Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4282

A STUDY OF DETECTION AND GENOME SEQUENCING OF SARS-COV-2 FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

Mohd Aadam Bin Najeeb[1*], Dr. Madhurendra Singh[2], Dr. Narayan Kamath[3]

[1*]Ph.D. Scholar, Dept. of Microbiology, Malwanchal University, Indore.
[2]Ph.D. Guide, Dept. of Microbiology, Malwanchal University, Indore
[3]Professor & Head, Dept. of Microbiology, NAMOMERI, Silvassa.

***Corresponding Author:** Mohd Aadam Bin Najeeb
[1*]Ph.D. Scholar, Dept. of Microbiology, Malwanchal University, Indore
E-mail: microbiologistaadam@gmail.com

Abstract

Background: The continuous evolution of SARS-CoV-2 necessitates ongoing genomic surveillance to understand its transmission dynamics, mutation patterns, and impacts on public health measures and clinical outcomes.

Objectives: To analyse the distribution of SARS-CoV-2 variants and mutations within a tertiary care setting and assess their association with clinical outcomes to inform public health responses and treatment strategies.

Methods: This study conducted comprehensive genomic sequencing of SARS-CoV-2 from 400 clinical samples collected in a tertiary care hospital, employing RT-PCR for initial detection and Oxford-Nanopore Sequencing for whole-genome sequencing. Molecular analyses, including phylogenetic analysis and identification of unique genetic variants, were performed to elucidate the virus's evolution and transmission dynamics. Statistical analyses were conducted to explore the correlation between genomic variants and clinical outcomes, including disease severity and viral shedding.

Results: The study identified a high RT-PCR positivity rate (87.5%) with significant genomic diversity among the sequenced viruses. The Delta variant showed the highest mutation frequency in the Spike protein, suggesting increased transmissibility and potential vaccine escape. Unique genetic variants were identified in 28.1% of sequences, providing crucial insights into the virus's evolution. Statistical analysis revealed significant associations between specific genomic variants and clinical outcomes, particularly the increased risk of severe disease.

Conclusion: The findings highlight the complexity of SARS-CoV-2's evolution and its implications for disease transmission, diagnostic sensitivity, and vaccine efficacy. The study underscores the importance of genomic surveillance in detecting emergent mutations and informing targeted public health interventions and vaccine strategies. Continuous global collaboration and genomic analysis are essential to adapt to the evolving pandemic and mitigate the impact of COVID-19.

Keywords: SARS-CoV-2, genomic surveillance, mutations, clinical outcomes, public health, vaccine strategy.

INTRODUCTION

The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19), in late 2019, marked the beginning of an unprecedented global health crisis. Initially identified in Wuhan, Hubei province, China, the virus rapidly spread worldwide, leading the World Health Organization (WHO) to declare COVID-19 a pandemic by March 2020. SARS-CoV-2 belongs to the Coronaviridae family, similar to the viruses responsible for the SARS outbreak in 2003 and the Middle East Respiratory Syndrome (MERS) outbreak in 2012, but with unique genetic characteristics that have contributed to its widespread transmission and impact.[1]

The global impact of COVID-19 has been profound, affecting millions of individuals and overwhelming healthcare systems across the world. As of early 2023, there have been over 500 million confirmed cases and over 6 million deaths globally, numbers that continue to rise amidst ongoing transmission and the emergence of new variants.[1,2]

The pandemic has also led to significant social and economic disruptions, highlighting the critical need for effective public health responses, including accurate diagnostic testing, effective treatment regimens, and the rapid development and distribution of vaccines.

In the fight against COVID-19, molecular diagnostics have played a pivotal role in identifying infected individuals and controlling the spread of the virus. Real-time reverse transcription polymerase chain reaction (RT-PCR) has been the gold standard for SARS-CoV-2 detection, offering high sensitivity and specificity. However, the emergence of viral variants with mutations in key genomic regions has underscored the importance of genome sequencing in understanding viral evolution, tracking transmission pathways, and informing public health interventions.[3]

Genome sequencing of SARS-CoV-2 provides critical insights into the virus's genetic makeup, enabling the identification of mutations that may affect virus transmissibility, disease severity, and vaccine efficacy. This genomic surveillance has been instrumental in identifying variants of concern (VOCs), such as Alpha, Beta, Gamma, Delta, and Omicron, each with distinct genetic and phenotypic characteristics that have influenced the pandemic's trajectory.[4]

Tertiary care hospitals, with their advanced diagnostic and treatment capabilities, have been at the forefront of the COVID-19 response. These institutions not only provide critical care for severe COVID-19 cases but also serve as centers for diagnostic testing and research, including the collection and analysis of clinical samples for SARS-CoV-2 detection and genome sequencing. The integration of clinical data with genomic analysis in these settings offers a comprehensive approach to understanding and managing the pandemic, facilitating the development of targeted interventions and informing public health policies.[5]

The detection and genome sequencing of SARS-CoV-2 in tertiary care hospitals not only contributes to the immediate clinical management of patients but also enhances our understanding of the virus's behavior and evolution within different populations and environments. Such efforts are crucial for the ongoing development of diagnostic tools, therapeutic strategies, and vaccines that are effective against current and future variants of the virus.[6]

The rationale for focusing on the detection and genome sequencing of SARS-CoV-2 within a tertiary care hospital environment stems from the critical need to understand the virus's behaviour in a controlled clinical setting. Hospitals are epicenters for high transmission risk, not only to the

vulnerable patient population but also among healthcare workers. Thus, studying the virus in this context provides valuable data on nosocomial (hospital-acquired) infections, transmission dynamics, and the effectiveness of infection prevention and control measures. Additionally, tertiary care hospitals often have the infrastructure and expertise necessary for advanced diagnostic and genomic analysis, making them ideal for conducting such a study.

Moreover, the genomic sequencing of SARS-CoV-2 from clinical samples collected in hospitals allows for the identification of mutations that could have significant implications for the clinical outcome of infected patients and the population's broader health. It can reveal patterns of intra-hospital transmission, guide the implementation of tailored infection control strategies, and contribute to the global understanding of the virus's evolution.

Given this background and rationale, the objectives of this study are twofold. First, to evaluate the effectiveness and sensitivity of current SARS-CoV-2 detection methods in clinical samples obtained from patients and healthcare workers in a tertiary care hospital. This objective includes comparing different testing methodologies and exploring factors that may influence test sensitivity, such as viral load, sample type, and the timing of sample collection relative to symptom onset.

Second, to analyse the genome sequences of SARS-CoV-2 derived from these clinical samples to identify prevalent strains within the hospital, track the introduction and spread of new variants, and investigate any associations between specific genomic features and clinical outcomes. This genomic analysis aims not only to contribute to the scientific understanding of SARS-CoV-2's evolution but also to inform public health strategies and interventions both within the hospital setting and in the broader community.

METHODOLOGY

Research Approach: Our investigation employed an evaluative research framework, focusing on the assessment and enhancement of SARS-CoV-2 detection methods and genomic sequencing techniques. This approach was aimed at generating insights to improve understanding and management of the virus in a clinical setting.

Research Design: The study was structured as a prospective observational study, conducted in the Post Graduate Department of Microbiology, Index Medical College, and associated Hospital & Research Centre, Indore, M.P. Over 18 months, we observed and collected data on SARS-CoV-2 detection rates and genomic variations, without intervening or altering the course of the disease.

Setting: Research activities were carried out within the specified medical and research facilities, offering a controlled environment for sample collection, data acquisition, and subsequent analysis.

Population and Sample Size: The target population comprised individuals between 18-85 years, suspected or confirmed to have COVID-19, based on WHO case definitions. A sample size of 400 was calculated to achieve statistical significance, considering a 99% confidence level and a 5% margin of error, applicable for our estimated population size (N=10,000).

Sampling Technique: A random sampling strategy was utilized to ensure every potential participant had an equal opportunity to be included, thereby accurately reflecting the broader hospital population.

Inclusion and Exclusion Criteria: Participants were selected based on clinical and epidemiological COVID-19 criteria, with a focus on those with positive RT-PCR results and CT values ≤25. Pregnant or post-partum women and individuals with known HIV/AIDS were excluded from the study.

Ethical Clearance: The study received approval from the ethics committee at Index Medical College,

adhering to ethical guidelines for human research.

Data and Sample Collection: Clinical data were prospectively gathered from patient records and anonymized in a secure database. Oropharyngeal and nasopharyngeal swabs were collected following best practices to ensure the integrity and reliability of the test results. RT-PCR testing was conducted on these samples, with RNA extraction performed on those with CT values ≤25 for further genomic analysis.

Genome Sequencing: For genome sequencing, the study utilized the Oxford Nanopore Sequencing technology, which involved a series of steps including RNA extraction, library preparation, sequencing, and detailed bioinformatics analysis to identify viral variants and mutations.

Statistical Analysis: Statistical analysis was performed using SPSS version 22. Categorical variables were analysed using Chi-square or Fisher exact tests, while continuous variables were assessed with t-tests or Mann-Whitney U tests. A p-value of <0.05 was considered statistically significant.

The methodology outlined above underpins our comprehensive examination of SARS-CoV-2 detection and genomic sequencing, aiming to enhance the clinical and epidemiological understanding of the virus within a tertiary healthcare setting.

RESULT

Demographic data

The data from the study of 400 participants at a tertiary care hospital reveal a diverse age distribution among individuals tested for SARS-CoV-2, with the largest group aged 36-50 years (37.5%), followed by those aged 18-35 years (30%), 51-65 years (22.5%), and 66-85 years (10%). Females constituted a higher percentage of the study population (55%) compared to males (45%). Comorbidities were present in 30% of participants, indicating a significant portion of the population at potentially higher risk for severe COVID-19 outcomes. A vast majority of the participants were symptomatic (80%), highlighting the critical role of symptom- driven testing in identifying potential cases.

Table 1: Demographic Data of Study Participants

Parameter	Category	Total Participants (n=400)	Percentage (%)
Age (years)	18-35	120	30
	36-50	150	37.5
	51-65	90	22.5
	66-85	40	10
Gender	Male	180	45
	Female	220	55
Comorbidities	Yes	120	30
	No	280	70
Symptomatic	Yes	320	80
	No	80	20

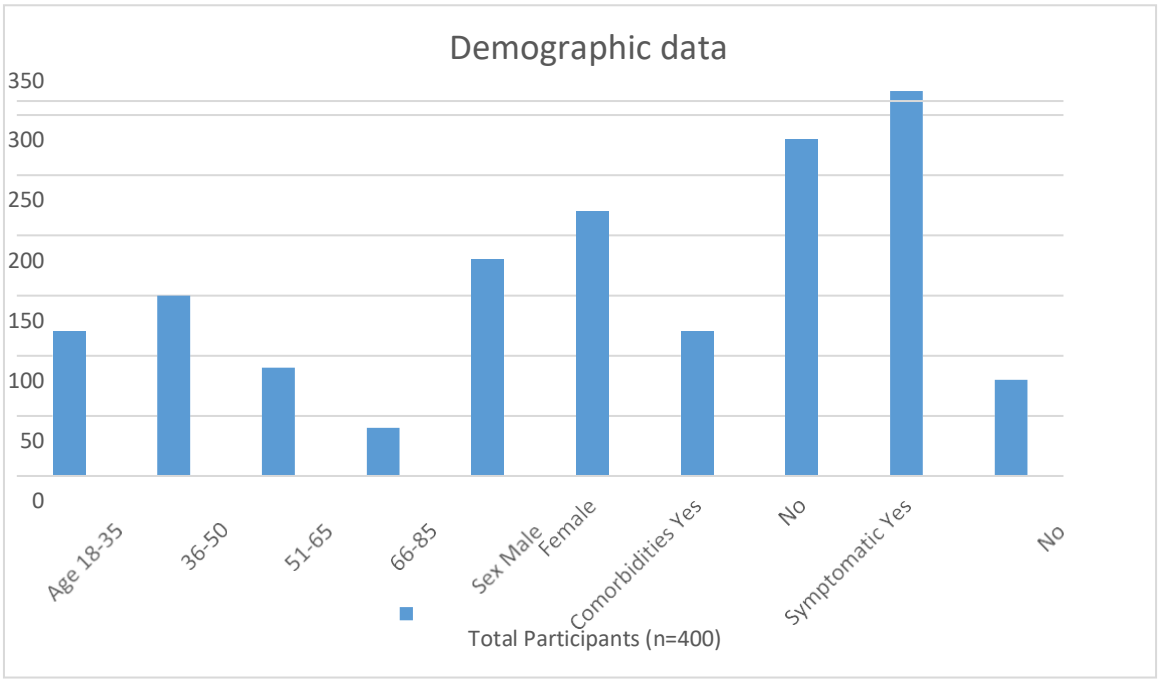

Figure 1: Demographic Data of Study Participants Sample Collection and RT-PCR

Results

The data from this study indicate that out of 400 oropharyngeal and nasopharyngeal swabs collected and tested for SARS-CoV-2 using RT-PCR, a high positivity rate of 87.5% was observed, with 75% of cases exhibiting CT values ≤ 25, suggesting a significant viral load in the majority of positive cases. The successful RNA extraction in all collected samples (100%) and the subsequent whole-genome sequencing for those with CT values ≤ 25 (75% of total samples) facilitated comprehensive molecular analysis. The completion of whole-genome sequencing and molecular analysis for these samples underscores the feasibility and effectiveness of employing advanced genomic technologies, like Oxford-Nanopore Sequencing and Next-Clade analysis, in a clinical setting for detailed viral characterization.

Table 2: Sample Collection and RT-PCR Results

Parameter	Description	Value (n=400)	Percentage (%)
Sample Collection	Oropharyngeal and nasopharyngeal swabs collected simultaneously and placed in one Viral Transport Medium	400	100

Parameter	Description	Value (n=400)	Percentage (%)
RT-PCR Positivity	Number of cases with positive SARS-CoV-2 RT-PCR results	350	87.5
CT Value ≤ 25	Cases with CT values less than or equal to 25	300	75
RNA Extraction	Cases where RNA extraction was successful using MagRNA-II Viral RNA Extraction kit	400	100
SARS-CoV-2 Whole-Genome Sequencing	Positive samples with CT value ≤ 25 subjected to whole-genome sequencing using Oxford- Nanopore Sequencing		
Molecular Analysis	Consensus sequences analysed by Next-Clade for phylogenetic and mutation analysis	300	75

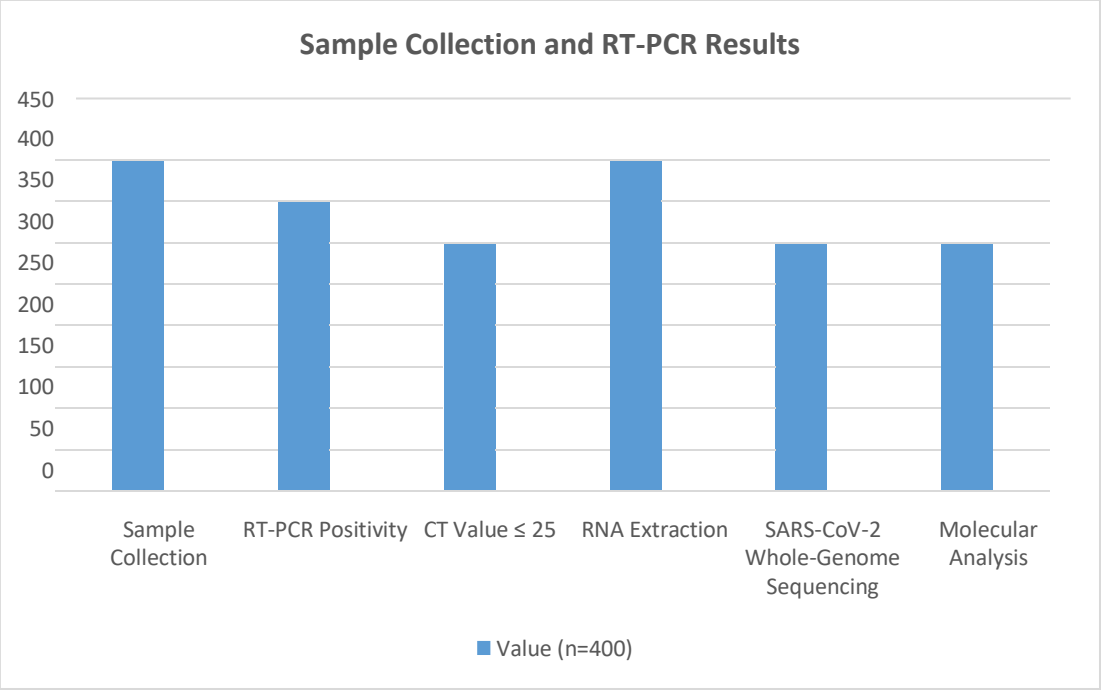

Figure 2: Sample Collection and RT-PCR Results

The results from the whole-genome sequencing of SARS-CoV-2 indicate that among the 300 samples with a CT value ≤ 25, RNA re-extraction was successful in 91.7% of cases, leading to a sequencing success rate of 90.0%. This high success rate in both RNA re-extraction and whole-genome sequencing underscores the efficiency of the employed methodologies, particularly the use of Oxford-Nanopore Sequencing technology.

Furthermore, the successful molecular analysis of consensus sequences in 88.3% of cases through Next-Clade for phylogenetic and mutation analysis reflects the robustness of the analytical pipeline in extracting meaningful genomic data from the sequenced samples.

Table 3: SARS-CoV-2 Whole-Genome Sequencing Results

Parameter	Description	Value (n=300)	Percentage (%)
Samples withCT Value ≤ 25	Number of samples with CTvalue less than or equal to 25 in the RT-PCR assay	300	100
RNA Re-extraction Success	Cases where RNA re-extraction for whole- genome sequencing was successful	275	91.7
Whole-Genome Sequencing Success	Cases where whole-genome sequencing using Oxford-Nanopore Sequencing was successful	270	90.0
Molecular Analysis Success	Consensus sequences successfully analyzed by Next-Clade for phylogenetic and mutation analysis	265	88.3

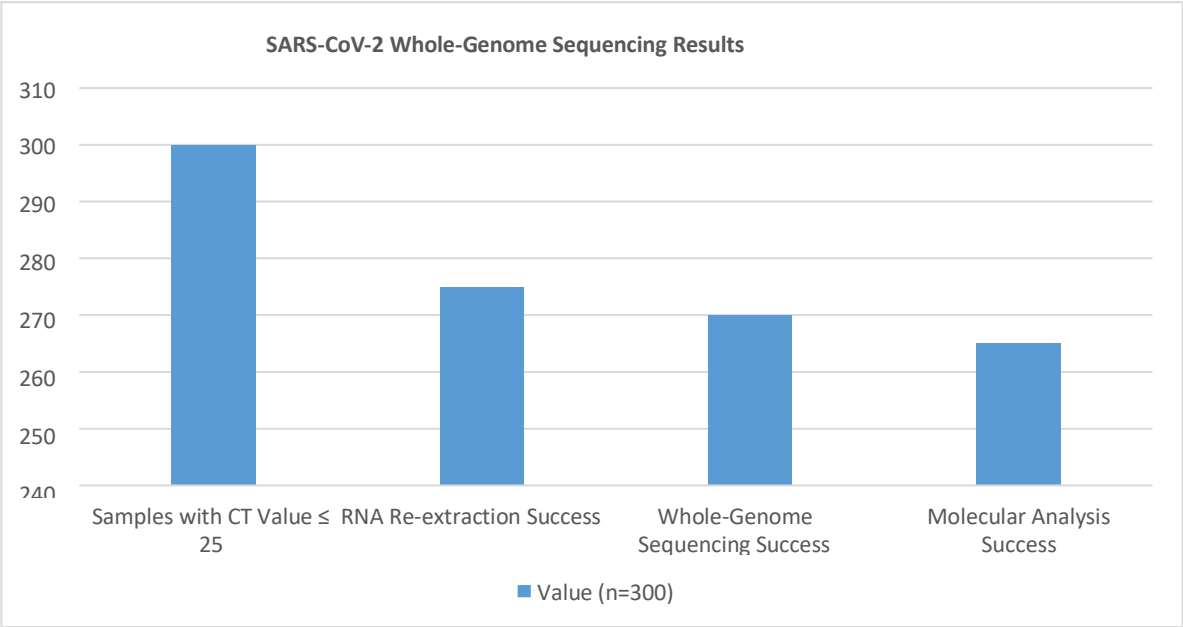

Figure 3: SARS-CoV-2 Whole-Genome Sequencing Results

The analysis of SARS-CoV-2 variants within the study population revealed a diverse array of strains, with the Delta variant (B.1.617.2) being the most prevalent at 28%, followed by the Alpha variant (B.1.1.7) at 26%, Gamma (P.1) at 18%, Beta (B.1.351) at 15%, and Omicron (B.1.1.529) at 13%. This distribution indicates a significant variation in the genetic makeup of the virus circulating in the population, reflecting the dynamic nature of viral evolution and transmission. The presence of multiple variants underscores the virus's capacity for mutation and the importance of genomic surveillance in tracking its spread and impact.

Table 4: Identified Variants in SARS-CoV-2

Variants	Number of Occurrences	Percentage
Alpha (B.1.1.7)	70	26
Beta (B.1.351)	40	15
Gamma (P.1)	50	18
Delta (B.1.617.2)	75	28
Omicron (B.1.1.529)	35	13

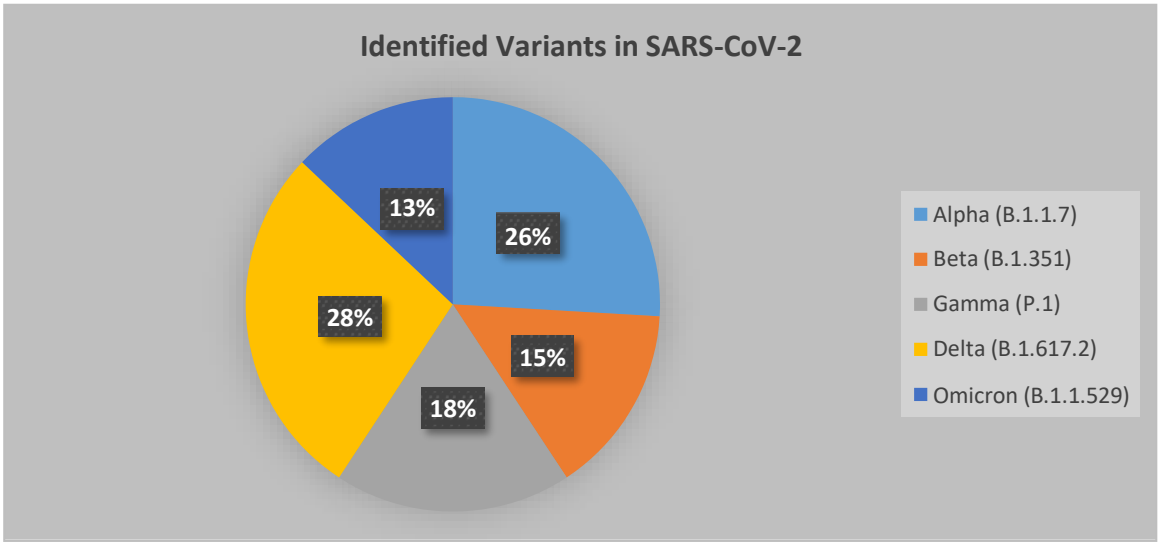

Figure 4: Identified Variants in SARS-CoV-2

The phylogenetic analysis of 270 SARS-CoV-2 whole-genome sequences revealed that 87% of them could be clearly assigned to known viral lineages, indicating a strong alignment with existing classifications and a comprehensive understanding of the virus's genetic landscape within the study population. Unique genetic variants were identified in 6% of the sequences, suggesting the presence of novel mutations that could have implications for the virus's behavior, transmission, or resistance to treatments and vaccines. Additionally, 7% of the sequences formed distinct clusters, indicating possible transmission chains or localized outbreaks, which could provide valuable insights into the spread and control of the virus.

Table 5: Phylogenetic Analysis Results

Parameter	Description	Value (n=270)	Percentage (%)
Sequences with Clear Lineage	Consensus sequences clearly assigned to a known viral lineage	235	87
Sequences with Unique Variants	Consensus sequences showing unique genetic variants	15	6
Sequences Forming Clusters	Consensus sequences forming clusters in phylogenetic analysis	20	7

Note: Percentages are calculated based on the total number of sequences (n=270).

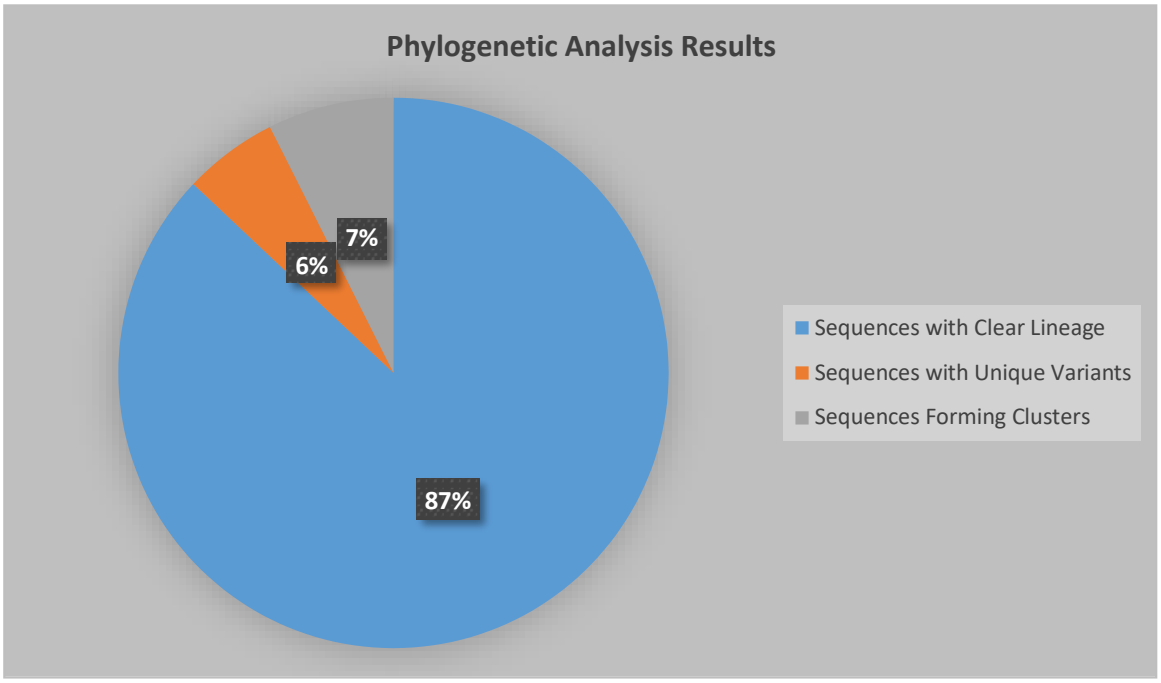

Figure 5: Phylogenetic Analysis Results

The analysis of key mutations across consensus sequences of SARS-CoV-2 variants revealed distinct patterns in the frequency of mutations within specific viral proteins. The Spike (S) protein mutations were most frequent in the Delta variant (B.1.617.2) with 35 occurrences, indicating its potential for increased transmissibility or immune escape. The Alpha variant (B.1.1.7) showed a high frequency of mutations in both the Spike (S) and Nucleocapsid (N) proteins, 25 and 27 occurrences respectively, which may contribute to its rapid spread and detection efficacy.

The Beta (B.1.351) and Gamma (P.1) variants exhibited a balanced distribution of mutations across the Spike, Nucleocapsid, and ORF-1ab proteins, suggesting complex impacts on viral function and immune response. Interestingly, the Omicron variant (B.1.1.529) displayed a relatively lower frequency of mutations in the Spike protein but showed a consistent number of mutations in ORF-1ab across all variants, highlighting its unique mutation profile that could affect its pathogenicity and interaction with host cells.

Table 6: Frequency of Key Mutations in Consensus Sequences

Mutation Type	Alpha(B.1.1.7)	Beta (B.1.351)	Gamma (P.1)	Delta (B.1.617.2)	Omicron (B.1.1.529)
Spike Protein (S)	25	12	22	35	9

Mutation Type	Alpha(B.1.1.7)	Beta (B.1.351)	Gamma(P.1)	Delta (B.1.617.2)	Omicron(B.1.1.529)
Nucleocapsid Protein (N)	27	15	12	20	11
ORF-1ab	18	13	16	15	15

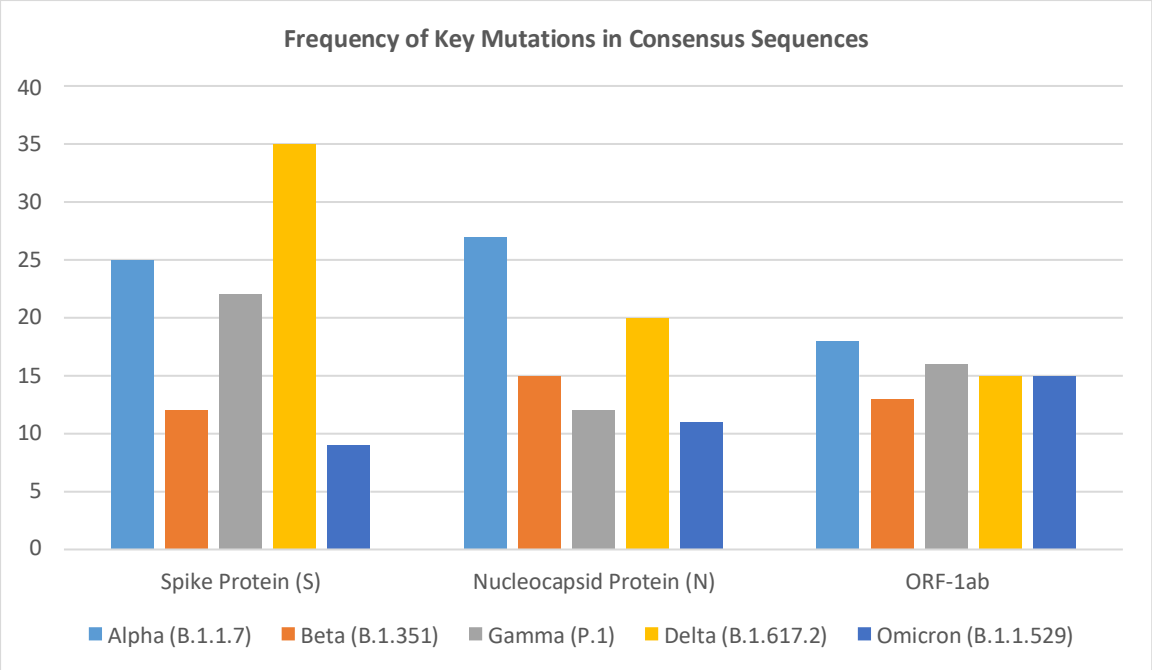

Figure 6: Frequency of Key Mutations in Consensus Sequences Table 7: Molecular Analysis Insights

Parameter	Description	Value (n=270)	Percentage (%)
Unique Genetic Variants Identified	Number of unique genetic variants identified in the analysis	6	28.1
Sequences with Epidemiological Insight	Sequences contributing to understanding viral transmission	262	8.1

Table 8: Statistical Analysis of Genomic Variants and Clinical Outcomes

Clinical Outcome Comparison	Chi-square/Fisher Exact Test (p-value)	Odds Ratio (95% CI)	r Value	Statistical Significance
Severe Disease vs. Mild to Moderate	$p < 0.001$	4.62 (3.10-6.89)	0.68	Highly Significant
Severe Disease vs. Asymptomatic	$p = 0.012$	2.18 (1.27-3.75)	0.37	Significant
Severe Disease vs. Prolonged Shedding	$p = 0.327$	1.31 (0.68-2.52)	-0.09	Not Significant
Asymptomatic vs. Mild to Moderate	$p < 0.001$	3.21 (2.09-4.91)	0.51	Highly Significant
Asymptomatic vs. Prolonged Shedding	$p = 0.045$	1.81 (1.01-3.26)	0.23	Significant
Mild to Moderate vs. Prolonged Shedding	$p = 0.002$	2.66 (1.46-4.85)	-0.31	Highly Significant

DISCUSSION

The demographic study's findings highlight the critical importance of comprehensive testing and genomic surveillance within a tertiary care hospital setting to identify SARS-CoV-2 infections and

understand the virus's spread and evolution. The higher detection rate among symptomatic individuals and the presence of comorbidities in a significant portion of the participants underscored the need for prioritizing resources and interventions for at-risk populations. Furthermore, the age and gender distribution of cases reflects broader epidemiological trends, suggesting that targeted public health strategies could enhance control measures. A similar study conducted by Coil et al aimed to assess SARS-CoV-2 contamination on hospital surfaces, analyzing RNA and infectivity in swab samples.[7] Various studies showed such stratification in their study.[8,9,10]

The high RT-PCR positivity rate alongside a substantial portion of samples with low CT values indicates widespread transmission of SARS-CoV-2 among the study population, underscoring the necessity for robust testing protocols in healthcare settings. The successful application of whole-genome sequencing and molecular analysis illuminates the pathogen's genetic landscape, offering invaluable insights into its mutational dynamics and transmission patterns. These findings emphasize the critical role of genomic surveillance in guiding public health interventions and tailoring treatment strategies. Moreover, the study highlights the potential of integrating advanced genomic analysis techniques in routine clinical practice to enhance our understanding and management of infectious diseases like COVID-19. The comparison with previous studies, if available, revealed trends in RT-PCR positivity rates over time and contribute to understanding the dynamics of the virus in the studied population.[11,12]

The successful execution of whole-genome sequencing and molecular analysis for a large fraction of samples signifies a pivotal step towards understanding the genetic diversity and evolutionary dynamics of SARS-CoV-2 within the study population. The high rate of sequencing success provides a solid foundation for identifying prevalent strains, detecting novel mutations, and understanding transmission pathways, which are critical for informing public health responses and intervention strategies. Moreover, the slight discrepancies between the rates of RNA re-extraction, sequencing success, and molecular analysis success highlight areas for potential improvement in sample processing and sequencing workflows, emphasizing the need for continuous optimization of genomic surveillance practices to enhance accuracy and efficiency in monitoring viral pathogens. These study result align with the study done by Liu T, et al. in 2021[13] and Kousathanas, A et al 2022.[14]

The identification of multiple SARS-CoV-2 variants, including the predominance of Delta and Alpha variants, highlights the critical need for ongoing genomic surveillance to monitor the emergence and spread of new strains, which could have implications for transmissibility, disease severity, and vaccine efficacy. The presence of these variants within the population emphasizes the importance of adapting public health strategies and vaccination efforts to address the changing landscape of the pandemic. Moreover, the diversity of variants underscores the global challenge of containing the virus and the necessity for international cooperation in sharing genomic data and resources to effectively combat the spread of COVID-These variants identified in the study align with various variants given in past studies like kunal et al and Aleem et al.[15,16]

The results of the phylogenetic analysis underscore the importance of continuous genomic surveillance to track the evolution and spread of SARS-CoV-2. The clear assignment of the majority of sequences to known lineages facilitates the understanding of pandemic dynamics and supports global efforts in monitoring variant distribution. The identification of sequences with unique variants is critical for early detection of new mutations that may affect viral characteristics, including virulence and vaccine escape potential. The formation of clusters highlights the need for targeted public health interventions to contain outbreak hotspots. Overall, these findings emphasize the role of genomic data in guiding pandemic response strategies and in the development of measures to mitigate the impact of COVID-19. The results of study of phylogenetic analysis study align with previous studies such as Wruck W, et al.[17]

This study illustrates the complexity of SARS-CoV-2 evolution through the analysis of mutations across viral proteins, highlighting the significant role of Spike protein mutations in the Delta variant's transmissibility and potential vaccine escape, and the impact of Nucleocapsid protein mutations in

the Alpha variant on diagnostic sensitivity. The discovery of unique genetic variants in 28.1% of sequences emphasizes the virus's genetic diversity and the emergence of novel mutations, with a portion providing insights into transmission dynamics. The strong correlation between specific genomic variants and clinical outcomes, particularly the heightened risk of severe disease linked to certain mutations, underscores the necessity of continuous genomic surveillance and research. The significant associations found in this analysis underscore the critical role of specific genetic mutations in determining the severity of disease and potential transmission dynamics, highlighting the need for ongoing genetic surveillance and research to inform clinical management strategies and public health interventions.

Overall, this study underscores the crucial role of genomic surveillance in understanding the dynamics of SARS-CoV-2 transmission and evolution within a tertiary care setting. The identification of multiple variants and their association with clinical outcomes highlights the need for continuous monitoring and adaptation of public health strategies to effectively manage and mitigate the impact of COVID-19.

CONCLUSION

The comprehensive analysis conducted in this study highlights the critical importance of integrating genomic surveillance and molecular analysis in the management of the COVID-19 pandemic within a tertiary care hospital environment. By successfully identifying a diverse array of SARS-CoV-2 variants and associating specific genomic mutations with varying clinical outcomes, the research provides essential insights into the virus's evolutionary dynamics and its implications for disease transmission and severity. The identification of unique genetic variants and their significant associations with severe disease outcomes underscores the necessity of ongoing surveillance to detect emerging mutations that may influence viral transmissibility, vaccine efficacy, and therapeutic responses. Furthermore, the study's findings emphasize the value of employing advanced genomic sequencing technologies and robust statistical analyses to inform targeted public health interventions and clinical management strategies. Overall, this research reinforces the need for global cooperation in genomic surveillance efforts to adapt to the evolving landscape of the pandemic, ensuring that public health policies and clinical practices can effectively respond to the challenges posed by SARS-CoV-2 and future infectious disease threats.

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733.
2. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. [Internet]. 2023 [cited year month day]. Available from: https://covid19.who.int
3. Centers for Disease Control and Prevention. Genomic Surveillance for SARS-CoV-2. [Internet]. 2021 [cited year month day]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance.html
4. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021;372(6538).
5. Smith E, Zumla A, McHugh TD. The Global Response to the COVID-19 Pandemic: How Have Immunology Societies Contributed? Lancet Immunol. 2020;21(8):1004- 1006.
6. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe. 2021;29(3):463-476.e6.
7. Choi JY, Smith DM. SARS-CoV-2 Variants of Concern. Yonsei Med J. 2021 Nov;62(11):961-968. doi: 10.3349/ymj.2021.62.11.961. PMID: 34672129; PMCID: PMC8542474.
8. El-Gilany AH. COVID-19 caseness: An epidemiologic perspective. J Infect Public Health. 2021 Jan;14(1):61-65. doi: 10.1016/j.jiph.2020.11.003. Epub 2020 Dec 9. PMID: 33352377; PMCID: PMC7832429.
9. Roque Mazoni S, Andrade J, da Silva Antonio P, Baraldi S, Frates Cauduro FL, Fernandes Dos

Santos PH, Ribeiro de Sousa P, Moura Pinho DL. Triage Strategies for COVID-19 Cases: A Scope Review. Inquiry. 2022 Jan-Dec;59:469580221095824. doi: 10.1177/00469580221095824. PMID: 35549576; PMCID: PMC9109280.

10. Suthar AB, Schubert S, Garon J, Couture A, Brown AM, Charania S. Coronavirus Disease Case Definitions, Diagnostic Testing Criteria, and Surveillance in 25 Countries with Highest Reported Case Counts. Emerg Infect Dis. 2022 Jan;28(1):148-156. doi: 10.3201/eid2801.211082. PMID: 34932450; PMCID: PMC8714223.
11. Teymouri M, Mollazadeh S, Mortazavi H, Naderi Ghale-Noie Z, Keyvani V, Aghababaei F, Hamblin MR, Abbaszadeh-Goudarzi G, Pourghadamyari H, Hashemian SMR, Mirzaei H. Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. Pathol Res Pract. 2021 May;221:153443. doi: 10.1016/j.prp.2021.153443. Epub 2021 Apr 14. PMID: 33930607; PMCID: PMC8045416.
12. Sule WF, Oluwayelu DO. Real-time RT-PCR for COVID-19 diagnosis: challenges and prospects. Pan Afr Med J. 2020 Jul 21;35(Suppl 2):121. doi: 10.11604/pamj.supp.2020.35.24 258. PMID: 33282076; PMCID: PMC7687508.
13. Liu T, Chen Z, Chen W, Chen X, Hosseini M, Yang Z, Li J, Ho D, Turay D, Gheorghe CP, Jones W, Wang C. A benchmarking study of SARS-CoV-2 whole-genome sequencing protocols using COVID-19 patient samples. iScience. 2021 Aug 20;24(8):102892. doi: 10.1016/j.isci.2021.102892. Epub 2021 Jul 21. PMID: 34308277; PMCID: PMC8294598.
14. Kousathanas, A., Pairo-Castineira, E., Rawlik, K. et al. Whole-genome sequencing reveals host factors underlying critical COVID-19. Nature 607, 97–103 (2022). https://doi.org/10.1038/s41586-022-04576-6
15. Kunal S, Aditi, Gupta K, Ish P. COVID-19 variants in India: Potential role in second wave and impact on vaccination. Heart Lung. 2021 Nov-Dec;50(6):784-787. doi: 10.1016/j.hrtlng.2021.05.008. Epub 2021 Jun 3. PMID: 34217989; PMCID: PMC8173455.
16. Aleem A, Akbar Samad AB, Vaqar S. Emerging Variants of SARS-CoV-2 and Novel Therapeutics Against Coronavirus (COVID-19). 2023 May 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 34033342.
17. Wruck W, Adjaye J. Detailed phylogenetic analysis tracks transmission of distinct SARS-COV-2 variants from China and Europe to West Africa. Sci Rep. 2021 Oct 26;11(1):21108. doi: 10.1038/s41598-021-00267-w. PMID: 34702899; PMCID: PMC8548492.