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Picornavirus, the Most Common Respiratory Virus Causing Infection among Patients of All Ages Hospitalized with Acute Respiratory Illness

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We evaluated the prevalence of respiratory virus infection (RVI) in 403 illnesses of 364 persons hospitalized over a 2-year period with acute respiratory conditions using virus-specific reverse transcription-PCR (RT-PCR) assays in addition to cell culture and serology. RVIs were identified in >75% of children under 5 years of age and 25 to 37% of adults. The molecular assays doubled the number of infections identified; picornaviruses were the most frequent in patients of all ages, followed by respiratory syncytial virus and influenza viruses.

Lower respiratory tract infections are the leading cause of infectious disease-related hospitalization in the United States (13). We previously determined the frequency of specific virus infections associated with acute respiratory tract conditions leading to hospitalizations (5). Patients enrolled in the study were admitted to a public or private hospital, and respiratory virus infections were identified by cell culture and serologic studies. Because the number of respiratory virus infections associated with respiratory illnesses can be increased by the application of reverse transcription-PCR (RT-PCR) assays (3, 6), we applied these assays to specimens collected and set aside for this purpose over a 2-year period to determine whether additional respiratory virus infections could be identified in this cohort.

The details of the clinical study have been described previously (5). Briefly, study participants were persons of all ages who were hospitalized at Ben Taub General Hospital or St. Luke's Episcopal Hospital in Houston, TX, with an acute respiratory condition (pneumonia, tracheobronchitis, bronchiolitis, croup, exacerbations of asthma or chronic obstructive pulmonary disease [COPD], or congestive heart failure). Respiratory samples (nasal wash or throat swab) and serum samples were collected within a median of 1 day of hospitalization. This substudy was conducted on participants enrolled from 1 September 1993 to the end of the study in May 1995. The respiratory sample was mixed with veal infusion broth as a stabilizer at the time of collection, transported to the laboratory on wet ice, and inoculated onto cell culture. The remaining sample was aliquoted and frozen at <-70°C until tested using molecular assays (described below). In general, samples underwent one or two freeze-thaws to generate cDNA for initial testing. A convalescent-phase serum sample was obtained 14 to 42 days later. All subjects provided informed consent under a protocol approved by the Baylor College of Medicine Institutional Review Board.

The methods used for cell culture and serology were described previously (5). The cell culture assays were tested for type A and B influenza viruses, parainfluenza virus (PIV) types 1 to 3, respiratory syncytial virus (RSV), rhinoviruses, enteroviruses, herpesviruses, and adenoviruses. Serological assays were performed as described previously (5) on paired serum samples for influenza A and B viruses, PIV types 1 to 3, RSV, and coronaviruses 229E and OC43.

RT-PCR assays were performed using previously described real-time RT-PCR assays for influenza A and B viruses and coronaviruses OC43 and 229E (3). PIV types 1 to 3 (PIV1, PIV2, and PIV3, respectively), RSV, human metapneumovirus (HMPV), and picornavirus infections were identified using previously described RT-PCR assays followed by Southern blot hybridization (3). Picornavirus-positive samples were further classified using rhinovirus- and enterovirus-specific real-time RT-PCR assays when sufficient sample remained for these assays (8, 11).

A total of 403 separate illnesses occurred during the 2-year study period in 364 persons. Seventy-six respiratory viruses (excluding herpes simplex virus [HSV] and cytomegalovirus [CMV]) were isolated from 75 illness specimens collected during the study period. These included 26 picornaviruses, 21 RSV, 19 influenza viruses, 4 adenoviruses, and 2 each of PIV1, PIV2, and PIV3 (Table 1). Thirty illnesses (of 136 paired sera tested) also had 33 infections identified by serology, 11 of which were identified by culture (6 influenza A subtype H3N2 [A/H3N2], 4 RSV, and 1 influenza B). Additional infections identified by serology but not by culture included seven A/H3N2, three each of RSV, PIV2, PIV3, and OC43, and one each of A/H1N1, PIV1, and 229E.

Respiratory samples from 386 illnesses (95.8%) were available for molecular testing. One hundred seventy-two (44.6%) samples were positive for at least one of the viruses assayed (Table 1), with a picornavirus ($n = 99$) being the most common infection identified. All viruses for which RT-PCR assays were performed were detected except PIV2; no PIV2 infections were identified by RT-PCR, while two infections were found using cell culture. Overall, 200 (49.6%) of the 403 illnesses were associated with at least one

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TABLE 1 Infections identified by culture or RT-PCR in a cohort of 403 illnesses evaluated between September 1993 and June 1995

Virus[a]	Total no. of infections	No. of infections that were:		
		PCR positive		PCR negative or ND[b]
		Culture positive	Culture negative	Culture positive
Picornaviruses				
Enterovirus	5	3	2	0
Rhinovirus	49	14	29	6
Unclassified	54	0	51	3
Paramyxoviruses				
PIV1	3	2	1	0
PIV2	2	0	0	2
PIV3	6	2	4	0
RSV	36	16	15	5
HMPV	12		12	0
Orthomyxoviruses				
Influenza A	21	8	5	8
Influenza B	3	1	0	2
Coronaviruses				
OC43	5		5	0
229E	2	0	2	0
Adenoviruses*	4			4
Herpesviruses				
HSV*	13			13
CMV*	3			3

[a] PIV, parainfluenza virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; HSV, herpes simplex virus; CMV, cytomegalovirus. *, PCR not done. Culture systems were not available for OC43 or HMPV at the time of the original study. Specimens positive for HMPV by RT-PCR were subsequently tested by cell culture and 1 was positive.
[b] ND, PCR not done.

virus infection. More than one virus infection was identified in 33 illnesses (31 with two infections and 2 with three infections). Twenty-two of the multiple infections included a picornavirus, with a picornavirus and RSV being the most common combination ($n = 10$); dual infection with RSV and influenza A virus was detected in 3 illnesses. Another respiratory virus was identified in all three illnesses from which CMV was isolated and in 6 of the 11 illnesses from which HSV was isolated.

A viral infection was identified in 77.6% of the children under 5 years of age (Table 2). Older children had an infection identified in more than 50% of their illnesses, and adults had an infection identified in approximately one-third of their illnesses. Multiple infections were identified most frequently in children less than 1 year of age.

The application of molecular methods to the diagnosis of respiratory viral infection has increased the frequency with which respiratory virus infection is identified in patients with exacerbations of asthma (1, 6), chronic obstructive pulmonary disease (3, 12), and bronchiolitis (4, 10) and in specimens submitted for general respiratory viral diagnostic studies (2, 9). We previously performed an epidemiological study examining the impact of respiratory virus infection on a community cohort using traditional (culture and serology) viral diagnostic methods. Application of RT-PCR assays to specimens collected in that study increased the number of viral infections identified by approximately 2-fold because of the increased sensitivity of the assay for some viruses (e.g., picornaviruses) compared to that of a cell culture and because of the ability to identify other viruses that are poorly or not cultivable (e.g., HMPV and coronavirus).

The family of viruses with the greatest frequency of detection was picornaviruses (enterovirus and rhinovirus species), being identified in approximately 25% of illness episodes. Because the rhinovirus- and enterovirus-specific primers used were not validated against all species of rhinovirus and enterovirus, it is possi-

TABLE 2 Distribution of respiratory virus infections by age group (combined results of culture, PCR, and serology)

Virus[a]	No. of infections in each age group (yrs) (*n*)						
	<1 (55)	1–4 (57)	5–17 (50)	18–44 (65)	45–64 (114)	≥65 (62)	All ages (403)
Picornaviruses							
Enterovirus	3	0	2	0	0	0	5
Rhinovirus	15	6	8	8	9	3	49
Unclassified	13	11	17	6	5	2	54
Paramyxoviruses							
PIV1	0	3	0	0	0	0	3
PIV2	2	3	0	0	0	0	5
PIV3	3	3	0	0	2	1	9
RSV	15	14	5	1	2	2	39
HMPV	2	4	0	2	2	2	12
Orthomyxoviruses							
Influenza A	0	4	2	6	11	3	26
Influenza B	0	2	0	0	0	1	3
Coronaviruses							
OC43	0	0	0	1	6	0	7
229E	0	0	0	0	1	2	3
Adenoviruses	1	1	0	1	1	0	4
Herpesviruses							
HSV	0	0	1	2	6	4	13
CMV	2	0	0	1	0	0	3
Any virus[b]	43	44	31	24	42	16	200
Multiple viruses	11	7	4	4	3	4	33

[a] PIV, parainfluenza virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; HSV, herpes simplex virus; CMV, cytomegalovirus.
[b] This category corresponds to persons with at least one virus.

ble that additional undetected picornavirus infections may have been missed. The increased identification of picornavirus infections using molecular assays is in keeping with findings in other studies of children less than 5 years of age and patients with asthma, COPD, and bronchiolitis (1, 6, 7, 10, 12).

In summary, the use of molecular diagnostic assays increased the frequency of identification of a respiratory viral infection in persons admitted to acute-care hospitals with an acute respiratory condition. In this cohort, more than 75% of respiratory illnesses in children less than 5 years of age were associated with the detection of a respiratory virus, compared to 25 to 37% of illnesses in adults. The prevalence of respiratory viral infections may have been even higher since we did not assay for NL63 or HKU1 coronaviruses or PIV type 4. Picornavirus (principally rhinovirus) infections were the most common infections identified in patients of all ages, followed by those caused by RSV and the influenza viruses. The use of molecular assays increases the frequency of identifying a respiratory viral infection compared to that of the classical viral diagnostic methods of cell culture and serology.

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