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Bacterial pathogens were detected from human exhaled breath using a novel protocol

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ABSTRACT

It is generally believed that influenza outbreak is associated with breath-borne transmission of viruses, however relevant evidence is little for that of respiratory bacterial infections. On another front, point-of-care infection diagnostic methods at the bedside are significantly lacking. Here, we used a newly developed protocol of integrating an exhaled breath condensate (EBC) collection device (PKU BioScreen) and Loop Mediated Isothermal Amplification (LAMP) to investigate what bacterial pathogens can be directly exhaled out from humans. Exhaled breath condensates were collected from human subjects with respiratory infection symptoms at Peking University 3rd hospital using the BioScreen. The screened bacterial pathogens included *Streptococcus pneumoniae, Staphylococcus aureus, Methicillin-resistant Stphylococcus aureus* (MRSA), *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, Haemophilus influenzae, Legionella pneumophila, Mycoplasma Pneumonia, Chlamydia pneumonia, and Mycobacterium tuberculosis.* The results were further compared and validated using throat swabs from the same patients by a PCR method.

Here, human bacterial pathogens such as *H. influenzae*, *P. aeruginosa*, *E. coli, S. aureus* and MRSA were detected in exhaled breath using the developed protocol that integrates the EBC collection and LAMP. For the patients recruited from the hospital, seven types of pathogens were detected from 36.5% of them, and for the remaining subjects none of those screened bacterial pathogens was detected. Importantly, some super resistant bacteria such as MRSA were detected from the exhaled breath, suggesting that breathing might be also an important bacterial transmission route. Results from throat swabs showed that 36.2% of the subjects were found to be infected with *H. influenzae*, *P. aeruginosa*, *E. coli, S. maltophilia*, *S. aureus* and MRSA. For the EBC samples, 33.3% were found to be infected with MRSA, *E. coli* and *P. aeruginosa*. Depending on the initial pathogen load in the sample, the entire protocol (EBC-LAMP) only takes 20–60 min to complete for a respiratory infection diagnosis. For different detection methods and pathogens, the agreements between the EBC and throat swabs from the same patients were found to range from 35% to 65%. Here, we have detected several bacterial pathogens including MRSA from exhaled breath, and the developed protocol could be very useful for the bedside pathogen screening particularly in remote areas where resources are significantly limited or prohibited.

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1. Introduction

Respiratory infection results in a tremendous toll on humans worldwide every year. Despite of significant progress in medical science, infectious diseases continue to affect millions of lives around the world, especially in low-income countries (Cohen, 2000; Mori & Notomi, 2009). According to WHO (2014), lower respiratory infections (such as pneumonia) was listed as the second killer and one of the top three causes of years of life lost (YLL) in 2012. In addition, Acute Respiratory Infections(ARI) caused 15% of death among children aged < 5 years in 2013 (WHO, 2015). Among them, pneumonia alone accounts for 16% of all deaths of children under 5 years old, killing 920,136 children in 2015 (WHO, 2016a, 2016b). The transmission of airborne pathogens further worsens the situation, e.g., influenza viruses (Fabian et al., 2008; O'Brien & Nonnenmann, 2016; Smith et al., 2009). Studies showed that the airborne transmission of viruses was the main cause for some outbreaks of respiratory infections (Jones & Brosseau, 2015; Pyankov, Pyankova, & Agranovski, 2012). For example, a previous study has detected viable severe acute respiratory syndrome(SARS) virus and its RNA in the ambient air, and the SARS outbreak was shown to be due to the airborne virus transmission (Booth et al., 2005; Yu et al., 2004). Another work also demonstrated that the H7N9 influenza viruses emerging back in 2013 were transmissible in ferrets through the air by respiratory droplets (Zhang et al., 2013). Kim et al. (2016) reported the detection of Middle East Respiratory Syndrome (MERS) Coronavirus in hospital air samples, suggesting possible airborne transmission of MERS. On the other hand, studies also revealed that *Klebsiella pneumoniae* spreading in the air caused high morbidity and mortality (Chandrashekar, Rathish, & Nagesha, 1997; Prazmo, Dutkiewicz, Skorska, Sitkowska, & Cholewa, 2003), and a review from Beggs (2003) concluded that airborne route transmission of infectious agents were both directly and indirectly underestimated, e.g. with respect to *Staphylococcus aureus* and meticillin-resistant *S. aureus* (MRSA), *M. tuberculosis*, *Acinetobacter spp*., *Aspergillus spp*., *Pseudomonas spp*. and *Legionella* spp. and so on. Nonetheless, less is known compared to viruses for breath-borne bacterial pathogen emissions and transmissions.

When infected, it is critical for patients to be diagnosed accurately and timely to receive earlier and proper treatments (Urdea et al., 2006). Currently, clinical doctors often rely on the empirical experiences for diagnosis (Caliendo, 2011), and those approaches often fall short of providing accurate diagnosis (Falsey et al., 2013). On the other hand, colloidal gold immunization method is widely used in the fever clinic to distinguish Influenza A with Influenza B as it only needs less than 30 min. But these methods usually lead to higher rates of false-negative or false-positive results (Singh, Vasoo, Stevens, Schreckenberger, & Trenholme, 2010). In the meantime, a variety of methods are being developed or used for detection of pathogens, including culturing, amplification of nucleic acid (i.e. polymerase chain reaction (PCR), multiplex PCR, real-time PCR and DNA microarray or nucleic acid sequence-based amplification (NASBA)) (Hu, Yu, Crosby, & Storch, 2013; Petric, Comanor, & Petti, 2006; Xu & Yao, 2013; Zaas et al., 2013), immunological-based method (i.e. immunofluorescence or enzyme-linked immunosorbent assay (ELISA)) (Shen et al., 2012; Usachev, Agranovski, Usacheva, & Agranovski, 2015; Wu, Shen, & Yao, 2010), serologic testing (i.e. cytokine makers, C-reactive protein or procalcitonin) (Falsey et al., 2013; Haran, Buglione-Corbett, & Lu, 2013) and biosensor-based methods (optical, electrochemical and mass-based biosensors). Although some of these methods are effective, they take a longer time or are cost prohibitive for detection, e.g., the time needed for isolating and culturing pathogens. Recently, a new nucleic acids amplification method, the loop-mediated isothermal amplification (LAMP), has attracted great attention as a result of being highly specific for the target sequence (Notomi et al., 2000). Some LAMP commercial kits were already approved by Food and Drug Administration (FDA) (Ratliff, Duffy, & Waites, 2014), e.g., for detecting Mycobacterium tuberculosis complex (MTBC) by WHO (WHO, 2016a, 2016b). Importantly, the LAMP detection results can be simply validated using naked eyes due to higher LAMP product concentration. This opens up an outstanding opportunity for those remote areas without access to modern facilities to screen infectious agents. In addition to nasal swabs, bronchoalveolar lavages, nasopharyngeal aspirates or sputum samples, exhaled breath condensate (EBC) on another front is increasingly being used for disease diagnosis and virus detection (Kostikas et al., 2011; Shen et al., 2012; Teng et al., 2011). Despite of these new developments, affordable point-of-care diagnostic methods are still lacking at the bedside (Niemz, Ferguson, & Boyle, 2011).

Here, we developed a new protocol that integrates an exhaled breath condensate (EBC) collection device (PKU BioScreen) and Loop Mediated Isothermal Amplification (LAMP) and further used it to investigate what bacterial pathogens can be directly exhaled out from humans. The detection results from exhaled breath were then compared and validated using the throat swab samples collected from the same patients together with a gold standard molecular method-qPCR. The results from this work contribute not only to our understanding of human emission of infectious bacteria via breathing, and but also to the development of rapid pathogen screening protocol that could be potentially made available in remote areas where resources are significantly limited or prohibited.

2. Materials and methods

2.1. Clinical specimens

2.1.1. Patients and sample collection

Subjects involved in this research were recruited as respiratory tract infection patients who visited a respiratory clinic of Peking University Third Hospital, Beijing. Those patients were diagnosed with respiratory tract infections if they had at least one of these symptoms: 1) fever > 38 °C; 2) cough; 3) pharyngalgia. A total of 150 specimens comprising 100 throat swabs (IDs: 1–100, and 50 exhaled breath condensate (EBC)) samples (IDs: 1–36 and 87–100) were collected from 100 patients (ID numbers 1–100; their White Blood Cell count and Neutrophil (%) as well as other information are listed in Table S1) to compare the respective efficiencies of qPCR and LAMP method. Throat swab and EBC collection methods are both non-invasive, however the samples were used to study different health problems, e.g., EBC for lower airway inflammation (Cathcart, Love, & Hughes, 2012; Kostikas, Papatheodorou, Psathakis, Panagou, & Loukides, 2003) and throat swabs for upper respiratory tract infection (Thornton, Hay, & Redmond, 2017).