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Original article

Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study

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ABSTRACT

Objectives: Automated point-of-care molecular assays have greatly shortened the turnaround time of respiratory virus testing. One of the major bottlenecks now lies at the specimen collection step, especially in a busy clinical setting. Saliva is a convenient specimen type that can be provided easily by adult patients. This study assessed the diagnostic validity, specimen collection time and cost associated with the use of saliva.

Methods: This was a prospective diagnostic validity study comparing the detection rate of respiratory viruses between saliva and nasopharyngeal aspirate (NPA) among adult hospitalized patients using Xpert® Xpress Flu/RSV. The cost and time associated with the collection of saliva and nasopharyngeal specimens were also estimated.

Results: Between July and October 2017, 214 patients were recruited. The overall agreement between saliva and NPA was 93.3% (196/210, κ 0.851, 95% CI 0.776–0.926). There was no significant difference in the detection rate of respiratory viruses between saliva and NPA (32.9% (69/210) versus 35.7% (75/210); p 0.146). The overall sensitivity and specificity were 90.8% (81.9%–96.2%) and 100% (97.3%–100%), respectively, for saliva, and were 96.1% (88.9%–99.2%) and 98.5% (94.7%–99.8%), respectively, for NPA. The time and cost associated with the collection of saliva were 2.26-fold and 2.59-fold lower, respectively, than those of NPA.

Conclusions: Saliva specimens have high sensitivity and specificity in the detection of respiratory viruses by an automated multiplex Clinical Laboratory Improvement Amendments-waived point-of-care molecular assay when compared with those of NPA. The use of saliva also reduces the time and cost associated with specimen collection. **K.K.W. To, Clin Microbiol Infect 2019;25:372**

Introduction

Respiratory tract infection is frequently caused by respiratory viruses [1,2]. Rapid and accurate detection of respiratory viruses is important in guiding antimicrobial treatment and infection control precautions to improve patient outcome and to prevent nosocomial transmission, respectively [3,4]. The World Health Organization has included the diagnosis of and diagnostic tests for severe acute respiratory infections as a key research agenda [5].

Automated multiplex molecular assay has significantly improved respiratory virus testing through its simplicity, short turnaround time and high accuracy [6–9], and has been shown to lower the risk of hospital admission, reduce length of hospital stay, optimize the use of antivirals, shorten duration of antimicrobial

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treatment and reduce radiological investigations [10–12]. Despite the favourable turn-around-time and accuracy of these automated assays, significant delay occurs in real-life clinical practice due to the delay in specimen collection, transportation and testing.

The delay in specimen collection is often due to the waiting time for healthcare workers to collect nasopharyngeal aspirate (NPA) or nasopharyngeal swab (NPS), which are currently the recommended specimen types [13]. The collection of nasopharyngeal specimens also causes significant discomfort to patients and is associated with infection control risk to healthcare workers [14,15]. Despite a lower sensitivity than nasopharyngeal specimens, nasal and throat swabs are also specimen types recommended by clinical guidelines [16–18]. The collection of nasal or throat specimens, though less invasive than nasopharyngeal specimens, is still uncomfortable and healthcare workers are required for their collection [14].

Saliva has been proposed as an alternative specimen type in the diagnosis of respiratory virus infection [19–22]. Saliva can be provided easily by the patient. The use of saliva instead of nasopharyngeal specimens would avoid patient discomfort, reduce the risk of nosocomial transmission to healthcare workers and other patients, and shorten the time to diagnosis. Kim et al. showed that the detection rate was comparable between NPS and saliva (77.5% versus 76.3%) among military recruits [20]. In our previous study, 92% of patients with respiratory virus detected in their NPA also had the same virus detected in their saliva [19].

Despite these advantages, saliva is not used widely in clinical practice. The major hurdle is that saliva is not a recommended specimen type for respiratory virus testing in approved rapid molecular assay platforms [6,7]. Furthermore, the diagnostic validity of saliva testing by automated molecular assays has not been reported. In this study, we compared the diagnostic validity of saliva and NPA in the detection of respiratory viruses using an automated multiplex molecular assay, which has recently received the Clinical Laboratory Improvement Amendments waiver. We also estimated the reduction in cost and time associated with the collection of saliva when compared with those of nasopharyngeal specimens.

Materials and methods

Study design, participants and setting

This was a prospective diagnostic validity study conducted in Queen Mary Hospital, a teaching hospital in Hong Kong with 1600 beds. Written informed consent was obtained from study participants. Research nurses screened patients for eligibility from Monday to Friday except for holidays during the study period. Saliva and NPA specimens from adult hospitalized patients with respiratory tract infection were tested for influenza A virus, influenza B virus and respiratory syncytial virus (RSV) using Xpert® Xpress Flu/RSV assay (GeneXpert System, Cepheid, Sunnyvale, CA, USA). Monoplex RT-PCR was performed when there were discrepant results between saliva and NPA in the Xpress Flu/RSV assay [19]. All amplification curves were inspected manually. Cycle threshold (C_t) values from the Xpress Flu/RSV assay were used as a surrogate for viral load.

Data collection was planned before patients were recruited. The results of the molecular diagnostic assays were not available to the attending physicians. Clinical information was not available to the performers of the laboratory tests. Patients were considered to have severe disease if they required oxygen supplementation, were admitted into the intensive care unit or coronary care unit, or died, as we described previously [23–25]. This study has been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 17-266). Please refer to supplementary methods (Appendix S1) for details on the inclusion and exclusion criteria, Xpress Flu/RSV assay, monoplex RT-PCR, and specimen and data collection.

Specimen collection time and cost

To estimate the time required for the collection of saliva, NPS and NPA, we have recorded the specimen collection time used by three different healthcare workers in collecting each of the specimen types. For cost analysis, resource use and associated costs, including staff costs, accrued to the hospital were taken into consideration, adopting a hospital setting perspective (see Supplementary material, Appendix S1).

Statistical analysis

The detection rate of paired saliva and NPA specimens was compared using McNemar's test. Agreement between saliva and NPA was performed using κ statistics [26]. For sensitivity and specificity, the reference standard for a positive result was either concordant result between saliva and NPA, or discordant result, but either saliva and NPA tested positive for the same respiratory virus by monoplex RT-PCR. C_t values were compared using Wilcoxon matched-paired signed rank test. The correlation of C_t values between saliva and NPA was assessed using Spearman correlation coefficient. A p value of <0.05 was considered statistically significant. All statistical analysis was performed using GraphPad PRISM® version 6.07 or SPSS 23.0.

Results

Patients' characteristics

Between 10 July and 6 October 2017, a total of 732 patients were screened, and 214 patients were eligible for the study (Fig. 1, and Supplementary material, Table S1). The median age was 71 years, and 55.6% (119/214) were women. The most common symptoms were cough and shortness of breath (79%; 169/214). Hypertension was the most common underlying disease (54.2%; 116/214), followed by chronic heart disease (33.2%; 71/214) and diabetes mellitus (33.2%; 71/214). Fifty-nine patients (27.6%) had severe disease and required oxygen supplementation.

Comparison of respiratory virus detection between saliva and NPA

Among the 214 eligible patients, 210 had valid results in their NPA and saliva specimens by Xpress Flu/RSV assay (Fig. 2). There was overall high agreement between saliva and NPA (93.3%; 196/210, κ 0.851, 95% CI 0.776–0.926). Among patients with concordant results, 32.7% (64/196) had the same respiratory virus detected in both saliva and NPA, including 21.9% (43/196) with influenza A virus, 1.5% (3/196) with influenza B virus, and 9.2% (18/196) with RSV. The detection rate of respiratory viruses in saliva was lower than that of NPA, but not reaching statistical significance (32.9%; 69/210 versus 35.7%; 75/210; p 0.146).

Fourteen patients had discordant results between saliva and NPA by Xpress Flu/RSV assay (Fig. 2 and Table 1), including three patients with virus detected in saliva but not in NPA (Patients 1–3 in Table 1), nine patients with virus detected in NPA but not in saliva (Patients 4–12 in Table 1), and two patients with multiple viruses in their NPA but only one virus detected in their saliva (Patients 13 and 14 in Table 1). For Patients 1–3, the viruses detected by Xpress Flu/RSV assay in their saliva were also detected by monoplex RT-qPCR for all three patients. The saliva testing results could have changed patient's antiviral treatment or infection control precaution measures. For Patients 4–12, the viruses

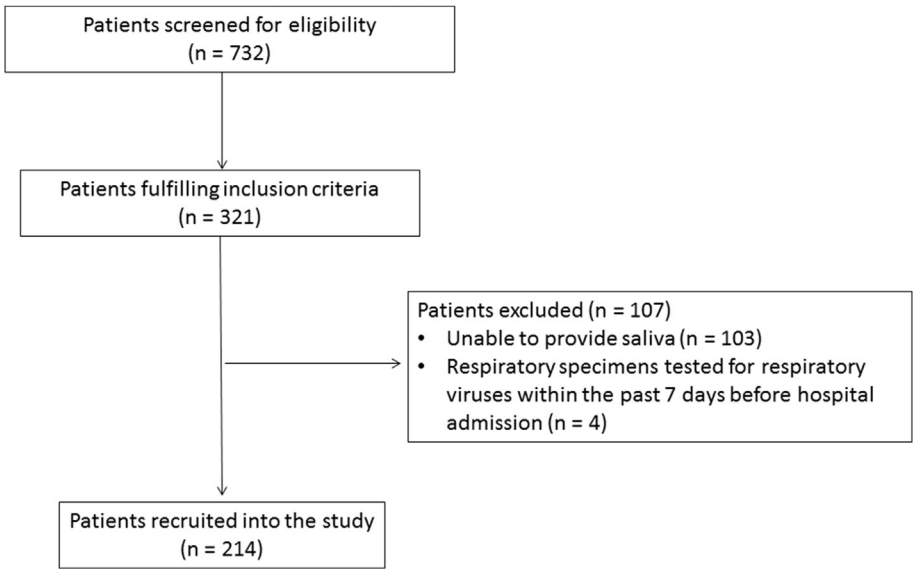

Fig. 1. Recruitment flow chart.

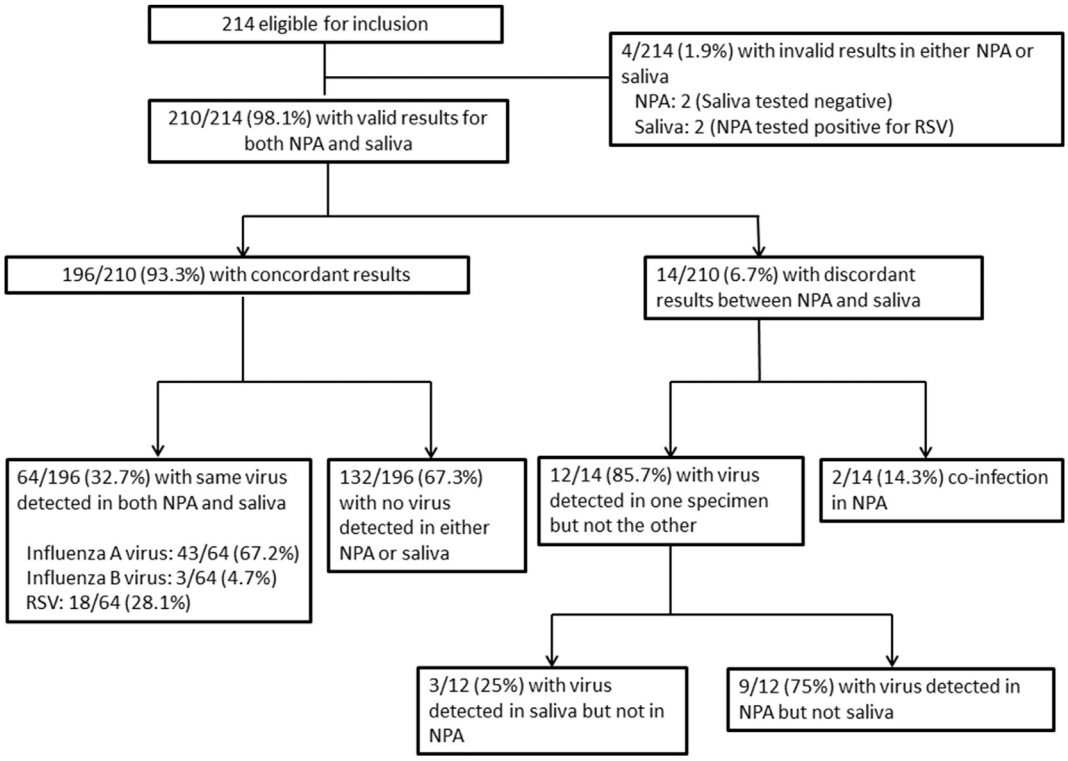

Fig. 2. Summary of saliva and nasopharyngeal aspirate results of Xpress® Flu/RSV in 214 patients.

detected in their NPA were also detected by monoplex RT-qPCR in seven patients. For Patient 12, for whom Xpress Flu/RSV assay detected RSV in NPA but not in saliva, monoplex RT-qPCR detected RSV in both NPA and saliva. For Patients 13 and 14, only the virus with the lowest C_t value (highest viral load) in the NPA specimen was detected in their saliva specimen by Xpress Flu/RSV assay.

We have further analysed the four patients with invalid/error results (see Supplementary material, Table S2). For the two patients with error results in saliva specimens (Patients 1 and 2 in Table S2), RSV was detected in the NPA specimens of both patients by Xpress Flu/RSV assay, and RSV was also detected in the saliva by monoplex RT-qPCR. For patient 1 in Table S2, manual inspection of the amplification curve of Xpress Flu/RSV assay showed that RSV RNA was amplified in saliva, indicating successful detection of RSV despite the machine indicating an error result. For the two patients with invalid results in their NPA specimens (Patients 3 and 4 in Table S2), respiratory virus was not detected by Xpress Flu/RSV assay in their saliva specimens; and monoplex RT-PCR for influenza A virus, influenza B virus and RSV were all negative in both of their NPA and saliva specimens.

Table 1
Patients with discordant results between saliva and nasopharyngeal aspirate by Xpress Flu/RSV assay

Patient no.	Gender/Age	Xpress Flu/RSV assay		Monoplex RT-PCR		Underlying disease	Diagnosis	Potential impact of testing result on patient treatment and infection control precautions
		NPA	Saliva	NPA	Saliva			
Only saliva tested positive by Xpress Flu/RSV								
1	M/61	ND	Influenza A virus	ND[a]	Influenza A virus	Hypopituitarism, gallstones	*Streptococcus pneumoniae* pneumonia and meningitis	Treatment with neuraminidase inhibitor
2	F/61	ND	RSV	ND[c]	RSV	Ischaemic heart disease, diabetes mellitus, hypertension, hyperlipidaemia	Acute coronary syndrome	Nil
3	F/68	ND	RSV	ND[c]	RSV	Acute myeloid leukaemia	Neutropenic fever	Contact precaution; treatment with ribavirin
Only NPA tested positive by Xpress Flu/RSV								
4	F/91	Influenza A virus	ND	Influenza A virus	ND[a]	Ischaemic heart disease, peripheral vascular disease, renal stones	Pneumonia	Treatment with neuraminidase inhibitor
5	M/82	Influenza A virus	ND	Influenza A virus	ND[a]	Renal stones	Pneumonia	Treatment with neuraminidase inhibitor
6	M/78	Influenza A virus	ND	ND[a]	ND[a]	COPD	COPD exacerbation	Treatment with neuraminidase inhibitor
7	F/81	Influenza A virus	ND	Influenza A virus	ND[a]	Hypertension, diabetes mellitus, hyperlipidaemia, stroke	Upper respiratory tract infection	Treatment with neuraminidase inhibitor
8	M/53	Influenza A virus	ND	Influenza A virus	ND[a]	Good past health	Nonarteritic anterior ischaemic optic neuropathy	Treatment with neuraminidase inhibitor
9	F/71	Influenza B virus	ND	Influenza B virus	ND[b]	Hypertension, hyperlipidaemia	Upper respiratory tract infection	Treatment with neuraminidase inhibitor
10	F/60	RSV	ND	ND[c]	ND[c]	Colonic polyp	Upper respiratory tract infection	Nil
11	M/66	RSV	ND	RSV	ND[c]	Diabetes mellitus, Schizophrenia	Upper respiratory tract infection	Nil
12	F/91	RSV	ND	RSV[d]	RSV[d]	Hypertension, atrial flutter	Pneumonia	Nil
Co-infection detected by Xpress Flu/RSV								
13	F/70	Influenza A virus[e] Influenza B virus[e] RSV[e]	Influenza A virus	Influenza A virus[g]	Influenza A virus[g]	Hypertension	Upper respiratory tract infection with vasovagal attack	Treatment with neuraminidase inhibitor
14	M/81	Influenza A virus[f] RSV[f]	RSV	RSV[h]	RSV[h]	Chronic rheumatic heart disease, atrial fibrillation	Acute bronchitis	Nil

Abbreviations: COPD, chronic obstructive pulmonary disease; F, female; M, male; ND, not detected; NPA, nasopharyngeal aspirate; RSV, respiratory syncytial virus.
[a] Monoplex RT-PCR for influenza A virus was performed.
[b] Monoplex RT-PCR for influenza B virus was performed.
[c] Monoplex RT-PCR for RSV was performed.
[d] The viral load in the NPA and saliva using monoplex qRT-PCR was 9.97×10^5 copies/reaction and 26 copies/reaction, respectively.
[e] The C_t values are: influenza A virus A 1: 27.4; influenza A virus A 2: 28.6; influenza B virus: 33.6; RSV: 35.3.
[f] The C_t values are: influenza A virus A1: 35.6; influenza A virus A 2: 39; RSV: 15.6.
[g] Influenza B virus and RSV were not detected by monoplex RT-PCR.
[h] Influenza A virus and influenza B virus were not detected by monoplex RT-PCR.

Analysis of sensitivity and specificity

Excluding the four patients with either invalid results or more than one virus detected in their NPA, the overall sensitivity and specificity for NPA was 96.1% (95% CI 88.9%–99.2%) and 98.5% (95% CI 94.7%–99.8%), respectively (Table 2). Excluding the two patients with error results in saliva, the overall sensitivity and specificity for saliva was 90.8% (95% CI 81.9%–96.2%) and 100% (95% CI 97.3%–100%), respectively.

Viral load analysis

Among the 64 patients with the same respiratory virus detected in both NPA and saliva, the C_t values were significantly lower in NPA than those in saliva for FluA1 ($p < 0.0001$), FluA2 ($p < 0.0001$) and RSV ($p < 0.004$) channel (see Supplementary material, Fig. S1a). Notably, 14.0% (6/43), 18.6% (8/43), 33.3% (1/3) and 22.2% (4/18) of patients had lower Ct values in their saliva than NPA for FluA1, FluA2, influenza B virus and RSV, respectively. There was no significant correlation between saliva and NPA for the C_t values for influenza A (FluA1, r^2 0.004; and FluA2, r^2 0.008) and RSV (r^2 0.039) ($p > 0.05$) (see Supplementary material, Figs S1b and S2).

Comparison of the time and cost associated with collection of saliva, NPS and NPA

The mean specimen collection time required for the collection of saliva (114 seconds) was 2.26-fold and 1.38-fold shorter than that required for the collection of NPA (259 seconds) and NPS (157 seconds), respectively (Fig. 3a). The mean cost of saliva collection ($1.16) was 2.59-fold and 2.09-fold lower than the costs of NPA ($2.77) and NPS ($2.03), respectively (Fig. 3b).

Discussion

Principal findings

In this study, we showed a high overall agreement (93.3%) between saliva and NPA specimens when tested by an automated multiplex molecular assay approved for point-of-care testing. The sensitivity of saliva was lower than that of NPA (90.8% versus 96.1%). However, the specimen collection time and the cost associated with the use of saliva were much lower than those of using nasopharyngeal specimens. Hence, saliva is a feasible specimen type for respiratory virus testing when used with automated molecular assays.

Comparison with other studies

Previous studies tested saliva for respiratory viruses using molecular assays that require a separate nucleic acid extraction step [19–22,27,28]. The current study tested saliva in a US Food and Drug Administration-approved automated multiplex molecular assay. The use of saliva in automated molecular assays can reduce the real-world turnaround time. One of the concerns of using saliva in automated molecular assays is that the viscous saliva may block the fluid channels within the system. However, in this study, the number of invalid/error results was the same for saliva and NPA. Therefore, saliva is a suitable specimen type for this kind of automated system.

The current study is also unique in that we estimated and analysed the time and costs associated with specimen collection. The shorter specimen collection time is important for busy clinical settings and the reduction in cost is important in resource-limited settings. This analysis has strengthened our recommendation to

Table 2
Results of Xpress Flu/RSV assay for saliva and nasopharyngeal aspirate when compared with patients' infection status

		Xpress Flu/RSV result	Patient with infection by A, B or RSV	Patient without infection by A, B or RSV	Total	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
NPA	Influenza A virus	Positive	48	2	50	98.0 (89.1–99.9)	98.8 (95.6–99.9)
		Negative	1	161	162		
		Total	49	163	212[a]		
	Influenza B virus	Positive	4	1	5	100 (39.8–100)	99.5 (97.4–100)
		Negative	0	207	207		
		Total	4	208	212[a]		
	RSV	Positive	23	2	25	92.0 (74.0–99.0)	98.9 (96.2–99.9)
		Negative	2	185	187		
		Total	25	187	212[a]		
	Total	Positive	73	2	75	96.1 (88.9–99.2)	98.5 (94.7–99.8)
		Negative	3	132	135		
		Total	76	134	210[b]		
Saliva	Influenza A virus	Positive	45	0	45	91.8 (80.4–97.7)	100 (97.8–100)
		Negative	4	163	167		
		Total	49	163	212[c]		
	Influenza B virus	Positive	3	0	3	75.0 (19.4–99.4)	100 (98.2–100)
		Negative	1	208	209		
		Total	4	208	212[c]		
	RSV	Positive	21	0	21	91.3 (72.0–98.9)	100 (98.1–100)
		Negative	2	189	191		
		Total	23	189	212[c]		
	Total	Positive	69	0	69	90.8 (81.9–96.2)	100 (97.3–100)
		Negative	7	136	143		
		Total	76	136	212[c]		

Abbreviations: A, influenza A virus; B, influenza B virus; NPA, nasopharyngeal aspirate; RSV, respiratory syncytial virus.
[a] Excluded two patients with invalid results from Xpert Xpress Flu/RSV assay in their NPA.
[b] Excluded two patients with invalid results and two patients with more than one virus detected by Xpert Xpress Flu/RSV assay in their NPA.
[c] Excluded two patients with error results from Xpert Xpress Flu/RSV assay in their saliva.

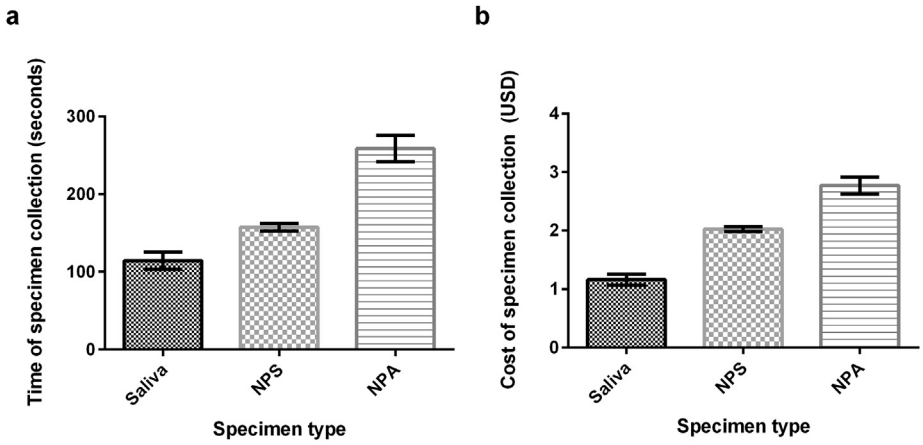

Fig. 3. Time (a) and cost (b) required for specimen collection per patient. The specimen collection time includes the time associated with the specimen collection, informing and instructing the patient about the procedure, and the procedure of putting on and removing masks and gloves. Data on the specimen collection time represents the mean ± standard deviation of the length of time measured for three healthcare workers. NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab.

implement the use of saliva for respiratory virus testing in the clinical setting.

Most of the previous studies on saliva testing were conducted in children or young adults, or at outpatient clinics or at emergency departments [20,21]. The current study enrolled hospitalized adult patients, including patients who developed severe disease during the hospitalization. This is important because patients with severe disease are likely to benefit most from laboratory confirmation.

The lower sensitivity of saliva, when compared with NPA, is probably due to the lower viral load in the saliva for most patients. In the current study, seven patients (9.2%) had respiratory viruses detected in their NPA but not in their saliva by both Xpress Flu/RSV assay and our in-house monoplex RT-PCR assay. Therefore, for patients with high clinical suspicion of respiratory virus infection but negative saliva result, NPA should be tested. However, there was a poor correlation in the viral load between paired saliva and NPA, and some patients had higher viral load in the saliva than in the NPA. Notably, respiratory virus could be detected in the saliva for three patients with negative NPA (3.8%). The saliva test results for these three patients have potential impact on the decisions on antiviral treatment and infection control precautions.

The sensitivity of saliva in the current study is much higher than that found in a previous paediatric study, which showed a much lower sensitivity (74%) for saliva specimens [21]. The higher sensitivity of saliva in the current study may be related to the assay used. In Xpress Flu/RSV, 300 μL of specimen in viral transport medium is used for nucleic acid extraction and all extracted nucleic acid is used for the PCR. However, in systems where nucleic acid extraction is performed as a separate step, the amount of nucleic acid used for the PCR is usually much less. Hence the high sensitivity of this automated multiplex assay has circumvented the problem of low viral load for some patients.

Limitations of this study

First, we only recruited adult patients. Further evaluation should be conducted in the paediatric population. Second, this study only included hospitalized patients with more severe disease. As viral load may be lower in patients with milder symptoms, further studies should be conducted in the outpatient setting. Third, Xpress Flu/RSV only detects influenza A virus, influenza B virus and RSV. Further studies should evaluate the accuracy for other respiratory viruses. Fourth, during the study period, there were very few patients with influenza B virus infection and no patients infected with avian influenza viruses. As the tissue tropism of avian influenza viruses can be different from that of seasonal influenza viruses [29], the use of saliva for these patients should be confirmed in future studies. Finally, among 321 patients fulfilling the inclusion criteria, 107 were excluded because these patients could not spit out a sufficient volume of saliva. Therefore, some patients would still require nasopharyngeal aspirate or swab for respiratory virus testing.

Conclusions and implications on clinical practice and research studies

The higher sensitivity of automated molecular assays has circumvented the problem of lower viral load in saliva specimens. As saliva can be collected easily with minimal equipment and manpower, saliva is a viable option for the detection of respiratory viruses. The use of saliva instead of nasopharyngeal specimens may also enhance recruitment of subjects in clinical studies. In community surveillance studies, the use of saliva allows self-collection. Saliva should be added to the list of recommended diagnostic specimen types for traditional or automated respiratory virus molecular assays in clinical or research settings.

Transparency declaration

All authors declare no conflict of interest.

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Contributions to authorship

KKWT, CCYY, CYWL, RWSP, IFNH and KYY designed the study. KKWT, CCYY, CYWL, DTYH, PKPP, ACKN and KHL acquired the data. KKWT and CKHW carried out the statistical analysis. All authors interpreted the data, revised the manuscript critically for important intellectual content and approved the final report.

Data sharing

The investigators will share data used in developing the results presented in this manuscript on request to the corresponding author. Anonymized record level data will be made available on proposal for analysis by those who have received ethical clearance from their host institution.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.06.009.

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