

Prevalence of Respiratory Viruses Detected by Multiplex Real-Time PCR in SARS-CoV-2-Negative Adults with Acute Respiratory Infection**Samit Sen¹, Nandini Duggal², Shipra Suyal³, Brijesh Sharma⁴, Mala Chhabra⁵**¹Department of Microbiology, Lady Hardinge Medical College, New Delhi, India^{2,5} Department of Microbiology, Atal Bihari Vajpayee Institute of Medical Sciences & Dr. Ram Manohar Lohia Hospital, New Delhi, India³Department of Microbiology, District Hospital Gopeshwar, Uttarakhand, India⁴Department of Medicine, Atal Bihari Vajpayee Institute of Medical Sciences & Dr. Ram Manohar Lohia Hospital, New Delhi, India

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Conflict of interest: Nil

Abstract**Background:** Diagnostic attention to acute respiratory infection (ARI) in adults has been dominated by SARS-CoV-2 testing since 2020, with less attention to other respiratory viruses circulating in SARS-CoV-2-negative patients.**Aims:** To determine the prevalence and distribution of non-SARS-CoV-2 respiratory viruses detected by multiplex real-time PCR on combined nasopharyngeal-orpharyngeal swabs in SARS-CoV-2-negative adults hospitalized with ARI.**Settings and Design:** Cross-sectional observational study at a tertiary care teaching hospital in New Delhi, India, June–November 2022.**Materials and Methods:** 500 adult inpatients with ARI who tested negative for SARS-CoV-2 by RT-PCR had a combined nasopharyngeal-orpharyngeal swab, pooled into a single viral transport medium tube, tested for 18 other respiratory viruses by multiplex real-time RT-PCR (Seegene Allplex Respiratory Panels 1–3).**Statistical Analysis Used:** Data were analyzed in SPSS; categorical variables were summarized as frequencies and percentages, with 95% confidence intervals where relevant.**Results:** At least one other respiratory virus was detected in 258/500 (51.6%). Rhinovirus was most frequent (11.8%), followed by coronavirus OC43 (11.4%) and adenovirus (10.0%); influenza A (5.0%), RSV A/B (5.4%), coronavirus 229E (4.0%), coronavirus NL63 (3.0%), and parainfluenza 1–4 (2.2%) were also found. Co-infections: RSV-A with OC43 (2.0%) and rhinovirus with OC43 (1.0%). Coronavirus HKU1, RSV-B, enterovirus, and parainfluenza 1 and 3 were not detected.**Conclusions:** Over half of SARS-CoV-2-negative adults with ARI had another identifiable respiratory virus, arguing against presumptive antibacterial therapy for a negative SARS-CoV-2 result without further testing.**Keywords:** Acute respiratory infection, adults, cross-sectional study, real-time PCR, respiratory viruses, SARS-CoV-2-negative.**DOI:** 10.25258/ijcpr.18.9.178

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Introduction

Respiratory pathogens remain a major cause of morbidity and mortality worldwide, with lower respiratory infections alone estimated to account for over a million deaths annually.[1] SARS-CoV-2 emerged as a novel human respiratory pathogen in December 2019. [2] Since then, diagnostic algorithms for patients presenting with respiratory symptoms have been dominated by SARS-CoV-2 testing. Non-pharmaceutical interventions adopted to control the pandemic — lockdowns, masking, and physical distancing — substantially disrupted the transmission and circulation of other endemic

respiratory viruses, and the net effect of the pandemic on non-SARS-CoV-2 respiratory virus epidemiology has been described as uncertain and setting-dependent.[3],[4] Importantly, a negative SARS-CoV-2 result does not exclude viral infection. Multicenter data from emergency departments across Europe, the Middle East, and Africa found that over one-third of SARS-CoV-2-negative patients with respiratory symptoms tested positive for at least one other respiratory pathogen by multiplex PCR, most commonly rhinovirus/enterovirus, non-SARS-CoV-2 coronaviruses, and

adenovirus. [5] Similar findings have been reported from Latin America, where half of patients with a clinically suspected but PCR-negative SARS-CoV-2 result tested positive for another respiratory virus, predominantly rhinovirus and influenza A.[6] In sub-Saharan Africa, a post-pandemic surveillance snapshot found human bocavirus, rhinovirus, and parainfluenza viruses to be the leading non-influenza, non-SARS-CoV-2 pathogens among patients hospitalised with severe acute respiratory infection.[7] Co-infection of SARS-CoV-2 itself with other respiratory viruses has also been documented in Indian patients, underscoring that the two are not mutually exclusive.[8]

Despite this evidence from other regions, dedicated data on the residual non-SARS-CoV-2 viral burden among SARS-CoV-2-negative Indian adults are limited; existing Indian etiological studies of adult ARI generally predate or straddle the pandemic rather than specifically characterising the SARS-CoV-2-negative population.[9] Clarifying this burden matters for antimicrobial stewardship: a negative SARS-CoV-2 result is often, without further testing, treated as grounds for empirical antibacterial therapy rather than as a prompt to consider an alternative viral etiology. We conducted this study to determine the prevalence and distribution of non-SARS-CoV-2 respiratory viruses detected by multiplex real-time RT-PCR on combined nasopharyngeal and oropharyngeal swabs among SARS-CoV-2-negative adults hospitalized with acute respiratory infection.

Materials and Methods

Study Design and Setting: This cross-sectional observational study was conducted jointly by the Departments of Microbiology and Medicine at a tertiary care teaching hospital in New Delhi, India, between 1 June 2022 and 30 November 2022. Data collection therefore predates the preparation of this manuscript by several years; where relevant, findings are discussed alongside more recently published literature to reflect the current state of evidence, and this interval is noted as a limitation below.

Participants: Adult inpatients with clinical features suggestive of ARI — fever, cough, rhinorrhea, sneezing, sore throat, body aches, headache, mouth breathing, fatigue, dyspnea, chest tightness, or haemoptysis — with symptom onset within the preceding 10 days were screened. Patients who tested positive for SARS-CoV-2 by RT-PCR were excluded from this analysis; only SARS-CoV-2-negative patients were included in the final study population.

Sample Size: Sample size was calculated using the standard single-proportion formula: $n = Z_{1-\alpha/2}^2 \times$

$p(1-p) / d^2$, where $Z_{1-\alpha/2} = 1.96$ (95% confidence level), p = anticipated proportion of SARS-CoV-2-negative ARI patients positive for at least one other respiratory virus, and d = absolute precision. Using $p = 36.3\%$, the positivity rate reported in a comparable multicenter SARS-CoV-2-negative cohort,[5] and $d = 5\%$, the minimum required sample size was 356. To allow more precise, narrower-confidence-interval estimates of prevalence for individual, less commonly detected viruses — consistent with the larger cohort sizes used in comparable studies (1,334 patients in Duclos et al.[5] and 475 in Kelly et al.[7]) — 500 SARS-CoV-2-negative adults were enrolled.

Specimen Collection: A combined nasopharyngeal and oropharyngeal swab was collected from each patient after informed consent: a nasopharyngeal swab and a throat (oropharyngeal) swab were taken sequentially using flocked swabs and pooled together into a single tube of viral transport medium. This combined specimen was transported to the laboratory and stored at -20°C until processed.

SARS-CoV-2 Screening: The combined swab specimen was first tested for SARS-CoV-2 using the LabGun COVID-19 RT-PCR kit, targeting the E and RdRp genes, with a cycle threshold (Ct) ≤ 40 considered positive. Only patients negative on this assay were carried forward for the multiplex viral panel described below, performed on the same specimen.

Molecular Testing for Other Respiratory Viruses: Nucleic acid extraction was performed on the bioMérieux NucliSENS easyMAG automated platform. Multiplex real-time RT-PCR was performed using Seegene's Allplex Respiratory Panels 1, 2, and 3 on a CFX96 Real-Time PCR System, with results interpreted using Seegene Viewer V2.0. These panels permit simultaneous detection of 18 non-SARS-CoV-2 respiratory viruses: influenza A (H1, H1pdm09, and H3 subtypes), influenza B, RSV A/B, adenovirus, enterovirus, human metapneumovirus, parainfluenza virus 1–4, rhinovirus, and the endemic human coronaviruses 229E, NL63, OC43, and HKU1.

Ethical Considerations: The study was approved by the Institutional Ethics Committee of the study hospital, and written informed consent was obtained from all participants prior to enrolment, in accordance with the Declaration of Helsinki. Approval details are on record and available on request.

Statistical Analysis: Data were entered in Microsoft Excel and analyzed in SPSS. Categorical variables were summarized as frequencies and

percentages, with 95% confidence intervals calculated where relevant.

Results

Of the 500 SARS-CoV-2-negative adults with ARI enrolled, at least one other respiratory virus was

detected by combined NP/OP swab RT-PCR in 258 (51.6%). Rhinovirus (11.8%) was the most frequently detected virus, followed by coronavirus OC43 (11.4%) and adenovirus (10.0%) [Table 1], [Figure 1].

Table 1: Prevalence of respiratory viruses detected by combined nasopharyngeal/oropharyngeal swab RT-PCR among SARS-CoV-2-negative adults with ARI (n = 500)

Virus	n	%
Rhinovirus	59	11.80
Coronavirus OC43	57	11.40
Adenovirus	50	10.00
Respiratory syncytial virus A/B	27	5.40
Influenza A (H1, H1pdm09, H3 combined)	25	5.00
Coronavirus 229E	20	4.00
Coronavirus NL63	15	3.00
Parainfluenza virus 1–4 (combined)	11	2.20
Influenza B	5	1.00
Human metapneumovirus	4	0.80
Coronavirus HKU1	0	0.00
Enterovirus	0	0.00
Co-infection: RSV-A + Coronavirus OC43	10	2.00
Co-infection: Rhinovirus + Coronavirus OC43	5	1.00
At least one virus detected	258	51.60
No virus detected	242	48.40

Note: Individual virus percentages do not sum to the overall 51.6% positivity rate because the two co-infection categories are subsets already reflected within their respective individual virus counts (e.g., the 10 RSV-A/OC43 co-infections are included within both the RSV A/B row and the coronavirus OC43 row), not additional patients.

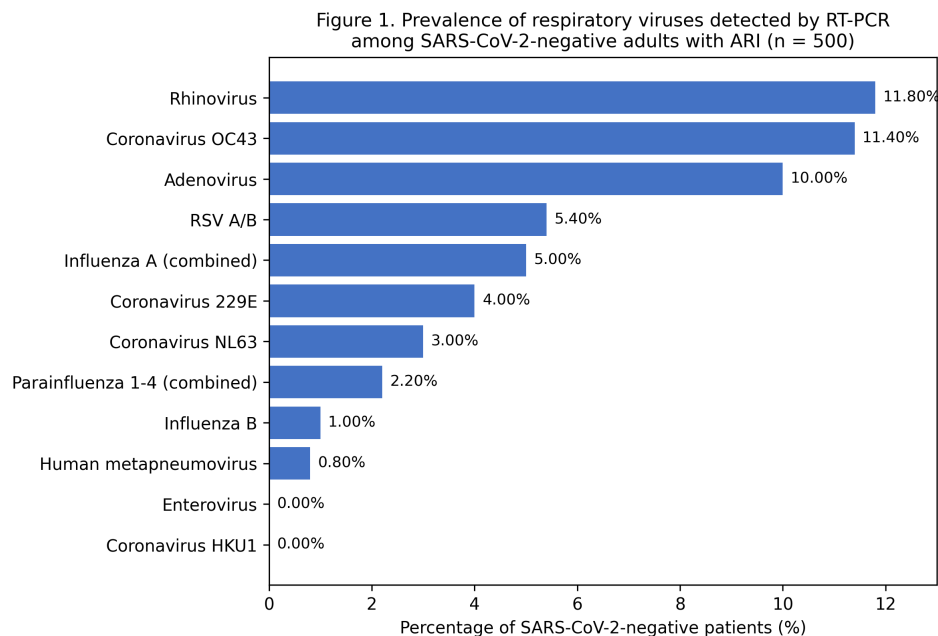


Figure 1: Prevalence of respiratory viruses detected by RT-PCR among SARS-CoV-2-negative adults with ARI (n = 500)

Discussion

Respiratory pathogens other than SARS-CoV-2 continue to circulate and cause disease in adults with ARI, but diagnostic attention has

disproportionately focused on SARS-CoV-2 itself since 2020. Data from a 13-country multicenter emergency department study found that 36.3% of SARS-CoV-2-negative patients with respiratory symptoms tested positive for another respiratory

pathogen, most often rhinovirus/enterovirus (56.5% of positive detections), followed by non-SARS-CoV-2 coronaviruses (11.0%) and adenovirus (9.9%). [5]

A Peruvian cohort of patients with a clinically suspected but PCR-negative SARS-CoV-2 result found an even higher positivity rate (50%), led by rhinovirus (54.4% of positive detections) and influenza A(H3N2) (22.8%).[6] A post-pandemic Tanzanian surveillance snapshot of SARS-CoV-2- and influenza-negative patients with severe ARI found human bocavirus (26.8%), rhinovirus (12.3%), and parainfluenza viruses (10.2%) to be the leading pathogens.[7]

In this study, 258 of 500 SARS-CoV-2-negative patients (51.6%) had at least one other respiratory virus identified, with rhinovirus (11.8%), coronavirus OC43 (11.4%), and adenovirus (10.0%) the leading pathogens. This overall positivity rate is closely comparable to the 50% reported by Gómez de la Torre Pretell et al. in Peru, and notably higher than the 36.3% reported in the multicenter European/Middle Eastern/African emergency department cohort by Duclos et al.[6],[5] The virus ranking in this study — led by rhinovirus, followed by a non-SARS-CoV-2 coronavirus and adenovirus — broadly mirrors both the Duclos et al. cohort (rhinovirus/enterovirus, then non-SARS-CoV-2 coronaviruses, then adenovirus) and the Peruvian cohort (also rhinovirus-led), rather than the bocavirus-led pattern reported from Tanzania.[5],[6],[7] The comparatively high burden of coronavirus OC43 specifically (11.4%) is notable and may reflect endemic seasonal coronavirus circulation resuming as SARS-CoV-2-specific public health measures were relaxed during the June–November 2022 study period.

These findings, taken together, support the broader point that a substantial proportion of SARS-CoV-2-negative ARI in adults has an identifiable, non-SARS-CoV-2 viral etiology rather than being presumptively bacterial. This has direct implications for antimicrobial stewardship, since a negative SARS-CoV-2 result should not, on its own, be taken as support for empirical antibacterial therapy — particularly given that community-based Indian data likewise point to a substantial and under-characterized burden of viral acute respiratory infection in adults.[9]

Limitations: This was a single-center study, which limits the precision of virus-specific prevalence estimates, particularly for less commonly detected viruses. As a single combined nasopharyngeal/oropharyngeal specimen was tested per patient, and lower respiratory tract sampling was not performed, the true burden of viruses with a predominantly lower-respiratory tropism may be

underestimated. As SARS-CoV-2-positive patients were excluded by design, this analysis characterizes the residual non-SARS-CoV-2 viral burden only. Finally, data collection took place between June and November 2022, and this manuscript was prepared several years later; respiratory virus circulation patterns can shift over such an interval, and the findings should be interpreted as reflecting the specific pandemic-era period studied rather than necessarily current circulation patterns.

Conclusion

Over half of SARS-CoV-2-negative adults hospitalized with acute respiratory infection in this cohort had another identifiable respiratory virus, most often rhinovirus, coronavirus OC43, or adenovirus. This substantial residual viral burden supports the case for multiplex respiratory virus testing, rather than presumptive antibacterial therapy, in SARS-CoV-2-negative adults with ARI, and adds India-specific data to a still-limited body of evidence on this question.

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