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A comparative study of nasopharyngeal aspirate and nasal swab specimens for the diagnosis of acute viral respiratory infection

Key Messages

1. Direct immunofluorescence (IF) testing of nasopharyngeal aspirate (NPA) is the standard method used for the rapid detection of influenza A and B, parainfluenza 1, 2, and 3, respiratory syncytial virus (RSV) and adenovirus in cases of acute respiratory tract infections admitted to hospitals in Hong Kong. More child-friendly nasal swab specimens could be used to replace NPA. However this would result in some loss of sensitivity, particularly for influenza A (0.40 vs 0.55), influenza B (0.17 vs 0.61) and RSV (0.68 vs 0.87).
2. New multiplex polymerase chain reaction diagnostic methods can reliably and quickly identify a wider range of 20 viral and atypical bacterial respiratory pathogens from nasal swab specimens. Further study is required to assess the potential time and cost advantages of this new diagnostic method over direct IF testing of NPA specimens.

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Introduction

Acute respiratory infection (ARI) accounted for about 40% of all paediatric admissions and two thirds of those admitted were under the age of 5 years.[1] Viral aetiologies play important roles in causing ARI in such young children. Early laboratory identification of the causative agent enables prompt implementaion of infection control measures and treatments, minimising the chance of cross-infection and the duration of symptoms and hospital stay. This matter has become more urgent and attracted more attention since 2003 following the outbreak of severe acute respiratory syndrome (SARS) that was caused by a novel coronavirus.

The standard investigations for the aetiology of suspected viral ARI in children admitted to the public hospitals in Hong Kong include direct immunoflurorescence (IF) of antigen and viral culture of nasopharygeal aspirate (NPA). The direct IF test rapidly detects influenza A and B, parainfluenza I, II, III, respiratory syncytial virus (RSV) and adenoviruses. However, the procedure of obtaining an NPA specimen is uncomfortable and frightening to children. It is also unpleasant for medical staff who have to carry out the process in struggling, crying and coughing children. In clinical practice, the optimal sampling methods must be balanced with patient's comfort, costs, effectiveness and risk to others. A few recent reports have shown that nasal swabs (NS) are as good as NPA for the detection of influenza virus using IF or enzyme immunoassay.[2,3] The sensitivity of using NS and IF for the diagnosis of RSV infection, however, is controversial.[3-5] The present study was undertaken to compare the usefulness of NS and NPA for the detection of a wide range of respiratory viruses using three different diagnostic tests (IF, culture, and polymerase chain reaction [PCR]) in children under 5 years of age.

Methods

This study was conducted from October 2005 to December 2006.

Setting and subjects

The study was conducted in a university hospital. One third of children under 5 years of age admitted for ARI were randomly enrolled, with written parental consent. The diagnostic criteria for ARI were: sudden onset (<36 hours) of one or more of the following symptoms and signs: rhinorrhoea, cough, sore throat, earache, hoarseness, stridor, wheeze, dyspnoea with or without fever.

Clinical specimens

The samples were taken by trained nurses. For NS, a cotton tipped swab (Copan, Italy) was placed 1 to 1.5 cm into the nostril and rotated 3 times against the surface of the nasal cavity. For NPA, the catheter was inserted into the opposite nostril to a depth of 5 to 7 cm and drawn back while applying gentle suction with an electric suction device.[2]

Immunofluorescence test

To screen for the presence of respiratory viruses, specimens were tested for

Table 1. Viral identification in 475 paired nasopharygeal aspirate (NPA) and nasal swabs (NS) specimens*

Viral detection	NPA (+) NS (-)	NPA (-) NS (+)	NPA (+) NS (+)	NPA (-) NS (-)	+ve in either or both samples
Adenovirus: +ve by any specimen and any method: 29 (6.1%)†					
IF	2	2	8	17	12 (2.5%)†
Culture	2	2	23	2	27 (5.7%)
PCR	5	0	20	4	25 (5.3%)
Influenza A: +ve by any specimen and any method: 40 (8.4%)					
IF	6	0	16	18	22 (4.6%)
Culture	3	4	22	11	29 (6.1%)
PCR	4	6	27	3	37 (7.8%)
Influenza B: +ve by any specimen and any method: 18 (3.8%)					
IF	9	1	2	6	12 (2.5%)
Culture	2	0	12	4	14 (2.9%)
PCR	2	1	15	0	18 (3.8%)
Parainfluenza (types 1-4): +ve by any specimen and method: 49 (10.3%)					
IF	9	2	15	23	26 (5.5%)
Culture	11	3	17	18	31 (6.5%)
PCR	9	3	37	0	49 (10.3%)
Respiratory syncytial virus: +ve by any specimen and method: 47 (9.9%)					
IF	12	3	29	3	44 (9.3%)
Culture	12	2	26	7	40 (8.4%)
PCR	10	2	28	7	40 (8.4%)
Subtotal of above: +ve by any specimen and method: 185 (38.9%)					
IF	38	8	71	68	126 (26.5%)
Culture	30	11	102	42	143 (30.1%)
PCR	30	12	129	14	171 (36%)
Other viruses: +ve by PCR: 73 (14.5%)					
PCR	21	13	39	-	73 (15.4%)
Grand total of viruses identified by any specimens and any methods: 258 (54%)					

* IF denotes direct immunoflurorescence test and PCR polymerase chain reaction
† % of 475 cases

influenza A, influenza B, parainfluenza (types 1 to 3), RSV and adenovirus by use of a direct IF test with fluorescein isothiocyanate (FITC)–conjugated monoclonal antibodies.

Virus culture

Virus culture was performed by inoculating approximately 200 μL of NS and NPA specimens onto HEp-2, MDCK and LLC-MK_2 cell monolayers.

Multiplex polymerase chain reaction

The multiplex PCR assays consist of five groups of multiplex nested fast PCR assays, targeting 20 respiratory pathogens (viruses and bacteria). Each multiplex method detected four microbes. Group 1 comprised influenza A and B group-specific and subtype H1, H3, H5-specific primers. Group 2 comprised parainfluenza virus types 1, 2, 3 and 4. Group 3 comprised respiratory syncytial virus A and B, rhinovirus, and enteroviruses. Group 4 comprised human coronavirus OC43, 229E, SARS-CoV, and human metapneumovirus. Group 5 comprised *Mycoplasma pneumoniae*, *Chlamydia pneumoniae, Legionella* and adenovirus. The nested PCR assays were run in a recently available fast thermal cycler (Applied Biosystems, US), which completes a 35-cycle PCR assay within 35 minutes, compared to the ~180 minutes of ordinary cyclers.

Statistical analyses

Agreement of the results by the two different sample collection methods (NPA, NS) was assessed using Cohen's kappa separately for each viral identification method (IF, culture and PCR). Sensitivities with NPA and NS specimens by IF, culture and PCR using any positive for any specimen by any diagnostic method as gold standard for influenza A, influenza B, parainfluenza, RSV and adenovirus according to the mode of sample collection were calculated and compared using the McNemar test.

Results

Paired samples were collected between November 2005 and October 2006 from 475 children under 5 years of age (253 boys, 222 girls). A summary of viral findings for the NPA and NS specimens using IF, culture and PCR methods is shown in Table 1. Multiplex PCR method detected additional viruses and bacteria in 11% and 13% of NS and NPA samples, respectively (Table 2). No SARS or H5 influenza viruses were detected. For the detection of respiratory viruses, agreement between IF, culture and PCR

Table 2. Detection of other respiratory pathogens in 475 paired nasal swab (NS) and nasopharyngeal aspirate (NPA) specimens by multiplex polymerase chain reaction methods

Respiratory pathogens	NPA (+) NS (-)	NPA (-) NS (+)	NPA (+) NS (+)	Either +ve/ total sample
Rhinovirus	8	0	15	22 (4.8%)
Coronavirus OC43	2	2	14	18 (3.8%)
Coronavirus 229E	1	0	2	3 (0.6%)
Severe acute respiratory syndrome	0	0	0	0
Metapneumovirus	4	4	3	11 (2.3%)
Enterovirus	0	1	1	2 (0.4%)
Chlamydia	2	1	0	3 (0.6%)
Legionella	0	0	0	0
Mycoplasma	4	5	4	13 (2.7%)
All	21	13	39	73 (15.4%)
Positive yield for NS: 52/475=10.9% Positive yield for NPA: 60/475=12.6%				

testing results of all NPA and NS specimens was substantial or perfect (Cohen's kappa, 0.72-0.92), except with the IF method that most positive results were from NPA (Cohen's kappa, 0.28). The sensitivities of using NPA/NS specimens and the three different virological methods to detect different respiratory viruses are shown in Table 3 (using any positive for any specimen by any diagnostic method as the gold standard). Reliance on NS specimens resulted in an overall 17% reduction in sensitivity compared with NPA specimens when IF method was employed. The sensitivities of IF method with NPA specimens were 15% (P=0.031), 44% (P=0.021), 19% (P=0.035) higher than that with NS for influenza A, influenza B and RSV, respectively. They were similar for parainfluenza and adenovirus. When PCR was used, the sensitivities between the two samples were similar for the detection of all studied viruses, except RSV (87% and 68% respectively for NPA and NS, P=0.039).

Discussion

Previous studies comparing NPA and NS used either viral culture or rapid testing (IF, enzyme immunoassay or enzyme-linked immunosorbent assay test), and all except one focused on RSV or influenza.[2-5] Our study used three different laboratory methods (IF, culture and PCR) to look for all important respiratory viruses and atypical bacteria. Our results showed that using NS specimens resulted in a 17% reduction in sensitivity compared to using NPA specimens when IF testing was employed. However, there were no significant differences in the sensitivity and specificity for the two specimens when PCR was used.

Among the diagnostic modes, viral culture has a high yield and has traditionally been the reference standard for diagnosis. However, it is usually irrelevant to clinical

Table 3. Comparison of nasopharyngeal aspirate (NPA) and nasal swab (NS) specimens for the detection of respiratory viruses by immunofluorescence (IF), culture and polymerase chain reaction (PCR) using any positive finding from any specimen by any method as the gold standard

Viral detection	NPA		NS		McNemar test*
	No. of +ve	Sensitivity	No. of +ve	Sensitivity	
Adenovirus: +ve for any specimen by any method: 29					
IF	10	0.34 (0.18-0.54)	10	0.34 (0.18-0.54)	0.999
Culture	25	0.86 (0.68-0.96)	25	0.86 (0.68-0.96)	0.999
PCR	25	0.86 (0.68-0.96)	20	0.69 (0.49-0.85)	0.063
Influenza A: +ve for any specimen by any method: 40					
IF	22	0.55 (0.38-0.71)	16	0.40 (0.25-0.57)	0.031
Culture	25	0.63 (0.46-0.77)	26	0.65 (0.48-0.79)	0.999
PCR	30	0.75 (0.59-0.87)	33	0.83 (0.67-0.93)	0.549
Influenza B: +ve for any specimen by any method: 18					
IF	11	0.61 (0.36-0.83)	3	0.17 (0.04-0.41)	0.021
Culture	14	0.78 (0.52-0.94)	12	0.67 (0.41-0.87)	0.500
PCR	16	0.89 (0.65-0.99)	16	0.89 (0.65-0.99)	0.999
Parainfluenza: +ve for any specimen by any method: 49					
IF	24	0.49 (0.34-0.64)	17	0.35 (0.22-0.50)	0.065
Culture	29	0.59 (0.44-0.73)	20	0.41 (0.27-0.56)	0.022
PCR	46	0.94 (0.83-0.99)	40	0.82 (0.68-0.91)	0.146
Respiratory syncytial virus: +ve for any specimen by any method: 47					
IF	41	0.87 (0.74-0.95)	32	0.68 (0.53-0.81)	0.035
Culture	38	0.81 (0.67-0.91)	28	0.60 (0.44-0.74)	0.013
PCR	38	0.81 (0.67-0.91)	30	0.64 (0.49-0.77)	0.039
Any of above: +ve for any specimen by any method: 180					
IF	108	0.60 (0.52-0.67)	78	0.43 (0.36-0.51)	<0.001
Culture	131	0.73 (0.66-0.79)	111	0.62 (0.54-0.69)	0.002
PCR	154	0.86 (0.80-0.90)	139	0.77 (0.70-0.83)	0.028

* McNemar test for the comparison of sensitivity of NPA and NS specimens for detection of respiratory viruses

decision making because of the long time required to obtain the results. The IF method using NPA specimens is known to be effective in diagnosing RSV and influenza and can yield results within a few hours and has been widely used in Hong Kong by both public and private hospitals. However, in most private clinics, collection of NPA has not been possible owing to the requirement of highly trained personnel, suitable suction devices, and a designated area with ventilation systems for infection control. The PCR methods are known to be the most sensitive and can yield results in a clinically relevant time frame, but they have not been used widely because they are technically demanding and expensive. The cost of a suction catheter and a mucus trap for the collection of an NPA specimen is HK$5 and a cotton-tipped swab for the collection of NS is HK$1. The laboratory cost for culture, IF and PCR varies in different settings. In general, reagent costs for IF range from HK$50 to $70 per sample, whereas for the five groups of multiplex PCR tests they range from HK$350 to $500 per sample. The turnaround time for IF is 2 to 3 hours, and for the fast-PCR is within 1 working day. Considering NS samples are much easier to collect and less intrusive to the patients than NPA, collecting NS samples for PCR test in a viorology laboratory with comprehensive facilities seems to be the best option for both public and private settings. This is especially true for surveillance of influenza outbreaks.

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