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Detection of SARS-CoV-2 in nasopharyngeal swab samples from COVID-19 patients in Riyadh, Saudi Arabia: a PCR-based study

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Abstract

This study involved laboratory experiments using conventional PCR to detect the *RNA-dependent RNA polymerase protein* (*RdRp*) and *Envelope* (*E*) genes in Forty-Seven nasopharyngeal swab samples from COVID-19 patients in Riyadh, Saudi Arabia. Gel electrophoresis results showed amplification of the *RdRp* gene in 85.1% of the samples and the *E* gene in 89.4%, confirming the widespread presence of these viral genes. The presence of bands in positive controls indicated the specificity of the primers whilst no bands were detected in the negative controls, indicating the absence of contamination. The study also included data collection from databases to explore the demographic and clinical characteristics of COVID-19 patients. The male to female infection ratio was 363:63, significantly favoring males ($P \leq 0.05$). Fever was present in 81.46% of patients ($P \leq 0.05$). A significant portion (60.56%) had not contacted positive cases or traveled outside Saudi Arabia ($P \leq 0.05$). The Saudi to non-Saudi ratio among patients was 24.65–75.35% ($P \leq 0.05$). Age distribution showed 62.21% of patients were under 50 years old ($P \leq 0.05$). ICU admission was required for 12.21% of patients ($P \leq 0.05$). Co-morbidities were present in 27.46% of patients ($P \leq 0.05$). The mortality rate was low, with a deceased to alive ratio of 1:141 ($P \leq 0.05$). Gel electrophoresis revealed that 85.1% of samples showed amplification for the *RdRp* gene, and 89.4% for the *E* gene, confirming the widespread presence of these viral genes among the samples tested.

Keywords COVID-19, Envelope (*E*) gene, Polymerase chain reaction (PCR), *RdRp* Gene, SARS-CoV-2

Introduction

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has imposed unprecedented challenges on global public health systems, economies, and societies. Rapid and accurate diagnosis of COVID-19 is paramount for effective disease management, containment, and mitigation strategies [1]. Among the various diagnostic modalities available, the detection of SARS-CoV-2 nucleic acid in respiratory specimens, particularly swab samples, remains the cornerstone for early identification and isolation of infected individuals [2].

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Since the emergence of the pandemic, significant efforts have been directed towards refining and improving diagnostic techniques for detecting SARS-CoV-2 in swab samples [3]. The urgency to develop reliable, sensitive, and high-throughput assays has spurred a wave of innovation in molecular biology, virology, and diagnostic technology [4]. From conventional reverse transcription polymerase chain reaction (RT-PCR) assays to advanced molecular and antigen-based methodologies, the landscape of COVID-19 diagnostics continues to evolve rapidly [5]. A deeper understanding of the strengths and limitations of existing methodologies, coupled with insights into emerging technologies, will be instrumental in enhancing diagnostic capabilities, improving patient care, and combating the ongoing pandemic [6].

Given the critical importance of accurate diagnostics in combating the pandemic, this study aimed to evaluate the effectiveness and applicability of conventional PCR-based methods for detecting SARS-CoV-2 in nasopharyngeal swab samples. The research focused on assessing the sensitivity, specificity, and clinical utility of RT-PCR assays targeting key viral genes such as the *RdRp* and *E* genes. Additionally, the study explored emerging diagnostic trends, and the challenges associated with sample collection, handling, and result interpretation (Fig. 1).

By synthesizing current diagnostic practices and identifying gaps in existing methodologies, this research seeks to inform clinicians, researchers, and policymakers about strategies to enhance diagnostic accuracy, improve patient care, and support global efforts to manage and mitigate the ongoing COVID-19 crisis.

In addition to discussing the technical aspects of diagnostic assays, this research also addressed the challenges and considerations associated with sample collection, handling, and interpretation of results.

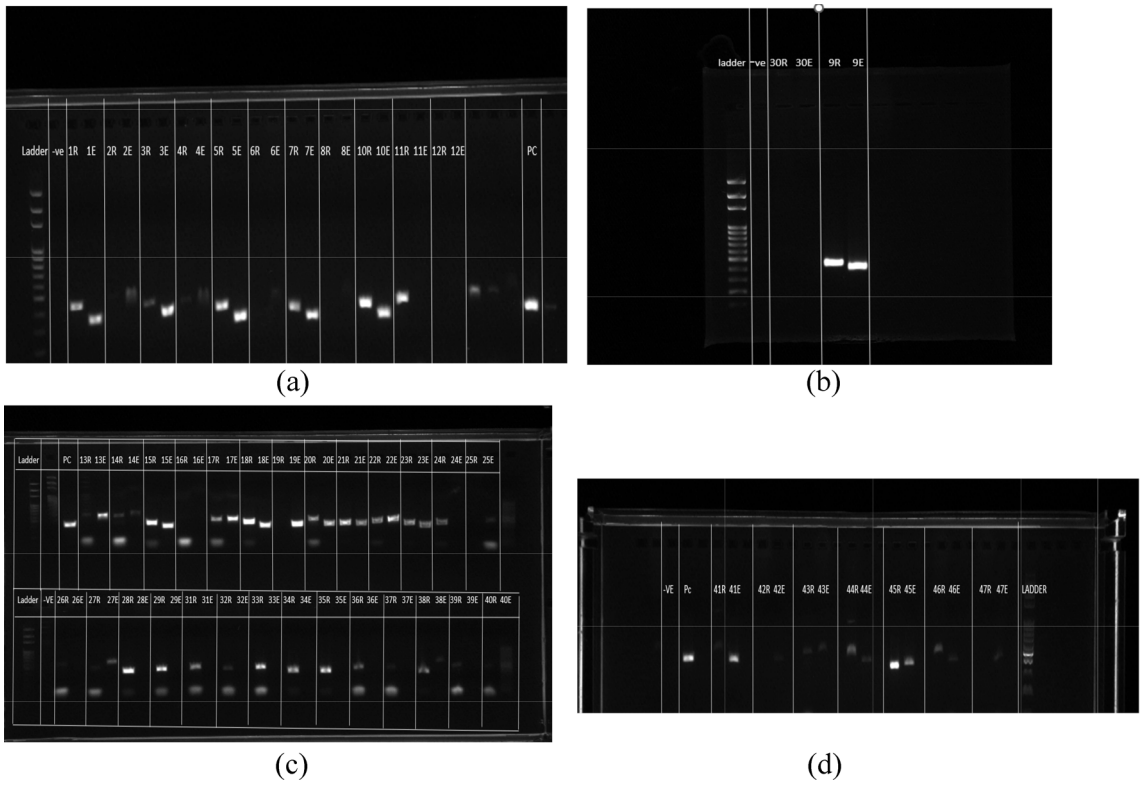

Fig. 1 **a** Visualization of positive amplicons of COVID-19 RD and E genes respectively on 0.8% agarose gel electrophoresis (Total $n=47$). Lane M shows a 110 bp DNA ladder, with bands at 100, 200, 300, 500, and 1000 bp, providing size markers for the PCR amplicons. Picture represents 1KB ladder, positive control, negative control, samples from 1–8 and 10–12. Lanes 1 (both genes),3 (E gene),5 and 7 and 9 (both genes),10 and 11 (RD gene) represents positive samples. **b** Negative control, positive control, Samples 30 and 9 (RdRp gene and E gene respectively), Lane M contains a 100 bp DNA ladder, with bands at 100, 200, 300, 500, and 1000 bp. Lane 9 indicated a positive sample for the E gene, as evidenced by the band appearing at approximately [specific size, e.g., 110 bp], when compared to the ladder. **c** Negative control, positive control, Samples 13–40 (RdRp gene and E gene respectively), Lane M contains a 100 bp DNA ladder, with bands at 100, 200, 300, 500, and 1000 bp. Lanes 13, 15, 17–23, 28–31, and 33–35 represent positive samples, as indicated by the presence of bands at approximately [specific size, e.g., 110 bp for RdRp or E gene], compared to the ladder. **d** Negative control, positive control, Samples 41–47 (RdRp gene and E gene respectively), Lane M contains a 100 bp DNA ladder, with bands at 100, 200, 300, 500, and 1000 bp. Lanes 41 and 45 represent positive samples, as indicated by the presence of bands at approximately [specific size, e.g., 110 bp for RdRp or E gene], compared to the ladder

Table 1 Demographic data of COVID-19 confirmed positive cases ($n=426$)

Demographic characteristics		
Sex ratio	Males	Females
	363 (85.21%)	63 (14.79%)
Common symptoms of COVID 19	**Without fever**	**With fever**
	79 (18.54%)	347 (81.46%)
Contact with positive or suspected cases and/or travel history	**No**	**Yes**
	258 (60.56%)	168 (39.44%)
Nationality	**Saudi**	**None- Saudi**
	105 (24.65%)	321 (75.35%)
Age	**Under 50**	**50 or above**
	265 (62.21%)	161 (37.79%)
Admission to ICU	**Yes**	**No**
	52 (12.21%)	374 (87. 79%)
Co morbidity with chronic diseases	**Yes**	**No**
	117 (27.46%)	309 (72.54%)
Mortality	**Alive**	**Deceased**
	423 (99.3%)	3 (0.7%)

Materials and methods

Study settings

Demographic data and nasopharyngeal swab samples were collected from patients presenting with symptoms consistent with COVID-19 at King Faisal Specialized Hospital and Research Centre (KFSHRC) in Riyadh, Saudi Arabia between October 2023 to January 2024. The study adhered to ethical standards established by Princess Nourah bint Abdulrahman University and received approval from the IRB committee (approval number: 24–0138). Prior to sample collection, informed consent was obtained from all participants or their legal guardians. Sample collection procedures were conducted following strict ethical guidelines to ensure the participants' rights, privacy, and safety. All experiments and analyses were carried out in accordance with institutional protocols and international ethical standards to maintain the integrity and reliability of the research findings.

Study design

This is a descriptive, retrospective analysis of demographic data of COVID-19 cases in Saudi Arabia. It aims to identify trends, risk factors, and the impact of demographic variables on the spread and outcomes of the disease.

Data source

The data for this study was sourced from the Ministry of Health (MOH) of Saudi Arabia, which maintains a comprehensive database of COVID-19 cases, including detailed demographic information (Table 1). Additional data was obtained from KFSHRC databases. The PCR results were obtained from experiments conducted at King Abduaalh hospital research Centre core laboratory.

Inclusion and exclusion criteria

Samples from confirmed COVID-19 cases (as per KFSHRC) as well as the availability of complete demographic data (age, gender, nationality, region) were included in the study while some cases and data were excluded according to certain criteria such as: Cases with incomplete demographic data as well as suspected but unconfirmed COVID-19 cases.

Data collection

Demographic variables

The collected data included demographic variables such as age, gender, nationality, and region of residence. Additionally, clinical variables were recorded, including the date of diagnosis, presenting symptoms, the presence of comorbidities, hospitalization status (whether the patient was admitted to a hospital or not), and the clinical outcome (categorized as recovered or deceased). These variables were systematically documented to facilitate a comprehensive analysis of the study population and their clinical characteristics.

Samples collection

Informed consent was obtained from all participants prior to sample collection and utilizing the coded demographic data of patients in KFSHRC. The research has then approved by the Ethical Committee in the research centre of King Abdullah Hospital where the molecular screening of the samples has been conducted. Data was anonymized to protect patient confidentiality. Swabs were inserted into the nostril to reach the nasopharynx, rotated gently, and then placed into viral transport medium (VTM) [7].

Study limitations

This study has several limitations that should be acknowledged. Due to limited funding, conventional PCR was utilized instead of RT-PCR, which may have impacted the sensitivity and specificity of the results. Additionally, sequencing was not conducted, restricting the ability to perform detailed genetic analyses and identify specific variants. The retrospective design of the study introduces the potential for incomplete or inaccurate data, as it relies on previously recorded information. Moreover, the study was limited to the available demographic variables, which may not capture all relevant risk factors. Lastly, the findings may not be generalizable to other countries or populations due to cultural and healthcare system differences, which could influence the applicability of the results in different contexts.

RNA extraction

Around 50 µg of RNA was extracted from collected 75 swab samples according to a recommended method using a commercial RNA extraction kit (QIAwave RNA Mini Kit (50), Cat. No. / ID: 74534) following the manufacturer's instructions [8]. Extraction was performed in a biosafety level 3 (BSL-3) laboratory equipped with appropriate personal protective equipment (PPE) and biosafety cabinets in King Abduaalh hospital research centre core laboratory [9].

Reverse transcription

Extracted RNA was subjected to reverse transcription to convert viral RNA into complementary DNA (cDNA) using a QIAGEN - QuantiTect Reverse Transcription Kit (Germany) according to the manufacturer's instructions. A total of 1–5 µg of total RNA or 10–100 ng of mRNA was added per reaction. The final RNA volume was limited to 8 µL in a 20 µL reaction. An amount of 1µL of reverse transcriptase enzyme and oligo (dT) primers were added in a 20 µL reaction, following the protocol provided with the kit [10].

PCR amplification

PCR amplification of the cDNA was performed using specific primers targeting the SARS-CoV-2 viral genome regions, such as the *E* and *RdRp* gene [11]. The amplification reaction mixture had a total volume of 20 µL, consisting of template cDNA (1–2 µL), PCR primers (forward and reverse, each at a final concentration of 0.1–0.5 µM), DNA polymerase enzyme (0.5–1 µL, depending on the enzyme), nucleotides (dNTPs at a final concentration of 200 µM each), and the reaction buffer (provided with the polymerase) to make up the remaining volume 12. All components were prepared according to the manufacturer's instructions for optimal amplification efficiency.

RdRp Gene Primers sequences were as follows:
Forward primer (*RdRp*_SARSr-F):
5'-GTGAAATGGTCATGTGTGGCGG-3'
Reverse primer (*RdRp*_SARSr-R):
5'-CAGACATTTTGCTCTCAAGTGT-3'
E Gene Primers sequences were as follows:
Forward Primer (*E*-Sarbeco-F):
5'-ACAGGTACGTTAATAGTTAATAGCGT-3'
Reverse Primer (*E*-Sarbeco-R):
5'-ATATTGCAGCAGTACGCACACA-3

PCR amplification was carried out in a thermal cycler with the following cycling conditions [13]: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at specific temperature of 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min.

Quality assurance of the test

Positive and negative controls containing known concentrations of SARS-CoV-2 RNA were included in each PCR run to validate assay. Negative controls containing no template RNA were included to monitor for contamination [14].

Gel electrophoresis

PCR products were analyzed by agarose gel electrophoresis to visualize the amplification products. The PCR products were analyzed using agarose gel electrophoresis. Gels were prepared with 2% agarose in 1X TAE buffer containing a DNA- ethidium bromide staining dye. The electrophoresis was carried out at 100 V specific voltage for a period of 30–60 min. Gel images were captured using a gel documentation system, and band sizes were compared with molecular weight markers to confirm the presence of the target amplicons [15].

Results

Demographic data results

In this study, the gender distribution of COVID-19 infected patients was observed. The male to female ratio of infected patients was found to be 363:63, corresponding to 85.21% males and 14.79% females. The chi-square test analysis for this male to female ratio yielded a value of 0.00012, indicating that the difference is statistically significant ($P \leq 0.05$).

Additionally, 81.46% of patients exhibited signs and symptoms combined with fever, while 18.54% of patients did not exhibit fever. The chi-square analysis value for this comparison was 0.009, which is also considered significant ($P \leq 0.05$).

Furthermore, 60.56% of patients had not contacted positive cases or traveled outside Saudi Arabia, whereas 39.44% of patients neither contacted positive cases nor traveled outside Saudi Arabia. The chi-square analysis for this comparison showed a value of 0.007, indicating a significant difference ($P \leq 0.05$).

The Saudi to non-Saudi ratio among the patients was 24.65–75.35%. The chi-square analysis for this ratio yielded a value of 0.0001, which is considered significant ($P \leq 0.05$).

In terms of age distribution, 62.21% of the patients were under 50 years old, while 37.79% were 50 years old or above. The chi-square analysis for this age distribution showed a value of 0.02, indicating a significant difference ($P \leq 0.05$).

On the other hand, 12.21% of the patients were admitted to the ICU, while the condition of 87.79% of the patients did not require ICU admission. The chi-square analysis for ICU admission yielded a value of 0.0003, which is considered significant ($P \leq 0.05$).

Around 27.46% of the patients exhibited co-morbidities with other diseases such as stroke, diabetes, and hypertension, while 72.54% of the patients did not have any co-morbidities. The chi-square analysis for the presence of co-morbidities showed a value of 0.001, indicating a significant difference ($P \leq 0.05$).

Results indicated that COVID-19 induces low mortality, with the ratio of deceased to alive patients being 1:141 (0.7% deceased and 99.3% alive). The chi-square analysis for this mortality ratio showed a value of 0.0009, which is considered significant ($P \leq 0.05$) (Table 1).

Gel electrophoresis results

The results were interpreted based on the presence or absence of specific amplicons corresponding to the target viral genes, namely the *RdRp* and *E* genes. To assess the sensitivity, specificity, and accuracy of the PCR assay for detecting SARS-CoV-2 in nasopharyngeal swab samples, a total of 47 samples were analyzed using 0.8% agarose gel electrophoresis.

The amplification products were visualized under UV light, and the bands corresponding to the *RdRp* and *E* genes were clearly present in the positive control samples, confirming that the primers effectively targeted the viral genes. No bands were observed in the negative control, indicating the absence of contamination and validating the specificity of the assay. The presence of bands in the experimental samples was used to confirm the presence of SARS-CoV-2 viral RNA.

These findings demonstrated that the PCR assay was both sensitive and specific in detecting SARS-CoV-2 in nasopharyngeal swab samples, with clear differentiation between positive and negative samples based on the presence of target amplicons.

Amplicon detection

The presence of amplicons specific to the *RdRp* and *E* genes was visualized under UV light after electrophoresis. Positive samples exhibited distinct bands at the expected sizes of approximately 110 bp for the *RdRp* gene and 72 bp for the *E* gene.

Positive samples

Out of 47 samples, 40 (85.1%) showed positive amplification for the *RdRp* gene. Similarly, 42 (89.4%) of the samples showed positive amplification for the *E* gene.

Representative Gel image The results of the agarose gel electrophoresis showed a 100 bp DNA ladder in Lane M, which was used as a molecular size marker. Lanes 1–47 contained PCR products from COVID-19 patient samples, demonstrating the amplification of the target regions. Lane 48 displayed the positive control for the *RdRp* gene, while Lane 49 showed the positive control for the *E* gene, confirming the validity of the amplification process. Lane 50, which contained the negative control with no template, showed no amplification, indicating the absence of contamination in the PCR reaction.

The bands corresponding to the *RdRp* and *E* genes were clearly visible in the positive controls, while the negative control showed no bands, indicating the specificity of the primers and the absence of contamination.

Interpretation Regarding the *RdRp* gene, out of the 47 samples tested, 40 showed bands at approximately 110 bp, confirming the presence of the *RdRp* gene. This resulted in a high positivity rate of 85.1%, indicating a widespread presence of the *RdRp* gene among the COVID-19 patient samples tested.

Discussion

The current study provides comprehensive insights into the demographic and clinical characteristics of COVID-19 patients, emphasizing significant differences in gender distribution, symptom presentation, exposure history, nationality, age distribution, ICU admission rates, co-morbidities, and mortality rates.

Our findings reveal a pronounced gender disparity among infected individuals, with males constituting 85.21% of the cases compared to 14.79% females. This significant gender difference ($P \leq 0.05$) aligns with previous reports suggesting that males may have a higher susceptibility to COVID-19, potentially due to differences in immune response, exposure risk, or underlying health conditions. The study corroborates findings from similar research indicating a significant gender disparity in COVID-19 mortality [16]. Specifically, of the deceased patients, 70.3% were men and 29.3% were women. This trend suggests that men are more prone to severe infections and death from COVID-19, despite having similar susceptibility and comorbidities as women ($P = 0.016$). This gender disparity in mortality is consistent with several studies reporting that men may experience more severe outcomes and higher mortality rates from COVID-19 compared to women. Possible explanations include differences in immune responses, underlying health conditions, and social factors affecting exposure and health-seeking behavior.

One of the most common symptoms observed in COVID-19 patients was fever, reported in 81.46% of cases in our study. This finding is consistent with the literature, which indicates that fever is present in 81.2% of COVID-19 patients, with a 95% confidence interval of 77.9–84.4% [17]. This prevalence underscores the importance of fever as a primary symptom in diagnosing and managing COVID-19. The high incidence of fever in our cohort aligns with this data, reinforcing fever's

role as a significant clinical marker. A notable finding in our study is that 60.56% of COVID-19 patients had neither contacted known positive cases nor traveled outside Saudi Arabia. This suggests a significant degree of local transmission and community spread, which is consistent with the epidemiological patterns observed in other studies. In contrast, 39.44% of patients neither had contact with positive cases nor travel history, indicating that even without these known risk factors, community transmission played a substantial role in the spread of COVID-19.

These findings resonate with a study that reported similar observations, noting that while the proportion of cases in the country was lower compared to global statistics, the percentage of the population affected was similar [18]. This study also highlighted that the intensity of COVID-19 varied across the 13 administrative areas, reflecting localized differences in transmission dynamics and healthcare responses.

Results of the current study found that 24.65% of COVID-19 patients were Saudi nationals, while 75.35% were non-Saudi nationals. This significant difference, as indicated by the chi-square analysis ($P \leq 0.05$), highlights the predominant impact of COVID-19 on non-Saudi populations. This observation is consistent with a previous study that reported 54.7% of COVID-19 cases were non-Saudi nationals [19]. The higher proportion of non-Saudi patients in our study may reflect various factors, including differences in living conditions, occupational exposures, and healthcare access.

One of the most notable differences observed was the age distribution of patients. These findings coincide with results of a previous study reported that COVID-19 patients were generally younger compared to those with seasonal influenza [20]. This finding contrasts with the typical seasonal influenza demographic, which often includes a higher proportion of older adults. The younger age of COVID-19 patients may influence the clinical management strategies and public health responses, as younger individuals may have different disease outcomes and healthcare needs compared to older populations more commonly affected by seasonal influenza.

Symptomatically, a substantial majority of patients (81.46%), underscoring its role as a key symptom in diagnosing and managing the disease. This finding aligns with the broader literature, where fever is frequently identified as a common symptom of COVID-19. Specifically, a study including 142 COVID-19 patients reported that 12.7% of cases had prolonged fever and 9.9% had saddleback fever[21]. These variations in fever presentation further illustrate the diverse clinical manifestations of COVID-19.

The exposure history of patients indicated that 60.56% had not contacted positive cases or traveled outside Saudi Arabia, while 39.44% had neither exposure nor travel history. This significant difference ($P \leq 0.05$) highlights the importance of community transmission in the spread of the virus and suggests that local transmission dynamics must be closely monitored.

Our results indicated that COVID-19 induces low mortality, with a deceased-to-alive patient ratio of 1:141, corresponding to 0.7% deceased and 99.3% alive. This low mortality rate underscores the effectiveness of current clinical management strategies and potentially the younger demographic of our patient cohort. These findings are consistent with other studies that report low overall mortality rates in COVID-19 patients compared to other respiratory infections [22]. Similar to the low mortality rate; 12.21% of the patients were admitted to the ICU, while 87.79% did not require ICU admission. This indicates that a majority of COVID-19 patients experienced mild to moderate illness that did not necessitate intensive care. These findings coincide with results of a research that examined ICU mortality among adult COVID-19 patients [23]. The primary outcome measure in these studies was death in intensive care as a proportion of completed ICU admissions. The meta-analysis revealed varying ICU mortality rates influenced by factors such as patient demographics, co-morbidities, and healthcare system capabilities. Our findings, which showed a low overall mortality rate and a relatively small proportion of patients requiring ICU admission, align with these broader trends observed in the meta-analysis.

Our study found that a majority of COVID-19 samples showed positive amplification for the *RdRp* and *E* genes, confirming the presence of COVID-19 genetic material. Specifically, 85.1% of samples showed positive amplification for the *RdRp* gene, and 89.4% for the *E* gene. These high detection rates validate the effectiveness of these molecular targets for diagnosing SARS-CoV-2 and support their use in diagnostic protocols. The reliability of these assays is crucial for accurate detection and management of COVID-19 cases.

Conclusion

This study highlights the accuracy and reliability of PCR-based detection of SARS-CoV-19 using nasopharyngeal swab samples, with a high proportion of positive results for *RdRp* and *E* genes confirming the presence of the virus in COVID-19 patients. The specificity of primers and absence of contamination in negative controls underscore the robustness of the method. Demographic and clinical analyses revealed significant patterns, including a higher prevalence of infection among males, a younger patient population, and a notable proportion of cases without known contact or travel history. Despite the presence of co-morbidities in some patients, the study reports a low mortality rate and a limited requirement for

ICU admission. These findings provide valuable insights into the epidemiological and clinical characteristics of COVID-19 patients in Riyadh, Saudi Arabia, while reinforcing the utility of PCR as a diagnostic tool in managing the pandemic.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02655-4.

Supplementary Material 1

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Author contributions
RA Designed the study, supervised the research, wrote and submitted the manuscript, and obtained the fund. SFA and ANS obtained the samples and purchased the kits, SYA and KA and SAA performed the experimental part and obtained the ethical approval for the study.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate
This study was conducted following ethical standards outlined in the Declaration of Princess Nourah bint Abdulrahman University and approved by the relevant ethics committee (24–0138). Informed consent was obtained from all participants or their legal guardians before sample collection.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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