

論文の内容の要旨

論文題目 Molecular epidemiology of acute respiratory infections by influenza virus, respiratory syncytial virus, human bocavirus and rhinovirus in Vietnamese children
(ベトナムの小児におけるインフルエンザウイルス、RS ウイルス、ヒトボカウイルス、ライノウイルスによる急性呼吸器感染症の分子疫学)

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Acute respiratory infections (ARIs) remain a leading cause of morbidity and mortality among children worldwide. However, limited information about etiologies of ARIs makes it difficult to improve the prevention, diagnosis and treatment in developing and tropical countries. This study was conducted to describe the molecular epidemiology and clinical characteristics of viral ARIs among hospitalized children in Vietnam.

From April 2010 to May 2011, clinical data and nasopharyngeal (NP) samples were collected from children with ARIs admitted to Children's Hospital 2 at Ho Chi Minh City, Vietnam. Four multiplex PCR assays were performed to detect 13 respiratory viruses in NP samples. The genetic characteristics of the most commonly detected viruses were further analyzed.

A total of 1,082 patients were enrolled. At least one virus was detected in 64.6% of cases, with 12% co-detection. The most frequent detected viruses were rhinovirus (HRV) (30%), respiratory syncytial virus (RSV) (23.8%), human bocavirus (HBoV) (7.2%) followed by parainfluenza virus (PIV) type 3 (5.3%), PIV type 1 (3.3%), and influenza A (FLU A) (3.2%). HRV was detected all year round, while RSV epidemic occurred mainly in the rainy season. FLU A was found in both the rainy and dry seasons with seasonal H3N2 in the former and pandemic H1N1 2009 (H1N1pdm09) in the latter. Other viruses were predominant in the dry season. HRV was detected in all age groups. RSV predominantly affected children < 6 months, PIV3 6 – 12

months, PIV1 and HBoV 12 – 24 months, and FLU A > 24 months. PIV1 detection was associated with croup ($p<0.005$) and RSV detection was associated with bronchiolitis ($p<0.005$). HBoV and HRV detections were associated with hypoxia and RSV detection was associated with chest retraction ($p<0.05$). These data show that a high incidence of virus-associated ARIs was found among hospitalized children in Vietnam. HRV, RSV, and HBoV were the most commonly detected viruses and associated with the severity of ARIs in children.

Phylogenetic analyses of FLU A showed that Vietnamese H1N1pdm09 strains in 2010-2011 formed the distinct cluster, with other contemporary Asian and 2012-American strains, suggesting a possible common ancestor. The Vietnamese H3N2 viruses in mid-2010 belonged to the emerging subclade Perth10/2010, which then spread worldwide in 2011. These results confirm that Southeast Asia may be the hot spot for influenza viruses to evolve before spreading worldwide. Although Vietnam locate in the Northern Hemisphere and are using the Northern Hemisphere vaccine formulation in private sectors, the Vietnamese influenza viruses in this study were well matched with the Southern Hemisphere vaccine formulation. Together with the bimodal seasonal pattern of influenza found in this study, this information is useful for policy-makers in development of the national vaccination program. Two strains carrying S247N and D199N in NA which reduced the neuraminidase inhibitor susceptibility were found. Mutations at antigenic sites were also identified in these viruses. Clinically, H1N1pdm09 viruses in the current study were not associated with severe symptoms. Interestingly, one case was detected with the swine H3N2 variant. Surveillance of influenza viruses in tropical countries is important not only for the development of their prevention and control strategies but also for earlier identification of the emerging new strains that may be selected for future vaccine.

RSV is a major cause of ARIs in children but information on the clinical and molecular characteristics of RSV infection in developing countries is limited. In this study, RSV A was the dominant subgroup, accounting for 91.4% (235/257), followed by RSV B, 5.1% (13/257), and 3.5% (9 cases) had a mixed detection of these subgroups. The phylogenetic analysis revealed that all group A strains belonged to the GA2 genotype. All group B strains belonged to the recently identified BA genotype, and further clustered into 2 new subgenotypes BA9 and BA10, firstly described in Japan. One GA2 strain had a premature stop codon which shortened the G protein length. This is the first time this mutant strain was identified directly from a clinical

sample. RSV detection was significantly associated with younger age and higher severity score than those without ($p<0.005$). Patients with RSV co-detection were more likely to have hypoxia than those with RSV single detection ($p=0.038$). RSV A was associated with more severe disease than RSV B, implying that determination of the subgroup at presentation would be useful for appropriate management and group A may have more priority than group B in choosing candidate strains for vaccine development. This is the first time the molecular features and disease severity of RSV were described in Vietnam. These results will not only contribute to the growing database on the molecular diversity of RSV circulating worldwide but may also be useful in clinical management and vaccine development.

HBoV, a novel virus, is recognized to increasingly associate with respiratory infections of previously unknown etiology in young children. In this study, HBoV was the third most common detected virus (7.2%). HBoV was associated with severe diseases, as the rates of hypoxia and pneumonia were higher, and hospitalization duration was longer in patients with HBoV detection than in those without ($p<0.05$). The phylogenetic analysis of partial VP1 gene showed minor variations and all HBoV sequences belonged to species 1 (HBoV1). These results imply that HBoV1 may have an important role in ARIs among children.

HRV has been recently identified as the leading cause in asthma exacerbation, severe bronchiolitis and pneumonia. In this study, HRV was the most frequently detected virus (30%). Among HRV mono-detected children, 70% were diagnosed as either pneumonia or bronchiolitis, and 12.4% had hypoxia that needed oxygen supplementation. Children with HRV were more likely to have difficult breathing and abnormal chest X-ray ($p<0.05$). Children hospitalized with HRV had less fever but more hypoxia and high blood eosinophil count than those detected with other viruses ($p<0.001$). Co-detection with HRV was associated with more difficult breathing than mono-detection ($p<0.05$). During the weak season of RSV (dry season), HRV replaced RSV to become the most common detected virus among patients with bronchiolitis and pneumonia. Among 58 randomly selected HRVs for sequencing, only 2 species were detected with HRV-A being more common (44/58) than HRV-C (14/58). The clinical symptoms of patients with HRV-C positive were similar to those with HRV-A positive. Phylogenetic analysis revealed diversity of circulating HRV types including 21 types of HRV-A and 10 types of HRV-C. One strain of HRV-A showed a high nucleotide difference exceeding the defined threshold and

proved to be new type. These results provide new insights into the burden and severity of HRV and highlighted the need for routine diagnosis in hospital settings.

In conclusion, this study confirmed the diversity and the importance of respiratory viruses associated with ARIs required hospitalization in Vietnamese children. Specific viruses were frequently associated with specific clinical syndromes, specific seasonal patterns and age distributions. The demographic, epidemiological and clinical information from this study is useful to improve the diagnosis, prevention and treatment activities especially in resource-limited countries. The molecular characterization of common respiratory viruses was determined for the first time in Vietnam, and some interesting results were found such as new type of HRV, new strain of RSV, and drug resistant strain of FLU A.