Supplementary Materials

Methods and Materials (Detailed)

Patients and clinical setting

Subjects were patients that were confirmed to be infected with the Omicron variant of SARS-CoV-2 by RT-qPCR sampling with nasopharyngeal swabs. The PICOS inclusion/exclusion criteria are listed in Table S1. All patients were tested daily (9:00 AM) for SARS-CoV-2 with an OPS and an SG immediately afterwards after the initiation of this study. A single flocked swab (TS-3, Shenzhen Shellman Bio-tech Co., China) was rubbed back and forth in the pharyngeal tonsils and the posterior pharyngeal wall three times and then placed into a collection tube containing a virus preservative (SM2102, Shenzhen Shellman Bio-tech Co.). Patients were asked to rinse their mouth with 8 mL of saline water for 10 seconds, tilt their head back and gargle for another 10 seconds, and then spit the water back in the 10 mL plastic tube. Samples were collected and stored in a refrigerator at -80°C. The time between sampling and refrigeration was limited to 4 hours.

The current study was strictly conducted per the guidelines of the Declaration of Helsinki of the World Medical Association (2000), and it was approved and supervised by the ethics committee of the Third People's Hospital of Shenzhen (approval number 2022-116-03). This study was registered with a Chinese clinical trial registry (ChiCTR2200063457). The study protocol was explained to all of the patients, who were asked to provide written informed consent to participation in this study.

RT-qPCR to detect SARS-CoV-2

Samples were first thawed at room temperature and then vortexed for 30 seconds. Each oropharyngeal swab and saline gargle sample (200 μL) was then transferred to the sample cells of the RNA/DNA Purification Kit (T200-96, Magnetic Bead, Zybio Inc., China), and nucleic acids were extracted according to the manufacturer's instructions (Nucleic acid isolation system EXM6000, Zybio Inc., China). Quantitative RT-PCR was performed using the BioGerm^{TM} 2019-nCoV Nucleic Acid Detection Kit (PCR-Fluorescence Probing, China) on the ABI7500 qPCR instrument, and results were analyzed with the accompanying software (Applied Biosystems, USA). Detection of the Nucleocapsid (N) Gene and open reading frame 1 (Orf1/ab) gene or the N-gene alone or the Orf1/ab gene alone was deemed to confirm COVID-19. With up to 45 cycles of amplification, cycle threshold (CT)-values ≤ 40 were deemed to constitute a "positive" result, CT values ≤ 40 for one gene alone (the N gene or Orf1/ab) were deemed to constitute a "single positive" result, and CT values > 40 were deemed to constitute a "negative" result.

Statistics

The software SPSS (ver23.00, IBM, US) was used for statistical analysis. Categorical variables were expressed as a percentage while continuous variables were expressed as a median with an interquartile range (IQR). The distribution of positive and negative patients was compared using a chi-squared test or

Fisher's exact test. A paired *t*-test was used to compare the difference in the CT values for the SG and OPS (CT values were only available for positive patients). Graphs were generated using the software Prism 9 (Prism 9 for macOS, GraphPad Software, LLC., US).

Table S1. The PICOS inclusion/exclusion criteria in the current study

Parameters	Inclusion criteria	Exclusion criteria
Patients	Patients infected with the Omicron variant of SARS-CoV-2 as confirmed by RT-qPCR sampling with nasopharyngeal swabs (less than 14 days later)	Patients under 18 years old; patients ≥ 70 years old; patients who were unable to perform a saline gargle without assistance; patients receiving other therapies such as antiviral therapy; patients with severe diseases of other systems; patients who did not provide informed consent. Duration of infection ≥ 14 days
Intervention	Sampling using a conventional oropharyngeal swab (OPS) and a subsequent saline gargle (SG)	Patients who chose not to undergo OPS or SG sampling
Comparison	OPS *vs*.SG	
Outcome	Distribution of the patients who were sampled with an OPS and SG; cycle threshold (CT) values.	
Study design	Non-randomized before-after study	

Table S2. Clinical characteristics of the patient with COVID-19 in the current study

	Male	Female	Total
Age, median (IQR, years)	37 (29.5, 48.25)	31 (27, 35)	33 (27.25, 46)
Symptomatic patients, *n* (%)	28 (73.68%)	20 (66.67%)	48 (70.59%)
Total	38	30	68
Times sampling was performed, *n* (%)			
1	7 (18.42%)	13 (43.33%)	20 (29.41%)
2	11 (28.95%)	11 (36.67%)	21 (32.35%)
3	8 (21.05%)	4 (13.33%)	12 (17.65%)
4	6 (15.79%)	1 (3.33%)	7 (10.29%)
5	4 (10.53%)	0 (0.00%)	4 (5.88%)
6	2 (5.26%)	0 (0.00%)	2 (2.94%)
7	0 (0.00%)	1 (3.33%)	1 (1.47%)

IQR, interquartile range.

Figure S1. Comparison of the internal reference gene (RNase P) in a saline gargle and oropharyngeal swab. CT, cycle threshold; OPS, oropharyngeal swab; SG, saline gargle. *** represents $p < 0.0001$