower airways are then forced through the filter, which collects the particles. The filter is then removed for microbiological assays.