Original Article

DOI: 10.7860/JCDR/2025/76307.21993

Microbiology Section

Genomic Insights into Vaccine Evasion: A Cross-sectional Study from a Tertiary Care Hospital of Northern India

UKSIM QADRI[1], BASHIR AHMAD FOMDA[2], SHAISTA NAZIR[3], YAWAR MIR[4], MEHVISH SALEEM[5], MUNEER BHAT[6]

ABSTRACT

Introduction: Coronavirus Disease-2019 (COVID-19) vaccines are a crucial tool in controlling and ultimately ending the pandemic, complementing other preventive measures. India launched its vaccination campaign on January 16, 2021, initially using two vaccines that received emergency authorisation: Covaxin (BBV152) and Covishield (ChAdOx1 nCoV-19). The vaccination effort began with elderly individuals (60+ years) and frontline workers, eventually expanding to include various age groups. During this period, India faced a severe second wave of COVID-19, marked by a notable rise in postvaccination breakthrough infections throughout 2021, driven by more transmissible and potentially more immune-evasive variants. To date, no study is available that details the rate of these breakthrough infections and the variants responsible in our region.

Aim: To determine the rate of breakthrough infections in our region and identify which Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) variants are responsible for these infections.

Materials and Methods: This cross-sectional study was conducted in a tertiary care hospital in Northern India over one year, from January 2021 to December 2021. Nasopharyngeal swabs from suspected COVID-19 patients were collected and subjected to Ribonucleic acid (RNA) extraction, followed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) testing. The breakthrough infection rate was defined as infections occurring in individuals who were fully vaccinated with two standard doses of the AZD1222/Covishield vaccine. Genome sequencing was performed to identify the SARS-CoV-2 variants responsible for postvaccination breakthrough infections. Data analysis was conducted using Microsoft Excel Software 16.

Results: Between January 2021 and December 2021, 513,197 nasopharyngeal swabs were analysed, with a total of 32,952 (6.42%) samples testing positive for SARS-CoV-2 by RT-PCR. Out of these, 2,043 (6.2%) samples were from cases in which individuals had been vaccinated with two doses of the vaccine. Genome sequencing of 11 such samples revealed that eight of the patients were infected with the Delta variant (Pango Lineage: B.1.617.2). One sample showed the Eta variant (Lineage B.1.575) with the E484K mutation. Unfortunately, two samples could not be processed due to inadequate quality.

Conclusion: A breakthrough infection rate of 6.2% was recorded, with the Delta variant (Pango Lineage: B.1.617.2) of SARS-CoV-2 being commonly associated with these infections. This study underscores the need for continued and rigorous genomic surveillance of emerging SARS-CoV-2 variants to assess their potential to evade immune responses.

Keywords: Breakthrough infections, COVID-19 Vaccines, Variants of concern

INTRODUCTION

The SARS-CoV-2 infection is an acute respiratory disease that can lead to severe illness and potentially fatal pneumonia. Originating in Wuhan, Hubei Province, China, in December 2019, it rapidly escalated into a global pandemic. To mitigate the catastrophic waves of COVID-19, there was a growing imperative for vaccine development. Among the vaccines developed and approved in India were AZD1222 (ChAdOx1-S, known as Covishield) and BBV152 (Covaxin). Covishield was produced by the Serum Institute of India under a licence from AstraZeneca-Oxford, while Covaxin was an indigenous vaccine created by Bharat Biotech in collaboration with the Indian Council of Medical Research (ICMR) [1,2].

In January 2021, India launched the world's largest COVID-19 vaccination campaign, implementing it in phases. After China, India became the second nation to reach the milestone of more than two billion doses of the COVID vaccine delivered [3]. The initial phase targeted healthcare workers, sanitation staff and essential frontline workers, followed by the elderly, individuals with co-morbidities, those over 45 years of age and eventually the entire adult population [4,5]. The efficacy of AZD1222 (ChAdOx1-S), following two doses, was reported to be 63.1% with potentially higher efficacy observed with longer intervals between doses [1]. It was anticipated that vaccination would decelerate the pandemic and significantly reduce hospitalisations, as the vaccines were proven to lessen disease severity, if not the transmission of the virus [5]. Despite this, no vaccine offers complete immunity and cases among fully vaccinated individuals were reported. Additionally, reinfections among those previously infected suggested either waning natural immunity or immune evasion by mutant strains of the virus.

As COVID-19 vaccines became a cornerstone in managing the pandemic, breakthrough infections- defined as infections occurring at least 14 days after the second vaccine dose-emerged as a notable concern [6,7]. Globally, postvaccination breakthrough COVID-19 infections were documented. By April 2021, the Centres for Disease Control and Prevention (CDC) had reported 10,262 breakthrough infections [8]. The ICMR noted that infection rates postvaccination were below 0.05% [9]. In April 2021, Hacisuleyman E et al., identified 417 breakthrough cases among individuals vaccinated with Pfizer and Moderna mRNA vaccines [7]. In India, breakthrough infections were reported in specific regions, including Kerala [10], Chennai [11] and Delhi [1]. Notably, Vaishya R et al., found that approximately 76% of healthcare workers in a New Delhi hospital who contracted the virus had breakthrough infections [12].

Studies regarding breakthrough infections from this region are not available; therefore, present study aimed to investigate SARS-CoV-2 infections among fully vaccinated individuals to determine the rate of breakthrough infections in this region and to understand which variants are responsible for these breakthrough cases. This study is the first of its kind from this region and will serve as a reference for future research in this area.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at the Sher-i-Kashmir Institute of Medical Sciences, a tertiary care hospital in Northern India, over a period of one year, from January 2021 to December 2021. The study was approved by the Institutional Ethics Committee (IEC) under IEC/SKIMS Protocol # RP 179/2021.

Inclusion criteria: Symptomatic cases that fulfilled the case definition and were fully vaccinated with two standard doses of the AZD1222/Covishield vaccine were included in the study.

Exclusion criteria: Asymptomatic individuals, unvaccinated individuals, or those who had received only a single dose of the vaccine were excluded from the study.

Sample size: The sample size was calculated using the proportion method, based on a reported breakthrough infection prevalence of 13.3% from Maulana Azad Medical College, Delhi, India. Assuming a 95% confidence level and 80% power, the required sample size was determined to be 154 [13,14]. All participants were included who fulfilled the inclusion criteria within the study period of one year.

Case definition: A breakthrough COVID-19 infection was defined as an individual testing positive for SARS-CoV-2 by rRT-PCR more than 14 days after receiving one dose of any of the licensed COVID-19 vaccines.

Study Procedure

Nasopharyngeal swab samples from symptomatic patients were taken as part of routine COVID-19 testing. The samples were aliquoted and RNA extraction was performed using a fully automated RNA extractor (Genetix). RT-PCR was conducted on a Bio-Rad machine following standard protocols with the Real-Q 2019-nCoV detection kit from BioSewoom, Seoul, Republic of Korea [15-17]. Positive samples were stored at -80°C until they were dispatched for sequencing to the National Centre for Disease Control (NCDC) in New Delhi and the Institute of Genomics and Integrative Biology (CSIR-IGIB) for whole-genome sequencing.

STATISTICAL ANALYSIS

Data analysis was performed using Microsoft Excel 2016 software.

RESULTS

Between January 2021 and December 2021, we analysed 513,197 nasopharyngeal swabs were analysed, with 32,952 (6.42%) testing positive for SARS-CoV-2 via RT-PCR. Of these positive cases, 2,043 (6.2%) occurred in individuals who had received both doses of the vaccine, indicating a breakthrough infection rate of 6.2%. Only the samples with a Cycle Threshold (Ct) value of <30 were recommended for genome sequencing and due to financial constraints, 11 such samples to the NCDC and IGIB for whole-genome sequencing. These samples were from male military personnel who had received two standard doses of the AZD1222/Covishield vaccine. The mean age of these patients was 40.36±11 years. Eight individuals received their first dose in February and the second in March, while the remaining three received their first dose in March and their second dose in April 2021. Five of these individuals had a history of contact with COVID-19-positive patients, while the other six had no known exposure. All 11 were referred for SARS-CoV-2 testing after developing mild influenza-like symptoms and tested positive on RT-PCR.

Sequencing results revealed the Delta variant (Pango Lineage: B.1.617.2) in eight of the eleven patients. One sample exhibited the Eta variant (Lineage B.1.575) with the E484K mutation. Two samples could not be processed due to poor quality. Eleven patients were vaccinated with an interval of 4-6 weeks between doses, and all experienced only mild COVID-19 symptoms following the second dose. Notably, none of the patients required hospitalisation or experienced severe illness. Demographic details and sequencing results are summarised in [Table/Fig-1].

S. No.	District	Age	Gender	Type of vaccine received	Date of first dose	Date of second dose	Date of testing	Report for SARS-CoV-2	VoC or mutation of interest detected? (Y/N)	Name of the variant if any
01	Srinagar	32	Male	Covishield	11/02/2021	23/03/2021	25/05/2021	Positive	Y	B.1.617.2
02	Srinagar	42	Male	Covishield	19/02/2021	23/03/2021	25/05/2021	Positive	Y	B.1.617.2
03	Srinagar	51	Male	Covishield	12/02/2021	13/03/2021	25/05/2021	Positive	Y	B.1.617.2
04	Bandipora	27	Male	Covishield	08/02/2021	13/03/2021	26/05/2021	Positive	N	Poor quality
05	Srinagar	39	Male	Covishield	11/02/2021	14/03/2021	30/05/2021	Positive	Y	B.1.617.2
06	Srinagar	26	Male	Covishield	15/02/2021	15/03/2021	31/05/2021	Positive	Y	B.1.617.2
07	Budgam	65	Male	Covishield	05/03/2021	30/04/2021	03/06/2021	Positive	Y	B.1.617.2
08	Srinagar	43	Male	Covishield	27/02/2021	30/03/2021	04/06/2021	Positive	Y	B.1.617.2
09	Srinagar	45	Male	Covishield	12/02/2021	16/03/2021	05/06/2021	Positive	N	Poor quality
10	Srinagar	36	Male	Covishield	03/03/2021	08/04/2021	09/06/2021	Positive	Y	B.1.575
11	Srinagar	38	Male	Covishield	17/03/2021	16/04/2021	09/06/2021	Positive	Y	B.1

[Table/Fig-1]: Demographic characteristics and genome sequencing results of the SARS-CoV-2 positive cases.

DISCUSSION

Globally, COVID-19 vaccines were granted Emergency Use Authorisation (EUA) and rapidly integrated into public health initiatives to prevent SARS-CoV-2 infections and reduce disease transmission, thereby protecting lives and livelihoods. However, the emergence of SARS-CoV-2 Variants Of Concern (VOCs) has heightened public health concerns due to their increased transmissibility and potential to evade the humoural immune response. Consequently, several countries have reported breakthrough infections in vaccinated individuals, even after completing their full vaccination regimen [7,18,19]. In the USA, over 10,000 breakthrough infections have been reported following full vaccination, although such cases represent a small fraction of the overall vaccinated population [8]. Recent studies have also noted mild symptomatic breakthrough infections in Kerala and Delhi, India [9,10,11]. Present study reported a breakthrough infection rate of 6.2%, which was lower than that observed in a study conducted by Singh CM et al., in Bihar [20]. This study identified that breakthrough infections were predominantly caused by the Delta variant (Pango

Lineage: B.1.617.2), followed by the Eta variant (Lineage B.1.575), which carries the E484K mutation. This suggests a period of significant community transmission of the Delta variant. Notably, none of the patients required hospitalisation, indicating that vaccination effectively mitigates disease severity, the need for hospitalisation, and mortality. According to the WHO classification, the Delta variant is categorised as a variant of concern due to its enhanced transmission and greater potential for immune evasion, while the other two sub-lineages of B.1.617- namely, B.1.617.1 and B.1.617.3, which carry the E484Q mutation- are classified as Variants Under Investigation (VUI) [12]. The B.1.617 variant and its lineage B.1.617.2 were largely responsible for the surge in COVID-19 cases in Maharashtra. During January and February 2021, Delta (B.1.617.2) and Kappa (B.1.617.1) were detected in 60% of clinical specimens collected in Maharashtra [19]. In India, there was a rapid rise in daily infections, with the Delta variant dominating, accounting for over 99% of all sequenced genomes in April 2021 [21]. A recent study in the UK showed that the Delta variant had a higher transmission rate compared to the Alpha variant [22]. The results of all these studies are in concordance with present study findings.

Postvaccination breakthrough infections have been reported globally, across various vaccines. Nevertheless, vaccination clearly provides significant protection against severe disease, hospitalisations and death. Therefore, ongoing monitoring of breakthrough infections and the clinical severity of these cases should be an integral part of vaccine deployment strategies in all countries. It remains unclear whether these breakthrough infections may be related to vaccine efficacy, immune evasion, or other factors. The findings from this study have significant clinical implications, particularly for understanding the real-world effectiveness of COVID-19 vaccines in the face of emerging variants. The identification of breakthrough infections, particularly those caused by variants such as Delta and Eta, underscores the importance of continued monitoring and vigilance regarding vaccine efficacy.

This study highlights the necessity of genomic surveillance for emerging SARS-CoV-2 variants to monitor their potential to bypass immunity. This can guide timely policy decisions and updates to vaccine formulations to ensure continued protection against evolving strains of the virus. Future research should explore the long-term efficacy of vaccines against breakthrough infections, especially in individuals who were vaccinated early in the campaign. Longitudinal studies can also examine the duration of immunity and factors influencing breakthrough infections, such as age, underlying health conditions and the presence of mutations in emerging variants.

Limitation(s)

Present study overlooked asymptomatic or mildly symptomatic cases, potentially leading to an underestimation of breakthrough infections. Only 11 samples were analysed for genome sequencing and these were from male patients residing in similar settings, which may limit the generalisability of the findings.

CONCLUSION(S)

A breakthrough infection rate of 6.2% was recorded in present study. The Delta variant (Pango Lineage: B.1.617.2) was most commonly identified as the cause of these breakthrough infections. Present study demonstrates that vaccines confer substantial protection and mitigate the severity of infections, thereby reducing morbidity, mortality and the need for hospitalisation.

Acknowledgement

Authors acknowledge Indian Council of Medical Research (IMCR), National Centre for Disease Control (NCDC) and the Institute of Genomics and Integrative Biology (CSIR-IGIB) for their support.

REFERENCES

[1] Tyagi K, Ghosh A, Nair D, Dutta K, Singh Bhandari P, Ahmed Ansari I, et al. Breakthrough COVID-19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India. Diabetes Metab Syndr. 2021;15:1007-08. Available from: https://pubmed.ncbi.nlm.nih.gov/33991805.

[2] Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): An exploratory analysis of a randomised controlled trial. Lancet. 2021;397(10282):1351-62. Doi: 10.1016/S0140-6736(21)00628-0.

[3] Bandre GR, Makade J, Bankar N, Hawale DV. An analysis of the COVID-19 vaccination campaign in India. J Clin Diagn Res. 2023;17(3):DM01-DM04. Doi: 10.7860/JCDR/2022/54897.16142

[4] Garg RK, Garg K, Gupta N, Chopra V, Gupta A. COVID-19 vaccine behaviour among people attending a tertiary care centre, Punjab, India. J Clin Diagn Res. 2022;16(3):LC26-LC32. Doi: 10.7860/JCDR/2022/54897.16142.

[5] World Health Organization. Tracking SARS-CoV-2 variants [cited 17 January 2023]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants.

[6] Centers for Disease Control and Prevention. COVID-19 vaccination of healthcare personnel measure specifications [cited 17 January 2021]. Available from: https://www.cdc.gov/.

[7] Hacisuleyman E, Hale C, Saito Y, Blachere NE, Bergh M, Conlon EG, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med. 2021;384:2212-18. Doi: 10.1056/NEJMoa2105000.

[8] COVID-19 Vaccine Breakthrough Case Investigations Team, Birhane M, Bressler S, Chang G, Clark T, Dorough L, et al. COVID-19 Vaccine Breakthrough Infections Reported to CDC- United States, January 1- April 30, 2021. MMWR Morb Mortal Wkly Rep. 2021;70:792-93. Doi: 10.15585/mmwr.mm7021e3.

[9] Gupta N, Kaur H, Yadav PD, Mukhopadhyay L, Sahay RR, Kumar A, et al. Clinical characterization and genomic analysis of samples from COVID-19 breakthrough infections during the second wave among the various states of India. Viruses. 2021;13(9):1782. Doi: 10.3390/v13091782.

[10] Philomina JB, Jolly B, John N, Bhoyar RC, Majeed N, Senthivel V, et al. Genomic survey of SARS-CoV-2 vaccine breakthrough infections in healthcare workers from Kerala, India. J Infect. 2021;83:237-79. Doi: 10.1016/j.jinf.2021.05.018.

[11] Thangaraj J, Yadav P, Kumar CG, Shete A, Nyayanit DA, Rani DS, et al. Predominance of delta variant among the COVID-19 vaccinated and unvaccinated individuals, India, May 2021. J Infect. 2021;2:23. Doi: 10.1016/j.jinf.2021.08.006.

[12] Vaishya R, Sibal A, Malani A, Prasad KH. SARS-CoV-2 infection after COVID-19 immunization in healthcare workers: A retrospective, pilot study. Indian J Med Res. 2021;153:550-54. Doi: 10.4103/ijmr.ijmr_1485_21.

[13] Karmacharya A, Rai K, Siwakoti S, Khanal B, Bhattarai NR. COVID-19 breakthrough infections in vaccinated individuals at BPKIHS, Nepal. BMC Infect Dis. 2024;24:1003. Available from: https://doi.org/10.1186/s12879-024-09902-z.

[14] Sharma P, Mishra S, Basu S, Kumar R, Tanwar N. Breakthrough infection with severe acute respiratory syndrome coronavirus 2 among healthcare workers in Delhi: A single-institution study. Cureus. 2021;13(10):e19070. Doi: 10.7759/cureus.19070. PMID: 34824945; PMCID: PMC8610433.

[15] Zhang Y-Z. Novel 2019 coronavirus genome. Virological. Available from: http://virological.org/t/novel-2019-coronavirus-genome/319.

[16] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045.

[17] Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infec. 2020;9(1):221-36.

[18] Loconsole D, Sallustio A, Accogli M, Leaci A, Sanguedolce A, Parisi A, et al. Investigation of an outbreak of symptomatic SARS-CoV-2 VOC 202012/01-Lineage B.1.1.7 infection in healthcare workers, Italy. Clin Microbiol Infect. 2021;27:1174.e1–1174.e4. Doi: 10.1016/j.cmi.2021.05.007.

[19] Mullen JL, Tsueng G, Latif AA, Alkuzweny M, Cano M, Haag E, et al. Center for viral systems biology. 2020. [Internet]. [cited 2021 Aug 30]. Available from: https://outbreak.info.

[20] Singh CM, Singh PK, Naik BN, Pandey S, Nirala SK, Singh PK. Clinico-epidemiological profile of breakthrough COVID-19 infection among vaccinated beneficiaries from a COVID-19 vaccination centre in Bihar, India. Ethiop J Health Sci. 2022;32(1):15-26. Doi: 10.4314/ejhs.v32i1.3.

[21] Keehner J, Horton LE, Pfeffer MA, Longhurst CA, Schooley RT, Currier JS, et al. SARS-CoV-2 infection after vaccination in healthcare workers in California. N Engl J Med. 2021;384(18):1774-75. Doi: 10.1056/NEJMc2101927. PMID: 33755376; PMCID: PMC8008750.

[22] Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al. A variant lineage of SARS-CoV-2 associated with rapid transmission in Manaus, Brazil, evolved in November 2020 with immune escape characteristics. Science. 2021;372:815-21. Doi: 10.1126/science.abh2644.

PARTICULARS OF CONTRIBUTORS:

1. Senior Resident, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
2. Professor and Head, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
3. Senior Resident, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
4. PhD Scholar, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
5. Scientist, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
6. Scientist, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Bashir Ahmad Fomda,
Professor and Head, Department of Microbiology, Sher-i-Kashmir Institute of
Medical Sciences, Soura, Srinagar, Jammu and Kashmir, India.
E-mail: bashirfomda@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Oct 16, 2024
- Manual Googling: May 27, 2025
- iThenticate Software: May 29, 2025 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 15, 2024**
Date of Peer Review: **Feb 05, 2025**
Date of Acceptance: **May 31, 2025**
Date of Publishing: **Nov 01, 2025**