RESEARCH ARTICLE

VIRAL CIRCULATION CAUSING SEVERE ACUTE RESPIRATORY INFECTION CONFIRMED BY MULTIPLE TESTING IN THE E.S.E. HOSPITAL UNIVERSITARIO ERASMO MEOZ

CIRCULACIÓN VIRAL CAUSANTE DE INFECCIÓN RESPIRATORIA AGUDA GRAVE CONFIRMADA POR PRUEBAS MÚLTIPLE EN LA E.S.E. HOSPITAL UNIVERSITARIO ERASMO MEOZ

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RESUMEN

Introducción: En el grupo de enfermedades infecciosas, la Infección Respiratoria Aguda (IRA) es la primera causa de morbimortalidad en la población global, los principales agentes causales de IRA comprenden virus como: Sars-CoV-2, Influenza virus tipos A, B y C, Parainfluenza tipos 1, 2, 3 y 4, Virus Sincitial Respiratorio, Coronavirus, Adenovirus, Rhinovirus, Metapneumovirus, Bocavirus y bacterias: Streptococcus pneumoniae y Haemophilus influenzae. La Sociedad Americana de Enfermedades Infecciosas (IDSA) recomienda el uso de pruebas moleculares como la Reacción de cadena polimerasa en tiempo real (rRT-PCR) por

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encima de otras pruebas, el objetivo del estudio fue caracterizar la circulación en infección respiratoria aguda grave (IRAG) a partir de pruebas diagnósticas moleculares para la detección de siete virus respiratorios: Influenza A, Infuenza A (H3N2), Influenza B, Adenovirus, Metapneumovirus, Sars-CoV-2 y Virus Sincitial Respiratorio (VSR). **Materiales y Métodos** Estudio descriptivo de corte transversal que incluyó a toda la población que ingresó al Hospital Universitario Erasmo Meoz por Infección Respiratoria Aguda Grave con criterios de hospitalización de manera consecutiva desde el 2021 hasta 2022. La circulación viral fue representada por ciclo vital mensualmente. Se identificó coinfección viral y se realizó emparejamiento para determinar la más común. **Resultados** Del total de 803 pacientes, se encontró una proporción más alta de IRAG en lactantes del 65% (n=523) con una distribución porcentual similar entre colombianos y migrantes. El siguiente grupo poblacional con mayor proporción fueron los adultos mayores con el 9% de los casos (n=76), teniendo una mayor distribución entre colombianos versus migrantes (11% vs 3%); los Hombres fueron los mayormente afectados, 55% y 54%, respectivamente. El régimen de seguridad social más atendido entre población colombiana fue el subsidiado con el 87% (n=542). Sin embargo, se notaron grandes diferencias en la población migrante en donde predominó el no aseguramiento en el 92% de los casos (n=165); lo cual indica el gran trabajo de responsabilidad social de la institución y la atención con enfoque diferencial. Al determinar los patrones de circulación por ciclo vital, el VSR y el adenovirus fueron especialmente prevalentes en lactantes menores y mayores durante la mayor parte de ambos años, mientras que SARS-CoV-2 en prescolares, escolares, adultos y adultos mayores hacia el final del 2022. Además, se identificaron patrones de coinfección virus-virus, siendo la del VSR y adenovirus las más frecuentes con los demás agentes virales. La mortalidad por IRAG disminuyó de 2.6 a 1.1 por cada 1000 egresos hospitalarios en colombianos y de 1.0 a 0.8 en migrantes entre 2021 y 2022. **Conclusiones** La RT-PCR Múltiple tiene una gran utilidad en los sistemas de vigilancia epidemiológica ya que permiten documentar patrones de circulación viral en Infecciones Respiratorias

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Agudas Graves causantes de morbimortalidad en nuestra región y patrones de posible coinfección virus-virus. Los modelos actuales de prevención deben estar dirigidos a pacientes menores de 5 años y mayores de 60 años en donde la posibilidad de hospitalización es mayor. Las edades con mayor reporte de infecciones y coinfecciones pertenecen a los ciclos de vida del lactante mayor y el prescolar, con una gran oportunidad para generar estrategias de reconocimiento temprano y seguimiento.

PALABRAS CLAVES: *Infección Respiratoria Aguda Grave, Virus respiratorios, RT-PCR*

SUMMARY

Introduction: In the group of infectious diseases, Acute Respiratory Infection (ARI) is the leading cause of morbidity and mortality in the global population. The main causal agents of ARI include viruses such as: Sars-CoV-2, Influenza viruses types A, B and C, Parainfluenza types 1, 2, 3 and 4, Respiratory Syncytial Virus, Coronavirus, Adenovirus, Rhinovirus, Metapneumovirus, Bocavirus and bacteria: Streptococcus pneumoniae and Haemophilus influenzae. The Infectious Diseases Society of America (IDSA) recommends the use of molecular tests such as Real-Time Polymerase Chain Reaction (rRT-PCR) over other tests, the objective of the study was to characterize the circulation in severe acute respiratory infection (SARI).) based on molecular diagnostic tests for the detection of seven respiratory viruses: Influenza A, Influenza A (H3N2), Influenza B, Adenovirus, Metapneumovirus, Sars-CoV-2 and Respiratory Syncytial Virus (RSV). **Materials and Methods** Cross-sectional descriptive study that included the entire population admitted to the Erasmo Meoz University Hospital for Severe Acute Respiratory Infection with hospitalization criteria consecutively from 2021 to 2022. Viral circulation was represented by life cycle monthly. Viral co-infection was identified, and matching was performed to determine the most common one. **Results** Of the total of 803 patients, a higher

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proportion of SARI in infants was found of 65% (n=523) with a similar percentage distribution between Colombians and migrants. The next population group with the highest proportion were older adults with 9% of the cases (n=76), having a greater distribution between Colombians versus migrants (11% vs 3%); Men were the most affected, 55% and 54%, respectively. The social security regime most served among the Colombian population was the subsidized one with 87% (n=542). However, great differences were noted in the migrant population where non-insurance predominated in 92% of the cases (n=165); which indicates the clever work of social responsibility of the institution and the attention with a differential approach. When determining circulation patterns by life cycle, RSV and adenovirus were especially prevalent in younger and older infants during most of both years, while SARS-CoV-2 in preschoolers, schoolchildren, adults, and older adults toward the end 2022. In addition, virus-virus coinfection patterns were identified, with RSV and adenovirus being the most frequent with the other viral agents. Mortality from SARI decreased from 2.6 to 1.1 per 1,000 hospital discharges in Colombians and from 1.0 to 0.8 in migrants between 2021 and 2022. **Conclusions** Multiple RT-PCR is especially useful in epidemiological surveillance systems since it allows documenting patterns of viral circulation in Severe Acute Respiratory Infections causing morbidity and mortality in our region and patterns of possible virus-virus coinfection. Current prevention models should be aimed at patients under 5 years of age and over 60 years of age where the possibility of hospitalization is greater. The ages with the highest reports of infections and coinfections belong to the life cycles of older infants and preschoolers, with a wonderful opportunity to generate early recognition and follow-up strategies.

KEYWORDS: Severe Acute Acute Respiratory Infection, Respiratory Viruses, RT-PCR

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INTRODUCTION

Worldwide, the main cause of infectious diseases that cause morbidity and mortality are acute respiratory infections (ARI) (1), mostly those affecting the lower respiratory tract (LRTI) such as the trachea and bronchial tree as opposed to the upper respiratory tract (URTI) such as the larynx, pharynx and nose. ARI is the third cause of mortality in children and the tenth in the population with 200,000 to 650,000 deaths annually affecting children, elderly and immunocompromised adults by means of micro aspirations with infectious particles that have viral microorganisms such as Sars-Cov-2, Influenza, Parainfluenza, Rhinovirus, Adenovirus, Metapneumovirus, Respiratory Syncytial; bacterial such as S. pneumoniae, H. influenzae, Moraxella Catarralis, Mycoplasma pneumoniae, Chlamydia pneumoniae and Mycobacterium tuberculosis, and fungi (2).

The etiology and pathogenesis is associated with the site of infection. The clinical picture of URTI includes rhinitis, sinusitis, otitis media, rhinorrhea, wet cough, headache and low grade fever; for IVRI the clinical picture is more severe presenting a clinical picture containing exacerbation of asthma, acute otitis media, pneumonia, bronchopneumonia, bronchitis, bronchiolitis, tracheitis and laryngitis. (3) The evolution time is less than 15 days and it may be mild with mostly viral etiology, of short duration and with symptoms such as odynophagia, headache, rhinorrhea, headache, muscle pain and adynamia; in this case, the management is purely symptomatic. On the other hand, Severe Acute Respiratory Infection (SARI) is related to clinical presentations that require hospitalization (2).

Health personnel require a diagnostic method to know the etiology of ARRI. In recent years, Nucleic Acid Amplification Test (NAAT) diagnostic methods have been developed; however, multiplex amplification has allowed the transition from traditional to molecular diagnostic methods. The Infectious Diseases Society of

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America (IDSA) recommends the use of molecular tests such as Real Time Polymerase Chain Reaction (RT-PCR) over other tests. (4,5) Likewise, the national protocol uses the rRT-PCR test in public health laboratories for SARI cases. (6)

The aim of this study was to characterize the population with confirmed ARRI for 7 viruses: 1) Influenza A, 2) Influenza B, 3) Respiratory syncytial virus, 4) Metapneumovirus, 5) SARS-CoV-2, 6) Adenovirus and 7) Influenza A (H3N2), by means of multiple RT-PCR technique. Incidence and in-hospital outcomes were also calculated.

MATERIALS AND METHODS:

The study included the entire pediatric and adult population of the E.S.E Hospital Universitario Erasmo Meoz who were admitted to the emergency department with severe acute respiratory infection and who met the following criteria: 1) All patients with severe acute respiratory infection with a history of fever and cough not more than 10 days of evolution requiring in-hospital management, 2) All patients who underwent nasopharyngeal swabbing for the detection of causative agents of severe acute respiratory infection in the E.S.E Hospital Universitario Erasmo Meoz. Patients with ARRI who requested voluntary discharge and those with insufficient sample collection or poor technique were excluded. All patients who participated in the study were notified through the event for severe acute respiratory infection 345. The study was approved by the institutional ethics committee.

Sampling and processing

Respiratory virus samples were taken during the first 10 days of symptom onset. Optimal conditions of conservation and storage of the samples were taken into account from the time of collection until their arrival at the laboratory for analysis; these conditions included the maintenance of the cold chain during transport, avoiding exposure to environmental factors that could interfere with the quality of the sample. The samples were stored at temperatures between

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2 and 8 ºC. Samples that did not meet the definitions established in the study protocol were not analyzed.

Multiple Open RT-PCR

The laboratory of the E.S.E. Hospital Universitario Erasmo Meoz uses the BioFire® RP2.1 cartridge, which corresponds to a closed disposable system containing all the reagents necessary for sample preparation, reverse transcription, polymerase chain reaction (PCR) and detection in order to isolate, amplify and detect nucleic acid of various respiratory pathogens in a swabbed nasopharyngeal sample. After the sample is collected, the hydration solution and sample combined with Sample Buffer are injected into the cartridge, the cartridge is placed into the BioFire® FilmArray® System instrument and a test is initiated; the entire process takes approximately 45 minutes. The sample is then smoothed by agitation (homogenization with beads) in addition to chemical lysis mediated by Sample Buffer. All nucleic acids are then extracted and purified from the sample using magnetic bead technology. Finally, a nested multiplex PCR is performed by reverse transcription, followed by a multiplex first-stage PCR reaction (PCR1); then, simultaneous PCR reactions are performed during the second stage (PCR2) on the array to amplify the sequences within the PCR1 products. Endpoint melting curve data were used to detect the amplicons.

Data analysis

The study took into account the following variables: sex, age, origin, nationality and in-hospital mortality. Likewise, we represented Severe Acute Respiratory Infection based on: 1) Respiratory syncytial virus, 2) Adenovirus, 3) Influenza A, 4) Influenza B, 5) Sars-Cov-2, 6) Metapneumovirus and 7) Influenza A (H3N2). Incidence was calculated for circulating viruses taking as reference population the hospital discharges of the years 2021 and 2022; viral coinfection was described for the seven referenced viral agents. Continuous variables were represented as median and

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interquartile ranges and categorical variables as absolute frequencies and percentages. All statistical analyses were performed using the latest version of R.

RESULTS

In the study conducted at the E.S.E. Hospital Universitario Erasmo Meoz between 2021 and 2022, a notable proportion of patients with Severe Acute Respiratory Infection (SARI) caused by seven viral agents was observed, affecting both Colombian citizens and migrants. In the Colombian population (n=623), the most affected groups were younger infants (1 to 11 months) with 42% and older infants (1 to 2 years) with 24%. The migrant cohort (n=180) showed a similar distribution, with younger and older infants being the most affected, with 34% and 29%, respectively. This pattern suggests variability in susceptibility to these viral agents according to age in relation to immunological maturity and risk of exposure.

In terms of gender, the distribution of ARI among Colombians showed a higher proportion of men (55%) compared to women (45%). Among the migrant population, this difference was similar, with 54% of men and 46% of women affected. In addition, the majority of affected Colombians were enrolled in the subsidized healthcare system (87%), while among migrants, the majority were uninsured (92%). These findings highlight significant differences in access to medical care between the two groups, which could influence the prevention and treatment of ARI. (see Table 1.)

The analysis of viral circulation in the E.S.E. Hospital Universitario Erasmo Meoz revealed interesting patterns throughout the different life cycles. These viruses showed significant variations in their prevalence and distribution from January 2021 to December 2022. Looking at viral circulation patterns among newborns, young infants and older infants, RSV had the highest prevalence with bimodal distribution towards the second and fourth trimesters of both

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years. With increasing age, Adenovirus accounted for a greater number of cases with a similar pattern of distribution to RSV. Between puberty, young adults and adults the viral circulation causing ARRI is much lower and the predominant viruses are Influenza A, RSV, Adenovirus and SARS-CoV-2. We observed an increase in Influenza A cases by January 2022 for most age groups. There is no evidence of prevalent circulation of SARS-CoV-2 because most of the patients admitted to the Hospital were confirmed by antigen testing and did not meet the inclusion criteria for this study (diagnostic confirmation through Multiple RT-PCR). In relation to older adults, circulation patterns are again similar compared to ages younger than 5 years. However, with a lower prevalence of adenovirus. In general, few cases of Influenza B were observed in all age groups. Metapneumovirus and H3N2 showed a more even distribution across the different life cycles, although with specific peaks in preschoolers and schoolchildren, respectively. These findings emphasize the changing dynamics of viral circulation in a diverse population, highlighting the need for surveillance and control strategies tailored to each age group for effective management of severe acute respiratory infections. (See Figure 1).

Viral co-infection

The secondary objective was to determine virus-virus co-infection by having the possibility of multiple RT-PCR in the initial nasopharyngeal swab collection in patients with ARRI criteria. A high co-infection of RSV with Adenovirus and SARS-CoV-2 was observed. This interaction between different respiratory viruses occurred predominantly in older infants and preschoolers, underlining the importance of an integrated approach to prevention, diagnosis and treatment.

In addition, the detailed analysis by virus pairing showed remarkable combinations, with Adenovirus taking

second place in coinfections with Metapneumovirus and SARS-CoV-2, also predominantly in infants and preschoolers. To a lesser extent, coinfection was found between Influenza A, B and H3N2 with other viruses; this could be due to the lower proportion of viral circulation for these viruses.

Intrahospital discharges

Analysis of the data collected revealed significant differences in in-hospital outcomes and the incidence of severe acute respiratory infection (SARI) between Colombian and migrant patients. The median hospital stay for Colombian patients with SARI was 5 days, with an interquartile range (IQR) of 3 to 8 days. On the other hand, migrant patients had a slightly longer median stay (6 days), with a wider IQR (3 to 11 days).

In terms of mortality rates due to ARI, a notable decrease was observed between 2021 and 2022. In 2021, the mortality rate per 1000 hospital admissions was 2.6 for Colombians and 1.0 for migrants. In 2022, these rates decreased to 1.1 and 0.8 respectively, reflecting an overall improvement in the management and treatment of ARIG. In addition, the incidence of ARTI per 1000 was documented to be higher in Colombian patients (12.7) compared to migrants (8.6).

Table 1. Sociodemographic Variables in Patients with ARI (seven viral agents) of the HUEM 2021 to 2022.

Variables	Colombians (n=623)	Migrants (n=180)
Ages by Life Cycle, n (%)		
Newborns (up to 29 days)	26 (4)	2 (1)
Youngest infant (1 to 11 months)	262 (42)	61 (34)

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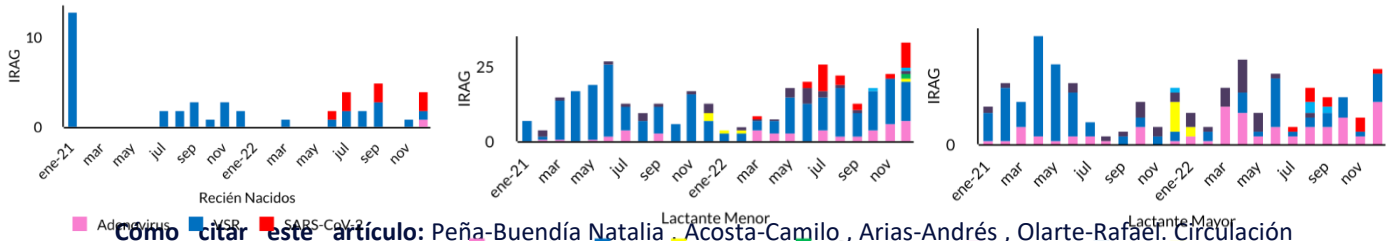
Older infant (1 to 2 years)	148 (24)	52 (29)
Preschool (3 to 5 years)	48 (8)	31 (17)
School (6 to 11 years old)	17 (3)	15 (8)
Puberty (12 to 17 years)	4 (1)	3 (2)
Young Adult (18 to 26 years old)	6 (1)	3 (2)
Adult (27 to 59 years old)	41 (7)	8 (4)
Elderly (60 or older)	71 (11)	5 (3)
Sex, n (%)		
Female	282 (45)	83 (46)
Male	341 (55)	97 (54)
Regime and Ethnicity, n(%)		
Subsidized	545 (87)	15 (8)
Uninsured	30 (5)	165 (92)
Contributory	31 (5)	--
Indigenous	10 (2)	14 (8)

Graph 1. Viral Circulation by Life Cycle 2021 to 2022



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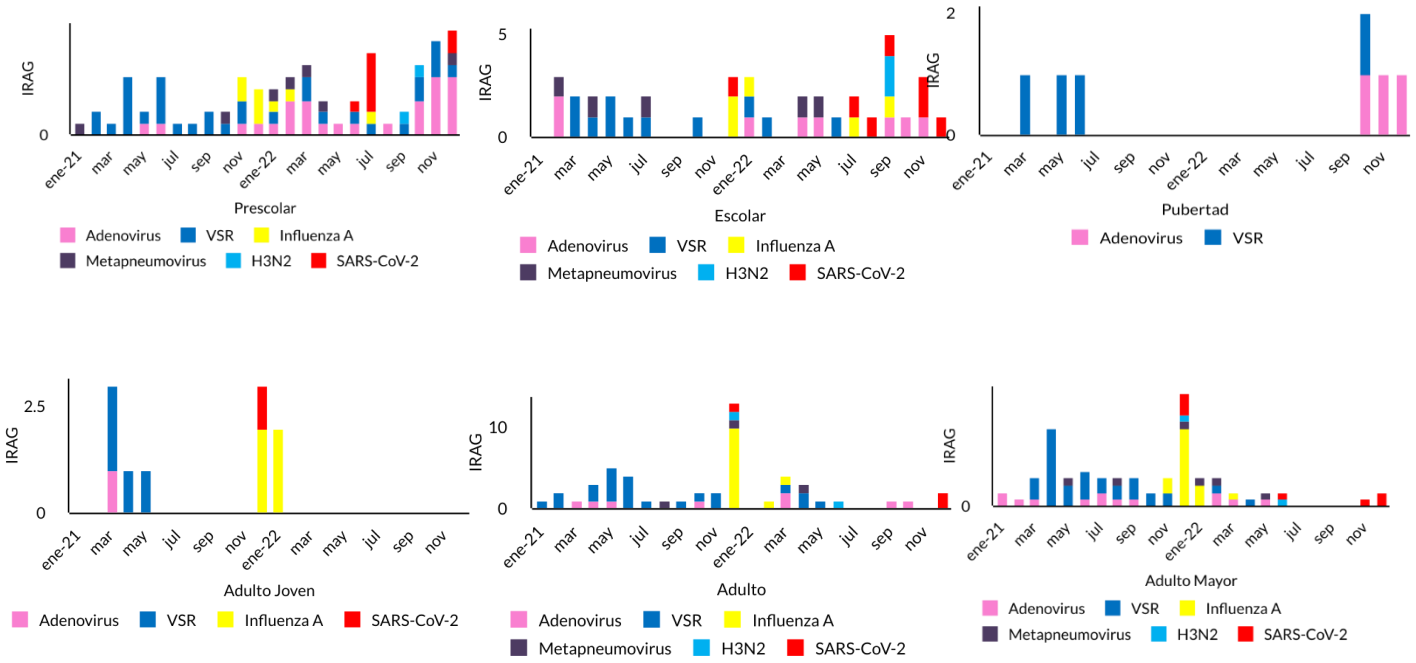
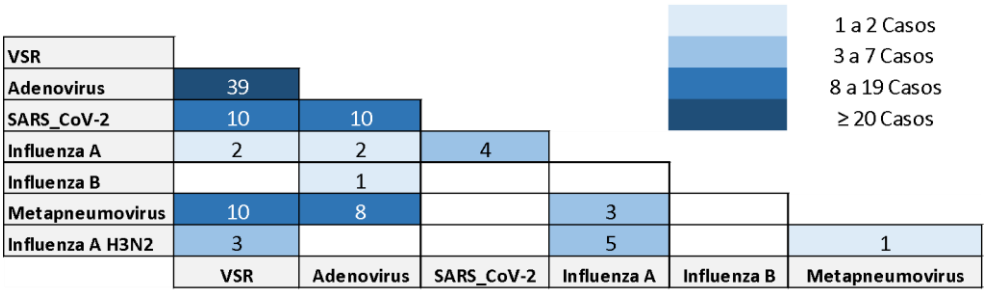


Viral co-infection causing ARI in the HUEM from 2021 to 2022.



DISCUSSION

In Colombia, ARI is monitored by the National Institute of Health (INS)

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through the strategies 1) sentinel surveillance of Influenza-like Illness and Severe ARI ESI-AIRAG, 2) surveillance of unusual ARIG, 3) surveillance of ARI mortality in children under 5 years of age and 4) surveillance of ARI morbidity in all age groups, in hospitalized and ambulatory patients. (7)

The INS in epidemiological week 38 of 2023 reports confirmation for isolated viruses such as respiratory syncytial virus (26.4%), influenza (20.0% with predominance of A(H1N1)pdm09 and influenza B lineage Victoria), rhinovirus (12.3%), enterovirus (10.6%), adenovirus (10.3%) and SARS-CoV-2 (7.4%) respectively.(8)

The E.S.E. H.U.E.M. participates in the sentinel surveillance of circulating viruses in Northeastern Colombia causing severe acute respiratory infection, serving as a source for decision making at institutional and national level in the control of these infections; this surveillance requires diagnostic confirmation of circulating viruses of patients with criteria for hospitalization under the definitions of confirmed and probable case established in the epidemiological surveillance protocol adopted by the institution and from the INS through molecular tests such as multiple RT-PCR allowing the conduct of such studies.

In the last years of the present century, as a result of the pandemic, viral respiratory infections became an important issue for public health, including hospitalization, symptomatology, diagnostic methods and treatment. The use of molecular tests as the gold standard has improved sensitivity in the diagnosis of viral agents, allowing the discovery of viral co-detection and the initiation of targeted management without antibiotic therapy. (9,10)

A systematic review concludes that viral coinfection predisposes to an increase in the hospitalization of patients compared to mono-infections, suggesting greater severity. In pediatric patients, multiple viral infection reaches approximately 30% of cases with acute respiratory infection, studies assume that this is

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due to an immature immune system. (11,12) In this study, it was found that the pediatric population of young and older infants, both in the national population and in the migrant population, are exposed to viral coinfections, generating a crucial role for promotion and prevention measures that allow the reduction of the disease.

CONCLUSION

Multiple RT-PCR is very useful in epidemiological surveillance systems as it allows documenting patterns of viral circulation in severe acute respiratory infections causing morbidity and mortality in our region and patterns of possible virus-virus co-infection. Viral co-detection in severe acute respiratory infection by means of multiple RT-PCR in the population of nortesantandereana admitted to the Erasmo Meoz University Hospital is important for viral confirmation as a sentinel institution. More health institutions that generate results through molecular testing are required in our region.

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