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.

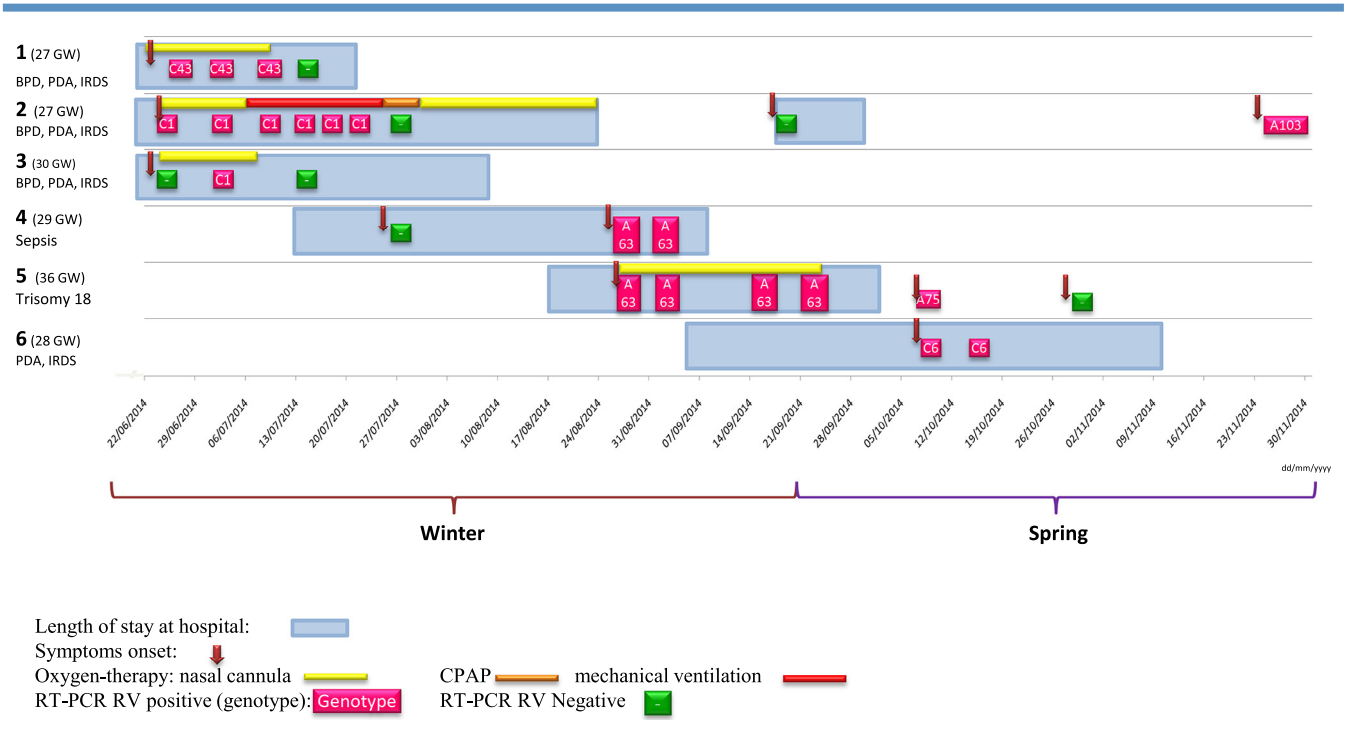

Figure 1. Sequence of virus detection and clinical events in 6 preterm infants with rhinovirus infection in the NICU. June-November 2014. *GW*, gestational week; *RT*, reverse transcription.

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