Identification of Candida Species from Different Respiratory Samples by Using the Phenotypic Method in a Tertiary Care Centre

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Abstract:

Background and Objective: In recent years, the incidence of Candidiasis has witnessed a concerning upsurge, resulting in a significant healthcare challenge. These infections are further enhanced by factors like the widespread use of broad-spectrum antimicrobials, chemotherapy-induced neutropenia, and the presence of medical devices. One factor that may be crucial in limiting disseminated candidiasis is the colonisation of Candida species in the respiratory systems of susceptible hosts. This study was designed to identify Candida species in all respiratory samples.

Material Method: Sampling was conducted from 2021 to 2023. A total of 86 clinical isolates of Candida species were obtained from the respiratory samples of both immunocompromised and immunocompetent patients. The samples were initially inoculated on SDA and examined under a KOH mount. The growth was identified using conventional microbiological techniques.

Result: In our study, Candida albicans (n=45 / %=54.65) was most isolated, followed by Candida tropicalis (n=26 / %=32.55and then Candida krusei (n=5 / %=8.13), Candida dublinensis (n=1 / %=2.32), and Candida glabrata (n=1 / %=2.32). In our study, 30–50 years is the most common affected age group.

Conclusion: In recent decades, non-Albicans candida has become more widely reported. Early and accurate diagnosis is very essential for the successful management of patients.

Keywords: Candida species, SDA, respiratory infection.

Introduction

Candida species are a part of the normal skin, mucous membrane, and gastrointestinal tract flora. They are often isolated from respiratory secretions in patients on mechanical ventilation because of aspiration of previously colonised oropharyngeal or stomach contents, or seeding of the lungs during hematogenous spread [1].

Significant mortality and morbidity have resulted from the alarmingly high prevalence of Candida infections in recent times. A number of Candida species are known to cause fungal infections in humans, including Candida albicans and non-albicans Candida spp. like C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. guilliermondii, C. dubliniensis, and C. auris [2]. Among the Candida species, Candida albicans is usually regarded as the main pathogen. Over the past few decades, there has been a noticeable rise in the frequency of non-albicans species [3, 4]. Both healthy people and those with compromised immunity are at significant danger from these fungal diseases. With the help of medical devices Candida invades tissues, subdues immune systems, and frequently results in serious infections [5].

Candida grows and invades due to a number of factors, including lower phagocytic activity, chemotherapy-induced neutropenia, parenteral feeding, and broad-spectrum antibiotics [6]. Phenotypic methods are used to routinely identify yeast species isolated from clinical samples. They consist of two parts: microscopic observation of fungal structures in the clinical samples and culture, and macroscopic observation of colony shape, size, and colour on an agar plate. These

methods are still regarded as the gold standard for identification. In order to identify the infecting species, clinical culture is typically followed by biochemical analysis based on chromogenic medium [7]. Regardless of the diagnostic technique employed, it is essential to early identification of Candida species causing an infection in order to promptly determine the best course of treatment, lower mortality, contain outbreaks, and conduct epidemiological research [8]

Material Method: This was a retrospective laboratory-based study conducted over a period of three years (January 2021 to December 2023) in the department of Microbiology at Mahatma Gandhi Memorial Medical College, Indore.

All the respiratory samples, like sputum, bronchial alveolar lavage (BAL), endotracheal secretions, and pleural fluid that were received in the mycology laboratory were included in this study. BAL, pleural fluid, and endotracheal secretions

were concentrated by centrifugation at 1500–2000 g for 10 minutes. Sputum samples were treated with N-acetyl cysteine prior to inoculation on Sabouraud Dextrose Agar (SDA). All samples were first observed under a KOH mount (10%) and then inoculated on 2 sets of plain SDA and SDA with cycloheximide. The tubes were incubated at both 25°C and 37°C. The tubes were observed on alternate days for the first week and then weekly for 4 weeks. Growth was isolated and identified using standard microbiological methods. Yeast identification included grams staining, germ tube testing, CHROM agar identification, and cornmeal agar (CMA) identification techniques.

Result

A total of 619 respiratory samples were evaluated in this study. Out of 619 respiratory samples, sputum 487 (79%) was the most common, followed by BAL 123 (20%) and pleural fluid 9 (1%). (Fig1)
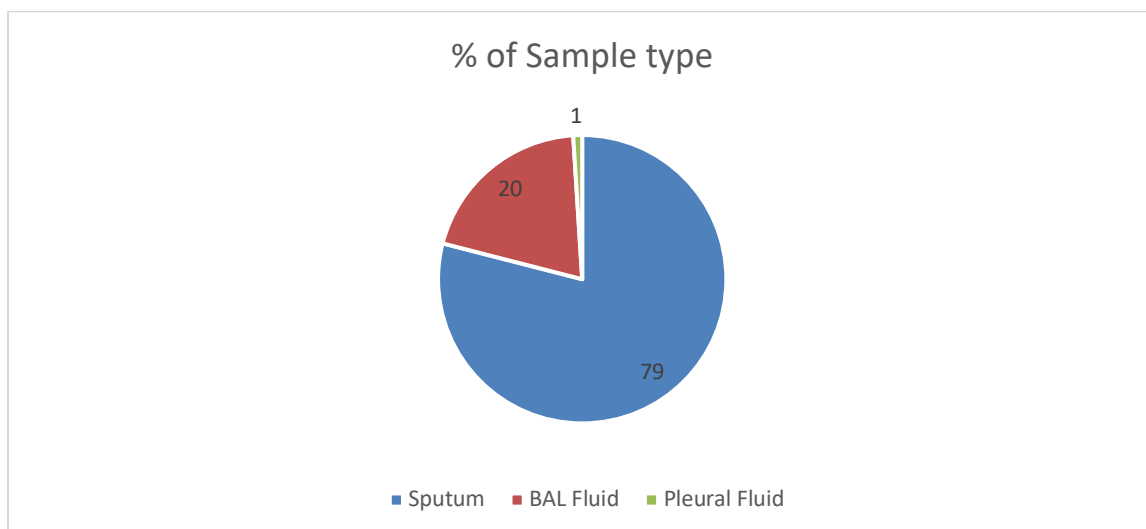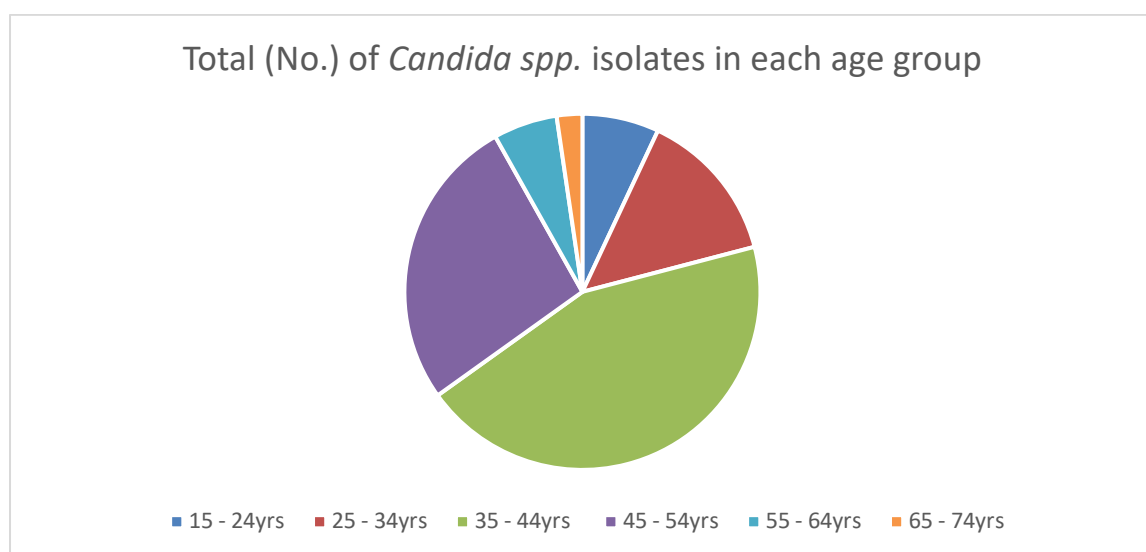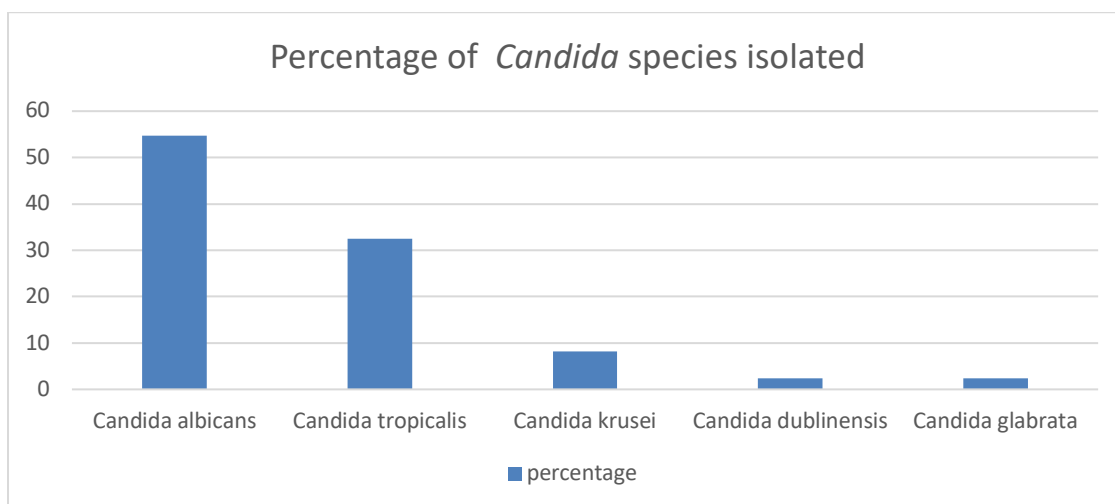

Figure 1:

Figure 2:

Out of 619, 86 (13.9%) were culture-positive for Candida spp. The most common age group affected was 30 to 50 years old. Males were more commonly affected than females.

Isolation rates from sputum were highest (69 samples; 80.2%), followed by BAL (15 samples; 17.4%) and pleural fluid (2 samples; 2.3%). A total of 7 samples were both KOH and culture-positive, but the rest of the samples were positive only by the culture method. In sputum samples, Candida albicans (37 isolates; 43.02%) was most isolated, followed by Candida tropicalis (20 isolates; 23.25%), and then Candida krusei (5 isolates;

5.81%), Candida dublinensis (2 isolates; 2.32%), and Candida glabrata (2 isolates; 2.32%). In BAL fluid samples, Candida albicans (10 isolates; 11.62%) was most isolated, followed by Candida tropicalis (7 isolates; 8.12%). In pleural fluid samples, one Candida tropicalis and two Candida krusei were isolated.

Overall, in respiratory samples, Candida albicans (45 isolates; 54.65%) was the most isolated, followed by Candida tropicalis (26 isolates; 32.55%), and then Candida krusei (5 isolates; 8.13%), Candida dublinensis (1 isolate; 2.32%), and Candida glabrata (1 isolate; 2.32%).

Figure 3:
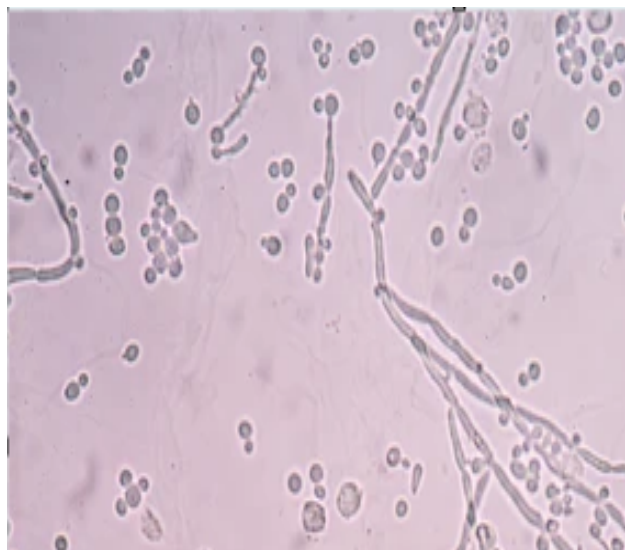
Figure 4: Figure showing Budding yeast cells and pseudohyphae in koh mount
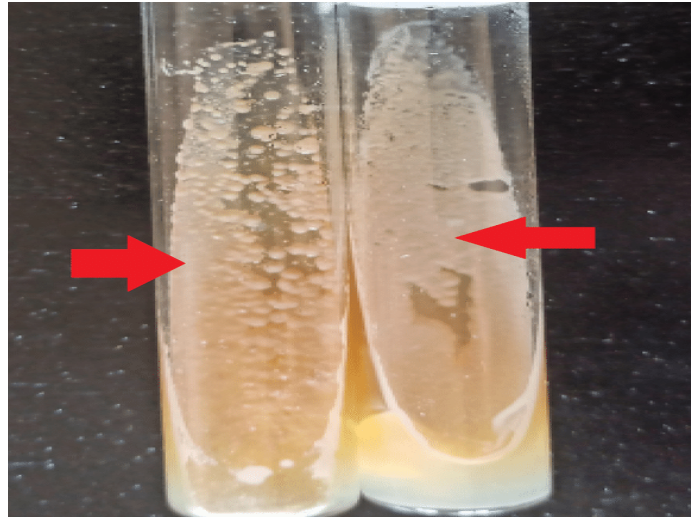
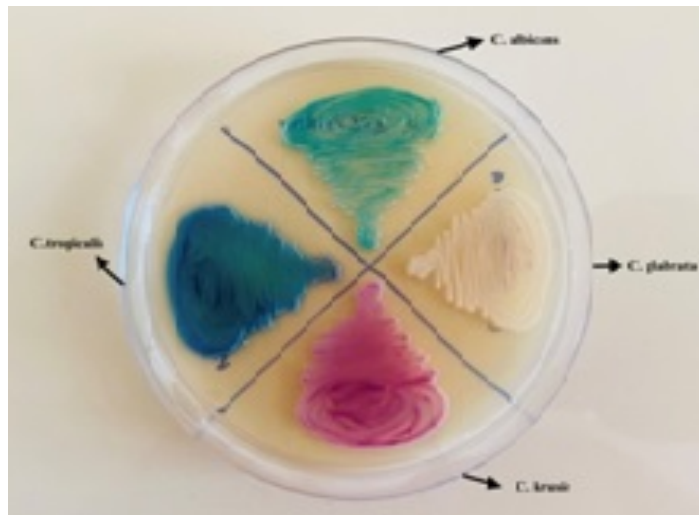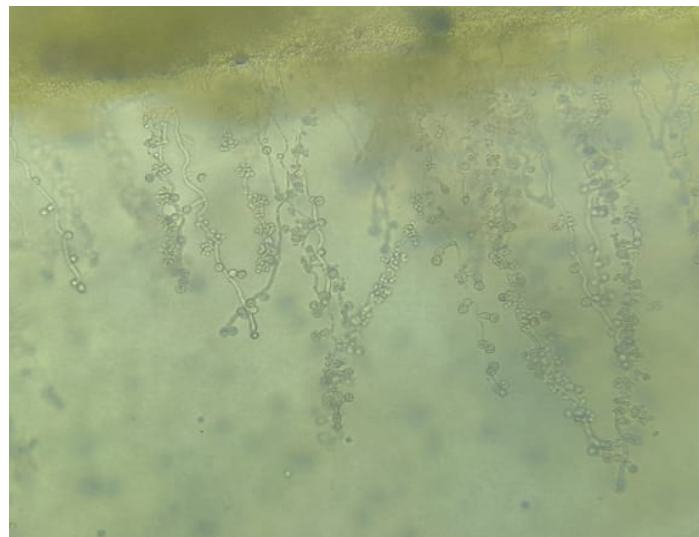**Figure 5: showing growth of Candida spp on SDA tubes.**

Figure 6: Chrom agar showing different Candida species growth

Figure 7: Germ tube test of Candida albicans.

Figure 8: Dalmau growth of Candida krusei

Figure 9: Dalmau growth of Candida albicans.

Discussion

In immunocompetent patients, invasive Candida pneumonia is so uncommon that its very existence is disputed. When it happens, it's thought to be caused by either aspirating colonized oropharyngeal or stomach contents, which is less common, or seeding the lungs from hematogenous dispersion. The identification of yeast and inflammatory cells in lung tissue through histologic analysis is necessary for the final diagnosis of pulmonary Candida infection.

The diagnosis of candida pneumonia is so challenging because histology is rarely accessible clinically, and less intrusive methods cannot differentiate between colonization and infection [9]. Treating fungal infections in hospitalised and immunocompromised patients is a major issue. Infections caused by Candida spp. have significantly grown over the past three decades, particularly by non-albicans species. Long-term use of antibiotics, which eventually changes the natural flora, corticosteroid use, surgical procedures, malnutrition, and hormonal imbalance all increase an individual's susceptibility to candidiasis in immunocompetent people.

Extended usage of commonly prescribed antifungal medications changes the occurrence of Candida spp [2, 10] In this study, out of all respiratory samples, 13.9% were culture-positive for Candida spp. and among all respiratory samples, sputum samples (80.2%) showed the highest positivity rate. Similarly, Athira Jayaram et al. showed the highest positivity from sputum samples [11].

In the present study, males were more affected, which is similar to many other studies related to respiratory tract infections [12, 13]. The most common affected age group is 30–50 years old. In this study, Candida albicans was the most common isolate from all respiratory samples, which is similar to the result shown by M.Taghizadeh et al. and El-Badrawy et al. [14, 15]. In the current study, C.

tropicalis was the most common non-Albican candida reported, which is similar to the results shown in many studies 16, 17, 18]

Conclusion

We have concluded that candida infections in the respiratory tract less commonly occur but remain important causes of morbidity. In recent decades, non-Albicans candida has become more widely reported. Early and accurate diagnosis is very essential for the successful management of patients.

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