ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Prevalence of *Candida* species and their antifungal susceptibility patterns among patients in tertiary care setting Butwal, Nepal: A cross-sectional study

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Submission: 02-08-2023 Revision: 27-02-2024 Publication: 01-04-2024

ABSTRACT

Background: *Candida* species, commonly present in the human body, can result in severe infections. Non-albicans *Candida* (NAC) species, with increasing antifungal resistance, pose a growing concern. **Aims and Objectives:** This study aims to evaluate candidiasis prevalence and antifungal susceptibility in isolated *Candida* species among suspected patients at tertiary care hospitals in Butwal, emphasizing early identification and appropriate treatment to avoid unnecessary use of toxic antifungal drugs. **Materials and Methods:** In a descriptive study on 303 patients with clinical symptoms of *Candida* infections, specimens underwent direct microscopic examination and culture on Sabouraud dextrose agar. Species identification involved phenotypic methods such as chromogenic character on CHROMagar *Candida* media, germ tube examination, and microscopic characteristics. Subsequently, isolated species were tested for antifungal susceptibility using the disc diffusion method. **Results:** Among the 303 samples tested, 80 (26.4%) were positive for candidal infection. NAC species were the most commonly isolated, with *Candida albicans* and *Candida krusei* being the most virulent. The isolates exhibited the highest sensitivity to fluconazole (77.5%) followed by itraconazole (75%), whereas amphotericin B showed the lowest effectiveness with 63.75% resistance. **Conclusion**: The rising prevalence of NAC species, particularly their growing resistance to Amphotericin B, has become a significant concern, as these species are frequently detected in various clinical samples. These findings underscore the importance of diligent monitoring and judicious selection of antifungal agents to ensure effective treatment strategies.

Key words: Antifungal agents; *Candida*; CHROMagar; Fungal infections; Non-albicans *Candida*

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v15i4.57264

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Fungi, a diverse group of eukaryotic organisms, inhabit various natural environments, with around 600 species known to cause diseases in humans, affecting an estimated 1.7 billion people worldwide.[1] The incidence of fungal infections has been steadily increasing over the past few decades, presenting significant challenges in diagnosis and treatment for healthcare professionals.[2] Among these infections, *Candida* species account for 66–80% of fungal infections and represent the most prevalent form of mycosis.[3] Although the *Candida* genus comprises over 200 species, only about 10% have been associated with human infections.[2,4]

Candida species are responsible for a broad spectrum of human infections, ranging from mild superficial conditions such as thrush, vaginitis, and diaper rash to severe invasive

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infections that can be life-threatening.[5] Superficial infections generally do not pose a significant risk to life, but invasive candidiasis, including conditions such as candidemia and disseminated infections, can be severe, especially in individuals with weakened immune systems.[3] Research indicates that approximately 75% of the population harbors *Candida* in various body areas, and it can cause vulvovaginal candidiasis in about 70% of women and contribute to 10–15% of nosocomial urinary tract infections.[3,6,7]

Candidemia, a bloodstream infection caused by *Candida* species, is a prominent nosocomial infection in the USA and Europe, associated with a mortality rate of approximately 50% among hospitalized patients.[3,6] Globally, there are estimated 400,000 cases of *Candida* bloodstream infections each year.[1] While *Candida albicans* remains the primary cause, non-albicans *Candida* (NAC) has been on the rise.[8] The five most encountered species, including *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*, account for over 90% of invasive infections.[3] Candidiasis ranks as the fourth leading cause of hospital-acquired infections, affecting both critically ill and immunocompromised patients, as well as otherwise healthy individuals.[9] The incidence of *Candida* infections is increasing due to the rise in immunocompromised patients from various medical interventions.[4]

Numerous studies have consistently found *C. albicans* to be the primary cause of severe infections, but the rise in NAC species and antifungal drug resistance can be attributed to various factors, including challenges in diagnosing fungal diseases, selective therapies with inadequate dosages, antifungal drug prophylaxis, and misuse of antifungal drugs without proper guidance.[11] With the changing landscape of candidiasis and emerging antifungal resistance, early identification, species determination, and antifungal susceptibility testing are vital for selecting appropriate treatment to prevent treatment failure, mortality, and prolonged hospital stays.[10-14] Thus, this study aims to evaluate *Candida* species prevalence and their antifungal susceptibility patterns among patients at a tertiary health-care facility in Butwal, Nepal.

Aims and objectives

To assess the prevalence of Candida species among patients in a tertiary care setting in Butwal, Nepal.

To determine the antifungal susceptibility patterns of identified Candida species in the study population.

MATERIALS AND METHODS

Study site and sample collection

A 6-month cross-sectional study was conducted at the Department of Microbiology, Crimson College of Technology, Butwal, Rupandehi, from February 2022 to September 2022. A total of 303 suspected specimens from various sites of fungal infection were randomly selected at Crimson Hospital, Butwal, following standard operating procedure guidelines. Prior consent was obtained from all participants, and their relevant medical history was recorded after getting approval from Crimson Hospital, Butwal, Nepal (Ref: 318/079/80). The collected specimens were transported to the Microbiology laboratory of Crimson College of Technology for further microbiological identification. Patients undergoing antifungal drug therapy were excluded from the study, while those with potential suspected fungal infections were included in the study.

Processing of specimens

A total of 303 clinical specimens, including urine (n=80), sputum, high vaginal swabs, pus, throat swab, nasal swab, and urethral swabs, were collected for laboratory investigation. The samples were aerobically inoculated on Sabouraud dextrose agar and incubated at 37°C for 7–14 days. Gram staining was performed to study Gram-positive yeast cells, and the Germ Tube Test was conducted microscopically to observe tubular elongation extending from the yeast cells, facilitating the differentiation between *C. albicans* and NAC species.

Further processing was conducted on yeast colonies exhibiting a yeasty, pasty, and creamy appearance, as well as Gram-positive budding yeast cells and pseudohyphae on microscopic examination. Negative results were obtained in the urea hydrolysis test for these colonies. *Candida* speciation was performed using CHROMagar *Candida* media, distinguishing different *Candida* species based on their growth type and color. After incubation at 37°C for 24–48 h, the observed colony colors on CHROMagar were as follows: *C. albicans* appeared as light green, *C. glabrata* as cream to white, *C. krusei* as purple and fuzzy, and *C. tropicalis* as blue to purple.

Antifungal susceptibility testing from isolates

Antifungal susceptibility testing was performed on all *Candida* isolates using the Kirby-Bauer disc diffusion method, following the guidelines provided in the Clinical and Laboratory Standards Institute (CLSI) M44-A document. A cotton swab was immersed in the fungal inoculum suspension and compared with a 0.5 McFarland standard. The swab was then streaked evenly onto Mueller-Hinton agar supplemented with 2% glucose and 5 μg/mL methylene blue. The interpretation of susceptibility testing results was based on the observed zone of inhibition around the antifungal discs.

Antifungal discs containing fluconazole (10 μg), ketoconazole (10 μg), clotrimazole (10 μg), Voriconazole (1 μg),

Itraconazole (10 µg), and Amphotericin B (100 units) manufactured by HiMedia Laboratories Pvt. Ltd., India were placed and incubated at 37°C for 24 h. If there was insufficient growth, the plates were reevaluated after 48 h of incubation. The zones of inhibition around the discs were measured and recorded once the colonies had grown. The susceptibility and resistance criteria of the antifungal discs were determined based on the standard protocol and categorized as S (sensitive), IM (intermediate), and R (resistant) following the CLSI guidelines M27-M44S.

Statistical analysis

Data collection was recorded in Microsoft Excel, and statistical analysis was performed using SPSS Statistics 22.0 version. The variables under study, the antifungal susceptibility pattern of *Candida* isolates were analyzed separately using one-way analysis of variance. A significance level of $P<0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population

Out of the 303 patients enrolled in the study, *Candida* spp. growth was observed in 80 isolates (26.4%), while 223 isolates (73.6%) showed negative results from various specimens. The majority of the patients were female, accounting for 199 individuals (65.68%), while males comprised 104 individuals (34.32%) (Table 1).

Distribution of *Candida* species isolated among different age groups of patients

In this investigation, *Candida* infection was most prevalent in the 20–40 years age group (n=33, 41.25%), followed by the 40–60 years age group (n=23, 28.75%), the above 60 years age group (n=20, 25%), and the 0–20 years age group (n=4, 5%), as presented in Table 2.

Prevalence of various *Candida* species from isolates

After analyzing the isolates (n=80), it was observed that NAC species (n=57; 71.25%) were more prevalent than *C. albicans* (n=23; 28.75%). Among the NAC species identified in the study, *C. krusei* (n=23; 28.75%) was the most predominant, followed by *C. glabrata* (n=19; 23.75%), *C. parapsilosis* (n=10; 12.5%), and *C. tropicalis* (n=5; 6.25%), as shown in Figure 1. The growth showing colony of different *Candida* species (a. *C. albicans*, b. *C. krusei*, c. *C. glabrata* d. *C. parapsilosis*, and e. *C. tropicalis*) on CHROMagar are depicted in Figure 2.

Distribution of *Candida* species according to specimens

In this research, a total of 80 *Candida* species were isolated from different specimens. Urine samples exhibited the highest percentage of *Candida* spp. isolates, accounting for 72.5% of the total. Sputum and pus samples followed equal percentage of 11.25% isolates, while high vaginal swabs showed 2.50%. Throat swabs and nasal swabs had the lowest occurrence, representing 1.25% each of the isolates. Further details can be found in Table 3.

Table 1: Distribution of *Candida* infection among the study population

Gender	No. of cases, (n, %)	Fungal growth	
		Negative, (n, %)	Positive, (n, %)
Male	104 (34.32)	79 (75.96)	25 (24.04)
Female	199 (65.68)	144 (72.36)	55 (27.64)
Total	303	223 (73.6)	80 (26.4)

Table 2: Age distribution of patients with *Candida* isolates

Age group (years)	Total samples	Positive growth (n)
<20	18	4
20–40	116	33
40–60	97	23
Above 60	72	20
Total	303	80

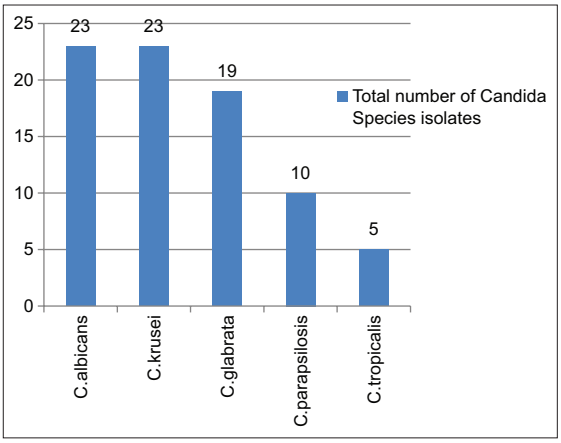

Figure 1: Bar diagram depicting the Prevalence of various Candida species from isolates

Antifungal susceptibility patterns of *Candida* species

The findings revealed that fluconazole was the most effective antifungal agent in this study (62 sensitive isolates, 77.5%), followed by Itraconazole (60 sensitive isolates, 75%), Voriconazole (51 sensitive isolates, 63.25%), Clotrimazole (34 sensitive isolates, 42.5%), ketoconazole (28 sensitive isolates, 35%), and finally Amphotericin B (23 sensitive isolates, 28.75%), as illustrated in Tables 4 and 5.

Pattern of sensitivity and resistance to antifungal agents

The susceptibility of antifungal discs is illustrated in Figure 3, and our study indicated that the most sensitive drugs were fluconazole (n=62 sensitive cases, 77.5%), followed by Itraconazole (n=60 sensitive cases, 75%), and

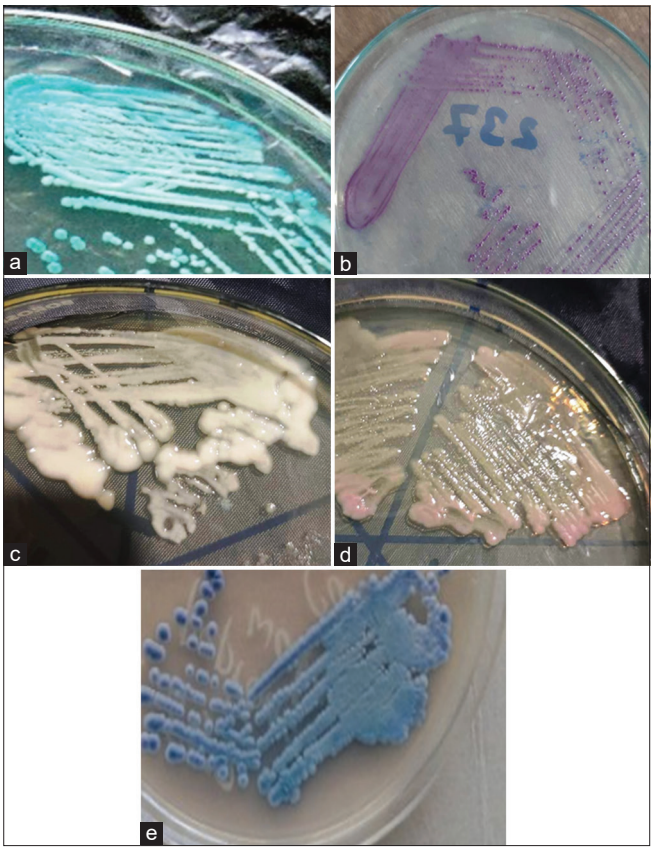

Figure 2: (a-e) *Candida* species colonies photograph seen on CHROMagar

Table 3: Distribution of *Candida* species according to specimens

Specimens	No. of cases	*Candida* spp. Isolates		
		Number	%	% of total
Urine	226	58	72.50	19.94
Sputum	16	9	11.25	2.97
Pus	37	9	11.25	2.97
Vaginal swabs	14	2	2.50	0.66
Throat swabs	2	1	1.25	0.33
Nasal swabs	6	1	1.25	0.33
Total	303	80	100	26.40

Voriconazole (n=51 sensitive cases, 63.75%). On the other hand, the most resistant drugs were Amphotericin B (n=51 resistant cases, 63.75%), followed by Ketoconazole (n=47 resistant cases, 58.75%), as presented in Table 5.

DISCUSSION

The rising incidence of fungal diseases and antifungal resistance poses a significant global health challenge, mainly attributed to the emergence of various *Candida* species, resulting in heightened mortality rates.[15,16] *Candida* species are ubiquitous in nature, frequently found in the gastrointestinal tract, and part of the normal skin flora. Out of the numerous *Candida* species known, at least 15 have been implicated in causing human diseases. Nevertheless, the majority of infections (approximately 95%) can be attributed to five main pathogens, namely, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*.[17,18]

The findings of the present study conducted during the years 2014 and 2015 revealed that while *C. albicans* remained the most frequently isolated species, NAC constituted a substantial proportion, accounting for 70.7% of total *Candida* isolates and 76.7% of candidemia cases.[11,19] The distribution of NAC among infected patients exhibited variations across different regions worldwide, influenced by factors such as the patients' medical condition, geographical location, age, and gender. Among the isolates, *C. albicans* represented a significant portion (49.36%), followed by various NAC species, including *C. tropicalis* (21.89%), *C. parapsilosis* (13.92%), and *C. glabrata* (11.37%). Notably, *C. tropicalis* emerged as the predominant non-albicans species in Asia, especially in tropical regions, compared to other regions such as Europe, the UK, and the USA. Conversely, an increase in the number of *C. glabrata* isolates was reported in Europe and the USA, potentially attributed to the extensive use of fluconazole for prophylaxis and treatment.[20] The most common yeast isolate in our study was *C. albicans*, with a notable prevalence in sputum samples, accounting for 28.75% of the total isolates. However, there was an increasing presence of NAC species in various samples, including *C. krusei* (28.75%), *C. glabrata* (23.75%), *C. parapsilosis* (12.5%), and *C. tropicalis* (6.25%). Overall, NAC species constituted a substantial proportion of 71.25%.[21]

The impact of age on immunocompromised conditions and disease susceptibility was evident in our study. *Candida* infection showed a higher prevalence in females (65.8%) compared to males (30.9%), consistent with previous findings.[18] In addition, our study demonstrated a higher distribution of *Candida* species among females (65.68%) compared to males (34.32%). This gender disparity in *Candida* species prevalence and virulence may be influenced by factors such as poor personal hygiene, limited access to healthcare, female reproductive hormones, and educational disparities. Regarding age-related trends, our findings align with previous research, where candidiasis was more common among patients in the age range of 31–40 years.[22] Similarly, our study indicated that the age group of 20–40 years was highly affected by *Candida* species (41.25%), followed by the age group of 40–60 (28.75%), and those above 60 accounted for 25%. In contrast, the age group below 20 had a lower incidence of 5%. The higher occurrence of *Candida* species in the 20–40 years age group may be attributed to various factors such as lifestyle habits, exposure to risk factors, and changes in immune status

Table 4: Antifungal sensitivity patterns of *Candida* species isolates

Candida species	Antifungal agent					
	Fluconazole Interpretation, n, %	Ketoconazole Interpretation, n, %	Clotrimazole Interpretation, n, %	Amphotericin B Interpretation, n, %	Itraconazole Interpretation, n, %	Voriconazole Interpretation, n, %
Candida albicans n = 23	S–16 (55.66) I–1 (3.48) R–6 (20.87)	S–12 (41.74) I–2 (6.07) R–9 (31.31)	S–14 (48.69) I–1 (3.48) R–8 (27.83)	S–12 (41.74) I–1 (3.48) R–10 (34.79)	S–18 (62.61) I–0 (0) R–5 (17.40)	S–17 (59.14) I–0 (0) R–6 (20.87
Candida krusei n = 23	S–20 (69.57) I–1 (3.48) R–2 (6.96)	S–3 (10.44) I–1 (3.48) R–19 (66.09)	S–5 (17.40) I–3 (10.44) R–15 (52.17)	S–2 (6.96) I–3 (10.44) R–18 (62.61)	S–17 (59.13) I–0 (0) R–6 (20.87)	S–16 (55.66) I–1 (3.48) R–6 (20.87)
Candida glabrata n = 19	S–15 (63.16) I–0 (0) R–4 (16.85)	S–8 (33.69) I–2 (8.43) R–9 (37.90)	S–8 (33.69) I–5 (21.12) R–5 (21.12)	S–4 (16.85) I–2 (8.43) R–13 (54.74)	S–13 (54.74) I–1 (4.22) R–5 (21.12)	S–10 (42.11) I–4 (16.85) R–5 (21.12)
Candida parapsilosis n = 10	S–7 (56) I–1 (8) R–2 (16)	S–4 (32) I–0 (0) R–6 (48)	S–5 (40) I–1 (8) R–4 (32)	S–4 (32) I–0 (0) R–6 (48)	S–9 (72) I–0 (0) R–1 (8)	S–4 (32) I–4 (32) R–2 (16)
Candida Tropicalis n = 5	S–4 (64) I–0 (0) R–1 (16)	S–1 (16) I–0 (0) R–4 (64)	S–2 (32) I–0 (0) R–3 (48)	S–1 (16) I–0 (0) R–4 (64)	S–3 (48) I–0 (0) R–2 (32)	S–4 (64) I–0 (0) R–1 (16)

S: Sensitive, I: Intermediate, R: Resistant

Table 5: Total antifungal susceptibility pattern of *Candida* isolates (n=80)

Name of the drugs	Total sensitivity, n,%	Total intermediate, n,%	Total resistance, n,%	P-value
Fluconazole	62 (77.5)	3 (3.75)	15 (18.75)	0.0007
Itraconazole	60 (75)	1 (1.25)	19 (23.75)	
Voriconazole	51 (63.75)	9 (11.25)	20 (25)	
Clotrimazole	34 (42.5)	11 (13.75)	35 (43.75)	
Ketoconazole	28 (35)	5 (6.25)	47 (58.75)	
Amphotericin B	23 (28.75)	6 (7.5)	51 (63.75)	

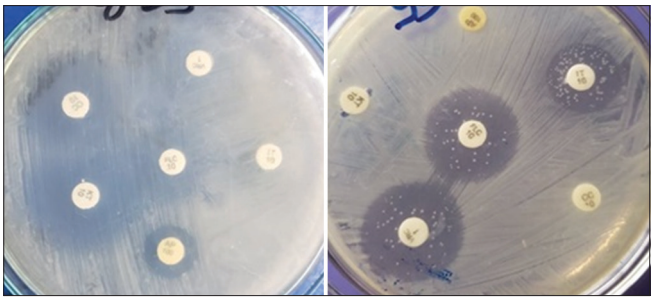

Figure 3: Antifungal susceptibility patterns photograph of fluconazole (10 μg), ketoconazole (10 μg), clotrimazole (10 μg), Voriconazole (1 μg), Itraconazole (10 μg), and Amphotericin B (100 units) with zone of inhibition on Mueller-Hinton agar supplemented with 2% glucose and 5 μg/mL methylene blue

during this period. Moreover, individuals in the older age groups (above 60) may experience compromised immunity due to age-related physiological changes, which could also contribute to the observed *Candida* species prevalence in this study.

The increasing challenges posed by fungal pathogens and the rise of resistant strains can be attributed to the lack of standardized practices and malpractice within healthcare settings. Among various *Candida* species, low susceptibility to the azole group of antifungal drugs was observed. For instance, *C. albicans* demonstrated susceptibility rates of 85.3% for ketoconazole and 91.6% for fluconazole.[20] Studies conducted in the United States, Latin America, Asia-Pacific regions, and Chile showed that approximately 90% of *Candida* isolates were susceptible to azoles, with *C. tropicalis* isolates exhibiting 97% susceptibility to fluconazole.[22,23] However, certain studies have reported differing susceptibility patterns, such as a study indicating *C. albicans* sensitivity to fluconazole (55.66%) and itraconazole (62.61%), with higher resistance observed against amphotericin B and ketoconazole.[21] The emergence of NAC species, particularly *C. glabrata*, has been associated with higher resistance to amphotericin B (54.74%). In our study, the most effective antifungal drugs were found to be fluconazole (sensitivity: 77.5%), followed by itraconazole (sensitivity: 75%), and voriconazole (sensitivity: 63.75%). Conversely, the most resistant drugs were Amphotericin B (resistance: 63.75%) and ketoconazole (resistance: 58.75%) (Figure 3). These findings emphasize the need for continuous surveillance of antifungal resistance patterns and the importance of choosing appropriate antifungal agents to effectively manage candidiasis and combat the rising challenges of antifungal resistance in clinical practice by following CLSI guidelines.[24]

The current study provides insights into the prevalence and characteristics of *Candida* species, with a particular focus on the emergence of NAC and their susceptibility to antifungal drugs. While *C. albicans* remains the most frequently isolated species, NAC was found to be significantly present, particularly in candidemia cases, consistent with earlier studies conducted during the years 2014 and 2015. The study also revealed a concerning trend of increasing antifungal drug resistance, with emerging species displaying multidrug resistance. As a result, it is crucial to implement mandatory antifungal susceptibility testing to ensure effective management of patients affected by candidiasis and combat the growing challenges of antifungal resistance.

Limitations of the study

This was a single-centered study.

CONCLUSION

This study highlights the crucial significance of accurately identifying *Candida* species in clinical specimens to enhance therapy and address the evolving epidemiology of *Candida* infections. The predominance of NAC species underscores the importance of species-specific identification, with *C. albicans* and *C. krusei* emerging as highly virulent species capable of causing various manifestations of candidiasis. Antifungal drug evaluation reveals notable sensitivity rates for fluconazole, itraconazole, and voriconazole, while Amphotericin B demonstrates limited efficacy against both *C. albicans* and NAC species. The utilization of chromogenic media, particularly Chromagar *Candida* agar, proves to be a valuable tool for differentiating *Candida* species, complementing traditional recognition methods in clinical microbiology laboratories. By providing insights into antifungal resistance, this study lays the foundation for the development of effective management strategies. Nevertheless, continuous monitoring of species distribution and antifungal susceptibility remains imperative to optimize therapy and enhance patient outcomes in *Candida* infections.

DATA AVAILABILITY

The data supporting this study are available from the corresponding author on request.

ACKNOWLEDGMENT

The authors extend their sincere appreciation to the Crimson Hospital and Laboratory Staff in Butwal, Nepal, for their invaluable support and collaboration during the course of this study.

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Authors' Contributions:
SBK, BB, BR, and BC- Were contributors of this research design and data analysis; **RBK, JP, KDK, and GPC-** Contributed role as manuscript preparation. All authors read and finalized the manuscript.

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Source of Support: Nil, **Conflicts of Interest:** None declared.