Original Article

Simultaneous Detection of 13 Key Bacterial Respiratory Pathogens by Combination of Multiplex PCR and Capillary Electrophoresis*

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Abstract

Objective Lower respiratory tract infections continue to pose a significant threat to human health. It is important to accurately and rapidly detect respiratory bacteria. To compensate for the limits of current respiratory bacteria detection methods, we developed a combination of multiplex polymerase chain reaction (PCR) and capillary electrophoresis (MPCE) assay to detect thirteen bacterial pathogens responsible for lower respiratory tract infections, including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Mycoplasma pneumoniae, Legionella* spp.*, Bordetella pertussis, Mycobacterium tuberculosis complex, Corynebacterium diphtheriae*, and *Streptococcus pyogenes*.

Methods Three multiplex PCR reactions were built, and the products were analyzed by capillary electrophoresis using the high-throughput DNA analyzer. The specificity of the MPCE assay was examined and the detection limit was evaluated using DNA samples from each bacterial strain and the simulative samples of each strain. This assay was further evaluated using 152 clinical specimens and compared with real-time PCR reactions. For this assay, three nested-multiplex-PCRs were used to detect these clinical specimens.

Results The detection limits of the MPCE assay for the 13 pathogens were very low and ranged from 10^{-7} to 10^{-2} ng/μL. Furthermore, analysis of the 152 clinical specimens yielded a specificity ranging from 96.5%-100.0%, and a sensitivity of 100.0% for the 13 pathogens.

Conclusion This study revealed that the MPCE assay is a rapid, reliable, and high-throughput method with high specificity and sensitivity. This assay has great potential in the molecular epidemiological survey of respiratory pathogens.

Key words: Respiratory pathogens; Lower respiratory tract infections; Multiplex PCR; Capillary electrophoresis

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INTRODUCTION

A lower respiratory tract infection (LRTI) is an acute illness usually presenting with cough as the main symptom, and with at least one other LRT symptom such as fever, sputum production, dyspnea, wheezing, or chest discomfort/pain and no alternative explanation[1]. LRTIs have a high mortality and morbidity worldwide, and The Global Burden of Disease Study reported that LRTIs are the second leading cause of deaths in 2013[2]. The age-standardized mortality rate of LRTIs has been reported to be 41.7 in 100,000 (95% *CI* 37.1-44.1)[2].

A large and growing number of microbial pathogens, including bacteria, atypical agents, viruses, and fungi, have been reported to be responsible for LRTI[3-5]. The clinical presentations of LRTI caused by different pathogens are very similar, making differentiation by clinical symptoms and pathology alone difficult.

Traditional methods used to identify respiratory pathogens include Gram stain of the sputum, culture of respiratory secretions and blood serological tests[6]. Although these traditional methods have their advantages in identifying respiratory pathogens to some extent, the disadvantages cannot be ignored. Such as time-consuming, labor-intensive and poor sensitivity. Molecular methods such as polymerase chain reaction (PCR) and real-time PCR have been developed for the rapid detection and identification for respiratory pathogens[7-10]. These methods have high sensitivity and specificity, and can be used to analyze clinical specimens, including blood, sputum, and urine. However, most molecular methods cannot detect more than five pathogens per reaction[7-10]. Therefore, designing a rapid, labor-saving, and high-throughput method to detect and differentiate respiratory pathogens in a single reaction would be greatly useful in enhancing rapid response for the prompt treatment and control for LRTI.

The Applied Biosystems 3730-XL 96-capillary DNA analyzer, which has been designed by Applied Biosystems (ABI, USA) Company, is based on the technology of capillary electrophoresis separation. It allows for multiplexed detection of molecules, and various molecular targets can be detected by using four types of fluorescent tags in 96 samples within a single 96-well plate. Additionally, fourteen 96-well plates could be placed in this analyzer at the same time, and the analyzer can automatically detect the different plates one by one. Compared with other methods, this is a high-throughput, rapid detection method with high specificity and sensitivity, and products differing by as little as 7-10 bp in size can be separated by this system[11].

In this study, a ABI 3730-XL DNA analyzer-based multiplex PCR and capillary electrophoresis (MPCE) assay was developed and applied for the simultaneous detection of 13 respiratory bacteria, including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Mycoplasma pneumoniae, Legionella* spp*., Bordetella pertussis, Mycobacterium tuberculosis complex, Corynebacterium diphtheriae, and Streptococcus pyogenes.* The sensitivity and specificity of the MPCE assay were tested, and 152 clinical samples collected from patients with LRTI were assayed for bacterial detection using this MPCE assay. The results using this method were then compared with results using the single real-time PCR assays mentioned below.

METHODS

Bacterial Strains, Simulative Samples, Clinical Specimens, and Controls

The 13 respiratory bacteria detected in this study are listed in Table S1 available in www.besjournal.com.

Simulative samples were composed by mixing sputum from individuals who didn't have respiratory infection symptoms and 11 target bacteria (from the 13 except for *M. tuberculosis and M. pneumoniae*). The sputum from these individuals were confirmed negative for the 13 target bacteria using 13 single real-time PCR assays. Serial tenfold dilutions (10^8-10^0 CFU/mL) of 11 bacteria (from the 13 target bacteria, except *M. tuberculosis and M. pneumoniae*) were mixed with the sputum samples (100 μL) separately. And the plate count method was used to measure bacteria in solution mentioned above (CFU/mL).

Then, 215 adult patients who were hospitalized in the Department of Respiratory Medicine, Shengjing Hospital, China Medical University, Shenyang, China, from May 2015 to January 2016, and were diagnosed with LRTI, including 180 cases of pneumonia, 14 cases of acute bronchitis, 17 cases of acute exacerbation of chronic obstructive pulmonary disease, and 4 cases of pulmonary abscess, were