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Respiratory viruses identified from hospitalized patients in an institution of higher complexity

Virus respiratorios identificados de pacientes hospitalizados en una institución de alta complejidad

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Summary

Introduction: Acute Respiratory Infections (ARI) are a group of diseases with similar respiratory symptoms, caused by bacteria or viruses that are acquired by direct contact or through the air.

Objective: To determine the prevalence of respiratory viruses identified by multiplex RT-PCR and microarray detection (CLART PneumoVir Genomica) at the Valle del Lili Foundation between June 2013 and December 2014.

Methods: We conducted a descriptive cross-sectional observational study, records of patients hospitalized in the LVF were evaluated between June 2013 and December 2014. The samples were evaluated by multiplex RT-PCR and microarray detection (CLART PneumoVir), and descriptive statistics were applied

Results: Of 161 samples, 96 (60%) were positive. The service with the highest proportion of positive patients was the Intensive Care Unit-ICU (56%). 32% of positive isolates were identified in patients older than 60 years. The most frequently detected viruses were: Rhinovirus (30%), Influenza (H1N1 / 2009) (12%) and Bocavirus (12%). Influenza A (H1N1 / 2009) was the most commonly isolated strain among influenza viruses (12%), followed by 11% (17 cases) with viral co-infection. The seasonal pattern was not identified.

Conclusion: Multiplex RT-PCR and microarray detection for the identification of the most circulating viruses in the world are useful, sensitive and fast tools. Unlike the one reported by the scientific literature, in this study a higher percentage of positive tests in adults was observed and seasonality was not observed for any of the viruses evaluated.

Resumen

Introducción: Las Infecciones Respiratorias Agudas (IRA) son un grupo de enfermedades con sintomatología respiratoria similar, causadas por bacterias o virus que se adquieren por contacto directo o a través del aire.

Objetivo: Determinar la prevalencia de virus respiratorios identificados por RT-PCR múltiplex y detección por microarreglos (CLART PneumoVir Genomica) en la Fundación Valle del Lili entre junio de 2013 y diciembre de 2014.

Métodos: Realizamos un estudio observacional descriptivo de corte transversal, se evaluaron registros de pacientes hospitalizados en la FVL entre junio de 2013 y diciembre de 2014. Las muestras fueron evaluadas por RT-PCR múltiplex y detección por microarreglos (CLART PneumoVir), se aplicó estadística descriptiva.

Resultados: De 161 muestras, 96 (60 %) fueron positivas. El servicio con la mayor proporción de pacientes positivos fue la Unidad de Cuidados Intensivos-UCI (56%). El 32 % de los aislamientos positivos se identificaron en pacientes mayores de 60 años. Los virus más frecuentemente detectados fueron: Rinovirus (30%), Influenza (H1N1/2009) (12 %) y Bocavirus (12 %). Influenza A (H1N1/2009) fue la cepa más comúnmente aislada entre los virus de la gripe (12%), seguido de un 11% (17 casos) con coinfección viral. No se identificó el patrón estacional.

Conclusión: La RT-PCR múltiplex y la detección por microarreglos para la identificación de los virus de mayor circulación en el mundo, son herramientas útiles, sensibles y rápidas. A diferencia de lo reportado por la literatura científica, en este estudio se observó mayor porcentaje de pruebas positivas en adultos y no se observó estacionalidad para ninguno de los virus evaluados.

Key contributions of the study

Objective	Determine the prevalence of respiratory viruses, detected by the CLART® Pneumovir test at the Valle del Lili Clinical Foundation, July 2013 to June 2014.
Design of the study	Observational cross-sectional study.
Sources of information	185 swab samples and nasopharyngeal aspirates were analysed for the detection of respiratory virus using two techniques
Population/sample	125 samples of swabs and nasopharyngeal aspirates were analysed by technique CLART Pneumovir® platform and 60 samples per Xpert® Flu (Cepheid)
Statistical analyses	Univariate to determine the Behavior of numerical variables, the Shapiro Wilk test was used. Temporal trend analysis to determine the frequency of each virus isolate.
Principle findings	Of 161 samples, 96 (60%) were positive. In the ICU it was where these viruses were most isolated (56%). 32% of positive patients were older than 60 years. The viruses with the highest detection were Rhinovirus (30%), Influenza (H1N1 / 2009) (12%) and Bocavirus (12%). Influenza A (H1N1 / 2009) was the strain that was most isolated among viruses of influenza (12%), followed by patients with 11% viral co-infections (17 cases). No seasonal pattern was found in the frequency of infections

Introduction

Acute respiratory infections (ARI) are a group of diseases that have a similar respiratory symptomatology and are caused by various microorganisms, either bacteria or viruses. They are transmitted by air or via the direct route via objects contaminated with secretions. Viruses are responsible for 80 to 90% of reported cases of ARI, both in children and adults (1). The most frequent causative agents of ARI, are the so-called "classic" viruses among which are influenza A, B and C viruses; Parainfluenza type 1, 2, 3 and 4 (PIV); Human Respiratory Syncytial Virus (hVSR); Human coronavirus (HCoV) OC43 and 229E; Adenovirus (AdV); Rhinovirus (hRV), some Enterovirus (Echovirus-EV), and other new agents that cause ARI such as Human Metapneumovirus (hMPV); Human Bocavirus (HBoV); some human mimiviruses and coronaviruses such as HKU1 (2).

The main viruses that cause ARI in the pediatric population are Respiratory Syncytial Virus (RSV) and Parainfluenza type 3 in low-income countries (3-7), with this being the etiologic agent in 20 to 25% of pneumonia cases and in 40-50% of broncholitis cases in hospitalized children (8).

These viruses are also responsible for other pathologies such as influenza, pharyngitis, trache-bronchitis and colds (9). In countries with seasons, these infections are more frequent during the winter and in countries without seasons they are more frequent during the rainy season. During these seasons, they are one of the first causes of consultation and hospitalization at all ages (10,11) especially those caused by the Influenza virus. In the United States, ARI has a lethality of 3% in children under two years of age, in Colombia it reaches 5% (12) especially that caused by Influenza A; In adults over 65 years of age, some co-morbidities such as immunosuppression, nutritional deficiencies, physical and mental limitations increase the risk of death due to ARI (12).

The identification of the etiologic agent of ARI is essential for the clinical management of the patient. Given the wide range of etiological agents, however, it is necessary to evaluate the most prevalent viruses in the hospital environment. The development of molecular techniques for the amplification of viral genetic material through multiplex PCR (CLART Pneumovir DNA array assay (Genomica, Coslada, Madrid, Spain)) allows the timely evaluation of the presence of the 19 most prevalent viruses associated with ARI in the world and also allows detection of concomitant viral infections (13-18). Despite the existence of these techniques, in most cases the causative agent is not identified, so the prevalence of different viruses is not known exactly.

The objective of this study was to determine the prevalence of respiratory viruses that are identified by multiplex RT-PCR and microarray detection (CLART PneumoVir Genomica) in patients hospitalized at the Valle del Lili Foundation between June 2013 and December 2014.

Methods

Study design and population

We conducted a descriptive cross-sectional observational study. Between June 1, 2013 and December 31, 2014, patients of both sexes, and of all ages, who were hospitalized in the intensive care unit, emergency and transplant areas of the Valle del Lili Foundation (FVL) were included in the study. These patients were all suspected of having ARI, caused by any of the 19 viruses that are detected through the multiplex RT-PCR (reverse PCR) and microarray detection (CLART® PneumoVir Genomics).

Those patients without clinical information or with incomplete clinical information were excluded.

Procedures

Samples were taken using swabs and nasopharyngeal aspirates, as described in the Guide for laboratory surveillance of influenza virus and other respiratory viruses of the National Institute of Health (19), preserved in transport medium (UTMTM COPAN reference 330C), stored at 4°C and sent immediately to the laboratory for processing. This procedure was performed for both platforms, CLART® Genomic PneumoVir and Xpert® Flu (Cepheid).

Nucleic acid extraction, amplification and detection

Nucleic acids (DNA/RNA) were extracted from the samples together with a negative control with the NucliSENS magnetic extraction reagents kit (Biomerieux reference 200293) using the Boom method with silica paramagnetic particles. In summary, this is based on the ability of silica to bind DNA and RNA in high salt concentrations (20).

The amplification was carried out by RT-PCR of a specific fragment of the viral genome between 120-330 bp, with the CLART® PneumoVir commercial kit containing two types of PCR mixtures, to cover all types and subtypes of viruses. PCR-1 for Coronavirus amplification; Metapneumovirus (subtypes A and B); Parainfluenza virus 1, 2, 3 and 4 (subtypes A and B) and RSV-A. PCR-2 for amplification of Adenovirus; Bocavirus; Enterovirus (Echovirus); Influenza virus A, B, C, and Influenza A H1N1 / 2009; Metapneumovirus, Rhinovirus and RSV-B. The PCR mixtures also contain an internal amplification control, to avoid false negative results. The PCR mixture favours the amplification of the viruses against the amplification control (20,21).

The detection with CLART® PneumoVir is based on the precipitation of an insoluble product in those areas of the arrangement in which hybridization of the amplified products with the specific probes occurs. During RT-PCR, the amplified products were labelled with biotin. After amplification, these products hybridized with their respective specific probes that are immobilized in specific and known areas of the array, after which it was incubated with a streptavidin peroxidase conjugate. The conjugate was bound through streptavidin with the biotin present in the amplified products (which in turn are attached to their specific probes) and the peroxidase activity causes the appearance of an insoluble product in the presence of the o-dianisidine substrate, with what is the precipitation of this in the areas of the arrangement in which hybridization occurs. The reading was done on the CAR platform (Clinical Array Reader) by reading optical density, compared to an algorithm for each virus. (20.21).

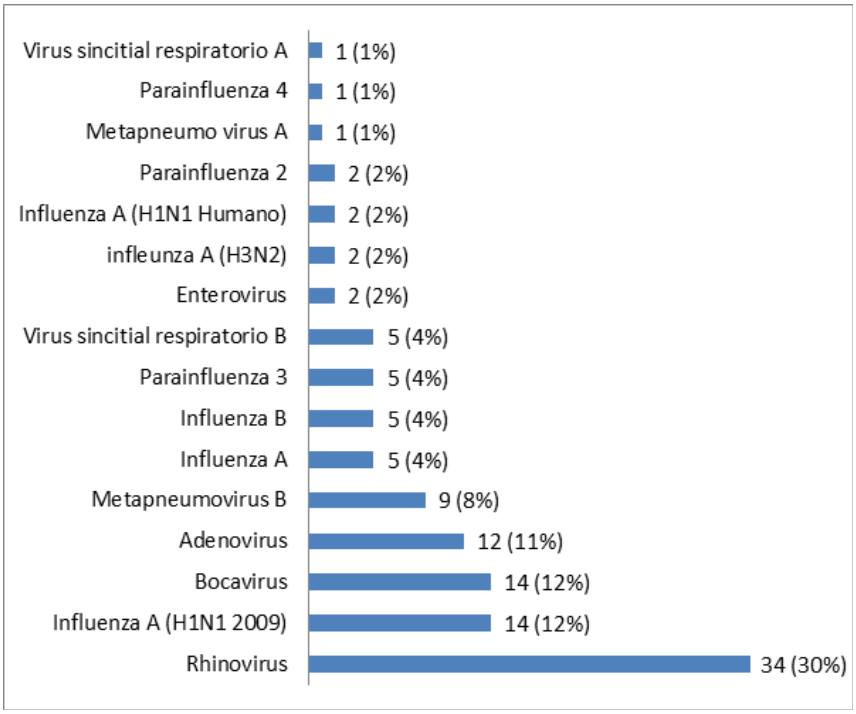

Figure 1. Infection frequency by virus type.

The CLART® PneumoVir platform is a standardized test by the commercial house, which was verified before its implementation in the laboratory.

The processing of the data obtained was carried out automatically by the CAR, from each of the analyses and a written report was generated in which the amplified viruses obtained in each of the samples were indicated (20,21,22).

Additionally, 60 samples were evaluated by the Xpert® Flu (Cepheid) technology that identifies Influenza A (H1N1 / 2009) and Influenza B. Automated platform in which the sample was added to a special closed cartridge along with an internal processing control the sample (SPC), this cartridge was inserted in the GeneXpert® equipment; Inside the cartridge a filter captures the sample and the SPC, the cells were ultrasonically lysed for DNA release; The solubilized DNA was mixed with the spheres of lyophilized reagents, the real-time PCR amplification and fluorescence detection are simultaneous, generating a written, positive or negative result for each virus (23).

Statistic analysis

A univariate analysis was performed, to determine the behaviour of the numerical variables and the Shapiro Wilk test was also used. Those with a $p>0.05$ were considered to have a normal distribution and were presented using averages and standard deviation. Those with different distributions than normal were presented using median and interquartile ranges as a summary measure. Categorical variables were presented as proportions. The institutional prevalence of each respiratory virus was determined as a proportion, taking as a numerator the number of CLART® Pneumovir positive tests for each virus and as a denominator the total CLART® Pneumovir tests performed. To assess the Behavior of the frequency of the viruses during each month in the year, a temporal trend analysis was performed, identifying the frequency of each virus isolated and comparing whether there was an increase or decrease in the frequency.

This study adhered to the international norms of investigation of the CIOMS (Council for International Organizations of Medical Sciences), to the declaration of Helsinki and the Nuremberg code, as well as to the Colombian norm outlined in Article 11 of the resolution 8430 of 1993. This research was approved by the Biomedical Research Ethics Committee of the Valle del Lili Foundation, according to the approval letter 797 of February 2015.

Results

During the study period, 161 patients with suspected ARI were evaluated, who had a critical medical condition, of which 96 (60%) were positive for at least one of the 19 viruses included in the panel. A total of 114 positive tests were obtained, the most frequently detected viruses were: Rhinovirus 34 times (30%), Influenza A (H1N1 2009), Bocavirus 14 times (12%), and Adenovirus 12 times (11%). In 11% of the patients a viral co-infection was reported (positive for two or more viruses), the most frequent observed in co-infection were Rhinovirus 11%, Bocavirus 9%, Adenovirus 6% and Influenza A (H1N1 / 2009) 2%. None of the samples evaluated were positive for Coronavirus, Influenza C or Parainfluenza 1 (Figure 1).

The patients with older than or equal to 61 was the age group with the most positive cases (32%), in this group, 12 of the 19 types of virus were identified, followed by the group of 15-45 years (25%) with 10 types of viruses identified. While in those under one year of age it had the lowest detection percentage of 8% with only 5 types of viruses (Table 1). The hospital services with the highest number of positive patients were the ICUs and the emergency room (Table 1).

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Table 1. Distribution of viruses by gender, age group and hospital service

	Hospitalization								ICU													Urgencies										Transplants					
Group age and gender	Metapneumovirus B	Parainfluenza 2	Parainfluenza 3	Bocavirus	Rhinovirus	Influenza A (H1N1/2009)	Adenovirus	Virus sincitial respiratorio B	Influenza A (H1N1 Humano)	Metapneumovirus A	Metapneumovirus B	Parainfluenza 3	Adenovirus	Rhinovirus	Bocavirus	Influenza A	Influenza A (H1N1/2009)	Influenza B	Enterovirus	Virus sincitial respiratorio A	Virus sincitial respiratorio B	Bocavirus	Influenza A (H1N1 Humano)	Parainfluenza 3	Metapneumovirus B	Rhinovirus	Influenza A (H1N1/2009)	Influenza B	Adenovirus	Influenza A	Enterovirus	infleunza A(H3N2)	Virus sincitial respiratorio B	Adenovirus	Bocavirus	Rhinovirus	Parainfluenza 4	Total general	%
<1																																					9	8
F														1							1															2	
M			1	1	1								1	1								1			1										7	
1-14																																					21	18
F		1				1		1			1			1	1							1			1								1	1	1	1	12	
M	1	1	1				1					1		1	1										2											9	
15-45																																					29	25
F											1			5	1		3		1	1							1	1								14	
M														1	2		2	1							4	1		1	2			1					15	
46-60																																					19	17
F				2			1			1			4	2			3	1					1														15	
M											1			1												2											4	
≥61																																					36	32
F				1			1		1		3	1		6		3		2			2	1		1	1					1	2						26	
M					1								1	2	1		1								1	2	1										10	
Total	1	2	2	4	2	1	3	1	1	1	6	2	6	21	6	3	9	4	1	1	3	3	1	1	2	10	4	1	2	2	1	2	1	1	1	1	114	100
Total Servicio	16	64	30	4	114																																	

Of the 60 samples that were evaluated by Xpert® Flu and CLART® PneumoVir, they were mostly negative for influenza A and B (Table 2). Only two samples were positive for both platforms (Xpert® Flu and CLART® PneumoVir), for Influenza A (H1N1 / 2009) and Influenza B. However, of the 58 samples (96.7%) that were negative for Xpert® Flu, 6 (8.6%) were positive for Influenza A (4 for Influenza A (H1N1 / 2009) and 2 for Influenza A) by the CLART® PneumoVir platform (Table 2). Of the 58 samples that were reported as negative by Xpert® Flu, 41 (70.7%) were positive for the CLART® PneumoVir platform for at least one of the 19 viruses evaluated by the platform and 17 (28.3%) samples were negative for both methodologies No seasonality was observed for any of the viruses detected.

Discussion

This is the first study - conducted in patients of all ages, who were critically ill and hospitalized in a high complexity medical centre in south-western Colombia - which describes the experience of using a platform that incorporates molecular techniques for the detection of the 19 most prevalent viruses in the world related to ARI.

In our population we found that the most prevalent viruses were Rhinovirus, Influenza A (H1N1 2009) and Bocavirus. Other studies conducted in different countries in which CLART PneumoVir was used showed differences in the prevalence of the most frequent viruses (7,24,25).

The behaviour of co-infections in our study population is different from other publications. In the present study, a percentage of co-infection was detected in 11% of the samples, the combination of Adenovirus and Rhinovirus being the most frequent virus, much lower than that found in an ICU in France (35%) (7) and 37% in Chile (26).

Our study showed a prevalence of 9% in the identification of Metapneumovirus B, a little higher than in other publications using the same methodology, 2.7% in Chile (26), 3.7% in Greece (27), viruses little studied because Routine methods did not include it. The first cases in Colombia describe them in Medellin, where the presence of this virus in our country begins to be studied (28).

In contrast to other reports in the scientific literature, a higher percentage of positive tests in adults were observed in this study (29,30). This difference can be explained by the use of other platforms for virus screening that are more frequent in the pediatric population of FVL (31).

The aging population forces health services to ensure that resources to meet the needs of the elderly population and that is why knowing the frequency of ARI-associated viruses in adults over 60 years of age is the first step in proposing prevention strategies. The virological demonstration is very important since the specific viral identification allows the initiation of an adequate antiviral therapy when appropriate, to avoid the unnecessary use of antibacterials and to implement infection control measures among hospitalized patients.

The molecular tests used are highly sensitive for the identification of viruses. The CLART Pneumovir DNA array test (Genomica, Coslada, Madrid, Spain) allowed us to identify 19 viruses more frequently that are associated with IRA, some of which could not be identified with the Xpert Flu platform. With the use of multiplex tests, co-infections that could go unnoticed with other molecular techniques can be detected.

The time to obtain the results is several hours and this can be a limitation for the clinician in terms of making timely decisions in critically ill patients, and the cost of the test is higher than other conventional methods, which could limit its use.

The increased sensitivity that these tests allow would help to make diagnoses more accurately and more routinely. Especially in the case of an infection in which low levels of the virus are present and which in these cases could be overlooked when non-molecular tests are used (29).

Conclusions

The viruses most frequently detected in this study were: Rhinovirus, Influenza A (H1N1 2009), Bocavirus and Adenovirus.

No patient with Parainfluenza A and B virus was detected during this period of time studied.

The highest percentage of positive patients were over 60 years old and female. The majority of patients with positive test were in the ICU service.

CLART®Pneumo Vir technology allows the detection and characterization of the presence of 19 human viruses (types and subtypes) that cause respiratory infection. It allows the detection of minimal amounts of nucleic acids from different viruses in swab or nasopharyngeal lavage samples quickly and highly specifically.

Table 2. Comparative samples processed by both platforms. Patients with suspected ARI in the FVL screened by CLART Pneumovir and Xpert® Flu (Cepheid) between June 2013 and December 2014 (n = 60)

Virus	CLART Pneumovir			Xpert® Flu (Cepheid)		
	Positive	Negative	Total	Positive	Negative	Total
Influenza A (H1N1)	8	52	60	2	58	60
Influenza B	2	58	60	2	58	60
Other viruses	41	17	58	N/A	N/A	N/A

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