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ORIGINAL ARTICLE

Viral etiology of bronchiolitis among pediatric inpatients in northern Taiwan with emphasis on newly identified respiratory viruses

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Purpose: Viral etiology of bronchiolitis in children in Taiwan has been fragmentary. We conducted a prospective study to figure out the viral epidemiology of bronchiolitis in Taiwan.
Materials and methods: From January 2009 to March 2011, a total of 113 children with bronchiolitis, aged <2 years, hospitalized in Chang Gung Children's Hospital were randomly selected for viral etiology investigation. Nasopharyngeal aspirates were obtained from each case and sent for viral detection by tissue culture, antigen test, and polymerase chain reaction.
Results: A total of 120 viruses were detected from 113 children. Positive viral etiology was identified in 86 (76%) children. Mixed viral pathogens were found in 28 cases (25%). Respiratory syncytial virus (RSV) was the most common pathogen and was identified in 43.4% of the cases. Human bocavirus (hBoV) was the second most common identified virus (in 19.5%), followed by human metapneumovirus (hMPV), rhinovirus, influenza viruses, and coronavirus OC43. In terms of clinical characteristics, no significant difference was found among the children with bronchiolitis either caused by different single or mixed viral infection.
Conclusion: RSV was the most common etiologic agent for children with bronchiolitis in Taiwan. Newly identified viruses, including hMPV and hBoV, were also among the common causative agents. Clinical characteristics were not significantly different among the children with bronchiolitis caused by different viruses.

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Introduction

Bronchiolitis is the most common lower respiratory tract infection and a major cause of hospitalization in infants around the world. It is characterized by acute inflammation, edema, and necrosis of epithelial cells lining small airways; increased mucus production; and bronchospasm. Clinical symptoms and signs of bronchiolitis typically include rhinitis, tachypnea, wheezing, cough, crackles, use of accessory muscles, and/or nasal flaring.[1,2]

With the development of molecular techniques and the availability of monoclonal antibodies for numerous viral species, detection of viral respiratory agents has been markedly improved in the past decades. These advances allowed re-evaluation of the role of various respiratory viruses in the pathogenesis of acute bronchiolitis. However, it is not infrequently seen that no causative agent can be identified in patients with bronchiolitis.

Respiratory syncytial virus (RSV) is the most frequently identified agent responsible for bronchiolitis worldwide.[3,4] However, many other respiratory viruses may also cause bronchiolitis. Until now, viral etiology of bronchiolitis in children in Taiwan has been limited, particularly the newly identified viruses, including human metapneumovirus (hMPV), human bocavirus (hBoV), and human coronavirus (hCoV) NL-63.[5,6] Therefore, we conducted a prospective study to figure out the viral epidemiology of bronchiolitis among pediatric inpatients in Taiwan.

Materials and methods

Patients and respiratory specimens

The study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, and informed consent was obtained from parents or legal guardians of the children. From January 2009 to March 2011, children (aged <24 months) with the initial diagnosis of acute bronchiolitis, hospitalized in Chang Gung Children's Hospital situated in northern Taiwan, were eligible for this study, and up to three cases per week were randomly selected for viral etiology investigation. Nasopharyngeal aspirates were collected prospectively from all patients within 1—3 days after hospital admission.

The diagnosis of acute bronchiolitis was made by an acute onset of respiratory distress with cough, tachypnea, retraction, and expiratory wheezes, often accompanied by rales. Patients with underlying chronic diseases, including cerebral palsy with bedridden status, tracheostomy, congenital heart disease, chronic pulmonary disease, and immunodeficiency, were excluded.

Medical records were reviewed for detailed demographic, clinical, and laboratory data; radiographic images; and underlying conditions of the patients. All the clinical symptoms and signs were recorded on a standardized form while the patients were hospitalized.

Virus detection

All the specimens were processed, and then nucleic acids (including DNA and RNA) were extracted by commercial kits and kept in a refrigerator at −70°C for further analysis. All specimens were sent for viral detection by conventional viral culture, immunofluorescent antigen detection for RSV, and multiplex reverse transcription-polymerase chain reaction (RT-PCR) for six viruses, including rhinovirus (RV), hMPV, hCoV-229E, hCoV-OC43, NL-63, and hBoV.

Sample preparation for virus culture and immunofluorescence

Nasopharyngeal aspirates were mixed with sterilized phosphate-buffered saline (PBS) followed by vigorous vortex. Then the cell suspension was centrifuged at 3000 rpm for 10 minutes to get the cell pellet. Finally, the supernatant was discarded and PBS was added to resuspend the cells again. The procedures were repeated for three times. After the third time wash, the cell pellet was resuspended in viral transport medium and treated with antibiotics for 30 minutes.

Virus isolation

Specimens of cell suspension prepared as described above were inoculated into MK2, MRC-5, and MDCK cells and incubated at 35°C for 2 weeks. Cytopathic effect (CPE) of all culture tubes was checked every 2 days. For CPE-positive tubes, a screening kit of immunofluorescence assay for respiratory virus (Chemicon Inc., Temecula, California, USA) was used for further examination of respiratory virus infection. Only the respiratory viruses identified, including RSV, parainfluenza (PIV)-1, PIV-2, PIV-3, adenovirus, influenza viruses A and B, were regarded as pathogens.

Immunofluorescent assay for the detection of RSV antigen

The cell suspension (50 μL) was added to a slide and fixed in acetone for 10 minutes. Monoclonal antibody that conjugated Fluorescein isothiocyanate (FITC) to RSV was used to detect RSV antigen.

RT-PCR assay

It is designed to amplify conserved region of each virus target. Sequence of primer for each virus target is given in Supplementary Table 1. Viral RNAs were extracted from 200 μL respiratory specimens using QIAmp Viral RNA kit (Qiagen, Chatsworth, CA, USA), and reverse transcription reactions were performed for complementary DNA synthesis using SuperScriptTM III One-Step RT-PCR kit (Invitrogen, Carlsbad, CA, USA). Each reaction included the following components: 0.2 M probe, 0.4 M primer, 12.5 l of 2× ABI Mater mixture (containing 0.4 mmol/L deoxyribonucleotide triphosphate (dNTP) and 2.4 mmol/L $MgSO_4$), and 5μL of specimen RNA extract or control. The final reaction volume was adjusted to 25 μL with PCR-grade water, and RT-PCR amplification was performed using the following conditions: an initial cDNA step at 50°C for 30 minutes, followed by at 95°C for 15 minutes, and 50 cycles at 95°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 30 seconds. RT-PCR products of hCoV-NL63 and hBoV for positive control were kindly provided by Professor Patrick C.Y. Woo (Hong Kong University).