

## Can Cycle Threshold Value of RT-PCR for SARS-CoV-2 Predict Secondary Bacterial Infections in Patients with Coronavirus Disease?

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### ABSTRACT

**Objectives:** This study aims to investigate the relationship between cycle threshold (Ct) values of RT-PCR for SARS-CoV-2, as a marker of viral load, and the occurrence of secondary bacterial infections in hospitalized COVID-19 patients.

**Methods:** A cross-sectional study was conducted among 141 COVID-19 patients at Sukraraj Tropical and Infectious Disease Hospital, Nepal, between July 2022 and June 2023. Bacterial cultures were performed for hospitalized patients. Ct values for SARS-CoV-2 N and Orf 1ab genes were compared between patients with and without secondary bacterial infections.

**Results:** Out of 32 hospitalized patients, 11 (34.37%) had secondary bacterial infections. The median Ct values of the N gene were slightly lower in patients with secondary bacterial infections (19.5) compared to those without (19.0), though this difference was not statistically significant ( $p = 0.2113$ ). Similar trends were observed for the Orf1ab gene (20.5 vs. 19.5;  $p = 0.3125$ ). The most frequently isolated bacterial pathogen was *Klebsiella pneumoniae*, while *Acinetobacter baumannii* and *E. coli* were associated with the lowest Ct values.

**Conclusion:** While patients with secondary bacterial infections tended to have lower Ct values, no statistically significant relationship was observed between Ct values and the occurrence of secondary bacterial infections. However, Ct values might still provide supplementary insights into disease severity and the risk of secondary infections.

**Keywords:** CT value, COVID-19, secondary bacterial infections

### INTRODUCTION

Since its emergence in late 2019, COVID-19, caused by the SARS-CoV-2 virus, has significantly impacted global health. Hospitalized COVID-19 patients are particularly

vulnerable to secondary bacterial infections, which can complicate their clinical course and contribute to increased morbidity and mortality (Langford et al., 2020; Rawson et al., 2020).

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In diagnostic testing for COVID-19, the cycle threshold (Ct) value from RT-PCR is often used as an indirect indicator of viral load. Lower Ct values correspond to higher amounts of viral RNA, suggesting a greater viral burden, which can be linked to more severe disease outcomes (Magleby et al., 2021). Despite the established use of Ct values for disease severity, their potential utility in predicting secondary bacterial infections in COVID-19 patients has not been well characterized.

Secondary bacterial infections are a known complication in patients with viral respiratory illnesses, including those with severe COVID-19, and are associated with factors like immune suppression, longer hospital stays, and intensive care admissions (Zhou et al., 2020). Recognizing early markers for these infections could be crucial for improving treatment outcomes and early antimicrobial therapies for at-risk patients (Sharma et al., 2021).

This study aims to determine whether the Ct value of RT-PCR for SARS-CoV-2 can predict secondary bacterial infections in COVID-19 patients. A clearer understanding of this relationship could improve clinical management, allowing for better allocation of medical resources and more targeted therapeutic interventions.

## METHODS

### Study design

This was a cross-sectional study conducted at Sukraraj Tropical and Infectious Disease hospital (STIDH), Teku, Kathmandu from July 2022 to June 2023. STIDH is the national referral hospital with the capacity of 100 beds inpatient service and receives patients referred from all over the country.

### Study population

Patients confirmed as COVID positive by laboratory through polymerase chain reaction (PCR) and admitted to STIDH were included in the study. Secondary bacterial infection was defined as the detection of bacteria in clinical sample collected after 48 hours of hospitalization in COVID positive cases (Langford et al., 2020). Specimen with insufficient volume and improperly labeled demographic information were excluded. Saliva

containing sample was excluded.

### Sample collection and transportation

For the detection of secondary bacterial infection, sputum sample was collected from the laboratory confirmed COVID positive case in a sterile leak proof container. Each sample was transported to the microbiology laboratory for further processing. Before accepting the sample, the sputum's quality was examined macroscopically for the presence of mucopurulent components.

### Isolation and identification of bacterial isolates

Each sputum sample was processed for bacterial culture by standard culture based techniques. Using an inoculating loop, the mucopurulent portion of sputum sample was immediately inoculated on MacConkey Agar (MA), Blood Agar (BA), and Chocolate Agar and incubated at 37 °C for 24 hours (Shakya et al., 2019). Identification of bacterial isolate was done by observation of colony characteristics, Gram's staining and biochemical tests including catalase, oxidase, indole, motility, Methyl Red Voges Proskauer (MRVP), Triple Sugar Iron (TSI), citrate utilization, urease production and others as required (Tille, 2020).

### Data analysis

All the collected data were entered into Microsoft Office Excel and subsequently into Statistical Package for Social Sciences (SPSS) (version 26.0). Mann-Whitney U test was used to compare the CT values between COVID cases with secondary bacterial infections and without secondary bacterial infection. A p-value of <0.05 was considered to be significant.

### Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Committee (IRC) of the Institute of Science and Technology (IOST), Tribhuvan University, Nepal (Reg. No. IRC/IOST 52/079/080). Written informed consent was obtained from all individual participants included in the study.

## RESULTS

### Demographic characteristics of patients

Altogether 141 COVID positive cases were enrolled in the study. Out of total COVID positive cases, 32 (22.69%) were admitted to hospital and 109 (77.31%) were OPD patients. The age of inpatients ranged from 1 to 90 years with a median age of 55 years and 15 (46.88%) were female. Out of 32 inpatients, 11 (34.37%) cases had secondary bacterial infection (Table 1).

**Table 1: Demographic characteristics of COVID positive cases**

Variable	Frequency, n (%)
<b>Type of infection</b>	
Secondary bacterial infection	11 (34.37%)
<b>Patient type</b>	
Hospitalized patients	32 (22.69%)
OPD patients	109 (77.31%)
<b>Age of patients in years</b>	
1	1 (3.13%)
24-35	3 (9.38%)
35-45	9 (28.13%)
45-60	7 (21.88%)
Above 60	12 (37.50%)
<b>Gender</b>	
Male	17 (53.13%)
Female	15 (46.88%)

### CT Values of COVID-19 Patients with and without secondary bacterial infections

Patients with secondary bacterial infections exhibited slightly lower CT values than those without, indicating a potentially higher viral load (Table 2).

**Table 2: Median CT Values of COVID-19 Patients with and without secondary bacterial infections**

	Median CT value		
	Secondary infection	Without secondary infection	p-value
<b>N gene</b>	19.5	19	0.2113
<b>Orf1ab gene</b>	20.5	19.5	0.3125

### CT values associated with specific bacterial pathogens in COVID-19 patients

Among 11 patients with secondary bacterial infections, seven different bacterial pathogens were isolated. Notably, *Acinetobacter baumannii* and *E. coli* were associated with the lowest CT values, indicating a higher viral burden in these cases. In contrast, *Enterobacter aerogenes* infections were associated with higher CT values, suggesting a lower viral load (Table 3).

**Table 3: Median CT values associated with specific bacterial pathogens in COVID-19 patients**

Type of bacteria (n=11)	Median CT value	
	N gene	Orf1ab gene
<i>Acinetobacter baumannii</i> (n=2)	15	16.5
<i>E.coli</i> (n=1)	14	15
<i>Enterobacter aerogenes</i> (n=2)	21.5	23.5
<i>Klbesiella pneumoniae</i> (n=5)	19	20
<i>Pseudomonas aeruginosa</i> (n=1)	18	19

## DISCUSSION

This study aimed to evaluate whether the cycle threshold (Ct) values of RT-PCR for SARS-CoV-2 could predict secondary bacterial infections in COVID-19 patients. Among the 32 hospitalized COVID-19 cases analyzed, 11 (34.37%) developed secondary bacterial infections, a prevalence consistent with findings from similar studies (Langford et al., 2020; Zhou et al., 2020).

Our results revealed that patients with secondary bacterial infections had slightly lower Ct values for both the N gene (19.5) and Orf1ab gene (20.5) compared to patients without secondary infections (19.0 and 19.5, respectively). Although these findings align with the hypothesis that higher viral load (lower Ct values) could compromise the immune system and predispose patients to secondary infections (Acharya et al., 2017; Rawson et al., 2020), the differences observed were not statistically significant (p = 0.2113 for N gene, p = 0.3125 for Orf1ab gene). This suggests that Ct values alone may not serve as a reliable predictor for seco-

ndary bacterial infections in COVID-19 patients.

The presence of bacterial pathogens such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *E. coli*, and *Pseudomonas aeruginosa* in patients with secondary infections underscores the importance of monitoring for nosocomial pathogens in hospitalized COVID-19 cases. Notably, *Acinetobacter baumannii* and *E. coli* were associated with the lowest Ct values (15 and 14 for the N gene), suggesting a potential association between high viral load and specific bacterial pathogens. This finding is consistent with prior evidence that high viral loads can exacerbate immune suppression, increasing susceptibility to opportunistic infections (Magleby et al., 2021).

Several factors could explain the lack of statistical significance in our findings. First, the relatively small sample size of hospitalized patients (n = 32) limits the power to detect subtle differences. Second, secondary bacterial infections are multifactorial, influenced by factors such as prolonged hospital stays, mechanical ventilation, and comorbidities, which were not fully accounted for in our study (Rawson et al., 2020; Zhou et al., 2020). Despite these limitations, our study highlights key trends that merit further investigation in larger, multicentric studies. Future research should integrate additional clinical parameters, such as inflammatory markers and comorbidity data, to better elucidate the relationship between viral load and secondary infections.

### Conclusion

While COVID-19 patients with secondary bacterial infections exhibited slightly lower Ct values, this difference was not statistically significant. Therefore, Ct values alone may not serve as a predictive marker for secondary bacterial infections in hospitalized COVID-19 patients. However, they could still provide supplementary information when combined with other clinical and laboratory parameters to guide the management of high-risk patients. Further studies with larger sample sizes are warranted to validate these findings.

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study or preparation of the manuscript and decision to publish.

### CONFLICT OF INTEREST

The authors declared no conflict of interest.

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