Arch Virol (2014) 159:65–72
DOI 10.1007/s00705-013-1794-4

ORIGINAL ARTICLE

Seasonality and prevalence of respiratory pathogens detected by multiplex PCR at a tertiary care medical center

Christine M. Litwin · James G. Bosley

Received: 22 April 2013 / Accepted: 11 June 2013 / Published online: 24 July 2013

Abstract Respiratory tract infections (RTIs) are a leading cause of mortality and morbidity. Seasonality has been reported for many viruses, including influenza virus, respiratory syncytial virus (RSV), and the recently described human metapneumovirus (hMPV). We hypothesize that the availability of rapid, multiplex PCR diagnostics will provide better clinical care and new insights into the etiology and clinical spectrum of RTIs. We conducted a retrospective analysis of the incidence of respiratory pathogens at a 500-bed adult and 154-bed pediatric hospital tertiary care center. A total of 939 specimens from patients with an age range of 5 days to 91 years (median, 2 years) were tested by a multiplex respiratory pathogen PCR from November 14, 2011 to November 13, 2012. Sixty-five percent of specimens were positive for at least one pathogen. As the age of the patient increased, the positivity rate for the PCR decreased proportionately. Rhinoviruses/enteroviruses (Rhino/Entero) were the most prevalent (34.3 %) followed by RSV (19.2 %) and hMPV (6.2 %). Twelve percent of the positive samples were positive for multiple analytes, with Rhino/Entero and RSV being the most common combination. The peak months were September and May for Rhino/Entero infections, January for RSV and February for coronavirus. hMPV peaked 2 months after RSV, as has been observed recently in other studies. Multiplex PCR provides rapid diagnostic information that can be used to make knowledgeable clinical decisions and potentially reduce the use of antibiotics. Active respiratory PCR surveillance could also predict seasonal respiratory epidemics to allow for adequate planning of additional infection control measures.

C. M. Litwin (✉) · J. G. Bosley
Department of Pathology, Medical College of Georgia, Georgia Regents University, 1120 15th St., Augusta, GA 30912, USA
e-mail: clitwin@gru.edu

Introduction

Acute respiratory tract illnesses are the most common illness in all age groups and are an important cause of hospitalization and mortality, especially in the winter months. For children less than 5 years of age, respiratory tract infections are the second leading cause of death [23]. Major causes of bronchiolitis and lower respiratory tract illnesses in children include respiratory syncytial virus (RSV), parainfluenza viruses, influenza virus, and human metapneumovirus (hMPV).

Understanding seasonal variations in these infections may allow optimal planning and utilization of resources in emergency departments, hospitals and clinics. Past epidemiologic studies used routine diagnostic methods such as culture for the detection of viral and bacterial respiratory pathogens [7, 38]. However, viral culture requires 3 to 5 days to detect most agents and detects rhinovirus or coronaviruses poorly or not at all. Multiplex reverse transcriptase PCR has been shown to be more sensitive than standard respiratory virus culture, bacterial culture, and antigen detection methods [5, 8, 18, 27]. Moreover, PCR is much faster. For the recently characterized hMPV, RT-PCR-based techniques are generally the method of choice for detection [35]. No antigen detection methods are commercially available for hMPV.

Expanded multiplex PCR panels now allow the detection of up to 14 different viruses along with influenza A subtyping and the detection of three common bacterial respiratory pathogens, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis* in one hour

[30, 31]. The high sensitivity and the expanded capability of these tests, therefore, may affect our understanding of the epidemiology of respiratory tract infections. This study explores the seasonality and prevalence of respiratory viral pathogens at a tertiary care medical center using the multiplex PCR respiratory pathogen panel.

Materials and methods

Study participants

Testing took place from November 14, 2011 to November 13, 2012 on nasopharyngeal specimens (NPS) originally sent to Georgia Health Sciences University (GHSU; its name was recently changed to Georgia Regents University) clinical microbiology laboratory (Augusta, GA) from the 500-bed adult and 154-bed pediatric hospital at GHSU for respiratory pathogen PCR assay by the FilmArray Respiratory Panel (RP) (BioFire Diagnostics, Inc., Salt Lake City, UT). NPS specimens were obtained from patients with symptoms of a respiratory infection, collected from the patients using standard technique, and placed in viral transport medium (Remel MicroTest M4RT Viral Transport tube). Specimens were tested as soon as possible after collection. The project was approved by the institutional review board of our institution; informed consent for the project was waived. Demographic data, such as initial symptoms, chief complaint, age, gender, and secondary diagnosis were obtained for each specimen tested.

FilmArray RP assay

The FilmArray assay was performed according to the manufacturer's instructions. In brief, 1 mL of purified water included in the kit was injected into the FilmArray pouch to rehydrate the reagents. Then, 300 µl of the viral transport medium that had contained the NPS specimen was mixed with 500 µl of sample buffer and injected into the sample port of the pouch. The pouch was then placed into the FilmArray instrument, and a respiratory PCR panel program was started. The first stage of the program consists of a multiplexed PCR, followed by an individual nested second-stage real-time PCR contained within a microarray chip. The FilmArray RP includes two internal controls: an RNA process control and controls for every step inside the pouch. Results are analyzed using melting curve data.

The organism/viruses detected by the FilmArray included adenovirus, influenza A virus (FluA), influenza B virus (FluB), parainfluenza virus 1 (Para 1), parainfluenza virus 2 (Para 2), parainfluenza virus 3 (Para 3), parainfluenza virus 4 (Para 4), respiratory syncytial virus (RSV), coronavirus 229E (CoronaV 229E), CoronaV NL63, CoronaV HKU1, CoronaV OC43, human metapneumovirus (hMPV), *Bordetella pertussis*, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. Due to genetic similarity between the human rhinoviruses and enteroviruses, a positive result with PCR primers to these viruses was listed as Rhino/Entero. Rhinovirus A, B and C and enterovirus A, B and C are detected in the assay. Both RSV subtypes A and B are detected in the assay, though not specifically assayed.

The FluA viruses could also be subtyped as FluA/H1, FluA/H3 or FluA-2009 if present.

Statistics

A two-sample Student's t-test between proportions was performed to determine whether there was a significant difference between the viruses with respect to the percentage of initial clinical symptoms in both single virus infections and mixed infections [6]. Statistical analysis was performed using the software package StatPac for Windows (Pepin, WI).

Results

Analysis of positivity rates and prevalence within age groups

Specimens from a total of 939 patients were analyzed by PCR. The age range of the patients was 5 days to 91 years of age (median, 2 years). The male:female ratio was 1.42. Rhino- and enteroviruses were the most prevalent (34.3 %), followed by RSV (19.2 %) and hMPV (6.2 %) (Table 1).

The data were divided according to age groups. The largest age group in our study was children $\leq$ 2 years of age, followed by the age group 3-21 years of age (Table 1). The older age groups, 22-49, and $\geq$ 50 years were small compared to the first two groups, with 24 and 52 patients in each group, respectively.

The highest rate of PCR positivity was observed in children $\leq$ 2 years of age, with a rate of 71.6 % (Table 1), followed by 62.5 % for the second-youngest age group, ages 3-21. The positivity rates for the 22-49 and $\geq$ 50 age groups were much lower, at 33.3 % and 21.2 %, respectively.

The prevalence of specific viruses and bacteria also differed between age groups (Table 1). Most viruses and bacteria showed the highest prevalence in children $\leq$ 2, with the exception of Flu A, for which the highest number of infections was in the 3-21 age group (Table 1). Rhino/Entero viruses were the most prevalent viruses in all age groups except in the 22-49 age group, where there were equal numbers of Rhino/Entero cases and Para 3 cases,

Table 1 Prevalence of respiratory pathogens tested in different age groups: November 14, 2011 to November 13, 2012. Results of 939 nasopharyngeal samples analyzed by multiplex real-time PCR in relation to age group

Analyte	≤2 years		3-21 years		22-49 years		≥ 50 years		All ages	
	# Pos	Prevalence (n= 570)	# Pos	Prevalence (n= 293)	#Pos	Prevalence (n= 24)	#Pos	Prevalence (n=52)	#Pos	Prevalence (n=939)
Rhino/Entero	196	34.4 %	117	40.8 %	3	12.5 %	6	11.5 %	322	34.3 %
RSV	148	26.0 %	30	10.2 %	1	4.2 %	1	1.9 %	180	19.2 %
hMPV	42	7.4 %	12	4.1 %	0	0 %	2	3.8 %	58	6.2 %
Para 1	6	1.1 %	2	0.7 %	0	0 %	0	0 %	8	0.9 %
Para 2	3	0.5 %	4	1.4 %	0	0 %	0	0 %	7	0.7 %
Para 3	34	6.0 %	7	2.4 %	3	12.5 %	0	0 %	44	4.7 %
Para 4	1	0.2 %	0	0 %	0	0 %	0	0 %	1	0.1 %
Parainfluenza total	44	7.7 %	13	4.4 %	3	12.5 %	0	0 %	60	6.4 %
CoronaV 229E	0	0 %	1	0.3 %	0	0 %	0	0 %	1	0.1 %
CoronaV HKU1	9	1.6 %	4	1.4 %	0	0 %	2	3.8 %	15	1.6 %
CoronaV NL63	14	2.5 %	2	0.7 %	1	4.2 %	0	0 %	17	1.8 %
CoronaV OC43	5	0.9 %	2	0.7 %	0	0 %	0	0 %	7	0.7 %
Coronavirus total	34	6.0 %	9	3.1 %	1	4.2 %	2	3.8 %	46	4.9 %
Adenovirus	6	1.1 %	5	1.7 %	0	0 %	0	0 %	11	1.2 %
Influenza A total	2	0.4 %	14	4.8 %	0	0 %	0	0 %	16	1.7 %
FluA/H1	0	0 %	0	0 %	0	0 %	0	0 %	0	0 %
FluA/H3	2	0.4 %	8	2.7 %	0	0 %	0	0 %	10	1.1 %
FluA/2009 H1	0	0 %	6	2.0 %	0	0 %	0	0 %	6	0.6 %
Influenza B	0	0 %	0	0 %	1	4.2 %	0	0 %	1	0.1 %
C. pneumoniae	2	0.4 %	0	0.7 %	0	0 %	0	0 %	2	0.2 %
M. pneumoniae	1	0.2 %	2	0.7 %	0	0 %	0	0 %	3	0.3 %
Total positive: One or more analytes	408	71.6 %	183	62.5 %	8	33.3 %	11	21.2 %	610	65.0 %

although there were only three cases in each group. No positive results were detected for *B. pertussis* during the study period.

All hMPV cases were detected in the ≤ 2 and 3-21 age groups, except for two cases. One case was in a 50-year-old female day-care worker admitted for asthma exacerbation, and the second was a 58-year-old female with an underlying hematologic malignancy.

All RSV cases were also detected in the ≤ 2 and 3-21 age group, except for two cases. One case of RSV was detected in a 33-year-male who also tested positive for Rhino/Entero, and a second case was detected in a 59-year-old male. The patient that tested positive for both Rhino/Entero and RSV was the only patient above the age of 18 who tested positive for more than one virus.

Comparison of initial symptoms/diagnoses with PCR diagnosis

The major initial clinical symptoms/diagnoses of the patients included respiratory distress, asthma/wheezing, pneumonia, fever or bronchiolitis. The percentages of the major initial symptoms/diagnoses for the more prevalent PCR results are presented in Fig. 1.

When compared to RSV, patients with hMPV showed significantly less respiratory distress (10.9 %, $p<0.05$) as a presenting symptom, but more fever (28.2 %, $p<0.05$). There were no significant differences in the percentages of initial presentations with pneumonia, asthma/wheezing or bronchiolitis between RSV and hMPV.

When single RSV or hMPV infections were compared to single rhino/enterovirus infections, there was a significantly higher percentage that presented with pneumonia (18.4 %, $p<0.001$ or 21.7 %, $p<0.05$, respectively) or bronchiolitis (9.5 %, $p<0.001$; 6.3 %, $p<0.05$). Mixed co-infections with Rhino/Entero and RSV or hMPV showed a significant lower percentage that presented with pneumonia (3.0 %, $p<0.05$), but not with bronchiolitis (6.5 %).

There was a significant increase in the number of patients that presented with respiratory distress with a mixed coronavirus and RSV or hMPV co-infection (61.5 %, $p<0.01$). There were only 13 cases for

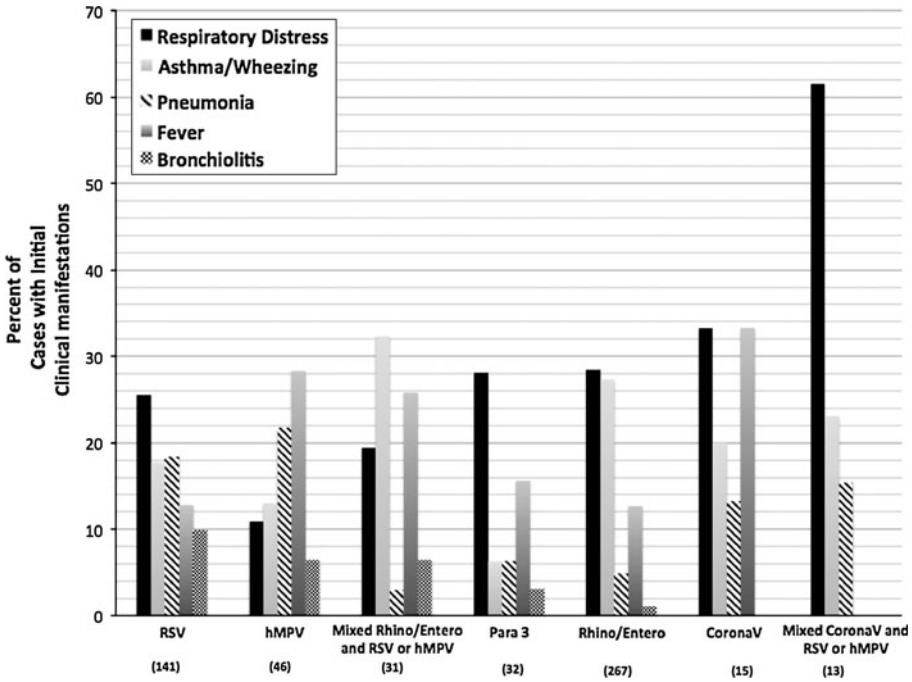

Fig. 1 Percent of cases with initial clinical manifestations. The number in parentheses is the total number of cases in the group

comparison, however. There were only two co-infections with both RSV and hMPV, one as part of a quadruple infection with two coronaviruses and one RSV and hMPV co-infection. The patient with the quadruple infection was admitted to pediatric intensive care with severe respiratory distress. The coinfection RSV and hMPV was an outpatient case seen for asthma exacerbation.

All three cases of *M. pneumoniae* involved co-infections (Table 2). Two of the cases were co-infected with Rhino/Entero, and the third was a triple infection with adenovirus and Rhino/Entero. With all three cases, the initial symptom was asthma/wheezing. One of the two *C. pneumoniae* cases was a co-infection with Rhino/Entero with an initial symptom of respiratory distress. The pure infection with *C. pneumoniae* had an initial diagnosis of pneumonia.

Analysis of multi-analyte-positive samples

Sixty-five percent (65 %) of all specimens were positive for at least one viral or bacterial organism. Of the 610 positive specimens, 12 % (73/610) were positive for more than one analyte (Table 2). The majority (72/73) of multi-analyte-positive samples were from patients $\leq$ 18 years of age. The most common viruses detected in multi-analyte-positive specimens were Rhino/Entero and RSV, which comprised 88 % of all multi-analyte-positive samples (64/73). Individually, Rhino/Entero and RSV were detected in all multi-analyte-positive samples, 75 % and 53 % of the time, respectively (Fig. 2). There were four triple-positive samples and two quadruple-positive samples (Table 2).

Additional viruses found in multi-analyte positive samples included hMPV, Para 3 and CoronaV (HKU1, NL63, OC43). The majority of the hMPV multi-analyte-positive samples were in conjunction with Rhino/Entero. There were only two samples in which hMPV was detected together with CoronaV (HKU1) or CoronaV (HKU1, NL63) and RSV. Also, the majority of the Para 3 multi-analyte-positive samples were also positive for Rhino/Entero. There were only two Para 3 positives that were positive for RSV.

Seasonal prevalence of respiratory pathogens from November 14, 2011 to November 13, 2012

The prevalence of Rhino/Entero remained relatively high throughout the year, with several incidence peaks, the highest occurring in August/September, with two minor peaks in May and December (Fig. 3). A distinct peak in RSV cases occurred in January, followed by a peak in total coronavirus cases in February, and a peak in hMPV cases in March. A distinct peak for Para 3 cases was observed in May.

The eight cases of Para 1 and seven cases of Para 2 all occurred in the fall, in September-November (data not shown). The one case of Para 4 was detected in November. The eleven cases of adenovirus occurred both in the spring and the fall months. Four cases of FluA type H1-2009 were detected February-April 2012. Two cases of FluA type H3 were detected March 2012, and eight cases were detected November 2012. One case of Flu B was detected February

Table 2 Viruses or bacteria detected in multi-analyte-positive samples

No. of multi-analyte-positive samples	Analyte 1	Analyte 2	Analyte 3	Analyte 4
24	Rhino/Entero	RSV		
10	Rhino/Entero	Para 3		
7	Rhino/Entero	hMPV		
4	Rhino/Entero	Para 1		
3	RSV	CoronaV NL63		
2	Rhino/Entero	Flu A		
2	Rhino/Entero	CoronaV NL63		
2	RSV	Para 3		
2	RSV	CoronaV HKU1		
2	RSV	Adenovirus		
2	Rhino/Entero	*M. pneumoniae*		
1	Rhino/Entero	Adenovirus		
1	Rhino/Entero	*C. pneumoniae*		
1	RSV	Para 4		
1	RSV	hMPV		
1	RSV	CoronaV OC43		
1	HKU1	CoronaV OC43		
1	HKU1	hMPV		
1	CoronaV NL63	CoronaV OC43		
1	HKU1	CoronaV OC43	RSV	
1	HKU1	CoronaV OC43	Corona V229E	
1	HKU1	NL63	RSV	
1	*M. pneumoniae*	Adenovirus	Rhino/ Entero	
1	HKU1	NL63	Rhino/ Entero	RSV
1	HKU1	NL63	hMPV	RSV

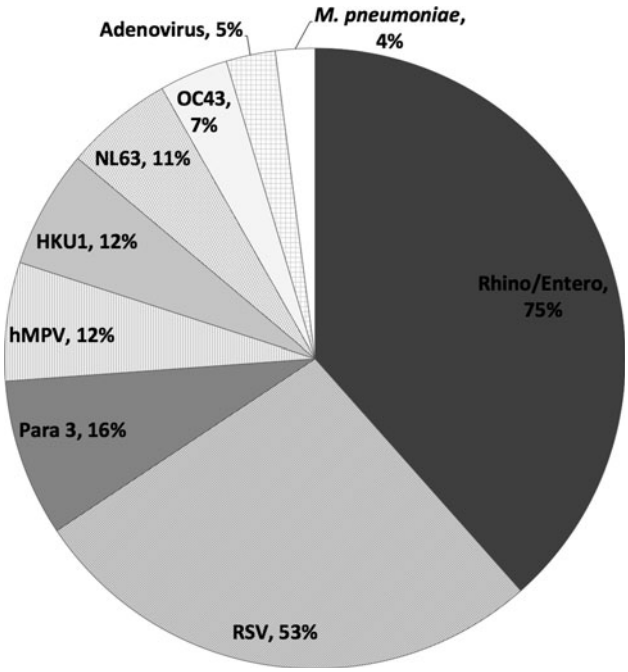

Fig. 2 Prevalence of viruses detected in multi-analyte-positive specimens. The percentage of multianalyte-positive samples positive for the specific analyte is shown

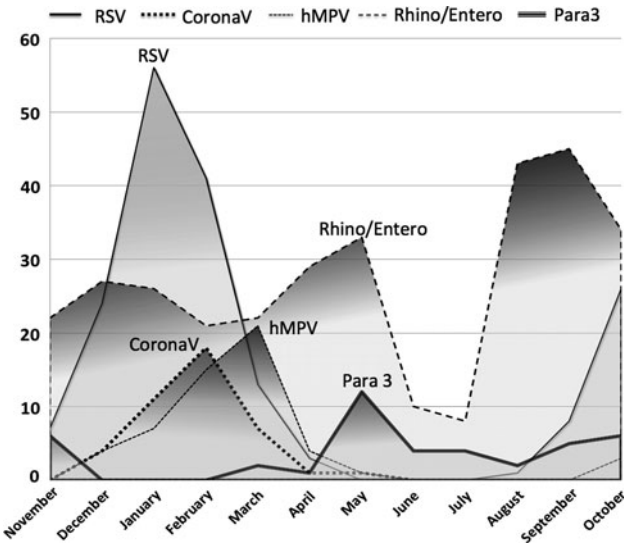

Fig. 3 Prevalence of respiratory viruses by month

2012. No seasonal variation was noted for the eleven adenovirus cases. All three *M. pneumoniae* cases occurred in September, and the two *C. pneumoniae* cases occurred in January and November.

Discussion

In our study, 939 specimens were analyzed over the course of a year using a respiratory pathogen multiplex PCR that yielded a positivity rate of 65 % with multiple analytes detected in 12 % of specimens, especially in children. As the age of the patient increased, the positivity rate for the PCR decreased proportionately. Other multiplex respiratory PCR studies have reported lower positivity rates for adults [9, 11]. Studies have shown that older adult patients shed lower titers of viruses, which demands the use of a highly sensitive methodology such as RT-PCR over

conventional culture or DFA [36]. It is possible that the multiplex PCR assays now available may still not be sensitive enough to detect the lower titers of virus shed by older adult patients. It is also possible that adult patients may have other bacterial infections causing the respiratory infections that are not detected by the PCR, such as *Legionella* or *Streptococcus pneumoniae*.

We noted a seasonal variation of the number of viral infections. Rhino/Entero was the most prevalent viral infection, with demonstrated peaks in the number of cases in May and September. The September peak appears to correspond to the start of school sessions. RSV was the second most prevalent virus, with a very large number of cases in January, followed two months later by an increased number of cases of hMPV.

Rhino/Entero was the predominant virus for all age groups and was superseded only by RSV from December to March. Brittain-Long et al. similarly observed that rhinovirus was the most common finding in the respiratory tract in their study, at 38.0 %, and was the predominant virus regardless of season [11]. Although the rhinoviruses are the most perennial of all the seasonal viruses, peaks of rhinovirus illness are well documented in September, after school starts, and again in the spring [24, 28, 39].

RSV and hMPV were the second- and third-most common causes of respiratory infections in our study. The majority of the RSV and hMPV infections in our study occurred in children. Recently, in a study of over 3,000 children less than 5 years of age, the burden of hMPV was determined to be 6 % in hospitalized children and 7 % of children in both outpatient clinics and emergency departments [14]. We also detected a similar positivity rate of 6.2 %.

RSV and hMPV PCR-positive cases were statistically much more likely than Rhino/Entero PCR-positive infections to initially present with pneumonia or bronchiolitis in our study. Huguenin et al. similarly showed that RSV was associated with more-severe disease in infants hospitalized for bronchiolitis [16]. RSV and hMPV are also known to cause severe respiratory infections in the elderly [17]. Some of these infections did occur in adults $\geq$ 50 in our study [13, 25]. Epidemiologic peaks for hMPV occurring 1 to 2 months later than that observed for RSV epidemics have been described in a number of studies [3, 4, 15, 20, 34]. Our study certainly supports this seasonality.

The parainfluenza viruses tend to have different patterns of seasonal variation based on type. Para 3, which can be predominant in spring in temperate climates, increased in the month of May [22]. Both Para 1 and Para 2 cases in our study were seen only in the fall months in our study, the typical season for these viruses [24]. The epidemiology of Para 4 is largely unknown. The number of coronavirus cases peaked in February in our study, indicating a winter seasonality. There is some evidence, however, that the seasonality of the coronaviruses may be dependent on geographic location [32].

There was an unusually low prevalence of influenza A virus (1.7 %) for the 2011-2012 season in our study. In a review of the 2011-2012 winter influenza season in the northern hemisphere by the WHO, the influenza season started unusually late in most of North America, the latest in nearly 30 years in the U.S. [1]. The season was remarkably mild in the U.S., where influenza A (H3N2) was the predominant virus circulating. Transmission remained low until the end of the year.

No *B. pertussis* cases were detected in our hospital setting. The state of Georgia has one of the lowest incidences of reported *B. pertussis* in the entire U.S., with an incidence of 2.7 per 100,000 [12].

Multiple respiratory pathogens were detected in 12 % of the respiratory specimens in our study; 75 % of the time, the second analyte was Rhino/Entero. Recently, Rand et al. [28] detected 15.9 % co-infections in a study of 200 patients by multiplex respiratory PCR. Other studies have reported slightly lower rates of multiple-analyte-positive specimens of around 10 % and 8.7 % [9, 21].

The significance of these multi-analyte-positive specimens is not clear, especially in conjunction with Rhino/Entero. It is likely that many of the dual-positive results with RSV and Rhino/Entero samples are due to viral shedding from a previous Rhino/Entero infection [10]. Rhinovirus is often found at a higher frequency than any other respiratory virus in asymptomatic carriers [26]. There was a significant increase in the number of patients who presented with respiratory distress (61.5 %) with a mixed coronavirus and RSV or hMPV co-infection. It is possible that these may have represented true co-infections with coronavirus rather than viral shedding. However, the significance of this observation is limited by having only 13 patients in this group.

Some studies that have concluded that more-severe disease occurs in infants with bronchiolitis with co-infections with RSV and hMPV, of which we detected only two cases, one as part of a quadruple infection and one mixed infection with RSV and hMPV [29, 33]. The quadruple infection was a severe respiratory case requiring intensive care.

A possible limitation of the study is that the subjects are biased toward the younger age groups, with only a modest representation of adults. However, the population reflects current physicians' choice of using PCR for the diagnosis of respiratory symptoms. Young children may seek healthcare earlier in the course of disease, and the reasons for testing in young children may be different in adults compared to children and young adults.

Multiplex respiratory PCR has been used in some cases to reduce nosocomial infections during specific seasonal

respiratory epidemics. For example, some investigators have advocated performing multiplex respiratory PCR on all symptomatic infants during the RSV season, recommending that the RSV-positive patients be cohorted and placed on gown and glove precautions, noting a reduction in nosocomial infection in newborn nurseries after putting such practices into place [19, 37]. Ideally, active respiratory PCR surveillance could help predict seasonal respiratory epidemics so that additional infection control measures can be instituted.

A distinct advantage of the rapidity of diagnosis with the multiplex PCR is the ability to quickly and specifically treat the respiratory pathogen and facilitate therapy. Some of the pathogens detected in the multiplex PCR have specific therapies [2]. The multiplex respiratory PCR detects three bacterial pathogens that can all be treated with specific antibiotics, including *C. pneumoniae*, *M. pneumoniae*, and *B. pertussis*. Adamantanes and neuraminidase inhibitors are available for the influenza viruses. Intravenous polyclonal immune globulin enriched against RSV and palivzumab, a monoclonal antibody against the RSV fusion glycoprotein, have been recommended for high-risk infants. Ribavirin has been also been approved for use with RSV and has had some apparent success in cases of infection with hMPV, adenovirus and parainfluenza viruses. Most conventional immunoglobulin preparations seem to contain sufficient amounts of neutralizing titers against hMPV.

In conclusion, molecular testing methods have significantly expanded our ability to diagnose respiratory infections because of their rapidity, sensitivity, and potential for the simultaneous detection of 15 or more respiratory pathogens. The use of a rapid and accurate diagnostic test enhances clinical decision making and safely promotes implementation of cost-effective treatment strategies, including limiting the unnecessary use of antibiotics.

Acknowledgments This study was supported by the Department of Pathology, Medical College of Georgia, Georgia Regents University, Augusta, Georgia.

Conflict of interest We declare that we have no conflicts of interest with respect to this study.

References

1. (2012) Review of the 2011-2012 winter influenza season, northern hemisphere. Wkly Epidemiol Rec 87:233-240
2. Abed Y, Boivin G (2006) Treatment of respiratory virus infections. Antiviral Res 70:1–16
3. Aberle JH, Aberle SW, Redlberger-Fritz M, Sandhofer MJ, Popow-Kraupp T (2010) Human metapneumovirus subgroup changes and seasonality during epidemics. Pediatr Infect Dis J 29:1016–1018
4. Aberle SW, Aberle JH, Sandhofer MJ, Pracher E, Popow-Kraupp T (2008) Biennial spring activity of human metapneumovirus in Austria. Pediatr Infect Dis J 27:1065–1068
5. Aguilar JC, Perez-Brena MP, Garcia ML, Cruz N, Erdman DD, Echevarria JE (2000) Detection and identification of human parainfluenza viruses 1, 2, 3, and 4 in clinical samples of pediatric patients by multiplex reverse transcription-PCR. J Clin Microbiol 38:1191–1195
6. Altman DG (1991) Practical statistics for medical research 1st edn. Chapman and Hall, London
7. Badger GF, Dingle JH, Feller AE, Hodges RG, Jordan WS Jr, Rammelkamp CH Jr (1953) A study of illness in a group of Cleveland families. II. Incidence of the common respiratory diseases. Am J Hyg 58:31–40
8. Balada-Llasat JM, LaRue H, Kelly C, Rigali L, Pancholi P (2011) Evaluation of commercial ResPlex II v2.0, MultiCode-PLx, and xTAG respiratory viral panels for the diagnosis of respiratory viral infections in adults. J Clin Virol 50:42–45
9. Bierbaum S, Konigsfeld N, Besazza N, Blessing K, Rucker G, Kontny U, Berner R, Schumacher M, Forster J, Falcone V, van de Sand C, Essig A, Huzly D, Rohde G, Neumann-Haefelin D, Panning M (2012) Performance of a novel microarray multiplex PCR for the detection of 23 respiratory pathogens (SYMP-ARI study). Eur J Clin Microbiol Infect Dis 31:2851–2861
10. Brittain-Long R, Westin J, Olofsson S, Lindh M, Andersson LM (2010) Prospective evaluation of a novel multiplex real-time PCR assay for detection of fifteen respiratory pathogens-duration of symptoms significantly affects detection rate. J Clin Virol 47:263–267
11. Brittain-Long R, Andersson LM, Olofsson S, Lindh M, Westin J (2012) Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay. Scand J Infect Dis 44:9–17
12. Ditt V, Lusebrink J, Tillmann RL, Schildgen V, Schildgen O (2011) Respiratory infections by HMPV and RSV are clinically indistinguishable but induce different host response in aged individuals. PLoS One 6:e16314
13. CDC (2012) 2012 Provisional Pertussis Surveillance Report. http://www.cdc.gov/pertussis/downloads/Provisional-Pertussis-Surveillance-Report.pdf
14. Edwards KM, Zhu Y, Griffin MR, Weinberg GA, Hall CB, Szilagyi PG, Staat MA, Iwane M, Prill MM, Williams JV (2013) Burden of human metapneumovirus infection in young children. N Engl J Med 368:633–643
15. Heininger U, Kruker AT, Bonhoeffer J, Schaad UB (2009) Human metapneumovirus infections–biannual epidemics and clinical findings in children in the region of Basel, Switzerland. Eur J Pediatr 168:1455–1460
16. Huguenin A, Moutte L, Renois F, Leveque N, Talmud D, Abely M, Nguyen Y, Carrat F, Andreoletti L (2012) Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. J Med Virol 84:979–985
17. Li J, Wang Z, Gonzalez R, Xiao Y, Zhou H, Zhang J, Paranhos-Baccala G, Vernet G, Jin Q, Wang J, Hung T (2012) Prevalence of human metapneumovirus in adults with acute respiratory tract infection in Beijing, China. J Infect 64:96–103
18. Liao RS, Tomalty LL, Majury A, Zoutman DE (2009) Comparison of viral isolation and multiplex real-time reverse transcription-PCR for confirmation of respiratory syncytial virus and influenza virus detection by antigen immunoassays. J Clin Microbiol 47:527–532
19. Madge P, Paton JY, McColl JH, Mackie PL (1992) Prospective controlled study of four infection-control procedures to prevent nosocomial infection with respiratory syncytial virus. Lancet 340:1079–1083

20. Madhi SA, Ludewick H, Kuwanda L, van Niekerk N, Cutland C, Klugman KP (2007) Seasonality, incidence, and repeat human metapneumovirus lower respiratory tract infections in an area with a high prevalence of human immunodeficiency virus type-1 infection. Pediatr Infect Dis J 26:693–699
21. Mahony JB (2008) Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 21:716–747
22. Monto AS (1973) The Tecumseh study of respiratory illness. V. Patterns of infection with the parainfluenzaviruses. Am J Epidemiol 97:338–348
23. Monto AS (2002) Epidemiology of viral respiratory infections. Am J Med 112(Suppl 6A):4S–12S
24. Monto AS (2004) Occurrence of respiratory virus: time, place and person. Pediatr Infect Dis J 23:S58–S64
25. Murata Y (2008) Respiratory syncytial virus infection in adults. Curr Opin Pulm Med 14:235–240
26. Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkaranta A (2002) Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. J Med Virol 66:417–420
27. Nolte FS, Marshall DJ, Rasberry C, Schievelbein S, Banks GG, Storch GA, Arens MQ, Buller RS, Prudent JR (2007) MultiCode-PLx system for multiplexed detection of seventeen respiratory viruses. J Clin Microbiol 45:2779–2786
28. Olofsson S, Brittain-Long R, Andersson LM, Westin J, Lindh M (2011) PCR for detection of respiratory viruses: seasonal variations of virus infections. Expert Rev Anti Infect Ther 9:615–626
29. Paranhos-Baccala G, Komurian-Pradel F, Richard N, Vernet G, Lina B, Floret D (2008) Mixed respiratory virus infections. J Clin Virol 43:407–410
30. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, Thatcher SA, Robbins T, Lingenfelter B, Amiott E, Herbener A, Daly J, Dobrowolski SF, Teng DH, Ririe KM (2011) FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. PLoS One 6:e26047
31. Rand KH, Rampersaud H, Houck HJ (2011) Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 49:2449–2453
32. Ren L, Gonzalez R, Xu J, Xiao Y, Li Y, Zhou H, Li J, Yang Q, Zhang J, Chen L, Wang W, Vernet G, Paranhos-Baccala G, Wang Z, Wang J (2011) Prevalence of human coronaviruses in adults with acute respiratory tract infections in Beijing, China. J Med Virol 83:291–297
33. Richard N, Komurian-Pradel F, Javouhey E, Perret M, Rajoharison A, Bagnaud A, Billaud G, Vernet G, Lina B, Floret D, Paranhos-Baccala G (2008) The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J 27:213–217
34. Robinson JL, Lee BE, Bastien N, Li Y (2005) Seasonality and clinical features of human metapneumovirus infection in children in Northern Alberta. J Med Virol 76:98–105
35. Schildgen V, van den Hoogen B, Fouchier R, Tripp RA, Alvarez R, Manoha C, Williams J, Schildgen O (2011) Human Metapneumovirus: lessons learned over the first decade. Clin Microbiol Rev 24:734–754
36. She RC, Polage CR, Caram LB, Taggart EW, Hymas WC, Woods CW, Schmader K, Petti CA (2010) Performance of diagnostic tests to detect respiratory viruses in older adults. Diagn Microbiol Infect Dis 67:246–250
37. Snydman DR, Greer C, Meissner HC, McIntosh K (1988) Prevention of nosocomial transmission of respiratory syncytial virus in a newborn nursery. Infect Control Hosp Epidemiol 9:105–108
38. Tsuchiya LR, Costa LM, Raboni SM, Nogueira MB, Pereira LA, Rotta I, Takahashi GR, Coelho M, Siqueira MM (2005) Viral respiratory infection in Curitiba, Southern Brazil. J Infect 51:401–407
39. Weigl JA, Puppe W, Meyer CU, Berner R, Forster J, Schmitt HJ, Zepp F (2007) Ten years' experience with year-round active surveillance of up to 19 respiratory pathogens in children. Eur J Pediatr 166:957–966