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Comparison of Sputum and Nasopharyngeal Swabs for Detection of Respiratory Viruses

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Diagnostic tests for respiratory viral infections use traditionally either nasopharyngeal washes or swabs. Sputum is representative of the lower respiratory tract but is used rarely for viral testing. The aim of this study was to compare the detection rates of respiratory viruses from nasopharyngeal swabs and sputum using a multiplex real-time reverse transcription-polymerase chain reaction (RT-PCR). Adults who were admitted or presented to the clinics of Gil Medical Center with acute respiratory symptoms were recruited from 1 November 2012 to 31 March 2013. Paired specimens of nasopharyngeal swabs and sputum were obtained from 154 subjects, and RNA was extracted and tested for 16 different respiratory viruses using the Anyplex II RV16 Detection kit (Seegene, Seoul, Korea). The positive rate was 53% (81/154) for nasopharyngeal swabs and 68% (105/154) for sputum ($P < 0.001$). One hundred thirty-four viruses were identified for 107 illnesses. Influenza A virus, RSV A, HRV, coronavirus OC43, and adenovirus were detected more frequently in sputum samples than in nasopharyngeal swabs ($P < 0.001$). Importantly, 12 of 44 (27%) influenza A infections and 11 of 27 (41%) RSV infections were positive in only sputum samples. The detection rates of respiratory viruses from sputum samples were significantly higher than those from nasopharyngeal swabs in adults using real-time multiplex RT-PCR. These findings suggest that sputum would benefit for the detection of respiratory viruses by nucleic acid amplification tests (NAATs) in patients who produce sputum. Further studies are needed to establish standardized RNA extraction methods from sputum samples. ***J. Med. Virol. 86: 2122–2127, 2014.*** © 2014 Wiley Periodicals, Inc.

KEY WORDS: nasopharyngeal swab; sputum; reverse transcription-polymerase chain reaction

INTRODUCTION

Respiratory viral infections, ranging from mild upper respiratory tract infections to more severe lower respiratory infections, are the major causes of morbidity, and disease severity can be affected by host- and viral-predisposing factors. The clinical impacts of respiratory viruses increase in young children, the elderly, immunocompromised hosts, and patients with underlying diseases [Monto, 2002]. Rapid identification of respiratory viral infection is critical to avoid the use of unnecessary antibiotics, the use of appropriate antiviral agents, minimize the risk of nosocomial transmission, and reduce the overall costs of patient management [Pérez-Ruiz et al., 2012].

The diagnosis of respiratory viral infections is performed traditionally using virus culture or direct antigen assays [Storch, 2000]. Culture, which is often considered the gold standard, is labor-intensive and time-consuming. Antigenic detection assays are simple but sometimes insufficiently sensitive. Molecular techniques have been used currently for respiratory virus detection with improved sensitivity, and multiplex real-time reverse transcription-polymerase chain reaction (RT-PCR) methods can detect simultaneously a number of viruses in a single assay.

Sputum is used rarely for traditional viral tests due to its viscous nature, which makes sample processing difficult. However, with the advent of molecular methods, sputum has been introduced for the diagnosis of respiratory viral infection. The aim of this study was to compare the detection rates of

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respiratory viruses in paired nasopharyngeal swabs and sputum samples from adult patients with respiratory symptoms using multiplex real-time RT-PCR.

MATERIALS AND METHODS

Subjects

Adults over 19 years of age who were admitted or presented to the clinics of Gil Medical Center with signs and symptoms of acute respiratory illness defined as cough, rhinorrhoea, nasal congestion, dyspnoea, sputum, or fever were recruited from 1 November 2012 to 31 March 2013. Paired specimens of nasopharyngeal swabs and sputum were obtained from 154 subjects. Each patient provided written informed consent, and the Institutional Review Boards of the Gil Medical Center approved the study. At enrolment, clinical, radiological, and laboratory information was collected.

Sputum

The sputum samples were expectorated spontaneously into sterile containers and delivered to the laboratory within 2 hr. The sputum samples were then diluted with an equal volume of phosphate buffered saline (PBS) and mixed by vortexing for 1 to 5 min, depending on the sputum viscosity. If the RT-PCR result was invalid, a retest was performed using a specimen diluted with twice the initial volume of PBS.

Nasopharyngeal Swab

Nasopharyngeal swabs were obtained from each patient by physicians using flocked swabs (Copan Diagnostics, Brescia, Italy) and were transported in 3.0 ml of universal transport medium (UTM; Copan Diagnostics). Prospectively collected sputum and nasopharyngeal swab samples were tested simultaneously.

Total RNA Isolation

RNA was extracted from 300 μl of samples with 10 μl of bacteriophage MS2 as an internal control using the GeneAll Ribospin kit (GeneAll Biotechnology, Seoul, Korea). The internal control was added to each specimen as an exogenous control to check the entire process from nucleic acid extraction to RT-PCR. The final elution volume of each sample was 50 μl. An automated protocol for extraction, RT-PCR, and PCR setup was implemented using the Nimbus (Hamilton Robotics, Reno, NV) automated liquid handling workstation to maximize the workflow and accuracy.

Multiplex Real-Time RT-PCR Detection Assay

Multiplex real-time RT-PCR was performed using the Anyplex II RV 16 Detection kit (Seegene, Seoul, Korea) according to the manufacturer's instructions. Sputum samples and nasopharyngeal specimens from each patient were tested in parallel for the following 16 respiratory viruses: human bocavirus (HBoV), human enterovirus (HEV), influenza virus A and B, parainfluenza virus 1, 2, 3, and 4 (PIV 1, PIV 2, PIV 3, and PIV 4), RSV A and B (RSV A and RSV B), adenovirus (Adv), metapneumovirus (MPV), coronavirus OC43 (OC43), coronavirus 229E (229E), coronavirus NL63 (NL63), and human rhinovirus A/B/C (HRV).

Statistical Analysis

The overall positive rates of viruses between nasopharyngeal swabs and sputum samples were compared using McNemar's test. The clinical characteristics of patients and diagnostic yields for detection of any virus between two specimens were compared using the Chi-square test or Fisher's exact test. Statistical significance was set at a P value of <0.05. Agreement of the results between nasopharyngeal swabs and sputum specimens was assessed using Kappa statistics (Cohen's kappa coefficient (K): <0 = poor, 0–0.2 = slight, 0.21–0.4 = fair, 0.41–0.6 = moderate, 0.61–0.8 = substantial, and 0.81–1 = almost perfect) [Landis and Koch, 1977]. All statistical analyses were performed using SPSS (version 17.0; SPSS, Inc., an IBM Company, Chicago, IL).

RESULTS

Paired nasopharyngeal swab and sputum samples were collected from 154 patients, and the clinical characteristics of the 154 patients are shown in Table I. The patients included 47 males (31%) and 107 females (69%), with a median age of 52 years (interquartile range, 35–62 years). Approximately 41% of the patients had an underlying chronic pulmonary disease, and patients had been troubled

TABLE I. Patient Characteristics

Characteristics	N = 154 (%)
Age (year)	
Median	52
Interquartile range	35–62
Sex (male/female)	47 (31)/107 (69)
Smoker	22 (14)
Underlying diseases	
Hypertension	35 (23)
Diabetes mellitus	9 (6)
Malignancy	9 (6)
Cardiac disease	11 (7)
Pulmonary disease	63 (41)
Respiratory symptoms	
Cough	145 (94)
Rhinorrhea	109 (71)
Dyspnea	29 (19)
Febrile sense	90 (58)
Myalgia	55 (36)
Duration of symptoms (days prior to sampling)	
Median	5
Interquartile range	3–7
Chest X-ray findings	
Abnormal findings	52 (34)
Not evaluated	52 (34)

TABLE II. Comparison of Real-Time RT-PCR Results between Nasopharyngeal Swab and Sputum Samples

	No. (%) of sputum result	
	Positive	Negative
No. (%) of nasopharyngeal swab result		
Positive	79 (51.3%)	2 (1.3%)
Negative	26 (16.9%)	47 (30.5%)

P value <0.001 by McNemar test.

with respiratory symptoms for a median of 5 days prior to sampling (Table I).

A respiratory viral infection was identified in 107 (69%) of 154 patients (Table II), and the positive rate was 53% (81/154) for nasopharyngeal swab and 68% (105/154) for sputum. Sputum showed a significantly higher positive rate than nasopharyngeal swabs in detecting respiratory viruses ($P < 0.001$), and viral co-infections were found in nasopharyngeal swabs and sputum (Table III). In nasopharyngeal swabs, a single virus was identified in 76 cases (94%), two viruses in four cases (5%), and three viruses in one case (1%). In sputum, a single virus was identified in 85 cases (81%), two viruses in 17 cases (16%), and three viruses in three cases (3%) (Table III).

The overall distribution of the detected respiratory viruses is shown in Table IV. One hundred thirty-four viruses were identified in 107 illnesses. Influenza A virus was the most common, followed by RSV A, HRV, and OC43. The diagnostic yields for detection of any virus were 87 (65%) of 134 for nasopharyngeal swabs and 128 (96%) of 134 for sputum. The distribution of positive results ranged from 46% to 77% of nasopharyngeal swabs tested and from 91% to 100% of sputum tested. Influenza A virus, RSV A, HRV, OC43, and Adv were detected more often in sputum samples than in nasopharyngeal swab ($P < 0.001$). Other viruses numbered too few for statistical testing. Importantly, 12 of 44 (27%) influenza A infections, 11 of 27 (41%) RSV A infection, 5 of 22 (23%) HRV infections, 5 of 13 (38%) OC43 infections, and 3 of 11 (27%) Adv were positive in only sputum samples. Substantial agreement between nasopharyngeal swab and sputum was observed for influenza A virus, RSV A, and OC43. HRV had nearly perfect agreement, but Adv and MPV had a poor agreement (Table IV).

Clinical characteristics of patients with positive nasopharyngeal swab RT-PCR results were not significantly different from those of patients with only positive sputum RT-PCR results (Table V).

DISCUSSION

Adequate specimen collection is important for the diagnosis of respiratory viral infections. A variety of upper respiratory samples, including nasal aspirates, nasal swabs, nasopharyngeal swabs, and oropharyngeal swabs, are used commonly for the detection of respiratory viruses. Early studies suggested that nasal aspirates were superior to swab specimens [Lieberman et al., 2009]; however, this might not be true for all respiratory viruses and all detection methods. In addition, several studies have shown that respiratory viruses are an increasingly recognized cause of lower respiratory tract infections. In those cases, lower respiratory specimens should be collected for the detection of respiratory viruses.

Sputum is the representative of lower respiratory secretions and is the most widely accepted specimen for the diagnosis of bacterial respiratory infection. However, it is used rarely for the detection of respiratory viruses using traditional methods such as the viral antigen test or viral culture. With nucleic acid amplification tests (NAATs), sputum samples have been used for the detection of respiratory pathogens. Currently, few data comparing the diagnostic yields of nasopharyngeal swabs and sputum samples for respiratory virus detection have been published. The

TABLE III. Distribution of Viruses in Patients with Co-Infections

Nasopharyngeal swab (no.)	Sputum (no.)
Influenza A + MPV + human bocavirus (1)	Influenza A + RSV A + coronavirus OC43 (1)
Influenza A + coronavirus OC43 (1)	Influenza A + RSV A + adenovirus (1)
Influenza A + adenovirus (1)	Influenza A + adenovirus, HRV (1)
RSV A + adenovirus (1)	Influenza A + coronavirus OC43 (1)
Adenovirus + coronavirus NL63 (1)	Influenza A + RSV A (5)
Total (5)	RSV A + coronavirus OC43 (2)
	RSV A + coronavirus NL63 (1)
	RSV A + adenovirus (1)
	HRV + coronavirus OC43 (1)
	HRV + adenovirus (2)
	HRV + PIV 1 (1)
	Adenovirus + coronavirus NL63 (1)
	Adenovirus + coronavirus OC43 (1)
	MPV + coronavirus OC43 (1)
	Total (20)

MPV, metapneumovirus; RSV A, respiratory syncytial viruses A; HRV, human rhinovirus.

TABLE IV. Distribution of 134 Viruses in 107 Patients with Respiratory Illness

Virus type	No. of positive nasopharyngeal swab and/or sputum	Nasopharyngeal swab (%)	Sputum (%)	*P* value[a]	Kappa statistics
Influenza A	44	32 (73)	43 (98)	<0.001	0.737
RSV A	27	16 (59)	25 (93)	<0.001	0.637
HRV	22	17 (77)	22 (100)	<0.001	0.854
Coronavirus OC43	13	8 (62)	13 (100)	<0.001	0.746
Adenovirus	11	5 (46)	10 (91)	<0.001	−0.045
MPV	5	1	4	NA	NA
Coronavirus NL63	4	2	4	NA	NA
PIV1	4	3	4	NA	NA
RSV B	2	1	2	NA	NA
Coronavirus 229E	1	1	1	NA	NA
Human bocavirus	1	1	0	NA	NA
Total	134	87 (65)	128 (96)		

RSV A, respiratory syncytial virus A; HRV, human rhinovirus; MPV, metapneumovirus; PIV1, parainfluenza virus 1; RSV B, respiratory syncytial virus B.
NA, not applicable for too small sample size.
[a]Chi-square test or Fisher's exact test.

present study found that the overall detection rate from sputum samples in adults was significantly higher than from nasopharyngeal swabs using multiplex real-time RT-PCR. This finding is consistent with the result of a previous study that sputum samples showed higher diagnostic yields than nose–throat swabs in adults using RT-PCR [Falsey et al., 2012].

The detection rates of respiratory virus from sputum and nasopharyngeal swabs were 68% and 53%, respectively. Generally, it is hard to compare detection rates of respiratory viruses because of differences in testing methods, specimen types (upper or lower airways, swabs or washes, nose or throat, flocked or regular swabs), and types of transport media. Our results are still relatively high compared with those of other reports [Lieberman et al., 2009; Falsey et al., 2012; Yu et al., 2012]. These differences can be explained by several factors. First, the Anyplex II RV16 Detection kit is a multiplex real-time RT-PCR assay that can detect simultaneously 16 different types of viruses. This kit offers higher sensitivity in the detection of traditionally diagnosed respiratory viruses in addition to the ability to detect newly recognized viruses, such as HBoV, PIV 1, and HRV C. Second, nasopharyngeal samplings were obtained using flocked swabs in the present study, and it is known that flocked swabs can collect significantly more epithelial cells, thus providing higher positive rates than conventional swabs [Loens et al., 2009]. Third, this study was performed during an influenza A (H1N1) outbreak season in Korea.

Influenza or RSV respiratory infection may manifest as severe lower respiratory tract disease in high-risk patients, and some investigators reported false-negative results in traditional upper airway samples from patients with respiratory viral infection [Bogoch et al., 2013]. A prior study compared the yields of paired nose–throat swabs and endotracheal aspirates

TABLE V. Clinical Characteristics of Patients with Real-Time RT-PCR-Positive Results

Characteristics		Positive in nasopharyngeal swab (N = 68)[a]	Positive in sputum only (N = 39)	*P* value[b]
Age (no. of (%) over 60 years)		16 (24)	14 (36)	0.186
Sex (no. (%) of women)		48 (71)	30 (77)	0.508
Smoking (no. (%) of smokers)		7 (10)	2 (5)	0.280
No. of (%) with underlying disease		37 (54)	17 (44)	0.319
Hypertension		10 (15)	10 (26)	0.200
Diabetes mellitus	4 (6)		3 (8)	0.704
Malignancy	4 (6)		1 (3)	0.651
Cardiac disease	2 (3)		5 (13)	0.097
Pulmonary disease	29 (43)		12 (31)	0.302
No. of (%) with respiratory symptom				
Cough		62 (91)	37 (95)	0.708
Rhinorrhea		46 (68)	30 (77)	0.379
Dyspnea		12 (18)	11 (28)	0.227
Febrile sense		45 (66)	22 (56)	0.407
Myalgia		28 (41)	9 (23)	0.090
Duration of symptoms ≥5 days		26 (38)	15 (39)	1.000

[a]The nasopharyngeal swab-positive group included subjects with positive and negative sputum RT-PCR results.
[b]Chi-square test or Fisher's exact test.

for the detection of influenza virus using RT-PCR. Among the 22 influenza A patients that were admitted to adult ICUs, this group detected influenza viruses from 14 (63.6%) nose–throat swabs and 20 (90.9%) endotracheal aspirates [Roa et al., 2012]. The current study also found that 32 of 44 (73%) influenza cases were detected in nasopharyngeal swabs, and 43 of 44 (98%) cases were detected in sputum samples. Similarly, 11 of 27 (41%) RSV A infections were positive in only sputum samples. These findings suggest that traditional nasopharyngeal diagnostic techniques can miss cases of lower respiratory tract infections.

Multiple respiratory viruses have been detected in 10–30% of patients with positive viral detection, with higher rates in young children [Drews et al., 2008; Kim et al., 2009; Gharabaghi et al., 2011; Zhang et al., 2012]. In this study, co-infections in sputum samples were more common than those in nasopharyngeal swabs (19% vs. 6%). One unanticipated finding was that influenza A and RSV A were found frequently in mixed infections. These results are contrary to other reports that HBoV and coronavirus were most commonly associated with multiple-agent infections, while influenza virus and RSV were least often detected in the presence of another virus [Gharabaghi et al., 2011]. These differences can be explained, in part, by the differences in specimen types and applied methods. The clinical significance of co-infections is still unknown, and further evaluation is required.

Some problems are encountered when handling sputum for NAATs. First, the highly viscous nature of sputum can make nucleic acid extraction difficult. Therefore, pre-treatment of sputum samples is needed, but no standardized pre-treatment procedure is available for viral detection. Dithiothreitol or NALC (N-acetyl-L-cysteine)-NaOH, which are used generally for the digestion of sputum in mycobacterial laboratories, may cause substantial loss of RNA due to the repetitive washing and pipetting steps in the procedures [Desjardin et al., 1996; Xiang et al., 2001]. In this study, sputum samples were diluted with PBS and mixed by vortexing, and the volume and time depended on the sputum viscosity. This procedure was sufficient for sputum homogenization. Another problem is that NAAT inhibitors in respiratory specimens may affect the sensitivity of the assay or lead to false-negative results. Inhibition of the amplification of the internal control was noted in 15% of the sputum samples, which is a high proportion compared with that of nasopharyngeal swabs (3%). A widely applied method for the removal of PCR inhibitors is sample dilution, which may lead to decreased inhibitors. In the present study, samples that showed invalid PCR results were diluted with twice the initial volume of PBS and re-extracted manually. In the author's experience, thoroughly mixing and homogenization of sputum samples might be important to obtain valid results. However, further evaluations of nucleic acid extraction methods in automated systems for sputum samples are needed.

Although sputum is more sensitive than nasopharyngeal swabs for respiratory viral detection, the use of sputum can be limited because not all patients with respiratory infections produce sputum, particularly elderly patients. Therefore, if a sputum sample is available, it might be the most reliable specimen for respiratory viral detection by NAATs.

This study has a limitation. The sputum samples were not screened microscopically to assess sputum quality. Therefore, contamination of sputum samples by upper respiratory secretions cannot be ruled out.

In conclusion, the detection rates of respiratory viruses from sputum samples are significantly higher than those from nasopharyngeal swabs in adults using multiple real-time RT-PCR. However, the use of sputum is limited to patients who can produce sputum. These findings suggest that sputum would benefit for the detection of respiratory viruses by NAATs in patients who produce sputum. Further studies are needed to establish standardized RNA extraction methods from sputum samples.

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