Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/dqd6v295

COVID-19 BREAKTHROUGH INFECTIONS AND DIAGNOSTIC PERFORMANCE OF RT-PCR AND RAPID ANTIGEN TESTS AMONG SYMPTOMATIC INDIVIDUALS: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: COVID-19 vaccination has significantly reduced global morbidity and mortality; however, breakthrough infections continue to occur, particularly with emerging SARS-CoV-2 variants possessing immune-escape characteristics. Accurate diagnostic evaluation of breakthrough infections is essential, especially comparisons between RT-PCR and rapid antigen tests (RATs).

Objectives:

(1) To determine the incidence of breakthrough infections among vaccinated individuals.
(2) To evaluate the diagnostic efficacy of RT-PCR and RATs in symptomatic COVID-19 patients.
(3) To compare clinical severity and haematological profiles in vaccinated versus unvaccinated infected individuals.
(4) To analyse the role of SARS-CoV-2 variants in breakthrough infections.

Methods: A prospective study was conducted (Nov 2021–Dec 2024) at Index Medical College, Indore. All symptomatic suspected COVID-19 patients and vaccinated RT-PCR–positive individuals meeting CDC's breakthrough criteria were included. Nasopharyngeal swabs, sputum and BAL specimens were collected. RT-PCR and RAT performance, clinical severity, and haematological markers were evaluated.

Results: Breakthrough infections accounted for a considerable proportion of positive cases, particularly during Delta and Omicron waves. Vaccination significantly reduced disease severity, hospitalization and inflammatory marker levels. RT-PCR demonstrated superior sensitivity, detecting low-viral-load infections that RATs frequently missed. RAT showed reduced sensitivity in vaccinated breakthrough cases.

Conclusion: Breakthrough infections remain an important epidemiological phenomenon but are predominantly mild among vaccinated individuals. RT-PCR remains the diagnostic gold standard, while RATs serve as adjunct tools with limited sensitivity. Vaccination continues to offer strong protection against severe outcomes.

Keywords: COVID-19, breakthrough infection, vaccination, RT-PCR, rapid antigen test, SARS-CoV-2 variants.

INTRODUCTION

Coronaviruses (CoVs) are large, enveloped, positive-sense RNA viruses within the family *Coronaviridae*, responsible for a spectrum of respiratory illnesses in humans and animals[1]. Their structural complexity and ability to undergo recombination contribute to emerging strains with pathogenic potential [2,3]. SARS-CoV-2, emerging in December 2019, rapidly disseminated across the globe causing substantial morbidity due to its efficient human-to-human transmission and wide tissue tropism [4,5].

The virus exhibits high viral loads in the upper respiratory tract during early infection, facilitating transmission through respiratory droplets and aerosols [6]. Environmental stability of SARS-CoV-2 contributes further to its spread, surviving for extended periods on non-porous surfaces[7]. Thus, early and accurate diagnosis has remained essential for containment.

RT-PCR is the gold standard diagnostic method due to its high sensitivity and ability to detect low viral loads [8]. However, rapid antigen tests (RATs) have gained prominence for quick screening despite their lower sensitivity and specificity [9].

India's vaccination programme began in January 2021 using Covishield and Covaxin, later supplemented with other vaccines [10,11]. Vaccines significantly reduce severe illness and death, but no vaccine has proven 100% effective; thus, breakthrough infections are expected [12,13].

Variants of concern (VOCs) such as Delta and Omicron exhibit increased transmissibility and immune escape properties, raising global concern regarding breakthrough infections[14–16]. Monitoring breakthrough infections, evaluating diagnostic tools, and understanding clinical outcomes in vaccinated individuals remain vital for ongoing pandemic control.

MATERIALS AND METHODS

Study Setting and Design

A prospective observational study was conducted at the Molecular Biology Laboratory and Central Clinical Laboratory, Index Medical College Hospital & Research Centre, Indore, from November 2020 to December 2024.

Study Population

- Symptomatic suspected COVID-19 individuals.
- Vaccinated individuals testing RT-PCR positive ≥14 days after completing recommended doses[12].

Exclusion Criteria

- Single-dose vaccinated individuals
- Non-consenting participants
- Incomplete clinical or vaccination data

Sample Collection

Nasopharyngeal Swabs

Collected using Dacron/polyester swabs and transported in VTM, which stabilizes viral RNA and inhibits microbial overgrowth[17,18].

Lower Respiratory Specimens

Sputum and BAL were collected in moderate to severe cases using standard biosafety precautions[19].

Diagnostic Procedures

1. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Performed using CDC-recommended EUA protocols, targeting SARS-CoV-2–specific genomic regions[8].

2. Rapid Antigen Test (RAT)

Performed using lateral-flow immunochromatographic assays for point-of-care testing [9].

Clinical Outcome Measures
- **Mild:** Upper respiratory symptoms only
- **Moderate:** Requiring hospital admission
- **Severe:** ICU admission or respiratory failure

Ethical Approval
Approval obtained from Institutional Ethics Committee. Verbal informed consent was obtained.

RESULTS

Incidence of Breakthrough Infection
Breakthrough infections accounted for a notable proportion of positive cases. Incidence was highest during periods dominated by Delta and Omicron VOCs, consistent with reported increased transmissibility and immune escape[14–16].
Factors contributing included:
- Waning immunity
- High-risk exposures
- Presence of comorbidities

RT-PCR vs. Rapid Antigen Test Performance
RT-PCR remained markedly superior, with high sensitivity even at low viral loads [8].
RAT performance showed:
- Lower sensitivity in symptomatic breakthrough cases
- High false-negative rates in low-viral-load infections
- Utility only for rapid, large-scale screening[9]

Clinical Presentation
Vaccinated individuals predominantly exhibited:
- Mild symptoms (cough, fever, sore throat)
- Faster recovery
- Reduced hospitalization and ICU admissions

Unvaccinated patients showed:
- Higher incidence of pneumonia and ARDS
- Elevated risk of sepsis and organ involvement[20]

Haematological Findings
Vaccinated breakthrough patients demonstrated:
- Lower neutrophil-lymphocyte ratio
- Reduced CRP and ferritin levels
- Better platelet profiles

These findings correlate with reduced inflammatory response and protection from cytokine storm[21–23].

DISCUSSION

Breakthrough infections, although expected, remain largely mild and pose significantly lesser clinical burden in vaccinated individuals. VOCs such as Delta and Omicron possess mutations that enhance transmissibility and reduce neutralizing antibody recognition [14–16].
RT-PCR continues to be indispensable for accurate diagnosis. RATs, although valuable for quick screening, displayed reduced detection capacity in breakthrough infections due to lower viral loads [8,9].
Vaccination substantially reduces severe outcomes by modulating systemic inflammation, reducing the risk of multi-organ involvement, and preventing exaggerated cytokine responses [21,22]. The

observed haematological differences align with global evidence of vaccine-mediated attenuation of disease severity.

The findings reinforce:

- Importance of booster doses
- Continued reliance on molecular diagnosis
- Ongoing variant surveillance
- Sustained public health vigilance

CONCLUSION

Breakthrough infections represent an important but manageable facet of the COVID-19 pandemic. Vaccinated individuals predominantly experience mild disease, with substantially lower rates of hospitalization, severe inflammation, and complications. RT-PCR remains the diagnostic gold standard, while RATs should be used cautiously due to poor sensitivity in breakthrough cases.

Vaccination continues to be the strongest defence against severe COVID-19 outcomes, even in the era of emerging variants.

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