Title

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Journal

Diagnostic Microbiology and Infectious Disease, 67(3)

ISSN

0732-8893

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Publication Date

2010-07-01

DOI

10.1016/j.diagmicrobio.2010.02.020

Peer reviewed

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Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 67 (2010) 246–250
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Virology

Performance of diagnostic tests to detect respiratory viruses in older adults

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Received 5 January 2010; accepted 18 February 2010

Abstract

The performance of 4 laboratory methods for diagnosis of viral respiratory tract infections (RTI) in older adults was evaluated. Seventy-four nasopharyngeal (NP) swab specimens were obtained from 60 patients with RTI at a long-term care facility over 2 respiratory seasons. Sixteen specimens were positive for a respiratory virus by at least 1 method. Multiplex reverse transcriptase polymerase chain reaction (RT-PCR) by the Luminex xTAG® Respiratory Viral Panel (RVP) detected 16 (100%) of the positive specimens, RVP of 24-h culture supernatant detected 8 (50%), direct fluorescent antibody testing detected 4 (25%), rapid culture detected 2 (12.5%), and rapid antigen testing detected none. For a comparison group, RVP was performed on NP swabs from 20 outpatient children with RTI. The mean fluorescence intensity by RVP was significantly lower for positive adult patients than pediatric patients ($P = 0.0373$). Our data suggest that older adult patients shed lower titers of viruses, necessitating a highly sensitive assay such as RT-PCR to reliably detect respiratory viral pathogens.

Keywords: RSV; Influenza; Diagnosis; Older adults

1. Introduction

Respiratory tract infections (RTI) cause considerable morbidity and mortality among the elderly population because of the general decline in the respiratory and immune systems with age (Falsey and Walsh, 2006). There is increasing recognition that many of these RTIs are viral in origin, with viruses contributing up to 80% of acute RTI (Falsey et al., 1995; Flamaing et al., 2003). Infectious outbreaks commonly occur among older adult communities (Graat et al., 2003; Hicks et al., 2006; Loeb et al., 2000; Strausbaugh and Joseph, 2000). Clinical clues are often unreliable because indicators of infection and traditional laboratory methods are cumbersome, slow, and variably insensitive, making most diagnoses retrospective (Falsey et al., 1996; Falsey and Walsh, 2006; Walsh et al., 2007). Emerging molecular diagnostic techniques now offer the potential to test for both a greater breadth of viruses and detect them with increased sensitivity and rapidity (Kuypers et al., 2006; Oosterheert et al., 2005; Templeton et al., 2005). Previous studies were often limited by design, knowledge of etiologic agents, or available diagnostic tests (Angeles Marcos et al., 2006; Flamaing et al., 2003; Jennings et al., 2008; Templeton et al., 2005).

Multiplex reverse transcriptase polymerase chain reaction (RT-PCR) for rapid diagnosis of viral RTI is a powerful diagnostic tool, providing clinical and financial benefits upon the health care system (Barenfanger et al., 2000; Gruteke et al., 2004; Marshall et al., 2007; Woo et al., 1997). With excellent sensitivity and specificity, multiplex RT-PCR can help make the diagnosis of viral respiratory infections in a timely and clinically relevant manner, allowing for more effective clinical care, more appropriate use of antimicrobial

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doi:10.1016/j.diagmicrobio.2010.02.020

therapy, and patient isolation to prevent viral transmission (Mahony et al., 2007).

Although testing characteristics of both traditional and newer diagnostic methods have been defined in diverse adult and children populations (Arden et al., 2006; Falsey et al., 2006; Jennings et al., 2008), little data exist on the optimal diagnostic test for older adults with viral RTI aside from influenza and respiratory syncytial virus (RSV) testing. For this population, there has not been a comprehensive comparison of current modalities for detection of respiratory viruses (i.e., direct fluorescent antibody testing [DFA], culture, multiplex nucleic acid amplification, and rapid antigen assays), and test performance is likely to differ because adults are known to shed fewer virus particles than children during RTI (Hall et al., 1976).

We seek to define the performance characteristics of 4 viral diagnostic tests in an older adult population and hypothesize that highly sensitive assays such as RT-PCR will be favored when considering speed and accuracy of diagnosis.

2. Materials and methods

2.1. Specimens

Older adult patients (age, ≥49 years) with RTI were prospectively enrolled from February 2007 to August 2008 at a 56-room, 120-bed long-term care facility (Durham VA Medical Center, Durham, NC). RTI was defined as the presence of at least 3 respiratory symptoms (new onset or increase in chronic cough, new onset or increase in sputum, dyspnea, chills, headache, myalgias, malaise, sore throat, or nasal congestion) or 2 respiratory symptoms and temperature ≥38.0 °C. Children with RTI presenting at an employee health clinic (ARUP Laboratories, Salt Lake City, UT) from December 2007 to March 2008 were also enrolled as a comparison group for multiplex RT-PCR by respiratory viral panel (RVP) testing. Nasopharyngeal (NP) specimens were obtained using flocked swabs (Diagnostic Hybrids [DHI], Athens, OH) and kept refrigerated in 3.0-mL universal transport media until analysis within 24 h of collection.

2.2. DFA, culture, and rapid antigen

DFA testing and culture were performed on all specimens for the detection of influenza A and B, RSV, parainfluenza 1 to 3, adenovirus (D^3 *Ultra* DFA Respiratory Kit, DHI), and human metapneumovirus (DHI). DFA and culture were performed using a standard laboratory protocol that includes inoculation of 0.2-mL patient specimen into 1 mL of R-mix refeed media (Dunn et al., 2004). R-mix cell lines (DHI) were cultured and stained at 20 to 24 h after inoculation. On a subset of specimens, rapid antigen testing for RSV and influenza A/B was performed by lateral flow (Binax NOW; Inverness Medical, Waltham, MA) according to the manufacturer's recommended protocol.

2.3. Respiratory Viral Panel by RT-PCR

ID-Tag™ RVP (Luminex, Austin, TX), a multiplex RT-PCR platform for detection of influenza A (H1/H3), influenza B, RSV A and B, parainfluenza 1 to 4, coronaviruses 229E, OC43, NL63, and HKU1, human metapneumovirus, enterovirus/rhinovirus, and adenovirus, was performed according to the manufacturer's instructions. Total nucleic acid was extracted from 0.14-mL transport media or 24-h culture supernatant (QIAamp Virus BioRobot 9604 kit; QIAGEN, Valencia, CA). Each 25-μL multiplex, 1-step RT-PCR reaction consisted of 5.75-μL molecular grade water, 2-μL QIAGEN OneStep RT-PCR enzyme mix, 6.25-μL 5× QIAGEN OneStep RT-PCR buffer, 1-μL QIAGEN 10 mmol/L deoxyribonucleotide triphosphate (dNTP) mix, 5-μL RVP RT-PCR primer mix (Luminex), and 5-μL template nucleic acid. Reverse transcription was performed at 50 °C for 30 min and inactivation at 95 °C for 15 min. PCR, target-specific primer extension, bead hybridization, and signal detection were performed as previously described (Mahony et al., 2007). Data were analyzed on the Luminex® xMAP® system using the Tag-It Data Analysis Software RVP-I. The mean fluorescence intensities (MFI) for positive samples were recorded. Statistical comparison between adult and pediatric patients was made using the 2-sample Wilcoxon rank-sum (Mann–Whitney) test. Proportions were compared using the 2-sample test for equality of proportions with continuity correction.

3. Results

Seventy-four NP swab specimens were obtained from 60 patients 49 to 95 years of age. There was a positive result from any of the test methods in 16 specimens (22%) from 15 patients. The distribution of positive results by patient is shown in Table 1. All specimens were tested by RVP (direct specimen), DFA, and culture. All but 2 specimens were tested by RVP on 24-h culture supernatant. Thirty-one specimens also underwent rapid antigen testing for RSV and influenza A and B. The distribution of positive results by type of respiratory virus is summarized in Table 2. For the pediatric comparison group (patient ages 8 month to 17 years), 10 (50%) of 20 samples were positive by direct specimen RVP.

3.1. DFA and culture

Of 74 NP swabs, 26 (35%) had inadequate cellularity for DFA interpretation, and of the 16 specimens found positive by any method, 5 (31%) had inadequate cellularity. There was no significant difference between these 2 proportions ($P = 0.99$). Four specimens were positive by direct specimen DFA: 2 RSVs, 1 influenza B, and 1 parainfluenza 3. Culture was positive in 2 cases (1 influenza B and 1 parainfluenza 3), which were also detected by RVP (direct specimen and post–24-h culture) and DFA.

Table 1
Results of all specimens with at least 1 positive test result

Patient no.	RVP—direct specimen	RVP—24-h culture	DFA	Culture	Rapid antigen
14	RSV B	Negative	Negative	Negative	Negative
21	Para 3	Negative	Negative	Negative	ND
25	Influenza B	Negative	Inadequate	Negative	ND
36	Corona, NL63	Corona, NL63	Negative	Negative	ND
37	Influenza B	Influenza B	Influenza B	Influenza B	ND
44	Para 1	Negative	Inadequate	Negative	ND
46	Para 3	Negative	Para 3	Para 3	ND
51-1	EV/Rhino	Negative	Negative	Negative	ND
51-2	RSV B	RSV B	RSV	Negative	Negative
62	Para 1	Negative	Negative	Negative	ND
83	RSV B	RSV B	Inadequate	Negative	Negative
86	RSV B	Negative	Inadequate	Negative	Negative
87	RSV B	RSV B	Negative	Negative	Negative
90	RSV B	RSV B	RSV	Negative	Negative
108	Corona, 229E	Corona, 229E	Negative	Negative	NA
113	EV/Rhino	EV/Rhino	Inadequate	Negative	NA

Corona = coronavirus; Para = parainfluenza; EV/Rhino = enterovirus/rhinovirus; ND = not done; NA = not applicable.

3.2. Respiratory Viral Panel by RT-PCR

RVP from direct specimen yielded 16 positive results. RVP after a 24-h culture detected 8 of these 16 positive results (50%). The 2 specimens that were not tested by RVP on 24-h culture supernatant were negative by direct specimen RVP, DFA, and culture. The 10 pediatric specimens positive by RVP included 3 RSVs, 2 influenza A, 1 influenza B, 1 human metapneumovirus, 1 enterovirus/rhinovirus, 1 coronavirus, and 1 dual enterovirus/rhinovirus and influenza A. The MFI of positive samples from adult patients with RTI was significantly lower than from pediatric patients with RTI (median, 1560 versus 3553 U, $P = 0.0373$).

3.3. Rapid antigen testing

Of 31 specimens, none were positive for RSV, influenza A, or influenza B by rapid antigen testing, including the 6 specimens that were RSV positive by at least 1 test method. The 2 specimens positive for influenza B by RVP were not tested by rapid antigen. No specimens during the study were positive for influenza A by rapid antigen or any other test method.

4. Discussion

Although many studies have examined the utility of the various methods used for diagnosis of respiratory virus infections, to our knowledge, none have systematically and concurrently examined the performance characteristics of DFA, culture, rapid antigen testing, and PCR on an older adult population (Barenfanger et al., 2000; Falsey et al., 1996, 1995; Lam et al., 2007; Templeton et al., 2004). We found that approximately one-fifth of older adult patients with an acute RTI had a detectable viral etiology. This is somewhat lower than the frequency seen in other studies, which have found a viral etiology in approximately one-third of elderly patients with RTI using various combinations of culture, serology, and antigen detection (Falsey et al., 1995; Flamaing et al., 2003). This study did not have a surveillance group of asymptomatic older adults to establish a causal relationship, but respiratory viruses are found generally in a low percentage (<5%) of adult surveillance specimens when PCR is used (Jartti et al., 2008).

The data in this study indicate that multiplexed RT-PCR is highly sensitive in detecting respiratory viruses in older adults compared to DFA, culture, and rapid antigen testing.

Table 2
Summary of positive results (no. of specimens with a positive result/total no. of specimens positive by at least 1 method)

	RVP—direct specimen	RVP—24-h culture	DFA	Culture	Rapid antigen
RSV	6/6	4/6	2/6	0/6	0/6
Influenza B	2/2	1/2	1/2	1/2	ND
Parainfluenza 1	2/2	0/2	0/2	0/2	NA
Parainfluenza 3	2/2	0/2	1/2	1/2	NA
Coronavirus	2/2	2/2	NA	NA	NA
Enterovirus/rhinovirus	2/2	1/2	NA	NA	NA
Total	16/16 (100%)	8/16 (50%)	4/12 (33%)	2/12 (17%)	0/6

ND = not done; NA = not applicable.

The MFI data from the RVP assay suggest that older adults shed less virus than children during RTI. Virus detection in the older adult population is therefore enhanced by highly sensitive methods such as RT-PCR. An additional advantage of the RVP assay is that viruses that are not routinely cultured or stained can be detected, such as rhinovirus and coronavirus. This is especially useful in elderly patients, for whom rhinovirus and coronavirus can cause severe to fatal respiratory disease (Greenberg, 2007; Hicks et al., 2006). Although it may seem intuitive that RVP testing after 24 h of culture would be equally if not more sensitive than RVP on the direct specimen, we found the opposite to be true. This may be due to the dilution of the specimen with culture media, particularly when there were low numbers of infective virus particles in the original sample. Furthermore, R-mix cells are optimized for rapid viral antigen detection at 24 or 48 h but not for propagation of virus (Wilkey et al., 2006). Our observation that RVP from the supernatant of a 24-h R-mix culture is associated with reduced sensitivity is an important finding, especially for developing diagnostic algorithms for the detection of respiratory viruses.

DFA on respiratory specimens remains a useful test in young children because they shed large amounts of virus, and DFA is quicker and simpler to perform compared to RT-PCR. In the older adult population studied here, DFA failed to detect a viral pathogen in 8 of 12 possible cases, whereas RVP did not miss any cases that were found by DFA. Culture was even less sensitive than DFA, contributing no additional information to the patients tested. Other studies have also found viral culture to be insensitive, especially in older adults (Falsey et al., 1996). Although convenient, rapid antigen testing for RSV is not recommended for testing in older adults because of its insensitivity in this patient population. This finding is corroborated by other investigators (Casiano-Colon et al., 2003; Falsey et al., 1996). No influenza A or B cases were available for testing by rapid antigen; thus, conclusions on rapid antigen testing for influenza in older adults cannot be made.

Dual infections were not found in older adults in this study, whereas in young children, they are found in 12% or more of patients tested in other investigations (Gruteke et al., 2004; Kuypers et al., 2006). The incidence of mixed viral RTI in older adults has not been studied using highly sensitive modalities such as RT-PCR but has been reported in very low frequency with use of other test methods such as serology and culture (Falsey et al., 1995; Flamaing et al., 2003). Our study did not include serologic testing, another sensitive testing modality, but serology is largely retrospective in contrast to the test methods used in this study.

We acknowledge that the results of this study may have been affected by the high number of inadequate specimens. However, specimens were collected by experienced nursing and medical staff. Moreover, this underscores the importance of having highly sensitive diagnostic assays for older adults and highlights the need for modified protocols for specimen collection, especially in the elderly population during dry winter conditions. The proportion of inadequate specimens overall and among specimens with positive results did not differ statistically. This finding suggests that PCR is sufficiently sensitive to detect virus even in specimens with poor cellularity. The low number of positive results from DFA or culture may has been due to specimen inadequacy. It should be noted that for PCR, culture, and rapid antigen testing, specimen cellularity is not routinely assessed and would be noted only if microscopy, e.g., DFA, was performed.

This study demonstrates that for older adults, a highly sensitive platform is necessary to minimize false-negative test results. Rapid antigen testing for RSV is not recommended for older adults. Negative DFA and culture results should be interpreted with caution because these methods are insensitive particularly in the older adult population. Nucleic acid amplification-based testing, with its superior sensitivity, serves as the most robust method to detect respiratory viruses in older adults, and further study is warranted to determine its clinical, epidemiologic, and financial benefits over traditional methods.

Acknowledgments

The authors thank Andrew Wilson for his performance of the statistical analyses, Jack Twersky, MD, Sara Hoffman, RN, and the Verterans Affair Extended Care and Rehabilitation Center patients and staff. DHI and Luminex provided reagents for this study. ARUP Institute for Clinical and Experimental Pathology, Atlantic Philanthropies, Infectious Diseases Society of America/National Foundation for Infectious Diseases, John A Hartford Foundation, and The Association for Specialty Professors supported this study. The authors declare no competing interests. This study was approved by the Durham Veterans Affair Medical Center and University of Utah institutional review boards.

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