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Effectiveness of oral fluid in pathogenic surveillance of acute respiratory infection

Running Head: Using Oral fluid to detect acute respiratory infection

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Abstract

Objective: As a new safe, non-invasive, convenient and efficient biological sample, oral fluid (OF) can be used for virus nucleic acid and antibody detection. Because of few studies on multiple respiratory pathogens surveillance, this study was to explore the application value of OF in this field.

Methods: OF and throat swabs were collected from acute respiratory tract infections in Beijing, from December 2020 to December 2021. Multiplex real-time PCR assay was performed. The detection performance of two samples was compared.

Results: A total of 769 OF and throat swab samples were collected. The detection rates of respiratory pathogens in throat swabs and OF were 29.26% (225/769) and 20.81% (160/769), respectively. Compared with the throat swab, the sensitivity and specificity of the OF assay were 71.11% (160/225) and 100% (544/544), respectively, and the two assays had an excellent agreement (kappa = 0.78). The consistency of different pathogens was different. For OF samples, the most common pathogen was the influenza B virus and the highest detection rate was in the ≤5-year-old group. The highest positive rate was in December 2021.

Conclusion: OF samples have great potential in the epidemiological surveillance of respiratory pathogens and would have application prospects in preventing and controlling infectious diseases.

Key words: oral fluid; throat swab; nucleic acid detection; respiratory pathogen

Introduction

Acute respiratory tract infection (ARTI) is a major cause of high morbidity and mortality globally [1]. There are many kinds of respiratory pathogens that cause ARTI, including influenza virus, respiratory syncytial virus (RSV), human coronaviruses (HCoVs), rhinovirus (RV), human

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parainfluenza viruses (HPIVs), and adenovirus (ADV). Currently, emerging pathogens continue to be detected and cause large-scale epidemics, such as severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, influenza A H1N1 virus in 2009, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) circulating globally now. The detection and surveillance of respiratory pathogens can not only make a timely clinical diagnosis and offer guidance for precision medicine, but also comprehensively and systematically help control the epidemic and identify the variation pattern of respiratory pathogens, providing a scientific basis for the early warning, prediction, prevention, and control of respiratory infectious diseases [2,3].

Presently, nasal or throat swabs are the dominant methods for collecting specimens for detecting and monitoring respiratory pathogens. The sampling of these swabs can cause severe nausea, retching, and other discomforts for subjects. Sample collection requires professional operation by trained medical staff who must wear personal protective equipment and bear a greater risk of infection. Therefore, it is necessary to explore a new method of collecting biological specimens that is safe, convenient, and efficient for the detection and surveillance of respiratory pathogens.

Oral fluid (OF) is a mixture of salivary gland secretions and gingival crevicular fluid that can be used to detect viral nucleic acids as well as plasma-derived IgM and IgG antibodies for antibody detection. OF can be self-collected, and thus is advantageous for safety, non-invasiveness, convenience, and efficiency. During the coronavirus disease 2019 (COVID-19) pandemic, several countries, such as the United States [4,5], England [6], and Japan [7], used OF-like samples for nucleic acid and antibody detection of SARS-CoV-2. In 2021, the Beijing Center for Disease Prevention and Control (BJCDC) [8,9] used OF for nucleic acid and antibody

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testing of SARS-CoV-2 in China, showing that OF has significant application value. However, there is no related study on OF samples for other respiratory pathogens in China.

Based on the Respiratory Pathogen Surveillance System (RPSS) in Beijing, we collected OF samples from ARTI cases and conducted a comparison with paired throat swabs to explore the detection performance of OF samples. This study provided valuable information for exploring the use of a new biological specimen and improving the level of prevention and control of critical respiratory tract infectious diseases such as COVID-19 and influenza.

Methods

Study population

Based on the RPSS, ARTI cases, including acute upper respiratory tract infection (AURTI) cases and community-acquired pneumonia (CAP) cases, were collected from Beijing Haidian Hospital, Beijing Luhe Hospital, and Beijing Tongren Hospital during two periods, from December 2020 to February 2021 and from October 2021 to December 2021. AURTI was defined as a fever (≥38℃) accompanied by cough or sore throat, nasal congestion, a runny nose, expectoration, and other upper respiratory symptoms. The diagnostic criteria of CAP were according to the Guidelines for the Diagnosis and Treatment of Community-Acquired Pneumonia in China [10,11].

Sample collection and processing

Paired OF and throat swabs were collected simultaneously from each individual. OF was self-collected using the Oracol collection device (Malvern Medical Developments, UK, Cat number: S10) following the manufacturer's instructions: the foam swab was removed from the collection tubes and used to swab the gum line for 90 seconds until the swab was completely

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soaked; the swab was then placed in the collection tube. Once sampling was completed, the collection tube was returned to the laboratory for processing within 24 hours. Elution buffer [9] was then added to each collection device for possessing, and the swab was removed and placed inside the cap rather than the tube. Next, the devices were centrifuged at 1,500 rpm for 1 min, and the supernatant was collected into 2 mL sterile tubes and stored at −20°C.

Throat swabs were collected according to the protocol on prevention and control of COVID-19 (Trial Version 8) [12]. All samples were stored at –20°C before laboratory testing.

Method optimization and laboratory test

Nucleic acid extraction**:** Total nucleic acids were extracted from the specimens using Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processors (Thermo Fisher).

Optimization of OF elution buffer quantities: OF samples of confirmed COVID-19 cases were collected. 0.6mL and 1.00mL elution buffer were used for OF samples processing, respectively. The SARS-CoV-2 nucleic acid test used PCR kits (Shanghai Berger Medical Technology Co. Ltd., Cat number: ZC-HX-201-2), and the PCR results of the different volumes were compared. The result was positive if the sample had an S-shaped amplification curve and cycle threshold (Ct) value of ORF1ab gene and N gene ≤38.00.

Optimization of PCR reaction system: 56 OF samples with paired throat swabs that were positive for respiratory pathogens were selected for PCR detection (Jiangsu Uninovo Biological Technology Co. Ltd., Cat number: CN12-33DA). 2μL and 5μL nucleic acid were added to PCR reaction mixture, respectively, and the PCR results were compared.

Nucleic acid detection: A multiplex combined real-time PCR detection kit (Jiangsu Uninovo Biological Technology Co. Ltd., Cat number: CN12-33DA) was used to detect respiratory

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pathogens for all paired OF and throat swab samples. The kit simultaneously identified the following 12 common respiratory pathogens: SARS-CoV-2, influenza A virus (Flu-A) (including H1N1 and H3N2), influenza B virus (Flu-B) (including Victoria and Yamagata lineage), HPIVs (HPIV-1/2/3/4), RSV, RV, ADV, human metapneumovirus (HMPV), enterovirus (EV), HCoVs (HCoV-NL63/OC43/229E/HKU1), *Mycoplasma pneumonia* (MP) and *Chlamydia pneumonia*. The result was positive if the sample had an S-shaped amplification curve and a Ct value ≤35.00. In contrast, the result was negative.

Quality control of sampling

The concentration of human IgG antibody (HIgG) was measured using HIgG antibody detection kits (Bioscience, Tianjin, China, Lot number: G202108003) to verify the quality of the OF samples. The sample was qualified when the HIgG antibody concentration was >0.3 μg/mL [9]; otherwise, the sample was removed.

Statistical analysis

Continuous variables were presented as mean or median (interquartile range, IQR) and categorical variables were presented as rate (%). Kappa analysis (95% confidence interval, 95%CI) was used to evaluate the consistency of the two samples, with a score ＞0.75 as an excellent agreement, 0.60 < kappa ≤0.75 as a high agreement, 0.40< kappa ≤0.60 as a medium agreement, and ≤0.40 as a poor agreement [13]. McNemar's test was used to compare the detection rates for the two sampling methods for the numbers of patients. A two-sided P value lower than 0.05 was considered statistically significant. Data were analyzed using Microsoft Excel 2019 and SPSS 19.0 (IBM, New York, USA).

Ethics statement

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The protocol of this study was approved by the Ethics Committee of the BJCDC. A written informed consent was obtained from each of the enrolled patients or their legal guardians.

Results

Optimization of OF detection method

Optimization of OF elution buffer quantities: Four OF samples from two confirmed COVID-19 cases were collected. PCR results showed that all samples were positive for SARS-CoV-2 when using 0.60 and 1.00 mL OF elution buffer for processing samples. For case 1, when 0.60 and 1.00 mL OF elution buffer were added, the Ct values for the ORF1ab gene for SARS-CoV-2 were 30.60 and 31.95, respectively, and Ct values for the N gene were 29.56 and 32.47, respectively. For case 2, when 0.60 and 1.00 mL OF elution buffer were added, the Ct values for the ORF1ab gene for SARS-CoV-2 were 33.60 and 34.23, respectively, and Ct values for the N gene were 34.21 and 34.47, respectively. The Ct value for 0.60 mL OF elution buffer was 0.26–2.91 lower than that for 1.00 mL elution buffer. Therefore, the 0.60-mL volume of OF elution buffer was selected for processing OF samples.

Optimization of PCR reaction system: 56 OF samples were selected for PCR detection. Addition of 2 and 5μL of nucleic acid resulted in the positive detection of 0 and 20 samples, respectively. Therefore, 5μL nucleic acid was selected for PCR detection with OF samples.

Comparison of respiratory pathogen detection

Study population: A total of 769 ARTI cases were enrolled in the study. The age range of the patients was from 3 to 100 years, with a median age of 28 years (IQR, 23–36). Enrolled cases included 387 males, 382 females; 5 children (≤14 years old), 762 adults (>14 years old), and 2 individuals without specified age information; a total of 709 patients had an AURTI, 60 patients

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had CAP.

Detection results of respiratory pathogens: Among the 769 cases, 225 (29.26%, 225/769) cases were positive for at least one pathogen on throat swabs, whereas for OF, 160 (20.81%, 160/769) cases were positive for at least one pathogen. The detection rate for the OF was significantly lower than that of throat swabs ($P < 0.001$). Using throat swabs as reference standard, OF had a sensitivity of 71.11% (160/225) and specificity of 100% (544/544). The total coincidence rate and kappa value were 91.55% and 0.78 (95% CI, 0.75-0.80), respectively (Table 1). According to the standard of kappa $>$ 0.75, the strength of agreement between OF and throat swab samples was excellent.

Comparison of different respiratory pathogens: Nine pathogens were detected in all cases, and the detailed results are shown in Table 2. The kappa values for Flu-B, MP, RSV, and ADV between throat swab and OF samples were 0.92 (95% CI, 0.90–0.94), 1.00, 0.89 (95% CI, 0.78–1.00), and 1.00, respectively. The results of the two sampling methods had an excellent agreement. The kappa value of HCoVs and EV were 0.64 (95% CI, 0.52– 0.75) and 0.61 (95% CI, 0.45–0.77), respectively. The results of the two methods had a high agreement. The kappa value for HPIVs was 0.47 (95% CI, 0.35–0.60), which was categorized as a medium agreement. A poor agreement was detected for RV (kappa = 0.40). There were three HMPV-positive cases for the throat swab but these had negative results in the paired OF samples.

Comparison of the Ct value: Among 225 positive cases, the Ct values of 10 cases were missing. The Ct value was between 12 to 35 among the 215 positive samples for the throat swab, with a median value of 24 (IQR, 19–26). The Ct value of the 150 positive samples for OF was between 15 to 35, with a median value of 26 (IQR, 24–28). The Ct value of the throat swabs was

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significantly lower than that of the OF ($P < 0.001$). When the Ct value of the throat swab ≤30, 70.53% (146/207), the paired OF samples were positive, whereas when the Ct value of the throat swab >30, 50.00% (4/8) of paired OF samples were positive (Table 3).

Epidemiological characteristics

Pathogen spectrum: For the OF samples, the most common pathogen was Flu-B (123/160, 76.88%), followed by RV (8/160, 5.00%), HCoVs (8/160, 5.00%), PIVs (6/160,3.75%), MP (5/160, 3.12%), EV (4/160, 2.50%), RSV (4/160, 2.50%) and ADV (2/160, 1.25%), HMPV was not detected. For throat swab samples, the most common pathogen was Flu-B (140/231, 60.61%), followed by RV (31/231, 13.42%), PIVs (19/231, 8.23%), HCoVs (17/231, 7.36%), EV (9/231, 3.90%), MP (5/231, 2.16%), RSV (5/231, 2.16%), HMPV (3/231, 1.30%) and ADV (2/231, 0.86%). The order of several respiratory pathogens was slightly different between the OF and throat swab samples, but the overall distribution of respiratory pathogens was fundamentally similar.

Age distribution: Among the 769 cases, two cases lacked age information. For the OF samples, the highest detection rate was observed in the ≤5-year-old group (2/2, 100%), followed by the 15–59-year-old (151/709， 21.30%) and ≥60-year-old (7/53, 13.21%) groups; no positive cases were detected in the 6–14-year-old group (0/3, 0.00%). Similarly, the throat swabs had the highest detection rates in ≤5-year-old group (2/2, 100%), followed by the 15–59-year-old (211/709, 29.76%) and ≥60-year-old (11/53, 20.75%) groups; no positive case was detected in the 6–14-year-old group (Figure 1).

Gender distribution: For the OF samples, the detection rate of males and females were 21.96% (85/387) and 19.63% (75/382), respectively. Similarly, the detection rate of males for the

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throat swab was 30.23% (117/387), which was slightly higher than that of females 28.27% (108/382).

Temporal distribution: From December 2020 to February 2021 and from October 2021 to December 2021, the monthly detection rates of OF samples over the six months tested were 7.17% (19/265), 10.34% (3/29), 0.00% (0/8), 8.93% (5/56), 17.97% (39/217), and 48.45% (94/194), respectively; the monthly detection rates of throat swab samples for these months were 18.11% (48/265), 10.34% (3/29), 25.00% (2/8), 8.93% (5/56), 26.27% (57/217) and 56.70% (110/194), respectively. The highest detection rates for both methods were in December 2021(Figure 2). From December 2020 to February 2021, there were low numbers of positive cases and low positive detection rates of respiratory pathogens. The positive rates of overall respiratory pathogens were 7.28% (22/302) and 17.55% (53/302) for the OF and throat swabs, respectively. From October 2021 to December 2021, the types of respiratory pathogens and the number of positive cases increased per month. The positive detection rates of overall respiratory pathogens increased to 29.55% (138/467) and 36.83% (172/467) for the OF and throat swabs, respectively, and the most prevalent pathogen was Flu-B with detection rates of 26.34% (123/467) and 29.98% (140/467) for the OF and throat swabs, respectively.

Discussion

In 1987, OF was first used for the detection of hepatitis A and HIV antibodies by Public Health England [14]. Subsequently, OF has been successfully used for the detection and surveillance of pathogens such as measles, rubella, and mumps [15,16]. During the COVID-19 pandemic, OF-like samples were shown to have high consistency with nasopharyngeal swabs and serum samples in the detection of SARS-CoV-2. Several studies [5,7]showed that OF samples had

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similar sensitivity with nasopharyngeal swabs in nucleic acid detection of SARS-CoV-2, with a high coincidence rate of 97.40%. Public Health England [6] had also shown that compared with serum, the SARS-CoV-2 antibody detection assay of OF samples had a sensitivity of 75% and specificity of 99%, which made this a tool suitable for population-based seroepidemiology studies. However, these studies have not yet been published on using OF samples for respiratory pathogens. Our study was the first time that OF samples had been used to detect respiratory pathogen in Beijing to explore their value for the detection and surveillance of nucleic acids from respiratory pathogens.

In this study, we optimized several experimental conditions to improve the detection rate of OF samples. Our results showed that compared with the throat swabs, the sensitivity and specificity of OF nucleic acid detection assay were 71.11% and 100%, respectively. These results were similar to a previous study where the sensitivity and specificity of saliva assay were 68.1% and 97.6%, respectively [17]. These variations in sensitivity and specificity may be due to different experimental conditions. Though the detection rate of OF was slightly lower than that of throat swabs, the level of agreement between the throat swabs and OF samples was excellent (kappa = 0.78). We speculated that the difference in the detection rate between OF and throat swabs may be related to different characteristics of pathogens.

We analyzed the Ct values of OF and throat swab samples and found that the Ct value of OF samples was generally higher than that of throat swabs. However, when the Ct value for the throat swab was ≤30, the paired OF samples had a high detection rate of 70.53%, and when the Ct value for the throat swab was >30, the detection rate of OF samples decreased to 50.00%. These data indicated that the detection rate of OF sample was associated with the viral load of the oral area

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and that patients with a high viral load had a high probability of OF detection. It was reported that the SARS-CoV-2 Omicron variant had several characteristics[18], such as high viral load and low Ct value, indicating that OF samples may likely have a high consistency with throat swabs and considerable potential to detect the SARS-CoV-2 Omicron variant.

The detection rate of OF samples was associated with pathogenic species. Recently, saliva has been suggested as an alternative sample for influenza virus diagnosis. Sueki et al. [19] detected influenza virus from 144 paired nasopharyngeal swabs and saliva samples and showed a 95.8% concordance for saliva and the nasopharyngeal swabs. Yoon et al. [20] performed PCR assays on 385 influenza-like cases and demonstrated coincidence rates of 93.5% and 97.1% for Flu-A and Flu-B between paired saliva and nasopharyngeal swabs, respectively. Similarly, our study demonstrated a coincidence rate of 97.79% for the influenza virus between the throat swab and OF with a kappa value was 0.92, indicating that the two sampling methods had an excellent agreement. Notably, all influenza virus samples belonged to Flu-B in our study, and no Flu-A-positive cases were detected. Studies showed that influenza viruses mainly infected respiratory epithelial cells and bound to sialic acid receptors[21], a large amount of sialic acid was found in the human trachea, bronchus, and saliva [22,23], and this could explain the excellent consistency for influenza virus between the two sampling methods.

Our study showed that throat swabs and OF samples had an excellent consistency for ADV, RSV, and MP and a high agreement for EV and HCoVs, although there was a poor consistency for RV and HPIVs. Differences in the consistency of respiratory pathogens may be related to possible heterogeneity in their replication sites. A previous study [24] performed multiplex RT-PCR on 236 ARTI cases, and the results showed the detection rate of ADV in saliva (15.8%) was higher

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than that in nasopharyngeal swabs (1.2%) ($P < 0.0001$). However, in this study, the throat swab and OF samples had an excellent agreement (kappa = 1), the difference may be because ADV had a low prevalence in our study and this could also prove that one major replication site of ADV is the oropharynx [17]. Furthermore, the consistency of the two sampling methods was poor for RV, and the detection rate of OF was lower than that of the throat swabs. The result was similar to Kim et al.[24] where there was a higher detection rate of RV in nasopharyngeal swabs (34.4%) compared to saliva (27.5%). RV mainly binds to intercellular adhesion molecule-1 (ICAM-1) and low-density lipoprotein receptor (LDLR) in the nasal epithelial cells and basolateral plasma membrane of the polarized airway to cause respiratory disease [25]. Therefore, we speculated that RV has high rates of detection in nasopharyngeal swabs. HPIVs can effectively replicate in the ciliated epithelial cells of the upper and lower respiratory tract [26]. HPIV-3 replication is mainly localized in the lower respiratory tract [27], and HPIV-3 is the most epidemic serotype in Beijing [28]. This may be the reason for the poor consistency of HPIVs and could explain the low detection rates of OF samples compared with those of throat swabs in several months in this study. Therefore, the application of OF samples in the detection of respiratory pathogens should consider the characteristics of the targeted pathogens.

Although the order of several respiratory pathogens slightly differed in the pathogen spectrum results between the throat swab and OF samples, the overall distribution of the respiratory pathogen spectrum was similar. We speculated that this may be associated with a lack of positive cases of several respiratory pathogens or the specific characteristics of several pathogens. Moreover, the distribution trend of respiratory pathogens in all age groups and gender were similar between OF and throat swabs. Among them, the highest detection rate was in the

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≤5-year-old group, followed by the 15–59-year-old and ≥60-year-old groups. Especially, OF samples have outstanding advantages, including safety, non-invasiveness, and convenience when used for young children and older adults with reduced mobility, as well as in cases of large-scale nucleic acid testing and shortages of medical staff. During the COVID-19 pandemic, self-collection of OF samples at home could solve problems related to inconvenient and uncooperative sampling and avoid large-scale exposure. Thus, OF would have considerable potential as a supplementary sample to detect SARS-CoV-2.

Public health measures implemented during the COVID-19 pandemic substantially reduced the prevalence of common respiratory pathogens, and consequently the detection rate of respiratory pathogens was significantly decreased. To study the epidemiological characteristics of respiratory pathogens at different times during the COVID-19 pandemic in Beijing, we analyzed the detection results of paired throat swabs and OF samples for ARTI cases. OF samples showed an overall 7.28% detection rate of respiratory pathogens from December 2020 to February 2021, which represented a low prevalence. This was significantly lower than the detection rates of respiratory pathogens reported in Beijing and other areas of China in the same season before COVID-19 [29-31]. Notably, from October to December 2021, the detection rate increased to 29.55% and Flu-B was the most prevalent pathogen with a detection rate of 26.34%. Other studies had reported a similar phenomenon. The detection rate of the influenza virus in northern China was reported to be 31.80% in week 52 of 2021, of which 98% of cases were Flu-B [32]. Many countries and regions reported higher influenza prevalence in winter 2021–2022 than that in winter 2020–2021, particularly in Europe, North America, Africa, and China [33]. The time trend of respiratory pathogens detected by throat swabs was similar with the trend detected by OF

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samples, the detection rate of Flu-B increased to 29.98% from October to December 2021. As the prevention and control of the COVID-19 pandemic become normalized, the detection rate of respiratory pathogens, especially influenza viruses, has increased, suggesting that we should be fully prepared for the co-epidemic of influenza viruses and SARS-CoV-2. Although OF is less sensitive than throat swabs in individual detection and clinical diagnosis, the epidemiological characteristics detected by OF samples are similar to those detected by throat swabs in the epidemiological surveillance of respiratory pathogens. Therefore, OF samples would be of considerable value in respiratory pathogenic surveillance.

There were several limitations to our study. During the COVID-19 pandemic, ARTI cases had been significantly reduced, and the respiratory pathogens subsequently exhibited low prevalence and detection rates, and the number of positive cases of several pathogens was minimal. Therefore, the application value of OF sampling could not be comprehensively evaluated, and further research should be conducted by expanding the sample size.

In summary, the epidemiological characteristics detected by OF samples are similar to those detected by throat swab samples, so OF samples have considerable application value in the diagnosis and epidemiological surveillance of respiratory infectious diseases. In addition, as a non-invasive, safe, and convenient new biological specimen, OF is especially suitable for children and older adults, as well as when large-scale nucleic acid detection is required and during medical staff shortages and would have an important application prospect in the prevention and control of COVID-19 and influenza.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1 Comparison of PCR results between throat swab and oral fluid samples

		Throat swab		
		Positive	Negative	Total
Oral Fluid	Positive	160	0	160
	Negative	65	544	609
	Total	225	544	769

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Table 2 Comparison of different respiratory pathogens between the throat swab and OF samples

	throat swab (detection rate%)	oral fluid (detection rate%)	Agreement (%)			Kappa value (95%CI)
			positive	negative	total	
Flu-B	140 (18.21)	123(15.99)	87.86	100.00	97.79	0.92(0.90-0.94)
RV	31(4.03)	8(1.04)	25.81	100.00	97.01	0.40(0.30-0.50)
PIVs	19(2.47)	6(0.78)	31.58	100.00	98.31	0.47(0.35-0.60)
HCoVs	17(2.21)	8(1.04)	47.06	100.00	98.83	0.64(0.52-0.75)
EV	9(1.17)	4(0.52)	44.44	100.00	99.35	0.61(0.45-0.77)
MP	5(0.65)	5(0.65)	100.00	100.00	100.00	1.00
RSV	5(0.65)	4(0.52)	80.00	100.00	99.87	0.89(0.78-1.00)
HMPV	3(0.39)	0	—	—	—	—
ADV	2(0.26)	2(0.26)	100.00	100.00	100.00	1.00
Total	231*	160	—	—	—	—

Note: * Since coinfections were presented in 6 cases and the number of each pathogen was calculated as 1 case, the sum of pathogen infections was greater than the total positive patients; "—" means no calculation; OF, oral fluid

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Table 3 Comparison of Ct value between throat swab and paired OF samples

Ct value of throat swab	Positive cases of throat swabs	Positive cases of paired oral fluid	The detection rate of paired oral fluid (%)
Ct ≤ 30	207	146	70.53% (146/207)
Ct>30	8	4	50.00% (4/8)

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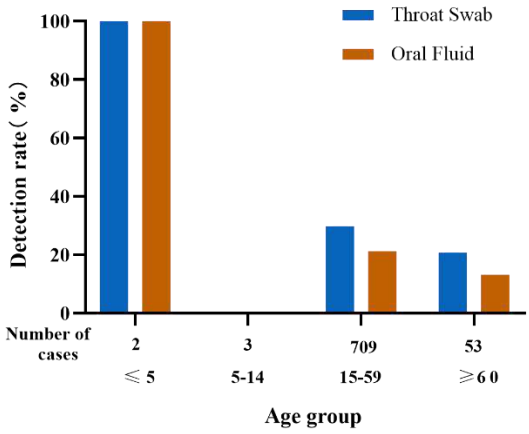

Figure 1 Distribution of respiratory pathogen infections by age group in Beijing from December 2020 to December 2021.

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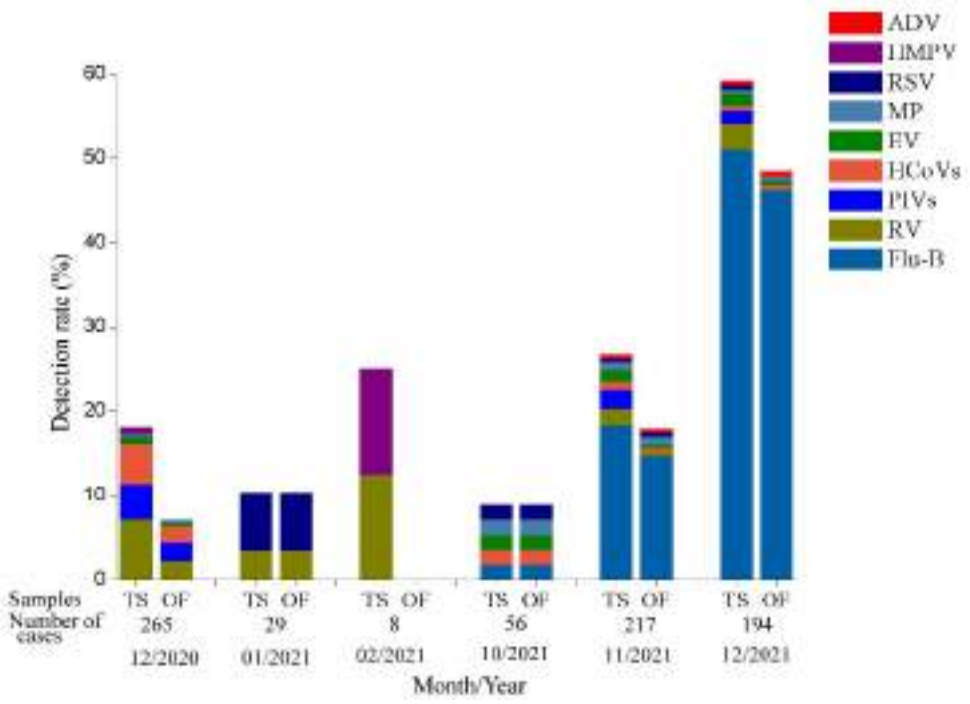

Figure 2 Distribution of multiple respiratory pathogens in different months in Beijing from December 2020 to December 2021

Note: TS, throat swab; OF, oral fluid