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ORIGINAL ARTICLE

Comparison of diagnostic tools with multiplex polymerase chain reaction for pediatric lower respiratory tract infection: A single center study

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KEYWORDS
Lower respiratory tract infection;
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Background/Purpose: Acute respiratory tract infections are a leading cause of morbidity and mortality in children worldwide. Most have a viral etiology, with pneumococcus as an important pathogen. This single-center study compared the use of conventional diagnostic tools and two multiplex polymerase chain reaction (PCR) examinations for determining pathogens in lower respiratory tract infections (LRTIs) among children aged <5 years.
Methods: From July to October 2010, 45 patients aged 2 months to 60 months and diagnosed as having LRTIs were enrolled. Their nasopharyngeal aspirates were evaluated through viral culture and two multiplex PCR examinations. The patients' clinical course, symptoms, signs, and laboratory findings were recorded and analyzed.
Results: Among the 45 patients, 38 (84.4%) had detectable pathogens. Conventional viral and blood cultures had 35.6% positive rate, which increased to 51.1% when the quick antigen tests (Influenza A+B test and respiratory syncytial virus) and urine pneumococcal antigen test were combined. The positive rate further increased to 84.4% when the two multiplex PCR methods were combined. Twelve patients had co-infection, including 10 detected by the multiplex PCR methods. The co-infection rate was 26.7% (12/45).

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Conclusion: Most LRTIs in children have a viral etiology. Multiplex PCR tests are rapid assays that can increase the diagnostic yield rate and detect slow-growing viruses and can detect more pathogens than conventional viral culture to enable, thereby helping clinicians to provide appropriate and timely treatment.
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Introduction

Acute respiratory infections are a leading cause of morbidity and mortality in children worldwide,[1,2] imposing significant disease burden.[3] Most respiratory tract infections among children aged under 3 years have a viral etiology and children with co-infection are hospitalized more frequently than those with a single viral infection.[4] Respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, and adenovirus are the most common causes of lower respiratory tract infections (LRTIs) in children,[5,6,7] whereas *Streptococcus pneumoniae* is a frequent cause of bacterial community-acquired pneumonia in Taiwan.

Conventional diagnostic tools such as viral, blood, and sputum cultures have high false negative rates such that some pathogens are often underestimated. Bacterial LRTIs require prompt diagnosis for early proper antibiotic therapy. The early recognition of pathogens in such infections helps in determining more appropriate treatment actions.

This study aimed to survey pathogens of LRTIs in children aged ≤5 years in a single center using two kinds of multiplex polymerase chain reaction (PCR) methods and to compare these with conventional diagnostic tools.

Methods

Participants

Children with LRTI admitted to the pediatric ward of Kaohsiung Veterans General Hospital from July to October 2010 were enrolled. In addition to detailed history taking and physical examinations, chest plain x-rays were taken to assist diagnosis. Lower respiratory tract infection was defined as a diagnosis of bronchitis, bronchiolitis, bronchopneumonia, or pneumonia. Children with asthma and laryngo-trachitis were excluded.

The hospital's institutional review board and ethics committee approved the study (VGHKS 99- CT-7-13) and the patients' parents or guardians provided written informed consent.

Specimens

Within 48 hours after admission, nasopharyngeal aspirates were collected from each patient and sent for viral culture and two kinds of multiplex PCRs. Other laboratory examinations included complete blood cells with differential counts, levels of C-reactive protein, blood urea nitrogen, serum creatinine, aspartate aminotransferase, and alanine aminotransferase, and sputum and blood cultures.

Pathogen identification

The specimens underwent conventional viral culture and nucleic acid extraction for multiplex PCR amplification examinations. The viral culture utilized a series of primary cell lines (human hibroblast, rhesus monkey kidney) and continuous cell lines (A549 human lung carcinoma) selected for their ability to support the replication of a wide variety of clinically relevant viruses. The specimens were inoculated onto these cell culture monolayers and monitored by light microscopy for the cytopathic effect, which were the visible cellular changes that occurred in response to viral infection.

The QIAGEN QIAamp RNA and DNA mini kits (Qiagen, Valencia, CA, USA) were used for extraction of nucleic acids from pathogens in the nasopharyngeal samples, and prepared these extracted nucleic acids for the two multiplex PCR methods: the Respiratory Viral Panel (RVP; Luminex Molecular Diagnostics, Totronto, Canada) for respiratory virus and the ResPlex I panel (Qiagen) for adenovirus and bacteria. These two multiplex PCR methods could detect pathogens from nasopharyngeal specimens within one day.

For RVP, the extracted nucleic acids from the QIAamp RNA mini kits were reverse transcribed to complimentary DNA for testing. Specific primers to each target virus were mixed for multiplex target-specific PCR and to rapidly create multiple copies of DNA. The amplified DNA was mixed with short sequences (TAG primers) of DNA that were specific to each viral target. If the target was present, the primer would bind and be lengthened through a process called target-specific primer extension. During this process, a label was incorporated. Amplified products were labeled while color-coded beads were added for the easy identification of tagged primers. They were then placed in the Luminex 200 instrument (Luminex, Austin, TX, USA) and the color-coded beads were analyzed by lasers (Luminex xMAP technology). The lasers identified the color of the beads (specific to a virus or subtype) and the presence or absence of the labeled primer. If a particular virus was present, it would generate a signal and be identified by the associated data analysis software (xPONENT software, Luminex) as positive. This method detected viral pathogens like adenovirus, entero-rhinovirus, influenza A and B, para-influenza 1, 2, 3, and 4, RSV, and human metapneumovirus.

In the ResPlex I panel method, the HotStarTaq Master Mix, ResPlex I SuperPrimers, extracted nucleic acids and RNase-free water were prepared for amplification reaction. A negative control (without template DNA) was also prepared. The GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA), running in 9600 emulation mode, was then used to program the thermal cycle. After

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