Respiratory Syncytial Virus incidence and typing in the last 6 seasons in the north of Spain (Asturias). Genetic characterization during the SARS-Cov-2 pandemic

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Abstract

Respiratory syncytial virus is associated with lower respiratory tract infections. As several types and genotypes can circulate at the same time, genomic characterisation is important for timely epidemiological control and treatment measures. In the last 6 seasons (2017-2023), 191236 nasopharyngeal swabs were processed for respiratory viruses. The incidence of RSV reached 7% in the pre-pandemic season. RSV was most frequent in children under 5 years of age (12.6%), but was also significant in those over 70 years of age (5.63%). The measures taken to control SARS-Cov2 infection were useful for RSV control and the incidence decreased to 1.8%, but caused a change in the types. Pre-pandemic, the majority circulating types were RSV-B/RSV-B/RSV-A and in pandemic it was RSV-B/RSV-B. In the last season, RSV-B and RSV-A were detected in the same proportion. Genetic characterization showed three new clades. This has been taken into account in order to take the correct measures.

Introduction

Respiratory Syncytial Virus (RSV) is a common viral pathogen that causes 64 million acute respiratory infections annually[1]. RSV is considered as a major cause of lower respiratory tract infections amongst young children, elderly and immunocompromised adults worldwide[2-5], and is a frequent cause of hospitalization[6,7]. In a recent systematic analysis, it was associated to 6.6 million of acute lower respiratory infection, where more than 1 million were hospitalized and 10000 dead in the hospital[8]. For example, in the United States, RSV infections cause between 58,000 and 80,000 hospitalizations each year in children under 5 years of age and between 60,000 and 160,000 in adults over 65 years of age[9].

Based on the differences in the nucleotide sequence in the G protein, RSV is divided into 45 genotypes. RSV-A has 15 genotypes whereas RSV-B has 30 genotypes. Different RSV genotypes exhibit different pathogenicities. In the same period and area, different RSV genotypes can be co-epidemic, but most epidemics are dominated by one of the subgroups or genotypes[10]. In the context of COVID-19 pandemic with resulting quarantine and barrier measures, a drastic reduction of RSV infections has been observed in Europe and North America[1]. Genetic characterization of RSV by direct sequencing of sub-genomic regions or full genomic sequencing will be an important part of RSV surveillance to monitor potential antigenic changes in the circulating viruses that might affect the efficacy of future immunization strategies. In general, the preferred RSV surveillance will be an active sentinel system, with both primary care and hospital patients being

systematically sampled and tested for RSV[11]. At present, data concerning the molecular epidemiological characteristics of RSV subtypes are limited[12].

No effective therapy or vaccine is available to date, but vaccine candidates and monoclonal antibodies are in late clinical development[5]. Lately, a new monoclonal antibody (nirsevimab) has been approved[13,14]. The effectiveness of these therapies may vary depending on the type of virus. Therefore, the reporting of RSV cases and the etiological diagnosis of the genotype are very important for planning future public health measures and allow appropriate treatment of the patient and avoidance of unnecessary therapies (such as antibiotics)[15].

Here, we conducted a study detecting and genotyping RSV, circulating in clinical samples in Asturias from 2017 to 2022. The aim of this study is to know the incidence and distribution of RSV types and to enrich the data of epidemiological molecular studies on RSV in Spain.

Materials and Methods

Samples and patients

Between September 2017 to January 2023, 191236 nasopharyngeal swabs were collected from the same number of patients to determine the etiology of acute respiratory infection, 50026 before SARS-CoV-2 pandemic (2017-19) and 141216 after (2020-22).

These samples correspond to 11301 children under one year of age, 36811 children aged 1 to 5 years, 17270 children aged 6 to 15 years, 80299 adults aged 16 to 70 years and 44918 adults aged 70 years and over.

Laboratory diagnosis

The samples were divided into two aliquots according to laboratory protocols. The first (1ml) was used for conventional monolayer cell culture (MRC-5 and mix of LLC-MK2, A549 and Hep-2), while the second (500 µl) was used for viral nucleic acid detection.

Nucleic acids extraction and virus detection.

Nucleic acids were extracted and purificated by using the automated nucleic acid purifier Magnapure (Roche Diagnostics SL, Swizertland) following manufacturer´s instructions. Extracted nucleic acids were resuspended in a final volume of 70 µl.

RSV genome was detected and quantificated by a multiplex real time reverse transcription polymerase chain reaction rt-RT-PCR for Influenzavirus A and B and RSV using type-specific primer pairs and MGB probes (Table 1) and the TaqMan Fast 1-Step Master Mix (Life technologies, CA). RT-PCR was performed with 5 µl of extracted nucleic acids in a final volume of 10 µl as follows: 50^{o}/10', 95^{o}/7', 45 cycles of 95^{o}/5" and 60^{o}/33".

In addition, the human ß-globin gene was quantified in each sample in order to evaluate sample quality and to calculate normalized viral load in copies/10^3 cells.

Table 1. Primers and probe used for detection of Influenza A (IA), Influenza B (IB) and RSV (types A and B) and sequencing

	Target	Primer/probe	Sequence (5' > 3')
Detection	IA	IA-TR-S	GACCRATCCTGTCACCTCTGAC
		IA-TR-A	AGGGCATTYTGGACAAAKCGTCT

	Target	Primer/probe	Sequence (5' > 3')
		IA-FAM	TGCAGTCCTCGCTCACTGGGCAC
	IB	IB-TR-S	AACATGGTAGTGAAACTGGGTGA
		IB-TR-A	CAGCATGCGCATTTTGGAT
		IB-NED	AYAACCAGATGATGGTYAAA
	RSV-A	RSVA-TR-S	GCCAGTGGCATTGCTGTAT
		RSVA-TR-A	CTGACTACGGCCTTGTTTGT
	RSV-B	RSVB-TR-S	GCAAGTGGTATAGCTGTAT
		RSVB-TR-A	CTGACTACAGCTTTGTTTGT
	RSV-A & RSV-B	RSV-VIC	AGAAGTGAACAAGATCAA
Typing	RSV-A	RSVA-TR-S	GCCAGTGGCATTGCTGTAT
		RSVA-TR-A	CGGCCTTGTTTGTGGATAGT
	RSV-B	RSVB-TR-S	GCAAGTGGTATAGCTGTA
		RSVB-TR-A	TGACTACAGCTTTGTTTGTAGAC
	RSV-A & RSV-B	RSV-VIC	AGAAGTGAACAAGATCAA
Sequencing	First round	VSR-TIP2021-G-S	GCAAATGCAACCATGTCCAA
		VSR-TIP2021-G-A	AACTGCACTACATGTCGATTGGT
	Second round	VSRA-G-Fwd	GAAGTGTTCAACTTTGTACC
		VSRB-G-Fwd	AAGATGATTACCATTTTGAAGT
		VSRA/B-G-Rev	CAACTCCATTGTTATTGCC

RSV typing

Two different RT-PCR assays, which include the VIC-labeled MGB probe and two primers for RSV-A or RSV-B separately (Table 1), were carried out to characterize RSV type (A and B) with the same amplification conditions that above.

RSV characterization

During 2021 and 2022, 116 RSV positive samples under 25 Ct were randomly chosen and genotyped by Sanger sequencing method. A fragment of the G gene was amplified by a nested-PCR. The first round of amplification was carried out using the OneStep RT-PCR kit (EURx, Poland) and specific outer primers (Table 3). Five microliters of extracted viral genome were added to 20 µl of a RT-PCR mixture according to the manufacturer's instructions. Amplification was performed using a GeneAmp PCR system 9600 thermal cycler (Applied Byosistems, USA) with the following conditions: 48^{o}/45', 95^{o}/2', 40 cycles of 95^{o}/30"-55^{o}/30"-72^{o}/30", and 72^{o}/10'.

To increase sensibility, a second round of amplification was carried out. Three microliters of the previous amplification reaction were added to 22 µl of a PCR mixture containing 0.5 pmol of specific inner primers (Table 1), 50 µM of each dNTPs (Gibco BRL, Carlsbad, CA, USA), and 1 U of *Taq* DNA polymerase (Gibco BRL) in PCR buffer (1x) supplied by manufacturer. The amplification protocol was as follows: 95º/5', 40 cycles of 95º/30"-55º/30"-72º/30", and 72º/10'.

PCR products were analyzed by agarose gel electrophoresis, extracted by using Montage DNA Gel Extraction Kit (Millipore, USA) and sequenced with Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) supplemented with inner primers using an ABI 3130 genetic analyzer (Applied Biosystems, USA).

Demographic data (age and sex) from those patients are in File S1.

Phylogenetic reconstructions

Nucleotide sequences were translated and aligned using the MUSCLE algorithm implemented in MEGA. Sequences (147 to 396 nucleotides) generated in this work have been deposited in GenBank with the following accession numbers:OR493270-OR493385.

For type characterization, phylogenetic trees were constructed using ModelFinder, tree reconstruction and ultrafast bootstrap (1000 replicates) with IQ-TREE 2.1.3. RSV reference sequences used were in File S2. The best-fit nucleotide substitution model GTR+F+G4 was identified according to Bayesian information criterion. Bootstrap values were estimated using the SH test and ultrafast bootstrap with 1000 replicates. To identify the phylogenetic relationship, 8373 G gene sequences type A and 5339 type B available in the GenBank database (one sequence of each type used for the Basic Local Alignment Search Tool (BLAST) in March 2023) were used (File S2). Transmission clusters were defined as viral sequences circulating in Asturias grouped into a single and well supported monophyletic clade with >90% bootstrap values. To search for mutations, the sequences were compared with ON1 (JN257693) and BA9 (DQ227395).

Statistical analyses

Statistical tests were performed using GraphPad InStat v.3 (GraphPad Software, USA). These tests were considered significant when p value was <0.05.

Results

Incidence and subtypes of RSV

Of the 191236 samples tested in the 6 seasons, 6203 (3.3%) were positive. From them, 1433 (23.1%) were typed as RSV-A, and 3492 (56,3%) as RSV-B. Table 2 and Figure 1 show the incidence by season and age, and types found.

Table 2. Number and Rate of RSV detected each season by age and types (A/B).

Seasons	**<1 year old**	**1-5 years**	**6-15 years**	**16-70 years**	**>70 years**	**Total**
S1 2017-18	335/1704 (19,7%) 6A/175B	375/3848 (9,7%) 2A/185B	75/1605 (4,7%) 0A/37B	207/3160 (6,6%) 4A/107B	183/1998 (9,2%) -/91B	1175/12315 (9,5%) 12A/595B
S2 2018-19	384/1991 (19,3%) 11A/344B	386/4944 (7,8%) 8A/341B	43/1958 (2,2%) -/32B	163/4040 (4,0%) 1A/128B	202/3083 (6,6%) 1A/171B	1178/16016 (7,4%) 21A/1016B
S3 2019-20	364/1664 (21,9%) 206A/45B	528/4600 (11,5%) 310A/90B	45/2395 (1,9%) 21A/3B	148/8642 (3,4%) 59A/20B	150/4414 (3,4%) 58A/26B	1235/21695 (5,7%) 655A/184B
S4 2020-21	65/1616 (4,0%) 3A/60B	269/7347 (3,7%) 12A/240B	3/2906 (0,1%) 2A/1B	12/3240 (0,4%) 0A /11B	9/1497 (0,6%) -/9B	358/16606 (2,2%) 17A/321B

Seasons	<1 year old	1-5 years	6-15 years	16-70 years	>70 years	Total
S5 2021-22	200/2901 (6,9%) 21A/170B	610/10770 (5,7%) 29A/538B	26/5549 (0,5%) 1A/20B	43/53568 (0,1%) 5A/33B	21/28372 (0,1%) -/20B	900/101160 (0,9%) 56A/781B
S6 2022-J23	327/1425 (22,9%) 157A/156B	638/5302 (12,0%) 343A/263B	93/2857 (3,3%) 52A/32B	117/7649 (1,5%) 41A/60B	183/5554 (3,3%) 79A/84B	1358/22787 (6,0%) 672A/595B
Total	1675/11301 (14,8%) 404A/950B	2805/36811 (7,6%) 704A/1657B	285/17270 (1,7%) 76A/125B	690/80299 (0,9%) 110A/359B	748/44918 (1,7%) 138A/401B	6203/190579 (3,3%) 1433A/3492B

S: Season; J: January

In the three pre-pandemic SARS-Cov-2 seasons 3587 (7.1%) of the samples had RSV, and in the pandemic seasons 2616 (1.8%) ($p<0.0001$).

On the other hand, RSV was detected in 3480 (9.1%) children under 6 years of age and in 1438 (1.14%) adults over 15 years of age ($p<0.0001$).

Finally, type A accounted for 1.97% in S1, 2.2% in S2, 78.6% in S3, 5.02% in S4, 6.69% in S5 and 53% in S6 ($p<0.0001$).

Figure 1. Incidence of RSV by season and age (A) or types (B).

RSV genotypes

Phylogenetic analysis of 116 sequenced RSV isolates showed that 51 RSV-A (9 from 2021 and 42 from 2022) viruses belong to the ON1 /genotype and 65 RSV-B (24 from 2021 and 41 from 2022) viruses belong to BA9 (Figure S1).

The ON1 was divided into two major clades and four small clades. The first major clades were related to strains from Kenia 2015, France 2021 and USA 2022 (30 patients), and the second to Netherlands 2021, Slovenia 2021 and France 2022 (15 patients). The small clades were related to strains from Bangladesh 2012 (1 patient), China 2018 (1 patient), Slovenia 2021(1 patients), USA 2022 (3 patients) (Figure 2A).

The BA9 was divided in one major clade related to strains from China 2019, Japan 2019, Italy 2021, USA 2021 Australia 2022, and Austria 2022 (22 patients 2021/ 41 patients 2022), and in two small clades all of 2021 related to strains from Germany 2017, Spain 2018, Thailand 2019 and China 2020 (1 patient), China 2021(1 patient) (Figure 2B).

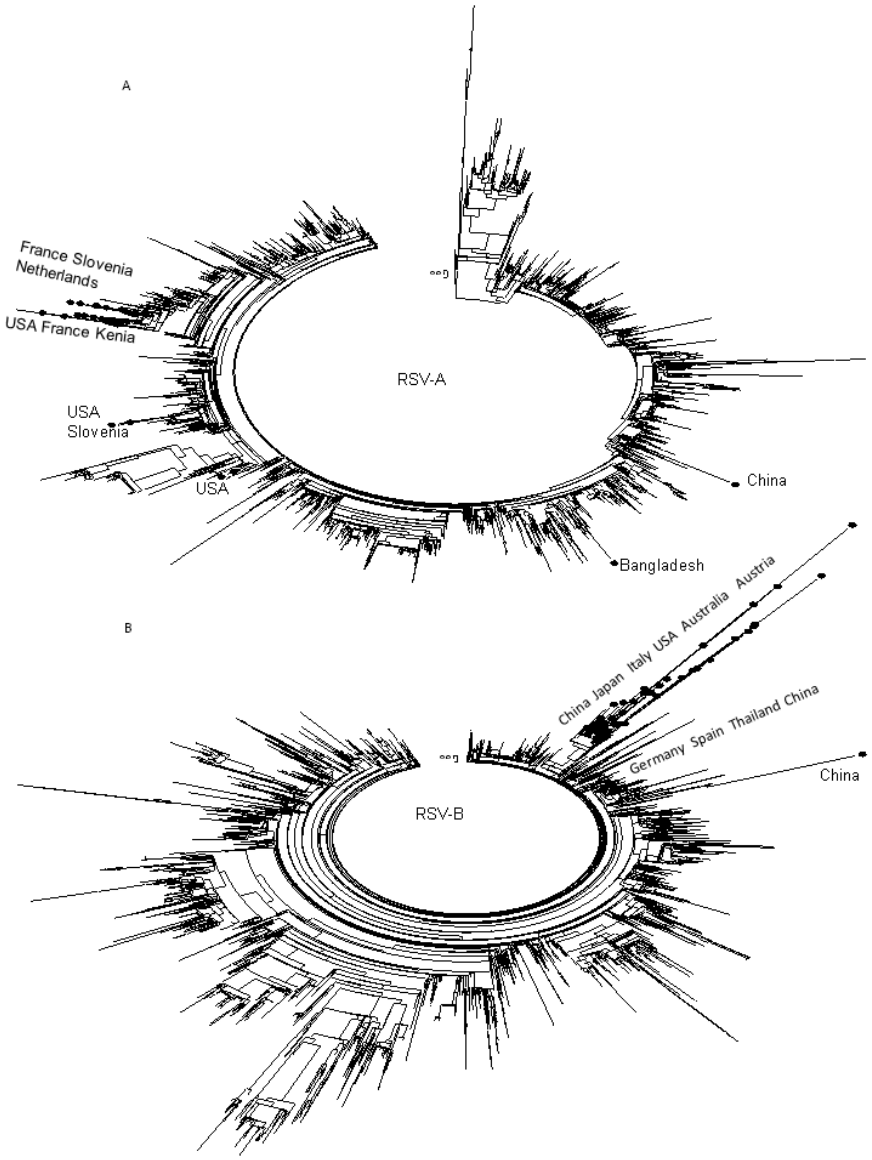

Figure 2 . Phylogenetic tree of genomes of RSV-A (A) and RSV-B (B) from Asturias (black circles) and NCBI. The geographical origins of the NCBI sequences phylogenetically related to those of Asturias are indicated.

In 2021, a new RVS-B sublineage spread in Asturias, characterized by the S263G mutation). In 2022, two new sublineages from ON1 spread in Asturias, one characterized by the S299I mutation (n=8) and the other by the K205N and Y280H mutations (n=26) (Table 3, Figure 3).

Table 3. Characteristics of the sublineages identified during 2021–22 in Asturias (Spain).

Sublineage	**n**	**Detection date**	**Sex**	**Sex**	**Age (years)**	**Age (years)**	**Age (years)**	**Age (y**
			Female	**Male**	**<1**	**1-5**	**6-15**	**16-70**
ON1+K205N+Y280H	26	31/10/2022	14	12	5	11	5	2
ON1+S299I	8	13/11/2022	2	6	2	2	2	1

Sublineage	n	Detection date	Sex	Sex	Age (years)	Age (years)	Age (years)	Age (y
BA9+S263G	13	06/10/2021	4	9	1	3		4

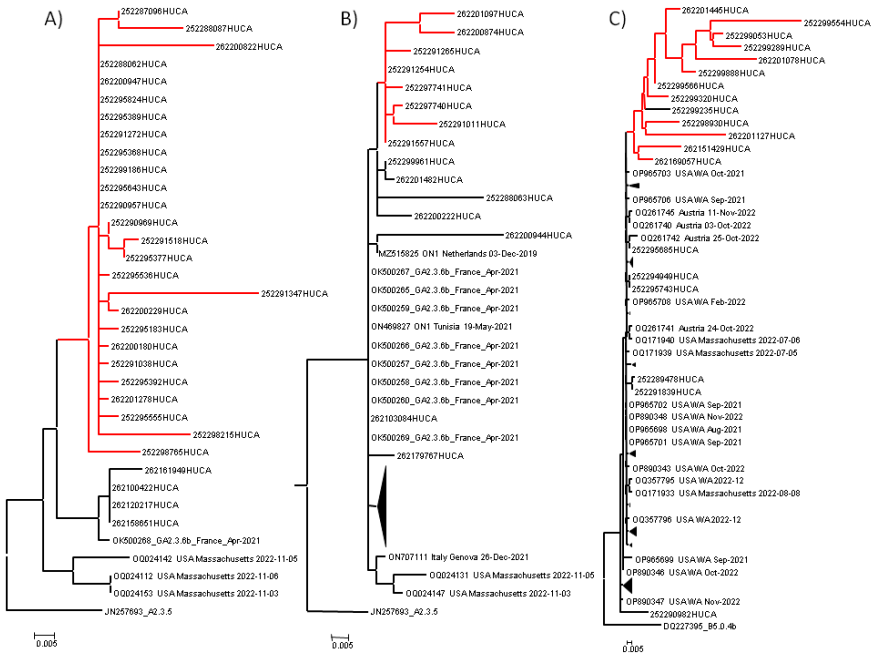

Figure 3. A) New ON1 sublineage that spread in Asturias, characterized by the K205N and Y280H mutations. B) New ON1 sublineage that spread in Asturias, characterized by the S299I mutation. C) New BA9 sublineage that spread in Asturias, characterized by the S263G mutation. All of them identified with red branches.

Discussion

Respiratory Syncytial virus is the most common cause of serious respiratory infection in infants. Reinfections occur commonly, including in older adults[16]. In recent years, the importance and severity of RSV infections in children as well as adults has been demonstrated and appreciated[1-7]. RSV-associated in-hospital mortality increases exponentially with age, posing a greater risk for older adults, particularly frail and high-risk patients[8,9,17,18] .Moreover, RSV infection may result in greater morbidity and mortality among older hospitalized adults than influenza[19]. RSV can also causes substantial outpatient illness with lower respiratory tract involvement. Belongia et al found the virus in the 11% of patients[20]. In a previous job in our Hospital this incidences reached 14% when children was principally studied[21].

It has been already reported that during the SARS-Cov2 pandemic, mitigation measures were associated with marked reductions in non-COVID-19 respiratory infections[9,22]. In this study, the overall incidence over 6 seasons was 3.3%, but a large difference was observed before and after the pandemic: in the first three seasons, the incidence reached 7.1% and decreased to 1.8% during the pandemic years

RSV was more common in children (9.1%) than in adults (1.14%), but can be found in any age group. Moreover, if we analyze the first two seasons (before the pandemic), the incidence in those over 70 years of age is similar to that in children aged one to five years. And the incidence is not negligible in the rest of the age groups. As noted above, the measures taken during the pandemic reduced viral transmission, especially in adults, where more pressure and control was exerted. The return to normality, even with the lessons learned, suggests that the incidence of RSV infection is likely to rise again.

RSV may be associated with escalation in respiratory support and an increased level of support in living situation at discharge[23]. RSV disease burden in adults aged more than 60 years in high-income countries is higher than previously estimated[24]. RSV hospitalizations were associated with increased attributable short-term and long-term healthcare costs[1]. Therefore, interventions that could prevent RSV may reduce healthcare burden. Understanding the clinical features and symptomatology of RSV infection can help to address the challenges related to case identification and management and allows for the implementation of appropriate preventive measures. For example, in this study incidence decreased from 5.6% pre-pandemic to 0.6% during SARS-Cov2 pandemic.

Since two types and several genotypes have been described and the behaviour of each may be different, an exact classification is necessary. Types RSV-A and RSV-B are simultaneously present in most outbreaks, but RSV-A is associated with severe disease. In RSV-A dominant years typically started, reached its peak and lasted than in RSV-B dominant years[8,25]. Several distinct genotypes within these types predominate within a community; the dominant strains shift yearly, perhaps accounting for frequent reinfections[25,26]. Previous RSV infection does not appear to protect against reinfection[27]. On the other hand, several substitutions in fusion protein reduce nirvesimab susceptibility[28], the new monoclonal antibody approved to immunize against RSV.

In this job, RSV-A and RSV-B were found simultaneously, but with very different incidence. In the first two seasons, RSV-B was predominant, but in 2019 (pre-pandemic SARS-Cov-2) there was a subtype shift and RSV-A was detected in 78% of cases, suggesting greater severity of symptoms. The measures taken in the pandemic significantly reduced respiratory infections, as discussed above[9,22] .In the first two years of the pandemic a new subtype shift was observed and again RSV-B became dominant. In the last season (2022-23) epidemiological control measures were again relaxed and both subtypes were observed to circulate in the same proportions.

Next season we will have to watch out whether RSV-A (or certain genotypes of this type) replaces RSV-B, as suggested by other studies[29,30], as a worsening of symptoms is to be expected. This is why

sequencing of viral strains is relevant not only for taxonomic purposes, but in order to better understand the epidemiology as well as the development of therapeutic and preventive strategies.

The genotyping analysis of RSV was used to characterize which genotypes circulated in Asturias and to investigate whether phylogenetic clusters occurred in the study period.

RSV genotypes ON1 (type A) and BA9 (type B) became the predominant genotypes worldwide from 2015 and were the only genotypes detected in the period 2021-2022 in Asturias. Variants of these genotypes are constantly generated, so it is necessary to identify them[31]. Numerous variants of the ON1 genotype have been reported worldwide, with various amino acid substitutions[32,33]. In a last study in USA in 2023, Goya et al did not find specific changes[34].

In Asturias, in the ON1 genotype, the gain of the K205N mutation that was only found in two NCBI strains (ON237320 Argentina 2017 and KX827403 Guatemala 2013) and the Y280H mutation (common in the world and found in Spain) formed a separate sublineage in 26 sequences. This variant was found in 2022 in a similar number of men and women, mostly being under 6 years of age. Besides, the gain of the S299I mutation in 8 sequences found in strains without duplication of ON1 (KT765684-KT 765686, OK458608-OK458615 Kenia 2006-2010) generated another new sublineage. This variant was found in 2022 mostly in men, also mostly in children less than 6 years of age, but not exclusively.

From VRS-B, in Europe the BA9 genotype is always the predominant, as was also reported in Catalonia (Spain)[32,35]. In Asturias, in 12 sequences the BA9 genotype, the gain of the mutation S263G, that was only found in two NCBI strains (OK078747 Pakistan 2010 and MH606068 Croatia 2017), formed a separate sublineage. This variant was found in 2021 in women under 6 years of age; it continued to be disseminated in 2022 in people over 65 years of age mainly in men.

Therefore these new sublinages can be found in any group of individuals. On the other hand, variants

without these mutations were previously detected in Asturias, supporting the idea that they were locally generated, in contrast to the finding of Goya et al[34].

When the GenBank sequences were examined, no new mutations were observed, but rare mutations appeared in new lineages, which could be limited to local transmission. New substitutions may contribute to antigenic escape, promote transmissibility, or be the result of the founder effect in a vulnerable population. Therefore, performing genotypic characterisation studies locally and sharing them with the rest of the scientific community should be a practice to be implemented in the control of this type of infections.

Limitations of the study are that few clinical data were available, that the number of patients sequenced is limited, that changes in the frequency of circulation of RSV genotypes in previous seasons cannot be addressed and that the analysis was based only on a fragment of the G gene.

Conclusion

In summary, although RSV was more frequent in children, it can infect at any age. Both subtypes (RSV-A and RSV-B) were found with B/B/A/B/B/A-B pattern. Genomic analyses did not detect any novel mutations, but did detect the emergence of new lineages with rare mutations in the data available to date. These lineages may influence the preventive treatments that are attempted.

This study provides an overview of the genetic variation of circulating RSV strains in Asturias with the identification of new ON1 and BA9 lineages. Ongoing and long-term molecular epidemiological studies for the detection of circulating and emerging genotypes in combination with clinical data are needed to gain a better understanding of the underlying genetic and antigenic mechanisms of RSV infection.

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References

1. Mac S, Shi S, Millson B, Tehrani A, Eberg M, Myageri V, et al. Burden of illness associated with Respiratory Syncytial Virus (RSV)-related hospitalizations among adults in Ontario, Canada: A retrospective population-based study. Vaccine. 7 de agosto de 2023;41(35):5141-9.

2. Fretzayas A, Moustaki M. Etiology and clinical features of viral bronchiolitis in infancy. World J Pediatr. agosto de 2017;13(4):293-9.

3. van Houten CB, Oved K, Eden E, Cohen A, Engelhard D, Boers S, et al. Observational multi-centre, prospective study to characterize novel pathogen-and host-related factors in hospitalized patients with lower respiratory tract infections and/or sepsis - the ≪TAILORED-Treatment≫ study. BMC Infect Dis. 7 de agosto de 2018;18(1):377.

4. Nam HH, Ison MG. Respiratory syncytial virus infection in adults. BMJ. 10 de septiembre de 2019;366:l5021.

5. Coppée R, Chenane HR, Bridier-Nahmias A, Tcherakian C, Catherinot E, Collin G, Lebourgeois S, Visseaux B, Descamps D, Vasse M, Farfour E. Temporal dynamics of RSV shedding and genetic diversity in adults during the COVID-19 pandemic in a French hospital, early 2021. Virus Res. 2022 Sep 29;323:198950. doi: 10.1016/j.virusres.2022.198950. Epub ahead of print. PMID: 36181977; PMCID: PMC9519364.

6. Ghazaly M, Nadel S. Characteristics of children admitted to intensive care with acute bronchiolitis. Eur J Pediatr. junio de 2018;177(6):913-20.

7. Meissner HC. More on Viral Bronchiolitis in Children. N Engl J Med. 22 de septiembre de 2016;375(12):1200.

8. Li T, Fang H, Liu X, Deng Y, Zang N, Xie J, et al. Defining RSV epidemic season in southwest China and assessing the relationship between birth month and RSV infection: A 10-year retrospective study from June 2009 to May 2019. J Med Virol. julio de 2023;95(7):e28928.

9. Hamid S, Winn A, Parikh R, Jones JM, McMorrow M, Prill MM, et al. Seasonality of Respiratory Syncytial Virus - United States, 2017-2023. MMWR Morb Mortal Wkly Rep. 7 de abril de 2023;72(14):355-61.

10. Chen G. Lan M, Lin S, Zhang Y, Zhang D, Weng Y, Zheng K. Genome analysis of human respiratory syncytial virus in Fujian Province, Southeast China. Infect Genet Evol. 2022 Sep;103:105329. doi: 10.1016/j.meegid.2022.105329. Epub 2022 Jul 3. PMID: 35788050.

11. Teirlinck AC. Broberg EK, Stuwitz Berg A, Campbell H, Reeves RM, Carnahan A, Lina B, Pakarna G, Bøås H, Nohynek H, Emborg HD, Nair H, Reiche J, Oliva JA, O'Gorman J, Paget J, Szymanski K, Danis K, Socan M, Gijon M, Rapp M,Havlíčková M , Trebbien R, Guiomar R, Hirve SS, Buda S, van der Werf S, Meijer A, Fischer TK. Recommendations for respiratory syncytial virus surveillance at the national level. Eur Respir J. 2021 Oct 1;58(3):2003766. doi: 10.1183/13993003.03766-2020. PMID: 33888523; PMCID: PMC8485062.

12. Staadegaard L. Caini S, Wangchuk S, Thapa B, de Almeida WAF, de Carvalho FC, Njouom R, Fasce RA, Bustos P, Kyncl J, Novakova L, Caicedo AB, de Mora Coloma DJ, Meijer A, Hooiveld M, Huang S, Wood T, Guiomar R, Rodrigues AP, Danilenko D, Stolyarov K, Lee VJM, Ang LW, Cohen C, Moyes J, Larrauri A, Delgado-Sanz C, Le MQ, Hoang PVM, Demont C, Bangert M, van Summeren J, Dückers M, Paget J. The Global Epidemiology of RSV in Community and Hospitalized Care: Findings From 15 Countries. Open Forum Infect Dis. 2021 Mar 30;8(7):ofab159. doi: 10.1093/ofid/ofab159. PMID: 34337092; PMCID: PMC8320297.

13. Strine MS, Wilen CB. Game over for RSV? Sci Immunol. 8 de junio de 2023;8(84):eadi8764.

14. Simões EAF, Madhi SA, Muller WJ, Atanasova V, Bosheva M, Cabañas F, et al. Efficacy of nirsevimab against respiratory syncytial virus lower respiratory tract infections in preterm and term infants, and pharmacokinetic extrapolation to infants with congenital heart disease and chronic lung disease: a pooled analysis of randomised controlled trials. Lancet Child Adolesc Health. marzo de 2023;7(3):180-9.

15. Menasalvas Ruiz AI. Respiratory syncytial virus infection in COVID-19 times: Trick or threat? Enferm Infecc Microbiol Clin (Engl Ed). 2023;41(6):319-20.

16. Falsey AR, Walsh EE. Respiratory syncytial virus Prefusion F vaccine. Cell. 20 de julio de 2023;186(15):3137-3137.e1.

17. Nguyen-Van-Tam JS, O'Leary M, Martin ET, Heijnen E, Callendret B, Fleischhackl R, et al. Burden of respiratory syncytial virus infection in older and high-risk adults: a systematic review and meta-analysis of the evidence from developed countries. Eur Respir Rev. 31 de diciembre de 2022;31(166):220105.

18. Heppe-Montero M, Gil-Prieto R, Del Diego Salas J, Hernández-Barrera V, Gil-de-Miguel Á. Impact of Respiratory Syncytial Virus and Influenza Virus Infection in the Adult Population in Spain between 2012 and 2020. Int J Environ Res Public Health. 9 de noviembre de 2022;19(22):14680.

19. Ackerson B, Tseng HF, Sy LS, Solano Z, Slezak J, Luo Y, et al. Severe Morbidity and Mortality Associated With Respiratory Syncytial Virus Versus Influenza Infection in Hospitalized Older Adults. Clin Infect Dis. 2 de julio de 2019;69(2):197-203.

20. Belongia EA, King JP, Kieke BA, Pluta J, Al-Hilli A, Meece JK, et al. Clinical Features, Severity, and Incidence of RSV Illness During 12 Consecutive Seasons in a Community Cohort of Adults [?]60 Years Old. Open Forum Infect Dis. diciembre de 2018;5(12):ofy316.

21. Gomez-Novo M, Boga JA, Alvarez-Arguelles ME, Rojo-Alba S, Fernandez A, Menendez MJ, et al. Human respiratory syncytial virus load normalized by cell quantification as predictor of acute respiratory tract infection. J Med Virol. mayo de 2018;90(5):861-6.

22. Agha R, Avner JR. Delayed Seasonal RSV Surge Observed During the COVID-19 Pandemic. Pediatrics. septiembre de 2021;148(3):e2021052089.

23. Hill-Ricciuti A, Walsh EE, Greendyke WG, Choi Y, Barrett A, Alba L, et al. Clinical impact of healthcare-associated respiratory syncytial virus in hospitalized adults. Infect Control Hosp Epidemiol. marzo de 2023;44(3):433-9.

24. Savic M, Penders Y, Shi T, Branche A, Pircon JY. Respiratory syncytial virus disease burden in adults aged 60 years and older in high-income countries: A systematic literature review and meta-analysis. Influenza Other Respir Viruses. enero de 2023;17(1):e13031.

25. Gilca R, De Serres G, Tremblay M, Vachon ML, Leblanc E, Bergeron MG, et al. Distribution and clinical impact of human respiratory syncytial virus genotypes in hospitalized children over 2 winter seasons. J Infect Dis. 1 de enero de 2006;193(1):54-8.

26. Robertson M. Eden JS, Levy A, Carter I, Tulloch RL, Cutmore EJ, Horsburgh BA, Sikazwe CT, Dwyer DE, Smith DW, Kok J. The spatial-temporal dynamics of respiratory syncytial virus infections across the east-west coasts of Australia during 2016-17. Virus Evol. 2021 Jul 22;7(2):veab068. doi: 10.1093/ve/veab068. PMID: 34532066; PMCID: PMC8438877.

27. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. J Infect Dis. abril de 1991;163(4):693-8.

28. Ahani B, Tuffy KM, Aksyuk AA, Wilkins D, Abram ME, Dagan R, et al. Molecular and phenotypic characteristics of RSV infections in infants during two nirsevimab randomized clinical trials. Nat Commun. 19 de julio de 2023;14(1):4347.

29. Otieno JR. Kamau EM, Agoti CN, Lewa C, Otieno G, Bett A, et al. Spread and evolution of respiratory syncytial virus A genotype ON1, coastal Kenya, 2010-2015. Emerg Infect Dis. 2017;23:264–71.

30. Viegas M. Goya S, Mistchenko AS. Sixteen years of evolution of human respiratory syncytial virus subgroup A in Buenos Aires, Argentina: GA2 the prevalent genotype through the years. Infect Genet Evol. 2016;43:213–21.

31. Luo Q. Li M, Li A, Gong C, Dong M, Huang Q, Luo M, Zhang H, Huang F. Genetic diversity and epidemiological features of respiratory syncytial virus, Beijing, 2015-2019: A multicenter and all-age groups study. J Infect. 2022 Jul;85(1):75-85. doi: 10.1016/j.jinf.2022.04.046. Epub 2022 May 6. PMID: 35533834.

32. Lin TY. Chi H, Kuo CY, Tsai HP, Wang JR, Liu CC, Shen CF. Outbreak of respiratory syncytial virus subtype ON1 among children during COVID-19 pandemic in Southern Taiwan. J Microbiol Immunol Infect. 2022 Dec;55(6 Pt 2):1168-1179. doi: 10.1016/j.jmii.2022.08.015. Epub 2022 Sep 13. PMID: 36137926.

33. Gaymard A,. Bouscambert-Duchamp M, Pichon M, Frobert E, Vallee J, Lina B, Casalegno JS, Morfin F. Genetic characterization of respiratory syncytial virus highlights a new BA genotype and emergence of the ON1 genotype in Lyon, France, between 2010 and 2014. J Clin Virol. 2018 May; 102:12-18. doi: 10.1016/j.jcv.2018.02.004. Epub 2018 Feb 6. PMID: 29471266.

34. Goya S, Sereewit J, Pfalmer D, Nguyen TV, Bakhash SAKM, Sobolik EB, et al. Genomic Characterization of Respiratory Syncytial Virus during 2022–23 Outbreak, Washington, USA - Volume 29, Number 4—April 2023 - Emerging Infectious Diseases journal - CDC. [citado 30 de agosto de 2023]; Disponible en: https://wwwnc.cdc.gov/eid/article/29/4/22-1834_article

35. Gimferrer L. Campins M, Codina MG, Martin Mdel C, Fuentes F, Esperalba J, Bruguera A, Vilca LM, Armadans L, Pumarola T, Anton A. Molecular epidemiology and molecular characterization of respiratory syncytial viruses at a tertiary care university hospital in Catalonia (Spain) during the 2013-2014 season. J Clin Virol. 2015 May;66:27-32. doi: 10.1016/j.jcv.2015.02.018. Epub 2015 Feb 26. PMID: 25866332.