Nosocomial Transmission and Genetic Diversity of Rhinovirus in a Neonatal Intensive Care Unit

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Rhinoviruses were detected as sole pathogens in 6 preterm infants who developed severe respiratory infections while hospitalized in a neonatal intensive care unit. We confirmed 2 nosocomial rhinovirus transmission episodes and describe the genetic diversity of rhinovirus strains that circulated simultaneously during a winter season. *(J Pediatr 2017;■■:■■-■■).*

Rhinoviruses are among the most frequent causes of upper respiratory tract infections. However, they also are associated with lower respiratory tract infections, including bronchiolitis,[1] pneumonia, asthma exacerbation,[2] and brief self-resolving unexpected events in infants.[3]

According to molecular methods, rhinovirus has been classified into 3 species within the genus *Enterovirus* of the *Picornaviridae* family, with more than 150 genotypes and as well as provisionally assigned types.[4]

Rhinoviruses are the most prevalent respiratory virus infections affecting preterm infants hospitalized in the neonatal intensive care unit (NICU).[5-7] However, few data exist about nosocomial rhinovirus transmission in these high-risk patients and genotyping analyses are scarce.[8,9]

We describe rhinovirus infections in 6 preterm infants hospitalized in a single NICU during a winter season. To detect potential nosocomial transmission, genotyping was performed on detected rhinoviruses from neonates as well as on circulating strains in the community.

Case Presentation

The neonatal unit at the Centro de Educación Médica e Investigaciones Clínicas University Hospital in Buenos Aires, Argentina, has a level III NICU equipped with 4 separate rooms with 17 incubators and cradles. An acute respiratory infection (ARI) is defined as the presence of rhinorrhea and/or cough with or without fever, which may lead to lethargy and poor feeding. All infants hospitalized in NICU who develop ARI are screened for respiratory viral and bacterial infections.

Nasopharyngeal aspirates for viral detection were obtained in viral transport media at symptom onset and once a week during the course of the disease. Samples were sent to the virology laboratory. For rhinovirus detection, total nucleic acids were extracted using MagnaPure Compact Kit Isolation (Roche, Mannheim, Germany), followed by a real-time reverse transcription polymerase chain reaction (PCR) that amplifies 207 nucleotides in the 5' noncoding region.[10] Other respiratory viruses, including respiratory syncytial virus, influenza A and B, adenovirus, and parainfluenza virus 1-3, were studied for antigen detection by immunofluorescence with monoclonal antibodies (Chemicon-Millipore, Temecula, CA). Routine blood and urine bacterial cultures were obtained from infants with suspicion of infection.

Rhinovirus genotyping was performed by using a reverse transcription PCR that amplifies the 5' noncoding region/VP4/VP2 partial region.[11] PCR products were purified and direct-sequenced (Macrogen, Seoul, Korea); partial rhinovirus sequences from 420 nt of the VP4/VP2 region were submitted to GenBank (accession number: KY288641-KY288653). Genotypes were assigned according to their clustering with reference strains.

During the winter season starting in June 2014, 6 preterm infants hospitalized in the NICU had rhinovirus detected during clinical acute respiratory illness and were included in this study. Empiric antibiotic treatment was started in all patients until etiologic diagnosis was made. These patients were negative for all other respiratory viruses tested and bacteria.

Five infants were male, 2 were extremely preterm (gestational age <28 weeks), 3 were very preterm (28-31 weeks), and 1 was late preterm (32-36 weeks) (**Table**). Median age at respiratory symptoms onset was 42 days (range 8-62). The most frequent underlying condition was bronchopulmonary dysplasia, followed by patent ductus arteriosus and infant respiratory distress syndrome. All infants had signs and symptoms of lower respiratory tract infection, and 4 required oxygen supplementation.

Temporal sequence of symptoms onset, rhinovirus diagnosis and genotype, oxygen-therapy requirement, and hospital discharge of the 6 newborns are shown in **Figure 1**. Rhinovirus shedding ranged from 10 to 28 days. Four different

ARI	Acute respiratory infection
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction

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Table. Demographic and clinical characteristics of 6 preterm infants with rhinovirus infection hospitalized in the NICU

Pts/sex	GA (wk)	BW (g)	Comorbidities	Age at symptoms onset (d)	Clinical signs	LOS (d)	Oxygen requirement during rhinovirus infection (d)	Rhinovirus genotype
1/M	27 + 1	1100	BPD, IRDS , PDA, twin 2	61	Rhinorrhea, cough, tachypnea, wheezing, retraction	90	nO_2:19	C43
2/M	27 + 1	759	BPD, IRDS, PDA, twin 1	62	Rhinorrhea, tachypnea, wheezing, retraction	124	MV: 18;CPAP:4;nO_2: 40	C1
3/M	30 + 2	1465	BPD, IRDS, PDA	40	Rhinorrhea, tachypnea, retraction	85	nO_2: 14	C1
4/F	29 + 0	1250	Sepsis	43	Tachypnea, retraction	57	No	A63-like
5/M	36 + 3	1920	Genetic disease (trisomy 18)	8	Tachypnea, wheezing, retraction, apnea	45	nO_2: 29	A63-like
6/M	28 + 5	1210	IRDS, PDA	30	Rhinorrea, tachypnea	66	No	C6

BPD, bronchopulmonary dysplasia; *BW*, birth weight; *CPAP*, continuous positive airway pressure; *F*, female; *GA*, gestational age; *IRDS*, infant respiratory distress syndrome; *LOS*, length of stay; *M*, male; *MV*, mechanical ventilation; *nO_2*, nasal cannula for oxygen supplementation; *PDA*, patent ductus arteriosus; *Pts*, patients; *RV*, rhinovirus.

genotypes were detected (**Figure 2**; available at www.jpeds.com). Three rhinovirus were species C (C43, C1, and C6) and only 1 was species A (A63-like). After discharge, infants were not systematically followed up for viral detection, unless they came to attention with a new episode of ARI. New respiratory infections occurred in 2 infants and were associated with different rhinovirus genotypes species A (A75 and A103).

The first 3 cases were housed in the same room in the NICU. They developed ARI and worsening of their respiratory condition, requiring oxygen supplementation. Rhinovirus was the only pathogen detected.

The first patient was positive for rhinovirus genotype C43 for 21 days. Simultaneously, patient 2 (patient 1 twin brother) was positive for rhinovirus genotype C1. He remained positive for 28 days. He first required oxygen supplementation by nasal cannula, but then required mechanical ventilation, followed by continuous positive airway pressure. When first hospitalized, patient 3 was in a cradle near patient 2, and was positive for rhinovirus with the same genotype C1. Patient 3 had a milder ARI and was virus negative 10 days later. The presence of the same genotype in 2 newborns whose cradles were close together suggests nosocomial transmission.

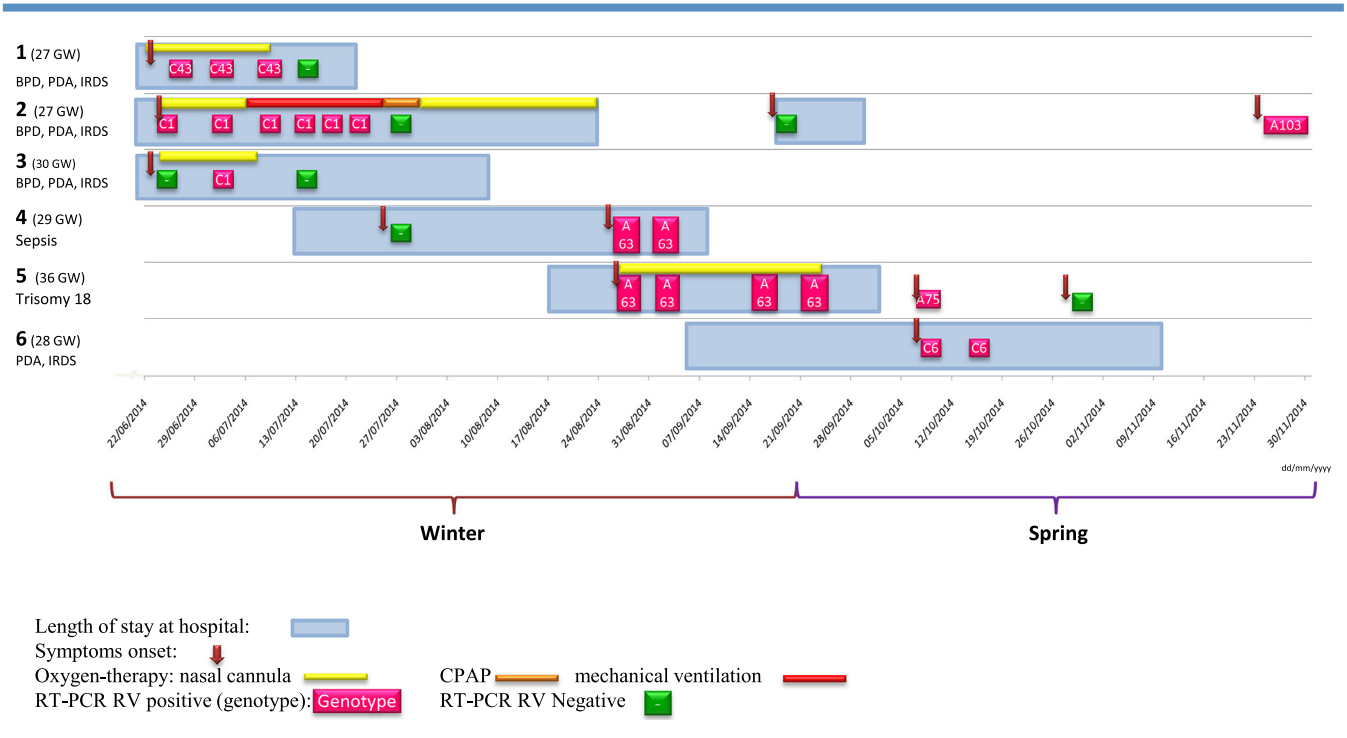

Figure 1. Sequence of virus detection and clinical events in 6 preterm infants with rhinovirus infection in the NICU. June-November 2014. *GW*, gestational week; *RT*, reverse transcription.

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One month later, another pair of infants housed near each other in the same room developed respiratory symptoms and were positive for rhinovirus genotype A63-like. The nucleotide divergence analysis of these strains using the VP4/VP2 coding region suggested the possibility of a new rhinovirus-A genotype. Further confirmation will use the VP1 coding region.[4] Five days after patient 5 was discharged, another infant (patient 6) developed ARI. A different rhinovirus species was detected (rhinovirus-C6).

Respiratory and contact isolation was indicated in all cases, until patients became rhinovirus negative or clinical improvement was evident. However, because there were no private rooms available for isolation, infants were placed in incubators.

After long lengths of stay (median: 76.5 days), all infants were discharged. However, patient 2, who had the worst clinical condition, required oxygen support at home.

At home, 2 patients developed new respiratory tract infections. Patient 2 developed bronchiolitis 1 month after discharge, requiring a short hospitalization; parainfluenza virus was detected. Two months later he developed another ARI; rhinovirus-A103 was detected. Patient 5 developed respiratory symptoms 4 days after discharge and was evaluated in the emergency department. Rhinovirus genotype A75, different from A63-like detected during his hospital stay, was detected.

Discussion

During the 2014 winter season, rhinovirus was detected as the sole pathogen in 6 preterm newborns who developed ARI while hospitalized in the NICU at the Centro de Educación Médica e Investigaciones Clínicas University Hospital, Argentina. Four infants required oxygen supplementation, 1 of whom required mechanical ventilation, followed by continuous positive airway pressure. After prolonged hospital stays, all patients were discharged, but 1 patient required oxygen support at home.

When the initial 3 rhinovirus cases were documented, phylogenetic analyses were performed to determine a potential outbreak at NICU. Genotyping allowed us to confirmed rhinovirus nosocomial transmission in 2 different episodes but also the absence of nosocomial transmission in other patients during the same study period. Without genotyping it might have been assumed that all cases were infected with the same rhinovirus genotype and nosocomially acquired. In addition, genotyping confirmed new rhinovirus infections because of different genotypes following hospital discharge.

The source of these nosocomial infections could not be determined. However, 1 nurse and 1 parent had mild respiratory symptoms during patients' symptom onset: they were not screened for rhinovirus. The identification of multiple rhinovirus genotypes in the NICU lead to emphasis on infection control measures, such as strict adherence to hand hygiene, decontamination and use of personal protective equipment, and restricted visitation of children and adults with respiratory symptoms to the NICU. These measures are especially important because rhinovirus can remain highly viable (from hours to days) in an indoor environment at ambient temperature, and transmission can occur via direct contact and aerosol.[12]

In our patients, rhinovirus shedding ranged from 10 to 28 days after symptoms onset. This finding is consistent with reports that detected rhinovirus shedding from 5 to 44 days in preterm infants[5] and for 30 days in infants without underlying conditions.[13]

In our study, most of the patients were infected with rhinovirus species C, including the most severe case, which required mechanical ventilation. Rhinovirus species C has been associated with higher acuity of illness. This is the main species in hospitalized infants with brief self-resolving unexpected events associated with ARI,[3] and in preterm newborns with ARI that require oxygen therapy.[9] In addition, rhinovirus-C caused a prolonged infection with severe pneumonia in a 3-week-old neonate with no comorbidities.[14] These data suggest that in this high-risk population, rhinovirus species C may be associated with greater severity of illness than species A or B.

The simultaneous detection of different rhinovirus genotypes highlights the genetic diversity of rhinovirus strains in NICU. Moreover, phylogenetic analysis permitted confirmation of reinfection with a different rhinovirus genotype rather than persistent rhinovirus shedding. Multiple rhinovirus respiratory infections because of different genotypes is common in children[15] because rhinovirus infections fail to provide protective immunity against other genotypes because of a low cross-neutralization between serotypes.[12]

The full extent of rhinovirus transmission in the NICU was unknown because asymptomatic infants were not screened in this study. ■

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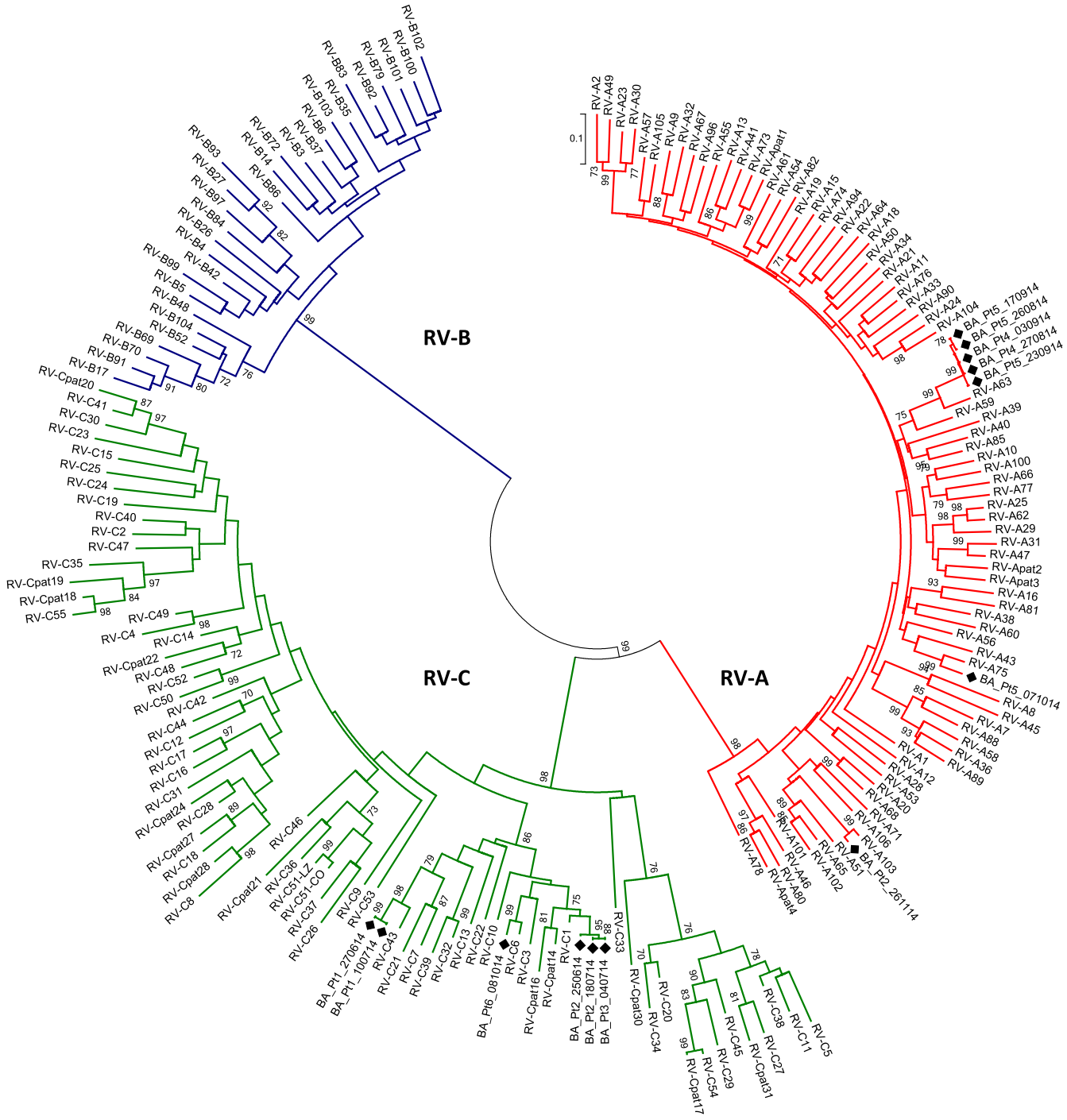

Figure 2. Phylogenetic analysis of VP4/VP2 coding region of Buenos Aires rhinovirus strains (♦) identified in preterm newborns hospitalized in NICU from June to November 2014 in Argentina. Study sequences were named Buenos Aires (BA) followed by the patient identification number and the sample collection date (ddmmyy). Reference sequences of all rhinovirus genotypes and provisionally assigned types of species A, B, and C[4] were downloaded from GenBank. Phylogenetic tree was constructed by neighbor-joining (MEGA 6.06) using the nucleotide substitution model selected in jModelTest. Branch support was assessed by bootstrap (1000 replicates); significant values higher than 70% are shown. Branch distance is indicated by the scale bar.

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