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Viral detection using a multiplex polymerase chain reaction–based assay in outpatients with upper respiratory infection[☆]

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ABSTRACT

We evaluated a commercial multiplex polymerase chain reaction (PCR) assay in a cross-sectional study among 81 adult and pediatric outpatients—40 cases with upper respiratory infection symptoms and 41 asymptomatic controls—from February to April 2008. Two specimens (throat swab and nasal swab) from each participant were tested using the EraGen MultiCode-PLx Respiratory Virus Panel that detects 17 viral targets. Throat swabs were also tested for Group A *Streptococcus* (GAS) by PCR. Respiratory viruses were detected in 22/40 (55%) cases and in 3/41 (7%) controls ($P < 0.001$). GAS was detected in 10 (25%) cases; GAS and respiratory virus co-infection was found in 4 (10%). Agreement between nasal and throat swabs for viral detection was 69% in cases and 95% in controls. Of 22 cases with a detectable virus, 12 (54%) were picked up by only 1 (throat or nasal) specimen, and the detection rate was increased by combining results of nasal and throat swab testing.

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1. Introduction

Etiologic agents of upper respiratory infection (URI) are difficult to ascertain based solely on clinical features; viral cultures are cumbersome, have long turnaround times, and have low yield (Makela et al., 1998). Several polymerase chain reaction (PCR)–based tests have demonstrated improvement in diagnostic yield and turnaround times compared with conventional cell cultures and antigen-based methods (Mahony et al., 2011; Wu and Tang, 2009). The EraGen MultiCode-PLx Respiratory Virus Panel (Luminex, Austin, TX, USA) is one such assay that couples multiplex PCR chemistry with high-throughput microsphere flow cytometry for simultaneous detection of 17 viruses: influenza A and B; respiratory syncytial virus A and B; parainfluenza 1, 2, 3, 4(a) and 4(b); metapneumovirus; adenovirus B, C, and E; coronavirus NL63, 229E, and OC43; and rhinovirus. Performance characteristics of the test have been previously described (Nolte, et al., 2007), with reported sensitivities for individual viruses ranging from 94% to 100% and specificities

ranging from 99% to 100%, when compared with viral culture (Arens, et al., 2010).

A potential limitation of PCR-based assays compared to viral culture is increased detection of respiratory viruses in both healthy and immunocompromised individuals in the absence of respiratory symptoms (Kuypers et al., 2009; Wright, et al., 2007). Reported rates of viral recovery from the respiratory tract of asymptomatic individuals using PCR-based assays vary from 2% to 30% depending on the population and test methodology (Brittain-Long, et al., 2010; Buecher, et al., 2010; Graat, et al., 2003; Sato, et al., 2009; van Gageldonk-Lafeber, et al., 2005), but comparable information regarding specificity of the MultiCode-PLx system (using symptoms as the reference standard) in outpatient URI diagnosis is not available. Second, in the case of URI, particularly pharyngotonsillitis in children, group A beta-hemolytic *Streptococcus* (GAS) is well established as the most common bacterial pathogen (Hable et al., 1971). Moreover, concurrent infection with a respiratory virus and GAS has been identified in prior studies (Brook and Gober, 2008; van Gageldonk-Lafeber, et al., 2005), suggesting that detection of a respiratory virus might not preclude antibiotic use in some cases. A third issue is determining the most appropriate sampling method and anatomical site for detection of respiratory viruses using this assay. In a recent study using multiplex real-time reverse transcription-PCR, investigators observed an increase in viral detection by combining different specimens (nasopharyngeal swab, oropharyngeal swab, nasopharyngeal washes) in adults hospitalized with lower respiratory tract infections

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(Lieberman, et al., 2009). In the outpatient setting, nasal and throat swabs are most frequently (and conveniently) obtained, especially in children. It is unknown whether the type of specimen (nasal versus throat swab) affects viral detection using multiplex PCR in URI.

The primary aim of the present study was to compare detection of respiratory viruses in outpatients (adults and children) with URI with that in asymptomatic individuals using the EraGen MultiCode-PLx Respiratory Virus Panel. Secondary aims were to 1) identify etiologic agents of URI among outpatients, including co-infection with a virus and GAS; and 2) compare viral recovery using this assay from throat and nasal swabs.

2. Materials and methods

2.1. Study design, setting, and participants

We performed a cross-sectional study among patients seen in outpatient facilities of Mayo Clinic, Rochester, MN, between February 1 and April 30, 2008. Patients with URI symptoms (“cases”) were prospectively enrolled using convenience sampling from several primary care settings including the Family Medicine Clinic, Community Pediatric Clinic, and Express Care (a walk-in clinic providing evaluation and treatment for straightforward and common conditions such as URI). Patients without symptoms of URI within 2 weeks prior to enrollment (“controls”) were concurrently recruited from the same clinics and also from the Travel Medicine Clinic. We enrolled a similar number of cases and controls in the pediatric- (≤ 18 years) and adult (>18 years) age groups.

2.2. Data collection

A study-specific questionnaire was used to collect the following information at the time of enrollment for all subjects: age, sex, history of asthma, presence of other chronic medical conditions (such as diabetes, kidney disease, and liver disease), immunosuppressive conditions or therapy, number of children in the household, daycare attendance (for pediatric participants and/or children in households of all participants), and smoking exposure. For cases, information regarding the date of symptom onset, clinical diagnosis, and prescription of antiviral and/or antibacterial therapy for the current illness was also collected. For controls, information regarding any respiratory illness in the last month, including the estimated date of the last symptom, was obtained.

2.3. Microbiologic methods

Two upper respiratory specimens (1 nasal and 1 throat) were obtained from each patient by study personnel, using the BBL™ CultureSwab™ (BD Diagnostic Systems, Sparks, MD, USA). Specimens were transported to the virology laboratory within 4 h after collection and placed in the refrigerator at 2–8 °C. Specimens were extracted daily, but testing was not performed in real time; instead, specimens were batched and tested weekly to increase efficiency. Swabs were broken off in Microtest™ M5® Transport (Remel; Thermo Fisher Scientific, Lenexa, KS, USA) prior to extraction using the MagNA Pure Compact instrument with the MagNA Pure Compact Total Nucleic Acid Isolation Kit–small volume (cat. no. 03 730 964 001) (Roche Applied Sciences, Indianapolis, IN, USA). Both nasal and throat swabs from each participant were tested with the EraGen MultiCode-PLx Respiratory virus panel using manufacturer recommendations. The assay utilizes an open system in which, following RNA/DNA amplification, the amplicon is transferred to the Luminex instrument for specific virus detection. All throat swabs were also tested for GAS by a separate, previously validated, LightCycler-based real-time PCR assay, with a sensitivity of 93% for GAS detection (Uhl, et al., 2003).

2.4. Statistical methods

With an estimated respiratory virus detection rate of 45% among cases, and 15% among asymptomatic controls, the sample size required for an alpha of 0.05 and 80% power would be 36 cases and a similar number of controls for the primary outcome. Two-by-two contingency tables were created, and data were analyzed using the chi-square test for difference between 2 proportions of interest. Agreement between results from nasal and throat swabs was calculated as the percentage of participants either testing positive for the same virus or testing negative on both specimens.

2.5. Human subject protection

The study was approved by the Mayo Clinic Institutional Review Board. Informed consent or assent, as applicable, was obtained for each participant. Results from the study were not reported to providers and did not influence treatment.

3. Results

3.1. Participant characteristics

Eighty-one participants comprising 40 cases (20 adult, 20 pediatric) and 41 controls (21 adult, 20 pediatric) were enrolled between February 1, 2008, and April 30, 2008. The median age (and age range) was 39 (19–65) years for adults and 10 (5–18) years for children. There were no significant differences between cases and controls in age, sex, history of asthma, number of children in the household, or proportion of children attending daycare (Table 1). There were very few participants with underlying chronic medical illnesses (other than asthma) or with immunosuppressive conditions (Table 1). Clinical diagnoses among symptomatic patients included pharyngitis or tonsillopharyngitis in 22 (55%), influenza-like illness in 8 (20%), URI not otherwise specified in 5 (13%), cough illness in 4 (10%), bronchitis in 2 (5%), and sinusitis in 1 (2%).

3.2. Test characteristics

The average total test time (extraction to result) for the MultiCode-PLx respiratory virus panel was 6 h.

3.3. Detection of respiratory viruses in cases and controls

The overall detection rate in cases using the MultiCode-PLx Respiratory virus panel and 2 specimens (i.e., the proportion of patients with URI symptoms with a virus detected on either the throat or the nasal swab) was 22/40 (55%). In contrast, the detection rate

Table 1
Participant characteristics in study of detection of viruses and group A *Streptococcus* in upper respiratory infection.

Characteristic	Cases (n = 40)	Controls (n = 41)	P value (chi-square)
Age, mean (SD)	24 (17)	26 (16)	0.67 ^a
Gender (% male)	15 (38)	18 (44)	0.56
Asthma ^b	11 (28)	6 (15)	0.15
Chronic medical condition ^b	2 (5)	2 (5)	^c
Immunosuppressive condition ^b	2 (5)	1 (3)	^c
≥2 children in household ^b	23 (58)	25 (61)	0.75
At least 1 child in daycare ^b	6 (15)	12 (30)	0.12
Current smoking ^b	2 (3)	2 (2)	^c
Exposure to smoker in household ^b	4 (5)	3 (4)	^c

Values are shown as n (%), unless otherwise specified.

^a P value based on t test for difference between 2 means.

^b Information missing for 2 participants.

^c Numbers too small to test for statistical difference.

was 3/41 (7%) in asymptomatic controls ($P < 0.0001$) (Table 2). Among cases, the detection rate was somewhat greater in pediatric (13/20, 65%) than in adult participants (9/20, 45%), although this difference was not statistically significant (Table 2). Controls in whom one or more viruses were detected by the assay were all in the pediatric age group (Table 2). With clinical symptoms as the “reference standard” for presence of URI, the clinical specificity of the assay (the proportion of controls with a negative test result) was 38/41 (93%) overall, with 100% clinical specificity among adults and 85% among children.

The viruses detected in cases were as follows: influenza A ($n = 6$), influenza B ($n = 6$), rhinovirus ($n = 5$), coronavirus ($n = 2$), RSV A ($n = 1$), parainfluenza 3 ($n = 1$), adenovirus B ($n = 1$), and adenovirus E ($n = 1$). The types and frequency of different viruses detected in the study population were different between adults and children (Fig. 1). While influenza A, influenza B, and coronavirus were the only viruses detected in adults, a greater variety of viruses were observed in the pediatric subjects, with rhinovirus being the most frequent (36%). The characteristics of the 3 pediatric controls in whom viruses were detected in the absence of symptoms were as follows: 1) adenovirus C detected from throat only; the subject had URI symptoms just over 2 weeks prior to enrollment; 2) influenza A detected from throat only; the subject had a history of asthma, attended daycare, and there were 3 young children in the subject's household, all in daycare; 3) rhinovirus, detected from both nose and throat swabs; there were 2 young children in the household, not in daycare.

3.4. Contribution of viruses versus GAS to URI

GAS was detected in 10 (25%) cases and in 2 (5%) controls ($P = 0.01$). Overall, a pathogen (either a virus or GAS) was identified in 28/40 (70%) cases: 18/40 (45%) had a viral infection alone (1 subject had 2 different viruses, rhinovirus and adenovirus), 6/40 (15%) had GAS infection alone, and 4/40 (10%) were co-infected with both a respiratory virus and GAS. GAS was more commonly detected among cases with a diagnosis of “pharyngitis” (7/22, 32%) compared to those with a different type of URI (3/18, 17%), although the difference was not statistically significant.

3.5. Performance of throat swabs versus nasal swabs

The overall agreement between results of nasal and throat swabs (either the same virus detected in both specimens, or both specimens

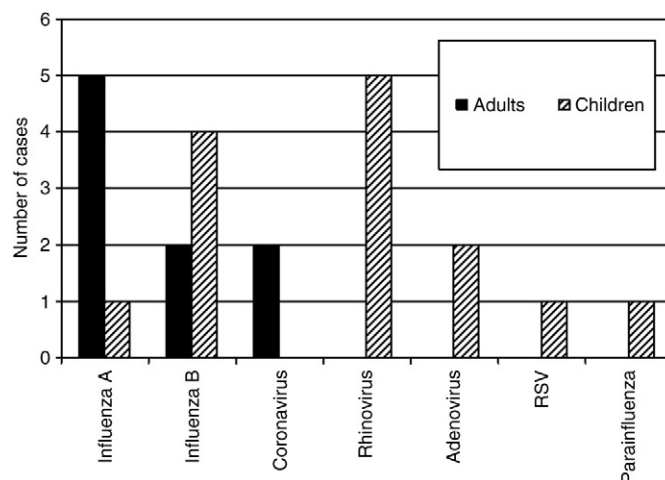


Fig. 1. Viral etiologic agents of upper respiratory infection in outpatients.

negative) was 27/39 (69%) for cases (68% in adults, 70% in children) and 39/41 (95%) for controls (100% in adults, 90% in children). There were no instances of detection of different viruses from 2 specimens from the same participant. Among cases, 12/22 (55%) (6/9 adult and 6/13 children) had only 1 specimen (throat or nasal) that tested positive using the MultiCode-PLx Respiratory virus panel. Combining results of both specimens increased the detection rate from 15/40 (37.5%) to 22/40 (55%) overall; from 5/20 (25%) to 9/20 (45%) among adult cases; and from 10/20 (50%) to 13/20 (65%) among pediatric cases. The numbers were too small to allow evaluation of any specific association(s) between the type of virus and type of specimen, or between the type of clinical syndrome and type of specimen yielding a positive assay.

3.6. Duration of symptoms and viral detection

Of 40 cases, the duration of symptoms could be determined for 31 patients. Of these, 14 (45%) had a positive result and 17 (55%) had a negative result using the MultiCode-PLx Respiratory virus panel. The mean and median duration of symptoms prior to specimen collection were 5.9 and 4 days (range 1–18 days), respectively. The detection rate among cases with duration of symptoms 6 days or less was 50% compared to 45% among those with duration of symptoms 7 days or more; however, this was not statistically significant.

3.7. Antibiotic treatment

Of 40 cases with symptoms of URI, 14 (35%) received antibiotic therapy. Among those who received antibiotics, 6 (43%) were positive for GAS, 4 (29%) were positive for a virus, and no pathogen could be identified in the remaining 4 cases.

4. Discussion

In this study, using multiplex PCR and 2 sampling sites (nasal and throat swabs), we were able to detect respiratory viruses in 55% of participants with upper respiratory infection (URI). This detection rate is consistent with that observed in other studies utilizing similar diagnostic platforms (Brittain-Long, et al., 2008, 2010). Although viral cultures were not performed for direct comparison in our study, this detection rate was higher than that reported for viral culture (10–35%) in previous published studies comparing multiplex PCR with viral culture (Arens, et al., 2010; Lee, et al., 2007).

With clinical symptoms as the “reference standard” for presence of URI, the assay demonstrated a high clinical specificity, particularly among adults (100%). We detected rhinovirus, influenza A, and

Table 2

Detection of viruses and group A *Streptococcus* in upper respiratory infection by age group and type of specimen.

	Cases	Controls	<i>P</i> value (chi-square)
<i>All participants</i>	<i>n</i> = 40	<i>n</i> = 41	
Virus detected by nasal swab ^a	15 (37.5%)	1 (2%)	<0.001
Virus detected by throat swab	16 (40%)	3 (7%)	<0.001
Virus detected by either specimen	22 (55%)	3 (7%)	<0.001
GAS ^b detected by PCR (throat swab)	10 (25%)	2 (5%)	0.01
<i>Adult participants only</i>	<i>n</i> = 20	<i>n</i> = 21	
Virus detected by nasal swab ^a	5 (25%)	0	0.03
Virus detected by throat swab	6 (30%)	0	0.007
Virus detected by either specimen	9 (45%)	0	<0.001
GAS ^b detected by PCR (throat swab)	2 (10%)	0	0.14
<i>Pediatric participants only</i>	<i>n</i> = 20	<i>n</i> = 20	
Virus detected by nasal swab	10 (50%)	1 (5%)	0.001
Virus detected by throat swab	10 (50%)	3 (15%)	0.02
Virus detected by either specimen	13 (65%)	3 (15%)	0.001
GAS ^b detected by PCR (throat swab)	8 (40%)	2 (10%)	0.03

^a One nasal swab was missing.

^b GAS = Group A *Streptococcus*.

adenovirus C in 3 asymptomatic children in our study. Despite the study being conducted during the “influenza season”, and influenza viruses being the predominantly recovered viruses in symptomatic adults, influenza viruses were not detected in adults in the absence of symptoms. The phenomenon of increase in viral detection among asymptomatic individuals with the use of more sensitive PCR assays has been previously demonstrated, and the rate of such “non-specific” detection using the MultiCode PLx assay in our study (7%) was in the lower range of previously published rates (2–30%) using similar platforms (Brittain-Long, et al., 2010; Buecher, et al., 2010; Fry, et al., 2011; Graat, et al., 2003; Sato, et al., 2009; Wright, et al., 2007). In contrast, viruses recovered in cell cultures have been associated with ill patients but not with asymptomatic individuals (Hable, et al., 1971). These data on clinical specificity are complementary to data on microbiologic specificity of the assay compared to viral culture and can help clinicians in interpreting the results of such assays in outpatient practice. As in our study, rhinoviruses appear to be the most frequently detected viruses in asymptomatic persons in several studies using molecular assays (Brittain-Long, et al., 2010; Buecher, et al., 2010; Graat, et al., 2003; Jansen, et al., 2011). Our data are also consistent with previous observations that asymptomatic carriage of respiratory viruses occurs more frequently in children than in adults (Buecher, et al., 2010; Fry, et al., 2011). In some instances, such detection could be due to an individual being in the convalescent phase of an acute illness—e.g., in 1 study, investigators were able to detect viruses from the nasopharynx of children for as long as 2 weeks after an acute infection (Jartti et al., 2004). We were careful to exclude individuals with respiratory illness within 2 weeks prior to enrollment. However, because of the small sample size, specific risk factors for asymptomatic carriage could not be determined in our study. Lastly, a higher rhinovirus viral load (using a molecular assay) was recently shown more likely to be associated with clinical symptoms, and the authors suggested that defining cutoff values should be considered for molecular tests used in the diagnosis of respiratory viral infections (Jansen, et al., 2011).

The ideal sampling method for detection of respiratory viruses in respiratory infection using multiplex PCR has not been established. This study adds to limited published data suggesting that there might be an increase in the diagnostic yield using 2 different sampling sites (Lieberman, et al., 2009) and is the first to demonstrate this in a sample of outpatients presenting with symptoms of URI. This finding was particularly notable in adults and is possibly related to the smaller quantities of virus shed by adults compared to children (She, et al., 2010). While sampling both sites is neither feasible nor necessary in every patient, it is important for clinicians to understand the limitations of sampling 1 site and to pursue an additional site if the clinical situation indicates. Future studies should aim to investigate whether one site is better than the other for a specific virus, clinical syndrome, or stage of illness.

A potential benefit of a sensitive, specific, and rapid diagnostic assay for community viral infections is in guiding appropriate antibiotic therapy. In our study, 35% of cases with symptoms of URI were prescribed antibiotics; however, in at least a third of these cases, antibiotic therapy was provided for a viral URI, indicating that routine use of such an assay has potential to curtail inappropriate antimicrobial therapy. On the other hand, co-infection with GAS was found in 4/40 (10%) symptomatic cases. Furthermore, clinical suspicion for GAS leading to either testing or treatment occurred in only 1 of those 4 patients. This would suggest that a testing strategy that combines the use of a rapid GAS test with a rapid viral diagnostic might be necessary for the optimal management of outpatient URIs. We were able to identify an etiology of URI in approx. 70% of patients with the combination of the 2 tests.

The main limitation of this study is the small number of participants which precluded evaluation of associations between detection of specific viruses, specimen types, and presenting clinical

syndrome. Second, we did not perform direct culture comparison. Third, the study was limited to a convenient sample of outpatients, and we were unable to enroll pediatric participants younger than 5 years or very elderly patients. The study was performed during the winter–spring season only; however, we believe that it was important to evaluate the clinical specificity of the assay during this season when the prevalence of circulating respiratory viral pathogens is high and nonspecific detection might be potentially increased. Only the BBL™ CultureSwab™ was used in this study, and since then, the use of nasal flocced swabs to improve the sensitivity of detection of respiratory viruses has been demonstrated, and combining nasal and throat swabs may not be required (Abu-Diab, et al., 2008). Despite several available multiplex assays, routine use in the clinical setting, particularly ambulatory clinics, is still limited by cost, the need to batch tests which prolongs the turnaround time, and accessibility. Lastly, although not a direct finding of the study, an important disadvantage of this “open system” assay that was noted in the laboratory is a higher risk for cross-sample contamination compared with closed real-time PCR systems.

In conclusion, this study provides preliminary data on issues that need to be addressed when considering routine clinical application of such multiplex respiratory viral diagnostic assays in the outpatient setting.

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