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Molecular epidemiology and molecular characterization of respiratory syncytial viruses at a tertiary care university hospital in Catalonia (Spain) during the 2013–2014 season

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ABSTRACT

Background: Human respiratory syncytial virus (HRSV) is the main cause of lower respiratory tract infections among infants and young children.
Objectives: The molecular epidemiology and characterization of HRSV strains detected at a Spanish tertiary hospital during the 2013–2014 season is reported.
Study design: Phylogenetic analysis and molecular characterization of HRSV laboratory-confirmed respiratory samples were performed, based on coding sequences of G and F proteins.
Results: HRSV infection was laboratory-confirmed in respiratory samples from 320 patients of which 223 (70%) were less than 2 years of age and none undergoing Palivizumab treatment. HRSV was detected at varying levels throughout the season with a maximum rate in the week 52/2013, right before the beginning of the influenza epidemic. Whilst both HRSV groups were found co-circulating, HRSV-B group clearly predominated. The phylogenetic analyses from 139 HVR-2 sequences revealed that most characterized strains belonged to ON1 and BA9 genotypes. Three different phylogenetic subgroups could be distinguished within these genotypes. In addition, three strains (out of the 52 randomly selected) were carrying amino acid substitutions in the epitope A of the F protein, one of them previously related to Palivizumab resistance.
Conclusions: The results of the present study highlight the importance of a continuous HRSV surveillance to monitor not only the introduction of new genotypes on circulation but also the emergence of viral variants with new genetic characteristics that can affect the antigenicity features and the susceptibility to the only current prophylaxis treatment and also for the future development of HRSV vaccines.

1. Background

Human respiratory syncytial virus (HRSV) is the most common respiratory pathogen and the main cause of lower respiratory tract infections (LRTI) among infants and young children. Primary infection usually affects infants under two years of age, and reinfections are common throughout life. HRSV is also recognised as a significant respiratory pathogen among immunosuppressed and elderly patients.

HRSV is classified in the genus *Pneumovirus*, family of Paramyxoviridae. Its genome is a non-segmented negative-strand RNA of approximately 15,000 nucleotides that contains 10 genes, which encode for 11 proteins. Two different groups, HRSV-A and HRSV-B, are described based on antigenic and genetic differences. Subsequently, twelve genotypes have been described for HRSV-A (GA1–GA7, SAA1, NA1–NA2, CB–A and ON1), and twenty for HRSV-B (GB1–GB4, SAB1–SAB3 and BA1–BA12, CB–B), using the hypervariable region 2 (HVR-2) in the *C*-terminal domain of the G protein. HRSV exhibits a clear pattern of seasonality; epidemics usually occur in the late fall and winter in temperate regions, or during the rainy season in tropical countries.

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To date, no safe and effective human vaccine has been licensed for prophylaxis. However, Palivizumab (HRSV-specific humanized monoclonal antibody) is currently used as a prophylaxis treatment for paediatric patients at high-risk for severe HRSV infection. Palivizumab binds the antigenic site A of the viral fusion protein (F), but some amino acid substitutions have already been associated with resistance despite being a highly conserved region [1,2]. As other RNA viruses, HRSV has a high capability to acquire point mutations in its genome, mainly in the coding regions of the envelope glycoproteins (G and F proteins) that are under the selection pressure from immunity [2,3]. Therefore, the emergence and selection of viral variants carrying new antigenic and genetic mutations can affect the diversity described among population and the viral features that are determinant to evade the recognition of Palivizumab and its prophylactic effectiveness.

2. Objective

To describe the genetic diversity of HRSV strains in respiratory samples collected from patients attended during the 2013–2014 season in the Hospital Universitari Vall d'Hebron (HUVH) in Barcelona (Spain), a tertiary 1200-bed university hospital, using the complete or partial sequencing of the coding sequences of the viral proteins G and F, respectively.

3. Study design

3.1. Patients and samples

From October 2013 (week 40/2013) to May 2014 (week 20/2014), upper (nasopharyngeal aspirates or swabs) and lower (bronchoalveolar lavages, bronchoaspirates and tracheal aspirates) respiratory tract samples were collected for laboratory-confirmation of respiratory virus infection from patients attended at the HUVH. Respiratory specimens were processed within the first 24 h, being kept at 2–4 °C in several aliquots until use.

3.2. Detection and typing of HRSV from respiratory specimens

The detection of HRSV was performed either by immunochromatography (Binax Now RSV Card, Allere Scarborough Inc., USA), immunofluorescence (D^3 Ultra 8™ DFA Respiratory Virus Screening & Identification Kit Diagnostic HYBRIDS, USA) or real-time multiplex RT-PCR (Anyplex II RV16 Detection Kit Seegene, Korea) assays. Total nucleic acids were extracted using NucliSense easyMAG (bioMérieux, Marcy líEtoile, France) according to the manufacturer's instructions and kept frozen until use. The determination of HRSV group (HRSV-A and–B) from all HRSV laboratory-confirmed samples was achieved using a specific real-time PCR with primers and probes that target a highly conserved genomic region of the nucleoprotein gene [4].

3.3. Phylogenetic analysis and molecular characterization of HRSV strains

For the phylogenetic analysis and the molecular characterization, the entire G gene of HRSV-A and HRSV-B was sequenced from a representative sampling that included at least 4 representative laboratory-confirmed specimens by week and by age groups (0–2, 2–4, 5–14, 15–65, >65 years old) when available. The amplification of the whole coding G protein sequence was carried out by a one-step RT-PCR assay using One-step RT-PCR Kit (Qiagen, Hilden, Germany), primers and PCR protocols, as shown in Table 1. PCR products were subsequently purified using Exo-SAP-IT (USB, Affymetrix Inc. Cleveland, Ohio, USA) and sequenced by the ABI Prism Big Dye Terminator cycle sequencing kit v3.1 on the ABI PRISM 3130XL sequencer (Applied Biosystems,

Table 1
Primers and specific conditions used for PCR amplification of complete G and partial F protein-coding sequences. The M13 primer binding sites used for sequencing are marked in bold.

Gene	HRSV group	Fragment	Primers	PCR conditions	Position (nt)
G	A	1	G-A-FWD1: **TGTAAAACGACGGCCAGT**TGGCCYTAYTTTACACTAATACAYATG	50 °C × 30′ –95 °C × 15′ – 45c:	4346–43715291–5270
			G-A-REV2: **CAGGAAACAGCTATGACC**GYTTGGTRGTGGTTTTCTTYCC	(95 °C × 15”–53 °C × 20”–72 °c × 1′30”)–72 °c × 10′	
		2	G-A-FWD2: **TGTAAAACGACGGCCAGT**GTACCYTGCAGCATATGCA	50 °C × 30′-95 °C × 15′– 45c:	5198–52165834–5812
			G-A-REV1: **CAGGAAACAGCTATGACC**GTTATRACACTRGTATACCAACC	(95 °C × 15”–53 °C × 20”–72 °c × 1′30”)–72 °c × 10′	
	B	1	G-B-FWD1: **TGTAAAACGACGGCCAGT**TGGCCYTAYTTTACACTAATACAYATG	50 °C × 30′-95 °C × 15′– 45c:	4343–43695275–5253
			G-B-REV2: **CAGGAAACAGCTATGACC**TTCTTYGGTTTRTTGCTTGGTAT	(95 °C × 15”-53 °c × 20”–72 °c × 1′30”)–72 °c × 10′	
		2	G-B-FWD2: **TGTAAAACGACGGCCAGT**GTGTTCAACTTCGTTCCCTG	50 °C × 30′-95 °C × 15′ – 45c:	5186–52055837–5815
			G-B-REV1: **CAGGAAACAGCTATGACC**GTTATRACACTRGTATACCAACC	(95 °C × 15”-53 °c × 20”–72 °c × 1′30”)–72 °c × 10′	
F	A&B		F-A-FWD: GGTTGGTATACYAGTGTYATAAC	50 °C × 30′-95 °C × 15′ –45c: (95 °C × 15”-53 °c × 20”–72 °c × 1′30”)–72 °c × 10′	5811–5833
			F-B-REV:GAGCTGCTTAYRTCTGTTTTTG	50 °C × 30′-95 °C × 15′ – 45c:	6875–6853
				(95 °C × 15”–53 °c × 20”–72 °c × 1′30”)–72 °c × 10′	