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American Journal of Infection Control 41 (2013) 868-73

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Detection of respiratory coinfections in pediatric patients using a small volume polymerase chain reaction array respiratory panel: More evidence for combined droplet and contact isolation

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Key Words:
Co-infection
Children
Respiratory virus
Isolation precautions
Molecular diagnostics

Background: In fall 2009, Children's Hospital of Michigan (CHM) instituted combined isolation precautions (contact and droplet isolation) for pediatric inpatients with upper respiratory infection (URI) symptoms to prevent health care-associated infection.

Methods: Pediatric patients with symptoms of URI had nasopharyngeal (NP) swab samples obtained prospectively between January and April and September and December 2010 for small volume polymerase chain reaction (SVPCR) array respiratory panel (RP) multiplex nucleic acid testing. NP swabs or nasal washes were obtained for viral culture and rapid antigen testing (RAT).

Results: Of 499 evaluable SVPCR array RP samples, 344 (69%) tested positive for at least 1 of the 21 tested organisms. The most commonly identified pathogen was rhinovirus/enterovirus (181/344 [53%]) for which no RAT exists at CHM. Of 344 positive specimens, 57 (17%) had at least 2 identified pathogens; 8 (2%) of these had 3. In 11% of patients, molecular testing detected pathogens or pathogen combinations requiring both contact and droplet precautions.

Conclusion: SVPCR array RP testing detected respiratory pathogens in pediatric patients with URI at rates higher than that of RAT and viral culture. Because of the pathogens and pathogen combinations detected, the study findings suggest that combined contact and droplet isolation precautions may be warranted to prevent health care-associated infection in pediatric inpatients with URI. Further studies will be needed to confirm these results.

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Pediatric upper respiratory infection (URI) and bronchiolitis are familiar disease entities.[1] Although guidelines and working evaluations of these guidelines have been developed, the best management for these illnesses remains unclear.[2-9]

In fall 2009, in light of the 2009 H1N1 influenza pandemic, Children's Hospital of Michigan (CHM) instituted a policy requiring combined contact (gown and glove) and droplet (mask) isolation precautions for all inpatients with URI symptoms. Determination of appropriate isolation precautions for hospitalized pediatric patients with potential respiratory coinfection is an important intervention to prevent health care-associated infections (HAI).[10] Cohorting of patients with clinical bronchiolitis symptoms has also been recommended in centers without private patient rooms for all admissions. However, because clinical bronchiolitis may be caused by more than 1 etiologic agent,[10] the use of rapid respiratory testing platforms gives clinicians information that may allow for more focused isolation and cohorting of patients in real time. The study hypothesis is that the use of combined isolation precautions

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This work was presented in part at the 49th Annual Meeting of the Infectious Diseases Society of America on October 22, 2011. Abstract 32206 (Poster 1231).

Supported by a research grant from Idaho Technology, Inc, for the initial clinical evaluation of the SVPCR array respiratory panel (to H.S.).

Conflicts of interest: E.J.M. investigator initiated Study Research Grant, Merck & Co. Rotavirus Vaccine research. H.S. has received study research grant support from Astellas and AdvanDx. The remaining authors disclose no conflicts.

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http://dx.doi.org/10.1016/j.ajic.2013.01.010

should be continued, even after the H1N1 pandemic, in pediatric patients with URI symptoms based on the detection of pathogen coinfections by use of a small volume polymerase chain reaction (SVPCR) array respiratory panel (RP) that requires both droplet and contact isolation precautions. This SVPCR array RP is the first US Food and Drug Administration (FDA)-approved molecular method capable of detecting 17 viral and 3 bacterial respiratory pathogens and has been compared with other molecular testing methods.[11,12]

METHODS

Patient enrollment

Detroit Medical Center was 1 of 3 US sites involved in clinical evaluation of FilmArray Respiratory Panel (BioFire Diagnostics, Inc; previously Idaho Technology Inc, Salt Lake City, UT). From January through April and again from September through December 2010 (no samples were collected May-August), pediatric patients (up to age 20 years) presenting to the emergency department (ED) at CHM, a tertiary pediatric medical center in Detroit, Michigan, were offered participation in the study if the treating clinician had ordered any testing for viral respiratory pathogens. Some admitted patients had specimens collected in the hospital. The protocol required study patients to have one or more of the following signs or symptoms suggesting respiratory infection: fever, cough, sore throat, runny nose, pain or pressure in ears, sinus pressure, and/or sneezing. The study coordinator confirmed with the treating clinician that the patient exhibited the recorded signs or symptoms before patient enrollment into the study. The study was approved by the Wayne State University Human Investigation Committee.

Standard respiratory virus testing

Our laboratory offers 2 rapid antigen tests (RAT) based on lateral flow Immunochromatographic methods for respiratory viruses: BinaxNow RSV (Binax, Scarborough, ME) and QuickVue Influenza A & B (Quidel, San Diego, CA). They were performed according to the manufacturers' instructions.

For respiratory virus cultures, patient specimens are inoculated into 4 monolayer cell culture tubes: rhesus monkey kidney, Hep-2, and 2 MRC-5 (human fetal lung fibroblast cell line; Binax, Scarborough, ME). All except 1 MRC-5 tube are incubated at 36°C and observed daily for the development of cytopathic effect (CPE). If CPE develops, depending on its appearance, the cells are reacted with the appropriate fluorescent antibody (influenza A; influenza B; parainfluenza viruses 1, 2, and 3; adenovirus, or respiratory syncytial virus (RSV); D^3 Ultra Respiratory Screening and Identification Kit, Diagnostic Hybrids). The 36°C MRC-5 tube of all respiratory cultures that are older than 3 days and do not show CPE are tested by hemadsorption using guinea pig red blood cells (ViroMed, Minnetonka, MN). Hemadsorbing viruses include influenza A and B and the parainfluenza viruses. Hemadsorption-positive cultures are tested with the fluorescent antibodies described above, potentially shortening the time to diagnosis. The remaining MRC-5 cell culture is incubated at 32°C. If CPE characteristic of rhinovirus is seen, the culture is signed out as presumptive rhinovirus. These techniques cannot detect coronavirus, human metapneumovirus (hMpV), or human bocavirus (HBCV). Viral cultures were monitored 14 days for CPE before being reported negative.

FilmArray respiratory panel testing

The FilmArray Respiratory Panel assay (BioFire Diagnostics, Inc) is a SVPCR assay and was designed to detect 21 respiratory pathogens simultaneously, from the same nasopharyngeal (NP) swab. Freeze-dried reagents for nucleic acid purification, reverse transcription, nested multiplex PCR, and high-resolution melting analysis are contained within a proprietary reagent pouch that is inoculated with the patient respiratory specimen.[13] FilmArray Respiratory Panel testing can identify 21 common and emerging bacterial and viral respiratory pathogens: adenovirus; HBCV; *Bordetella pertussis*; *Chlamydophila pneumoniae*; coronaviruses HKU1, NL63, 229E, and OC43; hMpV; rhinovirus and enterovirus (R/E); influenza A viruses A/H1, A/2009 H1, A/H3, influenza B; *Mycoplasma pneumoniae*; parainfluenza viruses 1, 2, 3, and 4; and RSV. As of May 2012, FDA clearance had been obtained for all but HBCV. FilmArray Respiratory Panel test results were compared with RAT and viral culture results as part of the clinical evaluation of this system. Results were analyzed locally for coinfection trends. Per study protocol, results of FilmArray Respiratory Panel testing were not shared with treating clinicians.

Isolation precautions

Suggested isolation precautions are recommended for specific respiratory pathogens based on methods of transmission to prevent HAI.[10,14] Respiratory isolation precautions are recommended for influenza viruses, R/E, *B pertussis*, and *M pneumoniae*.[10,14] Contact isolation precautions are recommended for RSV, hMpV, HBCV, and parainfluenza viruses.[10,14] Standard precautions are recommended for coronaviruses and *C pneumoniae*.[14] Dual contact and respiratory isolation precautions are recommended for adenovirus.[10,14]

Statistical analysis

The Pearson χ^2 test was used to compare the age-based differences in the detection of R/E, RSV, and hMpV. Fisher exact test was used to compare age-based differences between those with a pathogen detected compared with none detected and single and multiple detected pathogens.

RESULTS

Patient characteristics

Five hundred three NP swabs were obtained from 503 pediatric patients who were offered participation in the study because their physician had ordered testing for a respiratory pathogen. No patient parent/guardian offered participation in the study refused consent. Of the 503 enrolled, 499 had valid SVPCR array RP results (4 samples did not pass internal quality controls within the assay). There were 300 males (60%) and 199 females. Ages ranged from 1 week to 20 years (mean, 2.2 $\pm$ 3.2 years; median, 1 year). There were 377 patients aged $\leq$24 months (younger children) and 122 aged $\geq$25 months (older patients). This age designation was chosen because infants and young children cannot contain their secretions and require close physical contact for patient care, which may lead to an increased risk of transmission of pathogens. The most commonly reported symptoms were runny nose (358/499: 72%), cough (342/499: 69%), and fever (332/499: 67%). The median number of reported symptoms was 2 (range, 1-5 symptoms).

Test results

Standard technique results

Standard techniques utilizing RAT (for RSV and influenza A/B) and viral culture yielded positive results for at least 1 pathogen in 99 of 499 (20%) samples. RAT was positive in 66 (63 RSV, 3 influenza

Table 1
Frequency of specific respiratory pathogens identified by a small volume polymerase chain reaction array respiratory panel in 499 pediatric patients by age, n (%)*

	R/E†	RSV‡	hMpV†	CoV§	AdV	HBCV	PIV3	PIV2	PIV4	*B pertussis*	Inf B	*M pneum*	Inf A	None
0-6 mo (n = 162)	59 (36)	43 (27)	14 (7)	9 (5)	3 (2)	3 (2)	3 (2)	3 (2)	1 (1)	3 (2)	0 (0)	0 (0)	0 (0)	42 (26)
7-12 mo (n = 130)	45 (35)	29 (22)	12 (9)	9 (7)	12 (9)	11 (9)	1 (1)	1 (1)	3 (2)	0 (0)	0 (0)	1 (1)	0 (0)	30 (23)
13-24 mo (n = 85)	32 (38)	13 (15)	5 (6)	2 (2)	3 (4)	6 (7)	2 (2)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	30 (35)
≥25 mo (n = 122)	45 (37)	10 (8)	8 (7)	6 (5)	4 (3)	2 (2)	1 (1)	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)	1 (1)	53 (43)
All ages (n = 499)	181 (36)	95 (19)	39 (8)	26 (5)	22 (4)	22 (4)	7 (1)	4 (1)	4 (1)	4 (1)	2 (0.4)	1 (0.2)	1 (0.2)	155 (31)

AdV, Adenovirus; *B*, *Bordetella*; *CoV*, coronavirus; *HBCV*, Human bocavirus; *hMpV*, Human metapneumovirus; *Inf*, influenza virus; *mo*, months; *M pneum*, *Mycoplasma pneumoniae*; *None*, no pathogen identified; *PIV*, parainfluenza virus; *R/E*, rhinovirus/enterovirus; *RSV*, respiratory syncytial virus.
*Totals may exceed 100% because of coinfection (percentages calculated based on detection of pathogen in distinct age groups).
†There was no significant difference in the detection of R/E (*P value* = .871) or hMpV (*P value* = .551) in children aged <24 months (combined) compared with those >25 months. The other pathogens were not tested because their numbers were too small.
‡RSV detection differed significantly in all children aged < 24 months (combined) compared with those ≥ 25 months (*P value* ≤.001).
§Coronavirus values are combined totals within the age categories and included HKU1, NL63, OC43, and 229E.

A) samples. Viral culture was positive in 63 samples (29 RSV; 18 adenovirus; 5 enterovirus; 5 parainfluenza 3; 3 parainfluenza 2; 2 influenza A; and 1 influenza B). RSV was detected by RAT (alone) 38 times, by culture (alone) 4 times, and by both RAT and culture 25 times. Influenza A was detected 1 time in culture (alone) and 2 times by both RAT and culture.

False negative SVPCR array results

Only 6 samples that tested positive by standard techniques gave negative SVPCR array RP results: these included 3 viral cultures positive for adenoviruses, 1 for enterovirus, and 1 for influenza A virus (the last was confirmed by positive influenza A RAT). One additional sample positive for RSV by RAT was negative by viral culture and SVPCR array RP. One patient with a positive RAT for RSV had a discordant viral culture that grew only adenovirus. That patient's SVPCR array RP identified RSV as well as R/E but not the adenovirus.

SVPCR array RP results

Of the 499 samples tested by SVPCR array RP, 344 (69%; 3.4-fold higher than standard techniques) were positive for a total of 408 pathogens (Table 1), including 275 (73%) of 377 specimens from younger children and 69 (57%) of 122 from older patients. This difference is significant ($P = .001$). Excluding coronaviruses, hMpV, and HBCV, which our routine virologic diagnostic assays cannot detect, SVPCR array RP detected 318 viruses (3.2-fold higher than standard techniques). At least 1 pathogen detected by standard techniques correlated for at least 1 pathogen detected by SVPCR array RP results except for in the 6 cases noted above.

Only 5 bacterial infections were detected: 4 *B pertussis* and 1 *M pneumoniae*. The pathogens most frequently identified by SVPCR array RP were R/E in 181 of 344 (53%), RSV in 95 of 344 (28%), and hMpV in 39 of 344 (11%). RSV detection was significantly higher in younger children (95/377 or 25%) as compared with older patients (10/122 or 8%; $P \leq .001$). Frequency of the 3 pathogens most commonly detected by SVPCR array RP testing during the study months is shown in Figure 1.

Fever, as the only qualifying sign or symptom, was the single inclusion criterion for enrollment in only 28 (6%) of all participants having viral respiratory testing ordered by the treating clinician. Nine of these 28 (32%) had positive SVPCR array RP results. Those with positives results included 6 with R/E, 2 with RSV, and 1 with parainfluenza 2. One participant with R/E had coinfection with parainfluenza 3.

More than 1 pathogen was identified in 57 SVPCR array RP specimens (11% of the total and 17% of those testing positive). Of those, 49 (86%) tested positive for 2 and 8 (1.4%) for 3 (Fig 2). Multiple pathogens were detected in 49 younger children (13% of the total and 18% of those with a positive SVPCR array RP) but only in 8 older patients (6.6% of the total and 11.7% of those with a positive SVPCR array RP test). This age difference in the number of coinfections was not significant ($P = .277$).

Of those with coinfection, 36 of 57 (63%) would have required combined contact and droplet isolation precautions (gown, gloves, and mask) based on the pathogen combinations identified. Combined precautions are also recommended for adenovirus, which was detected in 17 singly infected patients. Therefore, 53 of 344 (15%) of those with at least 1 pathogen identified, or 11% of the

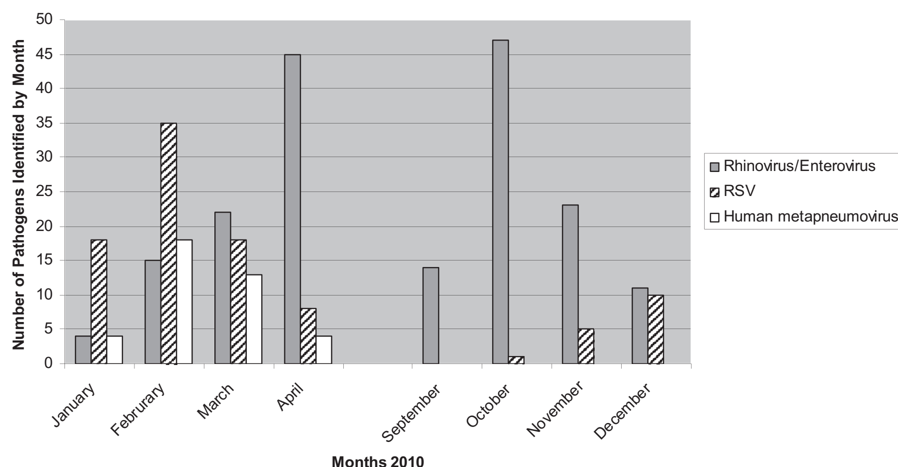

Fig 1. Seasonal frequency of rhinovirus/enterovirus, RSV, and Human Metapneumovirus (most frequently detected) by SVPCR array RP during study period (2010).

Pathogen	Co-Pathogen	Times detected (n=49)	Final Isolation Type
R/E	RSV	9	Combined
R/E	Adenovirus	6	Combined
R/E	Human Bocavirus	6	Combined
RSV	Human Bocavirus	4	Contact
R/E	Coronavirus NL63	2	Droplet
R/E	hMpV	2	Combined
RSV	Coronavirus NL63	2	Contact
RSV	Coronavirus OC34	2	Contact
RSV	hMpV	2	Contact
R/E	*B. pertussis*	1	Droplet
R/E	Coronavirus HKU1	1	Droplet
Adenovirus	hMpV	1	Combined
RSV	Bovavirus	1	Contact
RSV	Adenovirus	1	Combined
R/E	Parainfluenza 4	1	Combined
R/E	Parainfluenza 3	1	Combined
RSV	Coronavirus HKU1	1	Contact
RSV	Influenza B	1	Combined
hMpV	Coronavirus NL63	1	Contact
hMpV	Parainfluenza 4	1	Contact
hMpV	Coronavirus HKU1	1	Contact
Human Bocavirus	Coronavirus NL63	1	Contact
Human Bocavirus	Coronavirus HKU1	1	Contact

Pathogen	Co-Pathogen	Co-Pathogen	Times detected (n = 8)	Final Isolation Type
R/E	hMpV	Human Bocavirus	2	Combined
R/E	Adenovirus	Human Bocavirus	1	Combined
R/E	hMpV	Enterovirus (single)	1	Combined
R/E	RSV	Human Bocavirus	1	Combined
R/E	RSV	Coronavirus HKU1	1	Combined
R/E	hMpV	Coronavirus OC43	1	Combined
R/E	Adenovirus	*B. pertussis*	1	Combined

Fig 2. Dual and triple coinfections and recommended* isolation types. *hMpV*, Human metapneumovirus; *R/E*, rhinovirus/enterovirus; *RSV*, respiratory syncytial virus. Color designations: yellow: Contact Precautions; blue: Respiratory Droplet Precautions; purple: Standard Precautions; orange: Combined (Droplet and Contact) Precautions. *Data from Seigel et al[10] and American Academy of Pediatrics.[14]

499 tested, would have required combined isolation precautions (Fig 2).

To estimate the theoretical number of children who may have been assigned to the wrong level of isolation precautions, only the RAT and SVPCR array RP assays had results available in time to impact isolation precaution determinations as culture is significantly slower. Thus, only the RAT and SVPCR array RP results were used in the calculation. Only 66 RAT were positive compared with the 344 who had at least 1 pathogen identified by SVPCR array RP. The difference between the 2 assays is 278. Standard testing with RAT could have wrongly assigned isolation precautions (either no precautions for a false negative RAT or single precautions when there should have been dual precautions (for coinfection) based in increased yield from SVPCR array RP) in 278 of those 344 (81%) with a positive test result or 278 of the 499 (56%) total participants.

DISCUSSION

During the 8-month evaluation of this SVPCR array RP at a tertiary care children's hospital, participants were enrolled during the traditional months when respiratory pathogens infect children. This SVPCR array RP assay is rapid, takes 2 minutes of hands-on time/sample, and does not require special molecular training for the operator. This SVPCR array RP detected at least 1 pathogen in 344 (69%) of the 499 NP samples, whereas standard RAT and virus culture techniques detected only 99 (20% of the total and 29% of those with SVPCR array RP-detected pathogens). This rate is in the middle of the range of other studies using molecular methods for respiratory pathogen detection, which reported positive rates ranged from 46% to 93%.[5,15-18] Based on SVPCR array RP results, there was a significant difference in the number of children 24 months and younger with a detected pathogen compared with those aged 25 months and greater. This difference may be related to the developing immune system of the younger age group, lack of previous virus exposure during early life, or to possible colonization.[17] Those 25 months and older presenting with URI symptoms may have various other non-SVPCR array RP tested pathogens causing their symptoms. The FDA-cleared SVPCR array RP assay package insert reports 87.4% to 100% sensitivity and 94.6% to 100% specificity, based on pediatric and adult data (65% were children $\leq$5 years) obtained from the prospective clinical evaluation of the SVPCR array RP assay (excluding Bocavirus).[19]

In our patient population, coinfection with 2 or more pathogens, including the 3 bacteria included in the SVPCR array RP, was noted in 57 (17%) of 344 SVPCR array RP-positive specimens, with 8 of these having 3 detected pathogens. Other investigators have also reported "mixed" viral infection or coinfection in children. One study from Finland, utilizing both PCR and diagnostic assays, including serology, noted mixed viral infections in 57 of 293 (19%) samples. In their study, as in ours, respiratory rhinoviruses/enteroviruses were among the most commonly identified viruses in mixed infection.[16] A study from France found that 21.4% of PCR-positive nasal aspirate samples contained at least 2 viruses.[20] Additionally, a German group reported that 16.1% of their pediatric patients with acute respiratory tract infection had positive test results for more than 1 virus.[21] Two molecular studies from Seattle, WA, detected viral coinfection in 18% of their patients with at least 1 identified viral pathogen[17] and in 23% of their pediatric patients with symptoms of bronchiolitis.[18] Neither of these reports suggested that testing to identify respiratory picornaviruses was performed.[17,18] However, in a multicenter study, Mansbach et al reported that 28.9% of infants and children up to 2 years of age with bronchiolitis had multiple pathogens detected, including rhinovirus.[5]

The identification of viral coinfection has been associated with differing clinical outcomes. For example, Aramburo et al[22] used real-time PCR to detect respiratory viruses in critically ill children with respiratory tract disease and reported that 70.5% of the children sampled had 1 viral pathogen and that 14.5% of these patients had viral coinfections. Patients with viral coinfections had higher mortality rates, although this was not statistically significant.[22] In another study of patients with hematologic disorders, no significant differences were noted in presentation or outcome between episodes of febrile neutropenia caused by bacterial infection and those caused by mixed viral and bacterial infections.[23] Some combinations of pathogens, especially coinfection with RSV, have been associated with longer length of stay.[5]

Treatment of infants and young children with URI or bronchiolitis symptoms may not be altered by viral testing or pathogen identification.[2] However, for patients hospitalized with URIs, for whom private rooms are not available, cohorting has been recommended.[10] Additionally, isolation precautions are employed to prevent transmission of specific pathogens with known methods of transmission (ie, aerosols or contact) to minimize the risk of HAI.[10] RSV transmission may be optimally prevented by the use of contact isolation precautions (gown and gloves), whereas spread of rhinovirus and influenza is prevented by droplet precautions (mask).

Data provided from multiple investigations, including this study, add to the growing body of evidence suggesting that viral coinfections are common in infants and young children with URI symptoms.[15-18,24-27] The clinical significance of the multiple pathogens identified both by quantitative (viral load) and qualitative methods (present study) has been questioned before and has been variously reported to have different degrees of clinical importance.[17,26-28] The detection of dead or noninfectious virus particles by molecular assays is theoretically possible, as is the possibility of asymptomatic carriage.[1,5] For example, the SVPCR array RP detects *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*, which have been reported to persist long after active infection has resolved.[14] The clinical relevance and transmissibility of these 2 agents require clinical correlation. In contrast, *B pertussis* is thought not to cause prolonged NP colonization but may become undetectable, even by molecular techniques, shortly after the characteristic whoop develops. This suggests that positive results for *B pertussis* may indicate detection of a true pathogen, as was noted in 2 of our patients with coinfections. Heininger et al suggested that coinfections of viruses, such as RSV and hMpV, with *Bordetella* spp are rare.[25] If viral coinfection does truly increase disease severity, it may be that multiple infections are overrepresented in study samples from patients seeking evaluations in the ED and especially in those admitted to the hospital. Additionally, other agents both known and as yet unknown may also cause URI in children.

Based on review of the published pediatric literature using molecular techniques to identify respiratory pathogens, the reported rates of coinfection with pathogen combinations that would require combined isolation vary from an estimated 4% to 15% of the total samples tested.[5,17,18,22] Of note, combined isolation precautions are routinely recommended for some respiratory viruses, including adenovirus, because of known mechanisms of transmission.[10] This estimated rate of pathogens requiring combined isolation precautions would be even higher if detection of adenovirus alone is included. In our study, considering both mixed and adenovirus infections, 11% of all of the children tested had pathogens requiring both droplet and contact isolation precautions.

SVPCR array RP detected a large number of pathogens and multiple coinfections in children with URI seeking medical care at our children's hospital. Many of these required combined contact and droplet isolation precautions. Therefore, our center has continued the use of combined isolation (contact and droplet) precautions for hospitalized children with URI symptoms year-round. This is maintained for all in-patients with URI symptoms for the duration of the illness or until hospital discharge. Patients placed in combined isolation are cohorted upon admission when private rooms are not available. Although cohorting of patients should be based on symptoms because of the range of pathogens we cannot culture and for which no rapid diagnostic tests exist, the full impact of rapid respiratory testing platforms, such as the SVPCR array RP, may allow for more focused isolation and cohorting of patients in the future and needs further study.

Like many issues in hospital infection control, causation is difficult to prove because increases or decreases in HAI rates are multifactorial. Hospital epidemiologists at CHM track and report HAI rates for RSV, as detected by RAT or culture, during January through April for annual benchmarking. No other HAI respiratory

viruses were routinely monitored. For the 3 years prior to initiation of combined droplet and contact isolation (2006-2008), the average RSV HAI rate per 1,000 patient days was 0.25 (16 RSV cases; unpublished data). During 2009 to 2011, after the implementation of combined isolation, the rate was 0.15 (9 RSV cases; unpublished data). This difference is not significant ($P = .478$). However, targeted infection control interventions, including specific viral testing, isolation precautions, and cohorting, have been reported to be associated with prevention of HAI and cost savings at other centers.[4] The low number of influenza A strains detected may have reflected the timing of the influenza seasons. During the winter of 2009-2010, influenza peaked between October and December. During the winter of 2010-2011, almost all the influenza occurred during January through March. Thus, little influenza was detected by any methodology during our study periods.

Strengths of this study are the large sample size, the broad age range of enrolled pediatrics patients sampled for the study, and the use of the sensitive molecular method-based detection by SVPCR array RP. Limitations include that the data are obtained from a single center, that no samples were obtained or analyzed from May to August 2010, and that no further data on the patient's clinical course were recorded after enrollment. As noted above, with most respiratory molecular diagnostic sample testing including this study and others, there is a possibility of PCR tests from respiratory samples yielding positive results that may be due to dead virion detection as opposed to active viral replication.

CONCLUSION

In pediatric patients with URI symptoms, infections with common pathogens, including rhinoviruses, were detected when sensitive molecular assays are utilized. However, those same patients often had negative RAT and viral culture results because of limitations in sensitivity and in inherent detection capabilities of these assays. Nearly 70% of our patients with URI symptoms (73% of those aged ≤24 months) had positive SVPCR array RP results for at least 1 pathogen. Coinfection was present in 11% of all tested and 17% of those testing positive for at least 1 pathogen. Because of the pathogens and pathogen combinations detected in 11% of all participants tested, our study findings suggest that combined contact and droplet isolation precautions (gown, gloves, and mask) may be warranted to prevent HAI in pediatric inpatients with URI. Further studies will be needed to confirm these findings and to assess the effect of combined isolation precautions on prevention of HAI in pediatric hospitals.

Acknowledgment

The authors thank Lynn Semproch for providing the CHM epidemiology data for this analysis.

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