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Original article

Detection of respiratory viral and bacterial pathogens causing pediatric community-acquired pneumonia in Beijing using real-time PCR

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Abstract

Objective: The aim of this study was to determine the etiology and prevalence of pediatric CAP in Beijing using a real-time polymerase chain reaction (PCR) technique.

Methods: Between February 15, 2011 and January 18, 2012, 371 pediatric patients with CAP were enrolled at Beijing Children's Hospital. Sixteen respiratory viruses and two bacteria were detected from tracheal aspirate specimens using commercially available multiplex real-time reverse transcription PCR (RT-PCR) kits.

Results: A single viral pathogen was detected in 35.3% of enrolled patients, multiple viruses in 11.6%, and virus/bacteria co-infection in 17.8%. In contrast, only 6.5% of patients had a single bacterial pathogen and 2.2% were infected with multiple bacteria. The etiological agent was unknown for 26.7% of patients. The most common viruses were respiratory syncytial virus (RSV) (43.9%), rhinovirus (14.8%), parainfluenza virus (9.4%), and adenovirus (8.6%). In patients under three years of age, RSV (44.6%), rhinovirus (12.8%), and *Streptococcus pneumoniae* (9.9%) were the most frequent pathogens. In children aged 3−7 years, *S. pneumoniae* (38.9%), RSV (30.6%), *Haemophilus influenzae* (19.4%), and adenovirus (19.4%) were most prevalent. Finally in children over seven years, RSV (47.3%), *S. pneumoniae* (41.9%), and rhinovirus (21.5%) infections were most frequent.

Conclusions: Viral pathogens, specifically RSV, were responsible for the majority of CAP in pediatric patients. However, both *S. pneumoniae* and *H. influenzae* contributed as major causes of disease. Commercially available multiplexing real-time PCR allowed for rapid detection of the etiological agent.

Keywords: Real-time reverse transcription polymerase chain reaction (RT-PCR); Respiratory virus; Community-acquired pneumonia

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Introduction

The latest report from the World Health Organization attributed between 1.6 and 2.2 million deaths in children under 5-year-old to acute respiratory illness.[1] Community-acquired pneumonia (CAP) is a major cause of morbidity and hospitalization in young children worldwide. The annual incidence is 34−40 cases per 1000 children in Europe and North America.[1] The symptoms and signs of CAP are fever, cough, malaise and chest pain. The etiological agents of CAP are varied. Bacterial agents known to cause CAP include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*.[2−4] Viral agents, such as influenza virus A and B, respiratory syncytial virus, parainfluenza viruses 1, 2, and 3, adenovirus, and rhinovirus are also common.[5−7] CAP can also be caused by emerging respiratory viruses such as human metapneumovirus,[8] human coronaviruses NL63[9] and HKU1,[10] and human bocavirus[11] have been reported as etiological agents of CAP.

It is currently difficult to reliably identify the pathogen responsible for causing CAP based on the clinical signs and symptoms.[7] To be successful, most treatments for CAP need to be initiated within 24−48 hours of infection. Thus, developing rapid diagnostic tests for viral pathogens associated with CAP is of the utmost importance. The epidemics and pandemic caused by the swine influenza A virus (H_1N_1) infection in 2009 have also highlighted the necessity of developing diagnostic tools that are sensitive and rapid tests for use during ongoing surveillance studies.

The diagnostic methods that are currently used to detect respiratory infections include rapid antigen tests, virus culture, enzyme immunoassays, immunofluorescence, and conventional reverse transcription polymerase chain reaction (RT-PCR) assays.[12,13] Virus culture is considered the gold standard because of its broad applicability and high specificity. However, it is time-consuming and it takes 7−12 days to obtain a positive culture. Enzyme immunoassays and immunofluorescence give rapid results, but, their relative lack of sensitivity and the availability of reactive antisera can be limiting factors.[14] Advances in conventional RT-PCR and real-time quantitative PCR assays have greatly facilitated the etiological study of respiratory infections due to their higher sensitivity and specificity. These assays can also reduce labor and cost by detecting more than one pathogen in a single reaction by using multiple probes.[15] There are several commercial multiplex assays available; such as xTAG RVP from Luminex, Multicode-PLx RVP from EraGen Biosciences, and ResPlex II from Qiagen. These assays have been used for broad spectrum detection of respiratory viruses with high cost and they are time-consuming.

In this study, the prevalence and causative agents of CAP in pediatric patients was described using PCR techniques. Commercial multiplex real-time RT-PCR kits designed to detect common respiratory pathogens, including *S. pneumoniae*, *H. influenzae*, influenza viruses A (swine lineage influenza A virus H_1N_1, season influenza A virus H_3N_2) and B, respiratory syncytial virus, parainfluenza viruses 1 to 4, adenovirus, rhinovirus, human metapneumovirus, human coronavirus (NL63, OC43, 229E and HKU1) and human bocavirus were used. The viral and bacterial infections associated with CAP and clinical and the epidemiological characteristics of CAP in a pediatric population were analyzed in this study.

Materials and methods

Subject enrollment and sampling

Informed consent was obtained from the objects' parents or guardians. The participants, and their guardians, received information detailing the purpose of the study and their right to have any information determined remain confidential.

Tracheal aspirate specimens were collected from pediatric patients presenting with CAP at the Beijing Children's Hospital between February 15, 2011 and January 18, 2012. The patients were enrolled according to a set of criteria that included cough, a high fever, yellow mucus, lung consolidation, the number of white blood cells $>10 \times 10^9$/L or $<4 \times 10^9$/L, or one of the above-mentioned symptoms plus a spot piece shape shadow by chest X-ray. The samples were collected and transported to the Institute of Immunization and Prevention of Beijing Center for Disease Prevention and Control for viral and bacterial nucleic acid extraction and detection.

Extraction of total RNA/DNA

The tracheal aspirate specimens were first treated with an equal volume of Sputasol solution (Oxoid, Basingstok, UK) in a 37 °C water bath for liquefaction. The total RNA/DNA was then extracted from the liquefaction sample using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. The nucleic acids was stored in aliquots at −80 °C until use.