ORIGINAL ARTICLE

# API 20C: A RELIABLE AND RAPID DIAGNOSTIC TOOL FOR FUNGAL INFECTIONS

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## ABSTRACT

**Background:** Invasive candida infections pose a significant risk especially in intensive care units. Its early and accurate diagnosis is important for the timely administration of antifungal therapy. This study was conducted to evaluate the API 20C for the diagnosis of candidiasis.

**Material & Methods:** In this comparative study the samples were taken from 300 immunocompromised subjects. All the samples were processed in conventional way i.e. microscopy, culture and growth on special media and by API 20C as well. The API 20C method involved single step for filling tubes and results were read after 18 hours of incubation. The results were analyzed by SPSS version 16.

**Results:** Fungal infection was detected in 165 samples. Both methods had similar results in identifying *C. albicans* 51.5%, *C. glabrata* 23.6%, *C. tropicalis* 7.9%, and *C. krusei* 7.3%. Whereas the non-candida albicans spp. like *C. parapsilosis* 3%, *C. guiliermondii, C. keyfer, C. stellatoidea, Candida famata* each 1.2% were detected only by API 20C along with non-candidal yeasts like *Saccharomyces. cerevisiae, Geotrichum spp, Cryptococcus neoformans,* each 0.6%.

**Conclusion:** API-20C is a better procedure for the diagnosis of candidiasis and should be adopted as routine diagnostic procedure in the clinical microbiological laboratories.

**Key Words:** Candidiasis, API 20C, Fungal infection.

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## INTRODUCTION

Recent decades have seen a significant increase in the incidence of invasive fungal infections especially among the immunocompromised patients. A growing interest in *Candida* species (spp.) has been observed due to the emergence of *Candida* spp. from infrequent pathogens to the most important and frequent opportunistic microorganisms causing nosocomial infection in intensive care unit (ICU) patients. It is associated with high morbidity and mortality. Aging, underlying disease, invasive procedures and use of broad-spectrum antibiotics are the main risk factors for fungal infections. *Candida albicans* is still the main species, but the proportion of non-c*andida albicans* spp. is increasing year by year. Studies have documented the increased incidence of non-albicans spp. In the hospitalized and immunocompromised patients.[1,2]

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An early diagnosis is required for improvement of survival of such cases. Clinical diagnosis is complicated by lack of specific clinical signs and symptoms of disease. Conventional microbiological, histological and radiological techniques are still considered to be the cornerstone of diagnosis but are insensitive and have a limited impact on clinical decision-making as precise recognition of the causative agent is required. Advanced molecular techniques seem to be promising for improved sensitivity and specificity but in our setting these methods are not usually available. There is a need for efficient and easily applicable diagnostic method that should be fast and reliable with higher sensitivity.[3,4]

Many laboratories do not routinely identify *Candida* isolates to species level, but the growing importance of severe candidiasis has made it necessary to do so as species identification provides a diagnostic clue to the source of infection.

Standard approaches to the laboratory diagnosis of invasive fungal infections include microbiological examination by direct microscopic visualization of organisms in the freshly obtained body fluids, culture on Sabouraud Dextrose Agar (SDA) and Chromagar Candida, phenotypical methods (germ tube test, formation of chlamydospores), metabolic tests (fermentation pattern and carbohydrate assimilation) and histopathologic demonstration of fungi in the tissue sections. However, these approaches often are not sufficiently sensitive and/or specific to diagnose invasive fungal infections and sometimes require invasive procedures to obtain the required specimens.[5,6]

Identification of yeast by biochemical methods is not only complicated but also time consuming. Identification of yeasts by API 20C kit is easier and has greatly reduced the laboratory time involved in the speciation of *Candida* isolates.[7-10]

This study was conducted to evaluate the API 20C kit method for its reliability and usefulness as a rapid and reliable tool for the diagnosis of candidiasis in immunocompromised patients.

## MATERIAL AND METHODS

This comparative study was carried out at Basic Medical Science Institute, Jinnah Postgraduate Medical Center, Karachi. The duration of study was 6 months from October 2009 to March 2010. Inclusion criteria was immunocompromised subjects both males and females of all age groups, while patients with any acute infection were excluded. Samples from 300 immunocompromised patients were taken and processed with the conventional method of diagnosis i.e. direct microscopy, culture on SDA and sheep blood agar, by performing germ tube test. Candida albicans were diagnosed further. On cornmeal agar morphology, different species of Candida were identified and further confirmed by biochemical sugar fermentation tests.[11-13] All the samples were also processed on API 20C.

## RESULTS

In our study on 300 immunocompromised patients, 436 samples were processed and primary diagnosis up to species identification was done successfully in 300 cases. The nature of clinical samples obtained is shown in Table 1. Both the methods showed similar results in identifying *C. albicans, C. glabrata, C. tropicalis*, *C. krusei* and *C. stellatoidae*. (Table 2)

Whereas the non-candida albican spp. like *C. parapsilosis, C. guiliermondii*, *C.keyfer, Candida*

**Table 1: Various clinical samples taken from immunocompromised patients**

| Type of Samples | Number | Percentage |
|---|---|---|
| Oral swabs | 212 | 46.2 |
| Urine | 87 | 19.0 |
| High vaginal swabs | 55 | 12.0 |
| Skin scraping and nail clipping | 28 | 6.1 |
| Sputum | 27 | 6.0 |
| Tips of I/V cannula | 12 | 3.0 |
| Blood | 6 | 1.4 |
| Chest intubation | 6 | 1.4 |
| CSF | 2 | 0.5 |
| Pus from ocular lesion | 1 | 0.8 |
| Total | 436 | 100 |

**Table 2: Comparison of conventional and API 20C for identification of various species**

| Name of species | Conventional method | | API 20C | |
|---|---|---|---|---|
| | No. | %age | No. | %age |
| Candida albicans | 85 | 51.5 | 85 | 51.5 |
| Candida glabrata | 39 | 23.6 | 39 | 23.6 |
| Candida tropicalis | 13 | 7.9 | 13 | 7.9 |
| Candida krusei | 12 | 7.3 | 12 | 7.3 |
| Candida stellatoidea | 2 | 1.2 | 2 | 1.2 |
| Candida parapsilosis | 0 | 0 | 5 | 3.0 |
| Candida guilliermondii | 0 | 0 | 2 | 1.2 |
| Candida kefyr | 0 | 0 | 2 | 1.2 |
| Candida famata | 0 | 0 | 2 | 1.2 |
| Saccharomyces cerevisiae | 0 | 0 | 1 | 0.6 |
| Geotrichum spp. | 0 | 0 | 1 | 0.6 |
| Cryptococcus neoformans | 0 | 0 | 1 | 0.6 |
| Other yeasts | 14 | 8.5 | 0 | 0 |

*stellatoidea* and *Candida famata* were detected only by API 20C, along with non-candidial yeasts like *Saccharomyces cerevisiae, Geotrichum spp., Cryptococcus neoformans* but conventional method was unable to detect 8.5% subspecies.

## DISCUSSION

Invasive candidiasisis associated with high morbidity and mortality. Clinical diagnosis is complicated by a lack of specific clinical signs and symptoms. The genus *Candida* includes several species implicated in human pathology. *Candida albicans* is by far the most common species but the emergence of non-*albicans candida* spp. as significant pathogens has been well-recognized during the past decade.[14,15] Numerous records have documented the increased incidence of non-*albicans* spp. among hospitalized and immunosuppressed patients. The emergence of these opportunistic pathogens is favored by the change in host susceptibility due to the growing number of immunocompromised individuals in the population as a result of HIV pandemic and the use of long-term immunosuppressive therapy in cancer and organ transplant.[16-18]

Candida infections in immunocompromised patients are often severe, rapidly progressive, and difficult to treat as they are associated with strains which are often resistant to conventional antifungal therapy.[19] Despite the availability of new antifungal drugs, the overall survival for immunocompromised patients with invasive fungal infections remains too low, with large variations according to the underlying disease. Early diagnosis and subsequent early initiation of therapy improves the outcome. Serological tests have been the subject of much study but are difficult to interpret.[20] Histopathological evidence of tissue invasion can confirm a diagnosis but commercial reagents to identify and differentiate *Candida* spp. are not currently available.[21] In addition, patients at-risk for invasive *candidiasis* may be thrombocytopenic making tissue biopsy procedures hazardous. Newer molecular biological methods appear promising but are not yet readily available in most clinical laboratories especially in Pakistan. In addition, these newly developed methods may be too cumbersome for routine usage.[21-23] Diagnotic methods that can provide diagnosis of species earlier in the course of disease are the main challenge. The high mortality associated with IC is strongly associated to the diagnostic difficulties.[24] It has been reported that isolates identified by Randomly Amplified Polymorphic DNA (RAPID) technique showed a 95% agreement with API 20C and a 100% agreement with API 20 CAUX.[25]

## CONCLUSION

API 20C is a better procedure for the diagnosis of candidiasis and should be adopted as routine diagnostic procedure in the clinical microbiological laboratories.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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