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Genetic characterization of human respiratory syncytial virus detected in hospitalized children in the Philippines from 2008 to 2012

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ABSTRACT

Background: Human respiratory syncytial virus (HRSV) is the leading cause of acute lower respiratory tract infection in infants and young children. However, molecular characteristic of HRSV is still unknown in the Philippines.
Objective: To describe the molecular epidemiology of circulating HRSV detected in the Philippines.
Study design: From May 2008 to April 2012, nasopharyngeal swabs were collected from infants and children aged between 7 days and 14 years who were hospitalized with severe pneumonia. HRSV was detected by nested PCR targeting M2 gene, and C-terminus of the G gene was sequenced for phylogenetic analysis.
Result: Out of total 2150 samples, 19.3% ($n = 415$) were positive for HRSV, and 65.0% of them ($n = 270$) were identified as HRSV-A and 35.0% ($n = 145$) as HRSV-B. There were two major HRSV outbreaks: between June 2008 and February 2009, and between June and March 2012. Majority of HRSV strains detected during the former outbreak were HRSV-A (97.5%, 203/208) whereas during the later outbreak, both HRSV-A (54/158, 34.2%) and HRSV-B (104/158, 65.8%) were detected. All HRSV-A strains were classified as genotype NA1 and all HRSV-B as genotype BA, which had 60-nucleotide duplication in secondary hypervariable region of the G gene. Among HRSV-B positive samples, there were 2 distinct clusters with unique amino acid changes and low homology in compared to other strains in BA, suggesting emergence of new variant of HRSV-B.
Conclusion: The study provides an overview of the genetic variation in circulating HRSV viruses in the Philippines along with identification of possibly a novel variant of HRSV-B.

1. Background

Acute lower respiratory tract infection (ALRI) is a leading cause of death in children aged less than five years worldwide, and most of those deaths occur in developing countries.[1] The role of human respiratory syncytial virus (HRSV) in the etiology of ALRI has been well defined in developed countries[2,3] as well as in developing countries.[4–7] In the Philippines, several etiological studies on ALRI in children have been conducted, in which HRSV were detected in 7.1–18.9% of the ALRI cases.[8–12] Recently, we showed that HRSV is the second most common respiratory viral pathogen next to rhinoviruses among the children with severe pneumonia in the Philippines.[13] However, molecular characterization of the detected HRSV has not been yet studied.

HRSV has been divided into two subgroups (HRSV-A and HRSV-B) by reactivity with monoclonal antibody.[14,15] The HRSV envelope contains three transmembrane surface glycoproteins; the major attachment protein G, the fusion protein F, and the small hydrophobic SH protein. G protein is mainly involved in virus attachment

Abbreviations: HRSV, human respiratory syncytial virus; HRSV-A, human respiratory syncytial virus subgroup A; HRSV-B, human respiratory syncytial virus subgroup B; ALRI, acute lower respiratory tract infection; EVRMC, Eastern Visayas Regional Medical Center; NPS, nasopharyngeal swab; IMCI, Integrated Management of Childhood Illness; VTM, viral transport medium; RITM, Research Institute for Tropical Medicine; cDNA, complementary DNA; N, nucleocapsid; m-PCR, multiplex polymerase chain reaction; M-MLV reverse transcriptase, Moloney murine leukemia virus reverse transcriptase.

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to host cells and plays major role in the immunogenicity of the virus. Nowadays, HRSV subgroups can also be identified by genetic analysis.[16] G gene is the most variable among HRSV genes and has two hypervariable regions. The analysis of this region is commonly used for studying the molecular epidemiology studies of HRSV.[17,18] Currently, HRSV-A has 10 genotypes (GA1–GA7, SAA1, NA1, NA2) whereas HRSV-B has 9 genotypes (SAB1–SAB4, GB1–GB4, BA) with BA having 11 branches (BA1–11).[19–25]

2. Objectives

The objective of this prospective study was to describe the molecular epidemiology of circulating HRSV in the Philippines for the last four years between May 2008 and April 2012 by phylogenetic analysis of the secondary hypervariable region of G gene.

3. Study design

3.1. Patients and clinical samples

From May 2008 to April 2012, nasopharyngeal swabs (NPSs) were collected from infants and children aged between 7 days and 14 years who were hospitalized for severe pneumonia in Eastern Visayas Regional Medical Center (EVRMC) in Tacloban city of Leyte island, the Philippines.[13] The patients were diagnosed as severe pneumonia according to the case definition of Integrated Management of Childhood Illness (IMCI).[26] A total of 2150 patients were enrolled into the study. After sample collection, NPSs were stirred in 3 ml of viral transport medium (VTM) and transported with ice packs to the Research Institute for Tropical Medicine (RITM) in Manila for further analysis.

3.2. RNA extraction and complementary DNA synthesis

RNA was extracted from supernatant of clinical samples using QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany). Reverse transcription PCR to synthesize complementary DNA (cDNA) was performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase and random primers (all were from Invitrogen Carlsbad, CA, USA).

3.3. Detection of HRSV

For HRSV screening, multiplex polymerase chain reaction (m-PCR) was performed to amplify nucleocapsid (N) gene of HRSV together with influenza A virus, influenza B virus, and human metapneumovirus as previously described (Table 1).[13,27] The results of multiplex PCR were confirmed by PCR targeting M2 gene using Ex Taq (TaKaRa, Otsu, Japan) (Table 1).[28] From January 2010, we used PCR targeting M2 gene as an alternative screening method.

3.4. Subgrouping, sequencing and phylogenetic analysis of HRSV

To determine subgroups, we performed the nested PCR targeting C-terminus of the G gene, which included the secondary hypervariable region (HRSV-A: 270 bp, HRSV-B: 330 bp)[17,22] (Table 1). After purifying the amplification product using SUPREC™ PCR (TaKaRa, Otsu, Japan), nucleotide sequence was determined by BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and Genetic Analyzer 3130 (Applied Biosystems, Foster City, CA, USA).

Related sequences were searched by using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees based on sequences of the second hypervariable region of G gene were generated by the neighbor-joining method and maximum composite likelihood model with Molecular Evolutionary Genetic Analysis (MEGA) software version 5.0.[29] Since there is an insertion of 60 nucleotides in HRSV-BA, we used both complete and pairwise deletion method for treating the gap and confirming the consistency of formulated trees. Representative sequences of the corresponding region of each genotype were adapted from previous studies,[20–25,30,36–39] and the identical sequences were excluded from the analysis (Supplemental Table 1). *Homogeneity* among the strains was compared by pairwise distances (p-distance) estimated by MEGA software version 5.0. All sequences used in present study were deposited in the GenBank under accession numbers: AB749604–AB749760.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.01.001.

4. Results

4.1. Detection and subgrouping of HRSV

From May 2008 to April 2012, 2150 NPSs were collected from hospitalized children with severe pneumonia and 19.3% ($n = 415$) were positive for HRSV. HRSV positive rates were varied yearly with the highest rate in 2008 (33.0%, 203/615) and the lowest in 2009 (1.8%, 6/339) (Fig. 1). By subgroup specific nested PCR targeting G gene, HRSV-positive samples were classified into HRSV-A (65.0%, 270/415) and HRSV-B (35.0%, 145/415).

During the study period, there were two major HRSV outbreaks; one was in between June 2008 and February in 2009 and another one was in between June 2011 and March 2012. Majority of HRSV strains detected in the earlier outbreak were HRSV-A (203/208, 97.5%). On the other hand, the outbreak in 2011 to 2012 was caused by both HRSV-A (62/164, 37.8%) and HRSV-B (102/164, 62.1%) (Fig. 1). Notably, in the beginning of the second outbreak in 2011, a majority of detected HRSV was HRSV-A, but the numbers of HRSV-B cases were increased toward the end of the outbreak. From October 2009 to March 2010, only HRSV-A cases were detected; on the other hand, both HRSV-A and HRSV-B were detected between May 2010 and January 2011. It is noteworthy that increased numbers of total hospitalized cases with severe pneumonia coincided with these two HRSV outbreaks. Only sporadic cases were detected in between October 2009 and January 2011 with slight increase in January 2010.

4.2. Genetic characterization

The sequence of the second hypervariable region of C-terminus of G gene was determined for 96 out of 270 (35.6%) HRSV-A positive samples and 77 out of 145 (53.1%) HRSV-B positive samples (Supplementary Table 2).

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The phylogenetic tree of G gene's second hypervariable region (270 nucleotides) indicated that all HRSV-A were classified as NA1[24] (Fig. 2A). Based on the BLAST, the cases detected in 2008–2009 had high homology (>97.6%) with other NA1 strains detected in different parts of the world. NA1 strains detected in 2011–2012, except TTa-11-0405, and two strains in 2010 (TTa-10-0261 and TTa-10-0328) were appeared to form a unique cluster under NA1. Three amino acid substitutions, Asp^{237}, Leu^{274}, and Ser^{292} have been previously reported as specific amino acid substitutions for NA1.[24,25] We also found these substitutions in most of the NA1 strains analyzed in the present study such as Asp^{237} in 94.2% (82/96), Leu^{274} in 94.2% (82/96), and Ser^{292} in 100% (96/96) (Fig. 4A). Another specific substitution for NA1, an early stop codon at position 298, was also observed in 100% (96/96) of NA1 strains. In addition to these previously reported NA1 specific substitutions,