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Different Clinical Presentations of Human Rhinovirus Species Infection in Children and Adults in Mexico

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Background. Human rhinoviruses (HRVs) are a common cause of influenza-like illness, with the ability to infect the upper and lower respiratory tracts. In this study we aim to describe the clinical and molecular features of HRV infection in Mexican children and adults.

Methods. We performed a hospital-based, 4-year multicenter prospective observational cohort study of patients with influenza-like illness. Participants who tested positive for HRV were included. We described demographic, clinical, and laboratory characteristics and the association between HRV types, illness severity, and clinical outcomes.

Results. Of the 5662 subjects recruited, 1473 (26%) had HRV; of those, 988 (67.1%) were adults (≥18 years) and 485 (32.9%) were children. One hundred sixty-seven (11.33%) samples were sequenced; 101 (60.5%) were rhinovirus species A (HRV-A), 22 (13.2%) were rhinovirus species B (HRV-B), and 44 (26.3%) were rhinovirus species C (HRV-C). Among children and adults, 30.5% and 23.5%, respectively, were hospitalized (non–intensive care unit [ICU]). The odds of HRV-C are higher than HRV-A for participants in the ICU (compared to outpatient) and when platelets, lymphocytes, white blood cells, and lactate dehydrogenase are increased. The odds of HRV-C are higher than HRV-A and HRV-B with shortness of breath. The odds of HRV-A are higher than HRV-B, and the odds of HRV-B are higher than HRV-C, when mild symptoms like muscle ache and headache occur.

Conclusions. Rhinoviruses are a common cause of influenza-like illness. It is necessary to improve the surveillance, testing, and species identification for these viruses to understand different clinical presentations and risk factors associated with worse outcomes.

Clinical Trials Registration. NCT01418287.

Keywords. clinical presentation; HRV species; influenza-like illness; rhinovirus; severity.

Human rhinoviruses (HRVs) have been described as one of the most prevalent human respiratory viruses and a common cause for influenza-like illness (ILI) worldwide [1, 2]. HRVs are responsible for one-half of all acute upper respiratory illnesses and infect billions of people annually [3–5].

Furthermore, it is estimated that HRV infections represent a significant economic burden annually in terms of medical costs, work absenteeism, and inappropriate use of antibiotics [6, 7].

The most common clinical characteristics in patients with HRVs include rhinorrhea, cough, sore throat, headache, diffuse malaise, and nasal congestion. However, they may cause more serious manifestations including bronchiolitis and exacerbations of chronic pulmonary diseases, such as asthma [8, 9], particularly in children, the immunocompromised, and the elderly [10, 11].

HRVs are included in the Picornaviridae family, in the *Enterovirus* genus, and are specific to humans. HRVs are genetically very diverse and over 150 genotypes have been identified. They are classified into 3 main HRV species (HRV-A, HRV-B, and HRV-C) according to their distinctive genome, phylogenetic sequences, and molecular features [12]. Most HRV respiratory infections are due to HRV-A and HRV-C, and different

pathways of epidemiology, transmission, and disease outcomes among the 3 species have been described [13, 14].

Some studies suggest that progression to serious respiratory illness is more likely associated with HRV-A and HRV-C rather than with HRV-B. HRV-C is associated with an increased number of hospitalizations among children [15], especially in those with asthma [16] and cystic fibrosis [17].

Less is known about the epidemiology and clinical characteristics of HRVs in adults. Moreover, in Mexico and other Latin American countries, there is limited information regarding the clinical and molecular characteristics of HRVs as a cause of respiratory illness. Previously, our group described clinical characteristics and outcomes of ILI in an early cohort of 1065 subjects tested for different pathogens. We found that HRV was the most frequently isolated pathogen, infecting 15.3% of enrolled participants; 51% of the population infected with HRVs required hospitalization (62% of adults and 48% of children) [18]. The complete cohort enrolled a total of 5662 participants between 7 April 2010 and 4 April 2014, and HRV continued to be the most common cause of ILI.

In this study, we describe the clinical and molecular features of HRV infection in the Mexican population; we also explored the association between HRV type and more serious illness and worse clinical outcomes.

METHODS

Study Design and Settings

ILI002 was a hospital-based, multicenter prospective observational cohort study of ILI by the Mexican Emerging Infectious Disease Clinical Research Network (LaRed) between 7 April 2010 and 4 April 2014. The study was conducted at 6 hospitals, 5 of them in Mexico City and 1 in San Luis Potosí. Participating hospitals included 2 general hospitals (1 in Mexico City and 1 in San Luis Potosí), 2 tertiary care pediatric hospitals, and 2 tertiary care hospitals (1 is dedicated to the treatment of respiratory disorders; the other provides medical care in a wide range of medical specialties). The study was approved by the ethics committee of each institution (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, reference number 116; Instituto Nacional de Enfermedades Respiratorias Ref C12-10; Hospital General Manuel Gea González, reference number 36-50-2010; Instituto Nacional de Pediatría, reference number INP 014/2010; Hospital Infantil de México Federico Gómez, reference number HIM/2010/074; Hospital Regional Dr Ignacio Morones Prieto, reference number 88-12).

Study Population and Definitions

This study analyzed data from participants aged 1 month or older with ILI who sought medical attention and tested positive for HRV in nasopharyngeal swabs or nasal aspirates by real time polymerase chain reaction (rt-PCR). ILI was defined according to a modified case definition criteria from the World Health Organization [19] as having at least 1 respiratory symptom (ie, shortness of breath, cough) and either fever or 1 or more nonrespiratory symptom (ie, malaise, headache), with illness onset within the past 48 hours. By definition, those subjects seeking care for ILI and needing hospitalization are considered to have severe acute respiratory infection (SARI).

Study Procedures

All patients seeking care for ILI who met the inclusion and exclusion criteria at the emergency department or at the intensive care unit (ICU), depending on severity, were invited to participate in the study. After informed assent and consent forms were obtained, sociodemographic and symptom data were collected using previously standardized questionaries, and a complete physical examination was performed. A nasopharyngeal swab, or a nasal aspirate in children, was obtained at baseline for PCR detection of respiratory pathogens in all participants, and a blood sample for complete blood counts and chemistry analysis was collected. The final clinical decision regarding whether the subject would remain in the emergency department, require hospitalization, or receive treatment as an outpatient was made by the attending physician in charge. Follow-up information (symptoms, chronic medical conditions, previous treatment, impact on daily function, hospitalizations, and death) was obtained by phone at day 14 and 60 and at an in-person visit at day 28 through standardized case report forms.

Laboratory Diagnosis

Respiratory samples were tested with the RespiFinder 19 (April 2010 to May 2012) and RespiFinder 22 (previously RespiFinder Plus, June 2012 to March 2014) from PathoFinder BV, Maastricht, the Netherlands. RespiFinder 19 (multiplex rt-PCR test) detects and differentiate 15 viruses (coronaviruses NL63, OC43, and 229E; human metapneumovirus; influenza A, A/H5N1, and B; parainfluenza virus types 1–4; RSV A and B; picornavirus [enterovirus and HRV]; and adenovirus), as well as 4 bacteria (*Bordetella pertussis*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*). The RespiFinder 22 removed influenza H5N1 and added bocavirus (type 1), coronavirus HKU1, influenza A/H1N1v, and picornavirus (enterovirus and HRV). Sequenced samples were selected by convenience, clinician judgment, and resource availability to determine HRV species.

RNA Extraction

Total nucleic acids were extracted from 500 μL of nasopharyngeal swabs using the NucliSENS easyMAG system (bioMérieux, Netherlands) and eluted in 110 μL, according to the manufacturer's instructions.

Sequencing and Phylogenetic Analysis

To determine HRV type, a 549 bp fragment of the VP4/VP2 genomic region was amplified by RT-PCR and sequenced. RT-PCR was performed using 10 µL of nucleic acids with SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen, ThermoFisher Scientific, Carlsbad, California) with primers VP42F (GGGACCAACTACTTTGGGTGTCCGTGT) and VP42R (GCATCIGGYARYTTCCACCACCANCC) [20]. The amplification program was as follows: RT reaction at 50°C for 60 minutes, initial denaturation at 95°C for 2 minutes, 40 cycles of 95°C for 30 seconds, 55°C for 1 minute, 68°C for 1 minute, and the final extension at 72°C for 5 minutes. RT-PCR products were analyzed in horizontal 2% agarose gel stained with SYBR Safe (Invitrogen, ThermoFisher Scientific, Kiryat Shmona, Israel). All RT-PCR products were purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany); the concentration was determined by the Qubit fluorometer using the Qubit ds HS Assay kit (Invitrogen, ThermoFisher Scientific, Eugene, Oregon) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, California). The sequences obtained were aligned to reported sequences of HRV in GenBank.

Bioinformatic Analysis

To examine the associations between samples, sequences obtained were manually edited with Kodon software v2.04 (Applied Maths, Sint-Martens-Latem, Belgium). Alignment was performed using the ClustalW program, and phylogenetic relationships were constructed by the neighbor-joining method with bootstrap of 1000 replicates using the MEGA v6 software [21].

Viral Load

To measure viral load, a universal HRV quantitative RT-PCR assay was used. Primers qR447f (5′-GGCCCCTGAATGYG-GCTAA-3′), qR561r (5′-GAAACACGGACACCCAAAG-TAG-3′), and probe R529 (FAM 5′-AYGGRACCRACTACT-TTG-3′ MGB) were used to amplify a 114 bp fragment of the highly conserved region of the 5′ untranslated region as described previously [22]. RNA was amplified in an ABI Prism 7500 SDS Real-Time cycler with the following cycling program: RT reaction at 48°C for 30 minutes, initial denaturation at 95°C for 2 minutes, 45 cycles of 95°C for 15 seconds, and 60°C for 32 seconds. Serial dilution (1×10^1 to 1×10^7 copies/µL) of a plasmid containing the amplified fragment was used to construct a standard curve. All samples were tested in triplicates and viral load was determined by plotting cycle threshold values in the standard curve as viral copies per microliter of extracted RNA.

Statistical Analysis

Categorical variables were summarized by number and frequency and continuous variables by mean and standard deviation. A multinomial regression model was performed, restricted to participants who were sequenced and HRV-A, HRV-B, or HRV-C (with the dependent variable being disease group), to evaluate the univariate relationship of each clinical feature and virus type. Laboratory values were standardized for ease of comparison. A Kruskal-Wallis test was used to compare $\log_{10}$-transformed viral load between groups. To compare between participants who were sequenced and HRV-A, HRV-B, and HRV-C and all others, t tests were used for continuous variables and χ^2 tests were used for categorical variables. All analyses were done in R version 3.6.3 software; $P < .05$ was considered statistically significant. No corrections for multiple comparisons were performed.

RESULTS

A total of 5662 participants were in enrolled in the full cohort, of whom 1473 (26%) tested positive for HRV; the demographic and medical history data for these 1473 participants are described in Table 1. Nine hundred eighty-eight (67.1%) participants were adults (aged ≥18 years) and 485 (32.9%) were children; 69.4% of adults and 35.5% of children were outpatients. Of children and adults, 30.5% and 23.5%, respectively, were hospitalized (non-ICU); 15.3% of children and 6.1% of adults were in the emergency room (Supplementary Figure 1); and 18.8% of children and 1% of adults were in the ICU. One hundred thirty-seven (28.2%) children and 164 (16.6%) adults had a coinfection.

In children, 79% (383) of cases occurred in those ≤5 years of age. In adults, 868 (87.9%) cases were in those 18–59 years old, and 120 (12.1%) were in those ≥60 years old. The most common comorbidities in children were congenital syndromes, occurring in 89 (18.4%) children. In adults, common comorbidities were asthma (12.1%) and cardiovascular disorders (13.1%). Other reported comorbidities included chronic lung disease, human immunodeficiency virus infection, and immunodeficiencies, among others (Supplementary Table 1).

Antibiotic use was common, occurring in 262 (54%) children and 407 (41.2%) adults. Other medication usage included antihistamines (18.4% of children and 27.8% of adults), antipyretics (32.4% of children and 23.2% of adults), and systemic steroids due to an autoimmune or oncologic disease (17.7% of children and 22% of adults). Other medications included cough medicine, decongestants, and inhaled steroids (Supplementary Table 1).

One hundred sixty-seven (15.1%) samples were sequenced, of which 101 (60.5%) were HRV-A, 22 (13.2%) were HRV-B, and 44 (26.3%) were HRV-C. Fifty-seven children (34.1%) had sequencing data available; 26 children (45.6%) had

Table 1. Demographical Characteristics and Medical History of Enrolled Participants Among Children and Adults

Characteristic	Overall	Children	Adults
No.	1473	485	988
Status			
Emergency room	134 (9.1)	74 (15.3)	60 (6.1)
Hospitalized (non-ICU)	380 (25.8)	148 (30.5)	232 (23.5)
ICU	101 (6.9)	91 (18.8)	10 (1.0)
Outpatient	858 (58.2)	172 (35.5)	686 (69.4)
Age, y			
Mean (SD)	26.66 (21.34)	3.34 (4.1)	38.11 (16.51)
Median (IQR)	24.00 (5.00–41.00)	2.00 (0.00–5.00)	34.00 (24.00–49.00)
Categorical age, y			
0–5	383 (26.0)	383 (79.0)	0 (0.0)
6–11	64 (4.3)	64 (13.2)	0 (0.0)
12–17	38 (2.6)	38 (7.8)	0 (0.0)
18–59	868 (58.9)	0 (0.0)	868 (87.9)
≥60	120 (8.1)	0 (0.0)	120 (12.1)
Sex			
Female	895 (60.8)	220 (45.4)	675 (68.3)
Male	578 (39.2)	265 (54.6)	313 (31.7)
Smoking history			
Never smoked	923 (62.7)	361 (74.4)	562 (56.9)
Passive smoker	168 (11.4)	120 (24.7)	48 (4.9)
Current smoker	211 (14.3)	2 (0.4)	209 (21.2)
Former smoker	171 (11.6)	2 (0.4)	169 (17.1)
Coinfection	301 (20.4)	137 (28.2)	164 (16.6)
Influenza	55 (3.7)	20 (4.1)	35 (3.5)
Coronavirus	79 (5.4)	18 (3.7)	61 (6.2)
Parainfluenza virus	49 (3.3)	27 (5.6)	22 (2.2)
Respiratory syncytial virus	61 (4.1)	35 (7.2)	26 (2.6)
Adenovirus	44 (3.0)	24 (4.9)	20 (2.0)
Bordetella pertussis	8 (0.5)	7 (1.4)	1 (0.1)
Metapneumovirus	26 (1.8)	10 (2.1)	16 (1.6)
Mycoplasma pneumoniae	7 (0.5)	3 (0.6)	4 (0.4)
Comorbidities			
Asthma	165 (11.2)	45 (9.3)	120 (12.1)
Cardiovascular disorders	148 (10.0)	19 (3.9)	129 (13.1)
Congenital syndromes	92 (6.2)	89 (18.4)	3 (0.3)
Received influenza vaccine	131 (8.9)	40 (8.2)	91 (9.2)
Received pneumonia vaccine	473 (32.1)	332 (68.5)	141 (14.3)
Current medications			
Antibiotics	669 (45.4)	262 (54.0)	407 (41.2)
Antihistamines	364 (24.7)	89 (18.4)	275 (27.8)
Antipyretics	386 (26.2)	157 (32.4)	229 (23.2)
Systemic steroids	303 (20.6)	86 (17.7)	217 (22.0)
Death	29 (2.0)	6 (1.2)	23 (2.3)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ICU, intensive care unit; IQR, interquartile range; SD, standard deviation.

HRV-A and HRV-C, whereas 5 (8.8%) had HRV-B. One hundred ten adults (65.9%) had sequencing data available. Most adults (75 [68.2%]) with sequencing data available had HRV-A, whereas 17 (15.5%) and 18 (16.4%) adults had HRV-B and HRV-C, respectively.

Table 2 presents demographic and medical history data for the 167 cases of HRV that were sequenced and HRV-A, HRV-B, or HRV-C. Thirty-five of these subjects were admitted to the ICU, of whom 33 (94.3%) were children, and 35 subjects were hospitalized (non-ICU), of whom 24 (68.5%) were adults. Ten subjects were in the emergency room, of whom 6 (60%) were children; of the 87 subjects who were outpatients, 80 (92%) were adults.

Of the 26 children with HRV-A, 16 (61.5%) were admitted to the ICU, 4 (15.4%) were in the emergency room, and 3 (11.5%) each were hospitalized (non-ICU) and outpatient. Of the 5

Table 2. Demographical Characteristics and Medical History of the 176 Cases for Rhinovirus That Were Sequenced According to Virus Type and Age Group

Characteristic	Overall	Children			Adults		
		A	B	C	A	B	C
No. of participants	167	26	5	26	75	17	18
Status							
Emergency room	10 (6.0)	4 (15.4)	1 (20.0)	1 (3.8)	2 (2.7)	0 (0.0)	2 (11.1)
Hospitalized (non-ICU)	35 (21.0)	3 (11.5)	0 (0.0)	8 (30.8)	18 (24.0)	1 (5.9)	5 (27.8)
ICU	35 (21.0)	16 (61.5)	4 (80.0)	13 (50.0)	2 (2.7)	0 (0.0)	0 (0.0)
Outpatient	87 (52.1)	3 (11.5)	0 (0.0)	4 (15.4)	53 (70.7)	16 (94.1)	11 (61.1)
Age, y							
Mean (SD)	25.19 (20.13)	3 (3.69)	0.8 (1.3)	3.31 (5.08)	38.69 (15.07)	34.18 (15.4)	30.89 (10.36)
Median (IQR)	23.00 (4.00–41.00)	1.00 (0.00–4.75)	0.00 (0.00–1.00)	0.50 (0.00–5.50)	38.00 (24.00–50.00)	28.00 (23.00–47.00)	27.00 (22.00–37.75)
Categorical age, y							
0–5	44 (26.3)	20 (76.9)	5 (100.0)	19 (73.1)	0 (0.0)	0 (0.0)	0 (0.0)
6–11	8 (4.8)	4 (15.4)	0 (0.0)	4 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)
12–17	5 (3.0)	2 (7.7)	0 (0.0)	3 (11.5)	0 (0.0)	0 (0.0)	0 (0.0)
18–59	102 (61.1)	0 (0.0)	0 (0.0)	0 (0.0)	69 (92.0)	15 (88.2)	18 (100.0)
≥60	8 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	6 (8.0)	2 (11.8)	0 (0.0)
Sex							
Female	97 (58.1)	5 (19.2)	1 (20.0)	9 (34.6)	55 (73.3)	14 (82.4)	13 (72.2)
Male	70 (41.9)	21 (80.8)	4 (80.0)	17 (65.4)	20 (26.7)	3 (17.6)	5 (27.8)
Smoking history							
Never smoked	116 (69.5)	18 (69.2)	4 (80.0)	22 (84.6)	56 (74.7)	9 (52.9)	7 (38.9)
Passive smoker	20 (12.0)	8 (30.8)	1 (20.0)	4 (15.4)	3 (4.0)	1 (5.9)	3 (16.7)
Current smoker	15 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (9.3)	5 (29.4)	3 (16.7)
Former smoker	16 (9.6)	0 (0.0)	0 (0.0)	0 (0.0)	9 (12.0)	2 (11.8)	5 (27.8)
Coinfection	13 (7.8)	6 (23.1)	1 (20.0)	5 (19.2)	1 (1.3)	0 (0.0)	0 (0.0)
Influenza	4 (2.4)	1 (3.8)	0 (0.0)	2 (7.7)	1 (1.3)	0 (0.0)	0 (0.0)
Coronavirus	1 (0.6)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Parainfluenza virus	4 (2.4)	2 (7.7)	1 (20.0)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Respiratory syncytial virus	1 (0.6)	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Adenovirus	2 (1.2)	2 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bordetella pertussis	1 (0.6)	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Metapneumovirus	1 (0.6)	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Mycoplasma pneumoniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Comorbidities							
Asthma	20 (12.0)	1 (3.8)	0 (0.0)	2 (7.7)	12 (16.0)	0 (0.0)	5 (27.8)
Cardiovascular disorders	22 (13.2)	1 (3.8)	1 (20.0)	3 (11.5)	12 (16.0)	3 (17.6)	2 (11.1)
Congenital syndromes	9 (5.4)	5 (19.2)	2 (40.0)	2 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)
Received flu vaccine	13 (7.8)	1 (3.8)	0 (0.0)	3 (11.5)	6 (8.0)	0 (0.0)	3 (16.7)
Received pneumonia vaccine	50 (29.9)	18 (69.2)	4 (80.0)	12 (46.2)	10 (13.3)	1 (5.9)	5 (27.8)
Current medications							

Table 2. Continued

Characteristic	Overall	Children			Adults		
		A	B	C	A	B	C
Antibiotics	78 (46.7)	18 (69.2)	4 (80.0)	18 (69.2)	24 (32.0)	2 (11.8)	12 (66.7)
Antihistamines	35 (21.0)	3 (11.5)	0 (0.0)	3 (11.5)	17 (22.7)	5 (29.4)	7 (38.9)
Antipyretics	40 (24.0)	3 (11.5)	4 (80.0)	9 (34.6)	17 (22.7)	2 (11.8)	5 (27.8)
Systemic steroids	44 (26.3)	10 (38.5)	1 (20.0)	7 (26.9)	18 (24.0)	2 (11.8)	6 (33.3)
Death	4 (2.4)	2 (7.7)	0 (0.0)	0 (0.0)	2 (2.7)	0 (0.0)	0 (0.0)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ICU, intensive care unit; IQR, interquartile range; SD, standard deviation.

children with HRV-B, 4 (80%) were in the ICU and 1 (20%) was in the emergency room. Fifty percent of the 26 children with HRV-C were in the ICU; only 1 (3.8%) was in the emergency room, 8 (30.8%) were hospitalized (non-ICU), and 4 (15.4%) were outpatient. Of the 75 adults with HRV-A, most were outpatient (70.7%); 2 (2.7%) each were in the emergency room and in the ICU, and 18 (24%) were hospitalized (non-ICU). Similarly, 16 of the 17 (94.1%) adults with HRV-B were outpatient and 1 (5.9%) was hospitalized. Finally, of the 18 adults with HRV-C, 11 (61.1%) were outpatient, 5 (27.8%) were hospitalized, and 2 (11.1%) were in the emergency room. Hospitalization status overall was significantly different between the 167 sequenced participants with HRV-A, HRV-B, and HRV-C and the remaining 1306 participants ($P < .001$) (Supplementary Table 3). Of the 167 sequenced participants with HRV-A, HRV-B, and HRV-C, only 1 adult (HRV-A) had a coinfection, whereas 12 children had a coinfection (6 HRV-A, 1 HRV-B, and 5 HRV-C). Significantly fewer participants who were sequenced and HRV-A, HRV-B, and HRV-C had a coinfection compared to others (7.8% and 22.1%, respectively; $P < .001$). There was a significant difference in the distribution of enrollment year between the sequenced participants with HRV-A, HRV-B, and HRV-C and others; specifically, there were fewer sequenced subjects enrolled in 2014. There were no other differences between the group of participants that was sequenced and HRV-A, HRV-B, and HRV-C and all others in the cohort (Supplementary Table 3). Of the sequenced participants, 30.6% had comorbidities; 12% had asthma, 22% had cardiovascular diseases, and 5.4% had congenital syndromes (Supplementary Table 2). There were no statistical differences between HRV types for any of the comorbidities (data not shown). Figure 1 presents the odds ratios and 95% confidence intervals for the associations of each clinical feature and the HRV types from the univariate logistic regression model. For laboratory variables, the odds ratio reflects the effect of an increase of 1 standard deviation. Supplementary Tables 4 and 5 show the full data of the laboratory values of the participants with HRV infection by age, as well as the laboratory values for the participants who were sequenced according to the viral type.

The Figure 1 shows that the odds of HRV-A are higher than HRV-B in the presence of muscle ache, headache, and increases in body mass index and age. The odds of HRV-B are higher than HRV-A in participants who are current smokers (compared to those who have never smoked), and higher than HRV-C when displaying muscle ache, headache, and higher oxygen saturation. The odds of HRV-C are higher than both HRV-A and HRV-B when antibiotics are used and when shortness of breath is present. The odds of HRV-C are higher than HRV-A for participants in the ICU (compared to outpatient) and in the presence of increases in platelets, lymphocytes, white blood cells (WBCs), and lactate dehydrogenase (LDH).
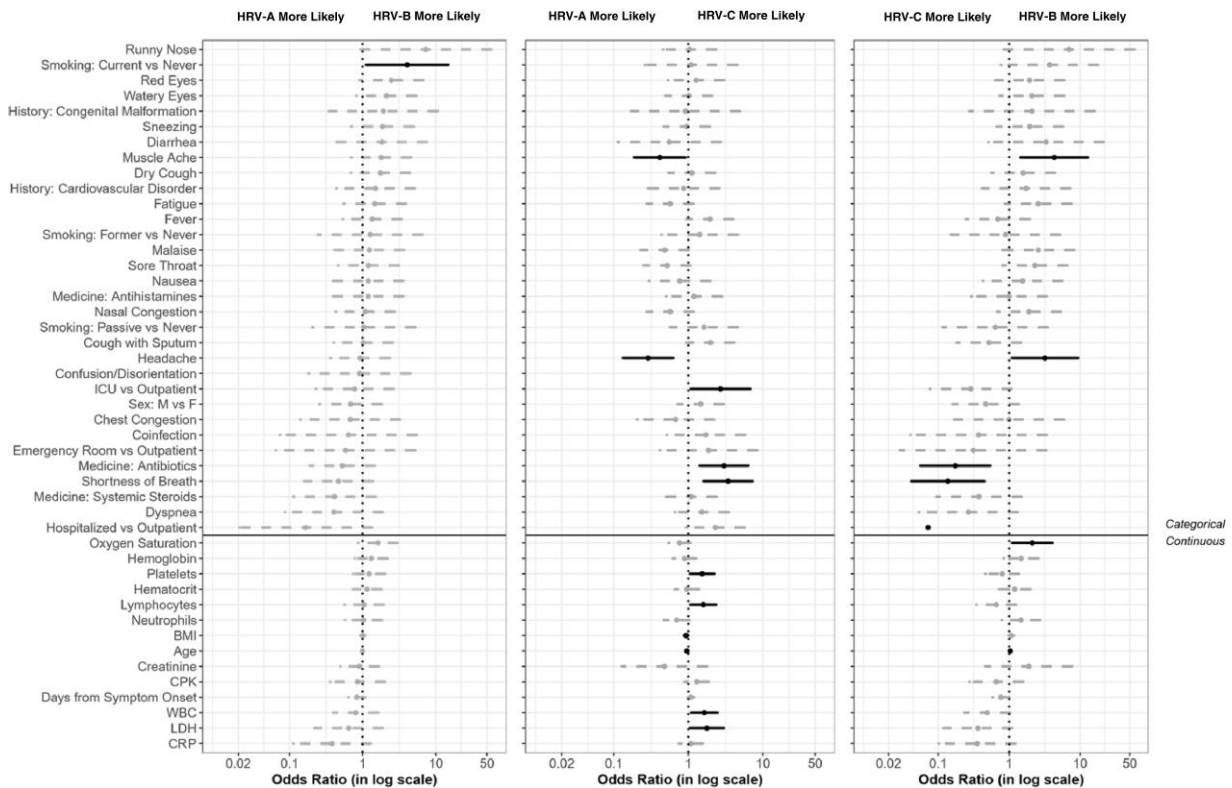
Figure 1. Odds ratios and associated 95% confidence intervals for the rhinovirus types for each characteristic in a univariate model. Characteristics below the solid black line are laboratory parameters, body mass index, and age. Abbreviations: BMI, body mass index; CPK, creatine phosphokinase; CRP, C-reactive protein; F, female; HRV, human rhinovirus; ICU, intensive care unit; LDH, lactate dehydrogenase; M, male; WBC, white blood cell.

The odds of HRV-C are higher than HRV-B for hospitalized (non-ICU) participants.

Of the 167 subjects with positive HRV tests who were sequenced, 94 (56.3%) had viral load information available. There were no significant differences in viral load between the HRV types or between hospitalization statuses (Figure 2).

Figure 3 presents the phylogenetic analysis of the sequenced samples. Phylogenetic analysis shows a high diversity of sequences in circulation among Mexican HRV species, and we could not find any relationship between the clades and seasonality or outbreaks.

DISCUSSION

Our study analyzed data from ILI002, where participants with ILI/SARI were prospectively included for 4 years. We identified enterovirus/HRV infection in 26% of the ILI002 participants (1473/5662) between 2010 and 2014, the most common pathogen isolated overall. Of these, 988 (67.1%) were adults and 485 (32.9%) were children. Sequencing and HRV species determination was performed in 167 samples.

The prevalence of enterovirus/HRV found in our study is similar to what has been previously observed. García et al

studied a large sample of children and young adults in several Central and South American countries. They reported that 16% of ILI cases were positive for HRV [23].

Clinically, HRV is characterized by symptoms compatible with ILI, and it can cause more severe disease, including pneumonia and bronchiolitis [24]. In Mexico, there is only 1 study of HRV in 306 children, characterizing HRV as an important cause of lower and upper respiratory tract infection [1]. In our study, the most affected group of children was those ≤5 years old (79%). Of note, 64.5% of the children who tested positive for HRV were either in the emergency room, hospitalized (non-ICU), or required ICU support. Only 28.2% had coinfection with another virus, supporting the hypothesis of the pathogenic role of HRV.

Worldwide, little has been described regarding HRV infection and severe disease in adults. Recently, Chiu et al found that 21.5% of adult patients with respiratory tract infections who visited Taiwan hospitals were positive for HRV [25]. In our study, most adults with HRV were outpatients, although 23.5% required hospitalization and 1 required the ICU. Golke et al found that chronic pulmonary conditions such as asthma and chronic obstructive pulmonary disease were comorbidities for HRV infection in both inpatient and outpatient adults [26]. In more critical patients, Fica et al found that chronic
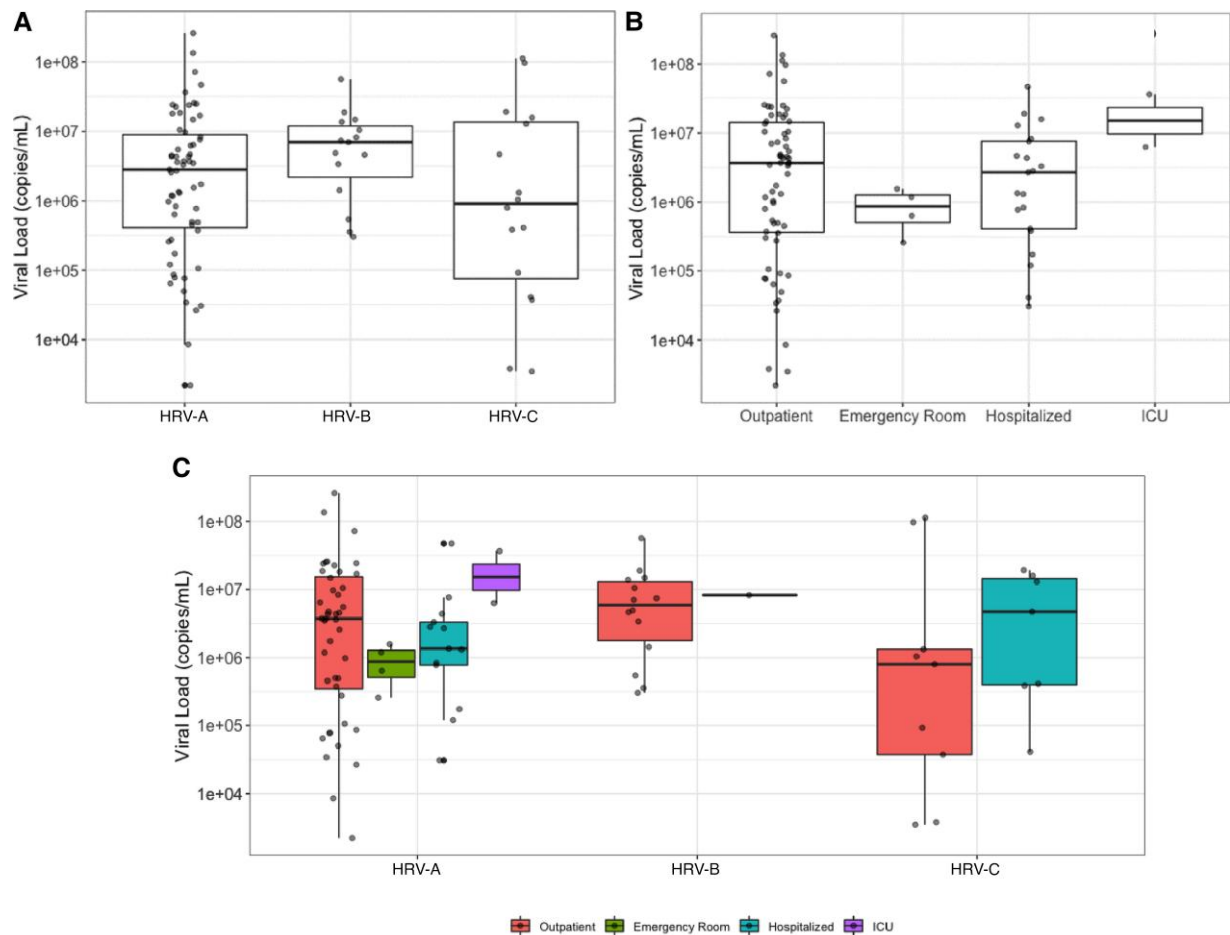
Figure 2. Viral load (copies/mL) by human rhinovirus (HRV) type and hospitalization status at baseline. No statistically significant differences associated with HRV type ($P = .243$) (A) or hospitalization status ($P = .318$) (B). P values are from a Kruskal-Wallis test for comparison between groups. Abbreviations: HRV, human rhinovirus; ICU, intensive care unit.

pulmonary conditions and heart disease were the most common comorbidities for HRV-positive subjects with SARI, along with diabetes mellitus and some neurologic diseases [27]. In our study, the most common comorbidities among adult HRV-positive cases were asthma (12.1%) and cardiovascular disorders (13.1%).

It has been reported that HRV-A and HRV-C may be associated with more severe disease compared with HRV-B in children [4]. More recently, it has been described that HRV-C was more frequently isolated in children aged <2 years [28]. In our cohort, from the genotyped samples, the most common species among children <5 years old were HRV-A and HRV-C.

Some studies have yielded divergent results regarding HRV severity [14, 26, 29]. This may be influenced by the nature of the study (prospective vs retrospective) or population type (children vs adults). For example, McCulloch et al did not find an association between hospitalized adults and genotype [29]. We assessed whether there was a univariate association

between symptoms, disease severity, and HRV species among the whole cohort (children and adults). We found that the odds of laboratory abnormalities (increases in platelets, lymphocytes, WBC, and LDH) increased with HRV-C infection when compared with HRV-A. We also found that HRV-C was associated with increased odds of being in the ICU. Interestingly, the odds of having shortness of breath were higher in subjects with HRV-C, compared to subjects with either HRV-A or HRV-B. The odds of muscle ache and headache were higher in subjects infected with HRV-A and HRV-B compared to HRV-C. These results support the hypothesis that HRV-C is associated with a more severe disease. Of note, there is a higher rate of ICU admissions in the sequenced group (21%) compared to all other participants (5.1%). This suggests that the sequenced subgroup represents a sicker group of patients (Supplementary Table 3).

Overall, we found a high frequency of antibiotic use in the treatment of HRV infection (41.2% of infected adults and 54% of children). Antibiotic use was more common in patients
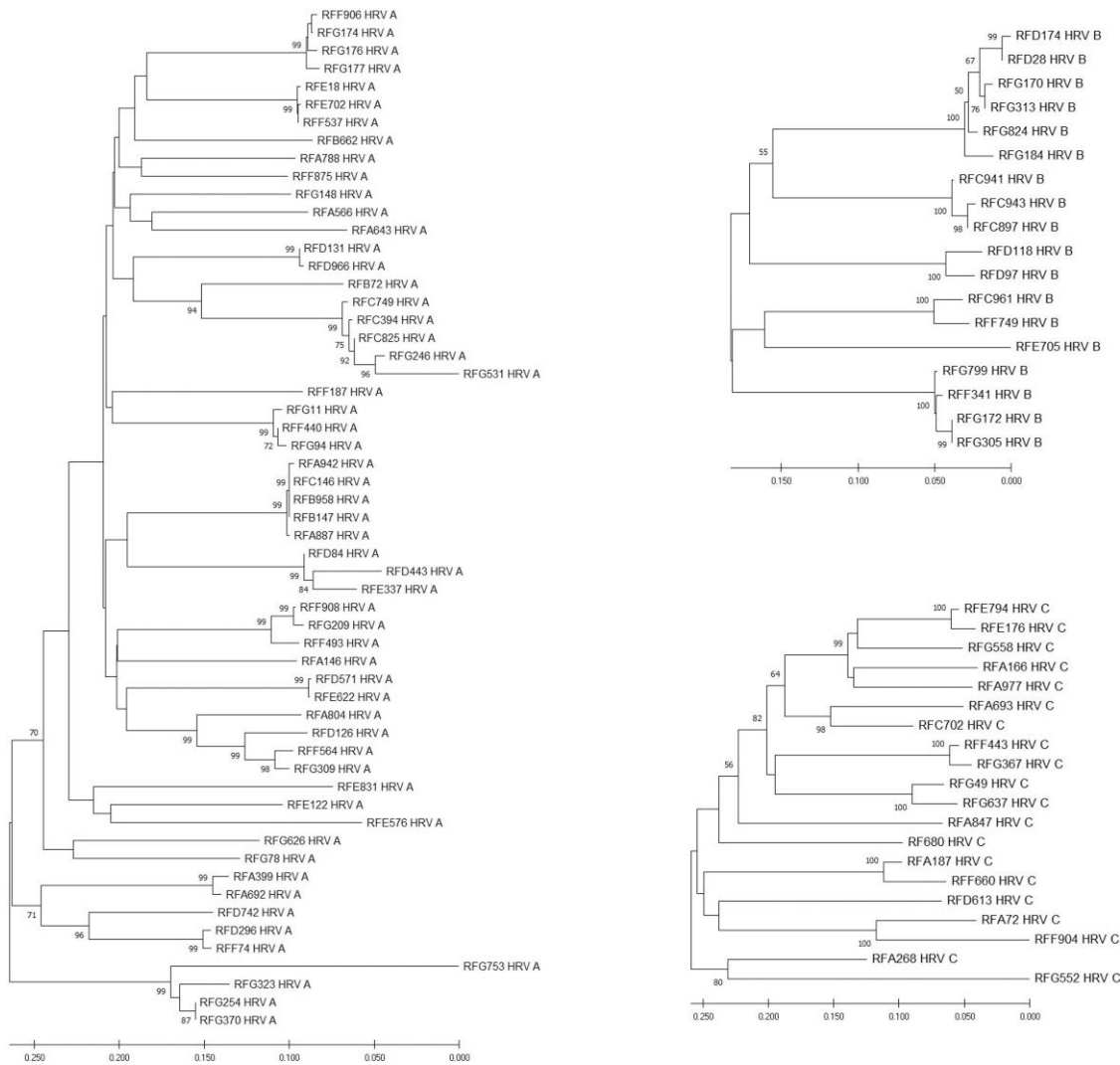
Figure 3. Phylogenetic analysis of human rhinovirus (HRV). The 558-bp fragment VP4/VP2 genomic region was analyzed by MEGA version 6 software. Associations between samples were done by the neighbor-joining method with bootstrap analysis of 1000 replicates for each HRV A, B, and C type.

with HRV-C compared to those with HRV-B. The overuse of antibiotics is a major health problem worldwide and exposes patients to potential harm for no benefit. The use of antibiotics in viral infections have been also reported by other authors: Li et al reported a 26.8% of antibiotic use in hospitalized patients with SARI [30], and Fica et al reported 90% antibiotic use in hospitalized adult patients with HRV infection [27].

We did not find any associations between viral load and hospitalization status nor viral load and HRV species, suggesting that there could be a pathogenic mechanism intrinsic to HRV-C, or host-response differences, that cause more severe disease and are not related to viral load. This hypothesis is supported by evidence that HRVs modulate and induce different immune responses [31–33], and it has been demonstrated that HRV-C can effectively evade and suppress the innate immune response [34].

This study is the first to describe the clinical and molecular features of HRV infection in the Mexican population, including both children and adults. The data were collected in a prospective manner, using previously standardized tools. Limitations of this study include the observational nature of the study and that most bacterial infections were not evaluated. In addition, RespiFinder multiplex PCR only detects enterovirus/HRV infection; however, after sequencing the available samples, HRV was confirmed as the causative agent.

Although our study took place from 2010 to 2014, the topic is still of interest because the role of HRVs as a cause of lower respiratory tract infections has been underestimated [35]. Moreover, recent data demonstrate the importance of studying and performing HRV epidemiological surveillance [35, 36]. It has been proposed that HRV delayed the development of the

2009 influenza A/H1N1 pandemic through viral interference, and interestingly during the coronavirus disease 2019 pandemic, the prevalence of most respiratory pathogens was unusually low. However, HRV remained the main virus co-circulating with SARS-CoV-2 [36].

This hospital-based study included patients seeking medical care by ILI/SARI, and this potentially would select for patients with more comorbidities and potentially more serious illness than the entire population. However, these results still show how HRV may cause severe disease in children and adults and that, in particular, HRV-C infection may cause worse outcomes.

In conclusion, this study highlights the frequency and importance of HRV as a cause of ILI/SARI and reflects the need to improve surveillance and testing for HRV. Testing may also favor appropriate use of antibiotics. Species identification will help to improve the understanding of the wide range of disease severity caused by different HRV species and subtypes and risk factors for outcomes in various types of patients.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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