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ORIGINAL ARTICLE

Effectiveness of Oral Fluid in Pathogenic Surveillance of Acute Respiratory Infection

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Abstract

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

Methods: OF and throat swabs were collected from December 2020 to December 2021 in patients with acute respiratory tract infections in Beijing. Multiplex real-time PCR was performed, and 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. The sensitivity and specificity of the OF assay, compared with the throat swab assay, were 71.11% (160/225) and 100% (544/544), respectively. The two assays had excellent agreement (kappa = 0.78). The detection consistency varied among pathogens. 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 positivity rate was observed in December 2021.

Conclusion: OF samples have excellent potential for the epidemiological surveillance of respiratory pathogens, and may have application prospects in preventing and controlling infectious diseases.

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

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INTRODUCTION

Acute respiratory tract infection (ARTI) is a major cause of high morbidity and mortality globally [1]. Many types of respiratory pathogens cause ARTI, including influenza virus, respiratory syncytial virus (RSV), human coronaviruses (HCoVs), rhinovirus (RV), human parainfluenza viruses (HPIVs), and adenovirus (ADV). Emerging pathogens continue to be detected and to 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), which is currently circulating globally. The detection and surveillance of respiratory pathogens not only enables timely clinical diagnosis and provides guidance for precision medicine, but also helps comprehensively

and systematically control epidemics and identify variants in respiratory pathogens, thus providing a scientific basis for the early warning, prediction, prevention, and control of respiratory infectious diseases [2,3].

Nasal or throat swabs are the dominant methods for collecting specimens for detecting and monitoring respiratory pathogens. The collection of these swab samples can cause severe nausea, retching, and other discomfort for patients. Sample collection must be performed by trained medical staff, who must wear personal protective equipment and are exposed to a risk of infection. Therefore, a new method of collecting biological specimens that is safe, convenient, and efficient is needed 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. OF can be self-collected and thus is advantageous in terms of 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 [8,9] used OF for nucleic acid and antibody testing of SARS-CoV-2 in China, thus demonstrating OF's substantial application value. However, no related study on OF samples for other respiratory pathogens in China has been performed.

Via the Respiratory Pathogen Surveillance System 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 provides valuable information for exploring the use of a new biological specimen type, and improving the prevention and control of critical respiratory tract infectious diseases, such as COVID-19 and influenza.

METHODS

Study population

Via the Respiratory Pathogen Surveillance System, 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: December 2020 to February 2021, and October 2021 to December 2021. AURTI was defined by a fever (≥38°C) accompanied by cough or sore throat, nasal congestion, runny nose, expectoration, and other upper respiratory symptoms. The diagnostic criteria of CAP followed 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 with an Oracol collection device (Malvern Medical Developments, UK, cat. number: S10) according to 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 soaked; the swab was then placed in the collection tube. After 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 for the prevention and control of COVID-19 (Trial Version 8) [12]. All samples were stored at –20°C before laboratory testing.

Method optimization and laboratory testing

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

Optimization of OF elution buffer quantities: OF samples of confirmed COVID-19 cases were collected, and 0.6 mL or 1.00 mL elution buffer was used for OF sample processing. 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 considered positive if the sample had an S-shaped amplification curve and threshold cycle (Ct) value for ORF1ab gene and N gene ≤38.00.

Optimization of the PCR reaction system: A total of 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). Volumes of 2 μL or 5 μL nucleic acid were added to the PCR reactions, 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 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 lineages), 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 considered positive if the sample had an S-shaped amplification curve and a Ct value ≤35.00. Otherwise, the result was considered negative.

Quality control of sampling

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

Statistical analysis

Continuous variables are presented as mean or median (interquartile range, IQR), and categorical variables are presented as rate (%). Kappa analysis (95% confidence interval, 95%CI) was used to evaluate the detection consistency between sample types, with scores as follows: >0.75 indicated excellent agreement, 0.60 < kappa ≤0.75 indicated high agreement, 0.40 < kappa ≤0.60 indicated moderate agreement, and ≤0.40 indicated 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 below 0.05 was considered statistically significant. Data were analyzed in Microsoft Excel 2019 and SPSS 19.0 (IBM, New York, USA).

Ethics statement

The protocol of this study was approved by the Ethics Committee of the Beijing Center for Disease Prevention and Control. Written informed consent was obtained from all enrolled patients or their legal guardians.

RESULTS

Optimization of the OF detection method

Optimization of OF elution buffer quantities: Four OF samples from two patients with confirmed COVID-cases were collected. PCR results indicated that all samples were positive for SARS-CoV-2 when 0.60 or 1.00 mL OF elution buffer was used for processing samples. For case 1, when 0.60 or 1.00 mL OF elution buffer was added, the Ct values for the ORF1ab gene for SARS-CoV-2 were 30.60 and 31.95, respectively, and the Ct values for the N gene were 29.56 and 32.47, respectively. For case 2, when 0.60 or 1.00 mL OF elution buffer was added, the Ct values for the ORF1ab gene for SARS-CoV-2 were 33.60 and 34.23, respectively, and the 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 the PCR reaction system: A total of 56 OF samples were selected for PCR detection. Addition of 2 or 5 μL of nucleic acid resulted in the positive detection of 0 or 20 samples, respectively. Therefore, 5 μL 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). The enrolled patients 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, and 60 patients had CAP.

Detection results of respiratory pathogens: Among the 769 cases, 225 (29.26%, 225/769) were positive for at least one pathogen in throat swabs, whereas 160 (20.81%, 160/769) cases were positive for at least one pathogen in OF samples. The detection rate for the OF samples was significantly lower than that for throat swabs (P < 0.001). With throat swabs as the reference standard, OF had a sensitivity of 71.11% (160/225) and a 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 respiratory pathogens: Nine pathogens were detected in all cases (detailed results in Table 2). The kappa values for Flu-B, MP, RSV, and ADV between the 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 excellent agreement. The kappa value for HCoVs and EV was 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 high agreement. The kappa value for HPIVs was 0.47 (95% CI, 0.35–0.60), thus indicating moderate agreement. Poor agreement was detected for RV (kappa = 0.40). Three HMPV-positive cases were identified through throat swabs, for which the paired OF samples yielded negative results.

Comparison of Ct values: Among 225 positive cases, the Ct values of ten cases were missing. The Ct value was 12–35 among the 215 positive samples for throat swabs, with a median value of 24 (IQR, 19–26). The Ct value of the 150 positive samples for OF was 15–35, with a median value of 26 (IQR, 24–28). The Ct value for the throat swabs was significantly lower than that for the OF samples (P < 0.001). When the Ct value of the throat swab was ≤30, 70.53% (146/207) of the paired OF samples were positive, whereas when the Ct value of the throat swab was >30, 50.00% (4/8) of the paired OF samples were positive (Table 3).

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

TABLE 2 | Comparison of different respiratory pathogens between 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: *Because coinfections were observed in six cases, and the number of pathogens infected in each patient was greater than 1, the sum of pathogen infections is greater than the total number of positive patients; "—" means no calculation; OF, oral fluid.

TABLE 3 | Comparison of Ct values between throat swabs and paired OF samples.

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

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 abundance of several respiratory pathogens showed slight differences 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 cases were detected in the 6–14-year-old group (Fig 1).

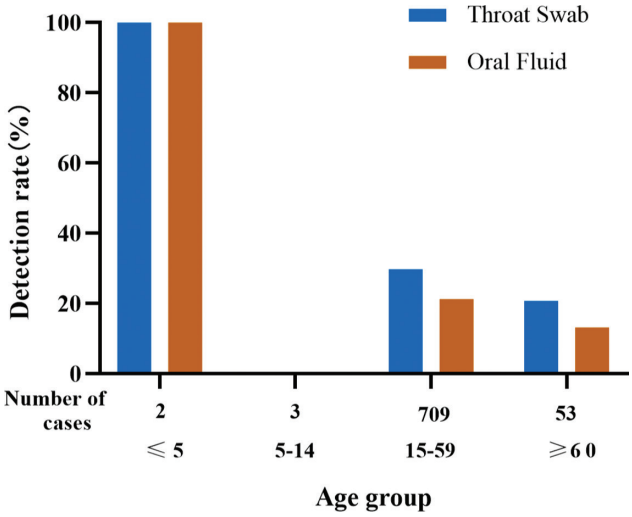

FIGURE 1 | Distribution of respiratory pathogen infections by age group in Beijing from December 2020 to December 2021.

Sex distribution: For the OF samples, the detection rates for males and females were 21.96% (85/387) and 19.63% (75/382), respectively. Similarly, the detection rates for throat swabs in males was 30.23% (117/387), a percentage slightly higher than that in females 28.27% (108/382).

Temporal distribution: From December 2020 to February 2021, and from October 2021 to December 2021, the monthly detection rates in OF samples over the 6 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 in 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

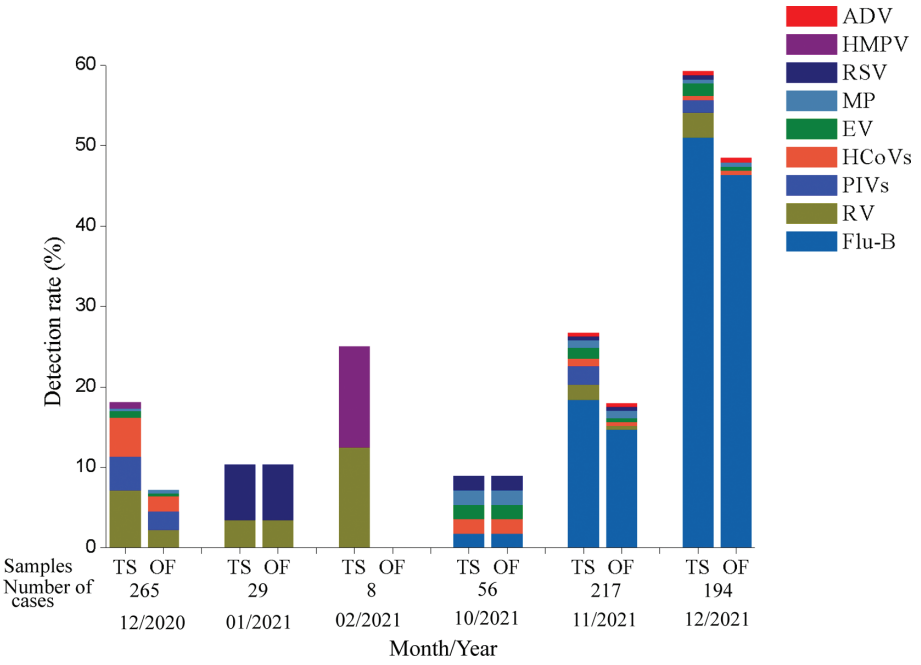

FIGURE 2 | Distribution of multiple respiratory pathogens in different months in Beijing from December 2020 to December 2021. Note: TS, throat swab; OF, oral fluid

observed in December 2021 (Fig 2). From December 2020 to February 2021, the numbers of positive cases and the positive detection rates of respiratory pathogens were low. The positivity 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 each 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. The most prevalent pathogen was Flu-B, which had 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]. OF has since 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] have indicated that OF samples have similar sensitivity to nasopharyngeal swabs in the 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 the SARS-CoV-2 antibody detection assays in serum, detection in OF samples has a sensitivity of 75% and specificity of 99%, thus making this tool suitable for population-based seroepidemiology studies. However, the use of OF samples for detection of respiratory pathogens has not been reported. This study provides the first report of using OF samples to detect respiratory pathogens in Beijing, to explore their value in the detection and surveillance of nucleic acids from respiratory pathogens.

We optimized several experimental conditions to improve the detection rate in OF samples. The sensitivity and specificity of the OF nucleic acid detection assay, compared with detection in throat swabs, were 71.11% and 100%, respectively. These results were similar to those of a previous study, in which the sensitivity and specificity of a saliva assay were 68.1% and 97.6%, respectively [17]. These variations in sensitivity and specificity might be due to differences in experimental conditions. Although the detection rate in OF was slightly lower than that in throat swabs, the 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 might have been associated with 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 swabs was ≤30, the paired OF samples had a high detection rate of 70.53%, and when the Ct value for the throat swabs was >30, the detection rate in OF samples decreased to 50.00%. These data indicated that the detection rate in OF samples was associated with the viral load in the oral area, and that patients with high viral load had a high probability of OF detection. Several characteristics of the SARS-CoV-2 Omicron variant have

been reported [18], such as high viral load and low Ct value, thus indicating that OF samples may be likely to have high detection consistency with throat swabs and considerable potential for detection of the SARS-CoV-2 Omicron variant.

The detection rate in OF samples was associated with the pathogenic species. Recently, saliva has been proposed as an alternative sample for influenza virus diagnosis. Sueki et al. [19] have detected influenza virus from 144 paired nasopharyngeal swabs and saliva samples and shown a 95.8% concordance for saliva and the nasopharyngeal swabs. Yoon et al. [20] have performed PCR assays on 385 influenza-like cases and demonstrated coincidence rates of 93.5% and 97.1% for Flu-A and Flu-B, respectively, between paired saliva and nasopharyngeal swabs. Similarly, our study demonstrated a coincidence rate of 97.79% for the influenza virus between the throat swabs and OF samples, with a kappa value of 0.92, thereby indicating that the two sampling methods had excellent agreement. Notably, all influenza virus samples contained Flu-B in our study, whereas no Flu-A-positive cases were detected. Studies have indicated that influenza viruses infect primarily respiratory epithelial cells and bind sialic acid receptors [21]. Large amounts of sialic acid have been found in the human trachea, bronchus, and saliva [22,23], thus potentially explaining the excellent detection consistency for influenza virus between sampling methods.

Throat swabs and OF samples showed excellent detection consistency for ADV, RSV, and MP, and high agreement for EV and HCoVs, although poor detection consistency was observed for RV and HPIVs. Differences in the detection consistency of respiratory pathogen detection may be associated with possible heterogeneity in replication sites. A previous study [24] has performed multiplex RT-PCR on 236 cases of ARTI and found that the detection rate of ADV in saliva (15.8%) was higher than that in nasopharyngeal swabs (1.2%) ($P < 0.0001$). However, in this study, the throat swab and OF samples had excellent agreement (kappa = 1). The difference between studies might be because ADV had a low prevalence in our study and also might have demonstrated that the oropharynx is a major replication site of ADV [17]. Furthermore, the detection consistency between sampling methods was poor for RV, and the detection rate of OF was lower than that of the throat swabs. The results were similar to of those reported by Kim et al. [24], who have observed a higher detection rate of RV in nasopharyngeal swabs (34.4%) than saliva (27.5%). RV binds primarily the intercellular adhesion molecule-1 (ICAM-1) and low-density lipoprotein receptor (LDLR) in nasal epithelial cells and basolateral plasma membranes of the polarized airway, and subsequently causes respiratory disease [25]. Therefore, we speculated that RV might have 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 localized primarily in the lower respiratory tract [27], and HPIV-3 is the most epidemic serotype in Beijing [28], thus potentially explaining the poor detection consistency for HPIVs, and the low detection rates of OF samples versus throat swabs for several months in this study. Therefore, in using OF samples for the detection of respiratory pathogens, the characteristics of the targeted pathogens should be considered.

Although the pathogen spectrum results showed slightly different orders of abundance of several respiratory pathogens between the throat swab and OF samples, the overall distribution of the respiratory pathogen spectrum was similar. We speculated that this finding might have been associated with a lack of positive cases of several respiratory pathogens or the specific characteristics of several pathogens. Moreover, the distribution trends of respiratory pathogens in all age groups and sexes were similar between the OF and throat swabs. The highest detection rate was in the ≤5-year-old group, followed by the 15–59-year-old and ≥60-year-old groups. OF samples provide outstanding advantages, including safety, non-invasiveness, and convenience, when used for young children and older adults with limited mobility, as well as in scenarios involving 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 associated with inconvenient and uncooperative sampling and avoid large-scale exposure. Thus, OF has considerable potential as a supplementary sample to detect SARS-CoV-2.

Public health measures implemented during the COVID-19 pandemic substantially decreased the prevalence of common respiratory pathogens; consequently, the detection rate of respiratory pathogens 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, thus indicating a low prevalence. This rate was significantly lower than the detection rates of respiratory pathogens reported in Beijing and other areas of China in the same season before the COVID-19 outbreak [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 have reported a similar phenomenon. The detection rate of the influenza virus in northern China has been reported to be 31.80% in week 52 of 2021, and 98% of cases were Flu-B [32]. Many countries and regions have reported higher influenza prevalence in the winter of 2021–2022 than in the winter of 2020–2021, particularly in Europe, North America, Africa, and China [33]. The time trend of respiratory pathogen detection in throat swabs was similar to that in OF 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 have become regular, the detection rate of respiratory pathogens, particularly influenza viruses, has increased, thus suggesting a need to 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 in OF samples are similar to those detected in throat swabs in the epidemiological surveillance of respiratory pathogens. Therefore, OF samples may have considerable value in respiratory pathogenic surveillance.

This study has several limitations. During the COVID-19 pandemic, ARTI cases significantly decreased, and the respiratory pathogens subsequently exhibited low prevalence and detection rates; moreover, 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 in OF samples are similar to those detected in throat swab samples. Therefore, OF samples may 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 particularly suitable for children and older adults, as well as in scenarios in which large-scale nucleic acid detection is required and during medical staff shortages. Thus, OF may have important application prospects in the prevention and control of COVID-19 and influenza.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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