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Identification of *Candida* Species: Conventional Methods in the Era of Molecular Diagnosis

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Abstract

Candida is unique among mycotic pathogens as it causes a broad spectrum of clinical manifestations ranging from mere mucocutaneous overgrowth to life threatening systemic infections. *Candida albicans* is generally considered as most pathogenic member of the genus and most common cause of different types of candidiasis. However, many recent studies from various parts of world have documented a shift from 'pervasive' *C. albicans* to 'cryptic' Non *Albicans Candida* (NAC) species. NAC spp. are closely related to *C. albicans* and cause similar clinical manifestations but differ with respect to epidemiology, virulence factors and most importantly the pattern of susceptibility to antifungal drugs. Although commercial systems and molecular diagnostic methods are rapid and reliable, high cost limits their use. Conventional techniques remain the mainstay of species identification of *Candida* isolates in most clinical microbiology laboratories.

Keywords: *Candida albicans*; Conventional techniques; Corn meal agar; Germ tube technique; Chromogenic media

Introduction

Infections due to fungi belonging to the genus *Candida* are increasingly reported in recent years. *Candida* spp. is the only opportunistic fungi that exist both as a commensal and pathogen. It is also unique among mycotic pathogens as it causes a broad spectrum of clinical manifestations ranging from mere mucocutaneous overgrowth to life threatening systemic infections [1].

The severity of candidias is ranges from moderate to fatal and is dependent on the site of infection, virulence of infecting strain and host's immune status. Cutaneous candidiasis is common and can occur in otherwise healthy individual. It is easy treat with basic hygiene and local treatment [2]. Mucocutaneous and invasive candidiasis is often opportunistic and manifests in patient with either acquired or induced immuno-suppressed conditions. Invasive *Candida* infections are one of major causes of morbidity and mortality in immunocompromised as well as critically ill immunocompetent patients [3].

Candida albicans is generally considered as most pathogenic member of the genus and most common cause of different types of candidiasis [4,5]. However, many recent studies from various parts of world have documented a shift from 'pervasive' *C. albicans* to 'cryptic' Non *Albicans Candida* (NAC) species [4,5]. NAC spp. are closely related to *C. albicans* and cause similar clinical manifestations but differ with respect to epidemiology, virulence factors and most importantly the pattern of susceptibility to antifungal drugs [4,5].

NAC is a heterogeneous group of *Candida* species with approximately 19 species implicated in human infections. *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* are most commonly reported NAC spp. [6]. *C. krusei* is innately resistant to fluconazole, in addition to intrinsic resistance nearly about 20% strains of *C. glabrata* can acquire resistance during course of therapy [4,6]. *C. tropicalis* is generally considered as a fluconazole-susceptible species however, recent studies have documented emergence of fluconazole resistance in this NAC spp [7,8]. *C. parapsilosis* is reported to have high minimum inhibitory concentration to echinocandins, the recent addition to antifungal arsenal [9].

Emergence of NAC spp. has highlighted importance of identification of the infecting species of *Candida* isolate for initiation of early and effective therapy, especially when antifungal susceptibility results are not readily available [10]. As NAC spp. significantly vary in their prevalence as per country and health-care setups within the country, species identification also plays an important role in formulation of local therapeutic guidelines [11].

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Table 1: Morphological features of medically important *Candida* species on corn meal agar [22, 23].

Candida spp.	**Morphological feature on corn meal agar**
C. albicans	Elongated pseudohyphae with grape-like clusters of blastoconidia at the septa. Chlamydospores are present at the end of the hyphae or their short, lateral branches.
C. tropicalis	Abundant branched pseudhyphae composed of elongated cells. Blastoconidia are seen singly or in small groups along mycelia and show characteristic "**pine forest arrangement**". True hyphae present in some strains and chlamydospores are produced, especially on initial inoculation.
C. parapsilosis	Pseudohyphae are long, thin and branched. Single or small clusters blastospores seen along the pseudomycelia. Large, mycelia elements, called giant cells is the characteristic feature.
C. guilliermondii	Abundant or sparse, very fine and short pseudohyphae. Small blastoconidia seen in small chains or in clusters. Absence of terminal chylamydospores.
C. krusei	Long, slender, straight cells showing tree-like branching and chains of blastoconidia arises from the point between cells resembling **"crossed matchsticks".**
C. kefyr	Abundant production of pseudohyphae. Cells are elongated and fall apart and lie parallel, like **"logs in a stream"**.
C. dubliniensis	Production of true hyphae on CMA helps to distinguish this species from *C. albicans*. Abundant chlamydospores often in clusters or contiguous pairs on the true hyphae. Presence of solitary or cluster of blastoconidia is an important characteristic feature.
C. glabrata	Formerly classified as "*Torulopsis glabrata*". Absence of hyphae or pseudohyphae is the characteristic feature.
C. lusitaniae	Ovoid yeast cells arranged in pairs and chains. Abundant branched pseudohyphae may be seen. Pseudohyphae are curved. Some strains have rudimentary to no pseudohyphae.
C. rugosa	Pseudohyphae well developed with abundant blastospores at internodes.

In recent years, the field of laboratory medicine has undergone a sea change from conventional methods to rapid commercial systems and from rapid commercial systems to molecular diagnosis. Although commercial systems and molecular diagnostic methods are rapid and reliable, high cost limits their use [12,13]. Conventional techniques remain the mainstay of species identification of *Candida* isolates in most clinical microbiology laboratories.

The present review article is focused on various conventional techniques for species identification of *Candida* isolate.

Species Identification of *Candida* Isolates: An Overview

Various conventional, kit based commercial and molecular methods are applied for species identification of *Candida* isolates. Some of these techniques necessitate prior isolation of *Candida* whereas other methods can be directly applied to clinical specimens for species identification.

Conventional techniques are based on morphological or physiological characteristic [13]. Rapid commercial kit based systems are simplified, miniaturized version of conventional tests [13]. These tests require less time and minimal technical expertise as compared to conventional and molecular techniques [13].

Molecular techniques are highly reliable and precise. However, high cost and requirement of technical excellence limits its use in great majority of diagnostic microbiology services [12] Various Polymerase Chain Reaction (PCR) and non PCR based methods are employed for identification of *Candida* spp. Conventional, semi-nested and nested PCR, PCR-enzyme immunoassay, various types of real-time PCR and multiplex PCR are examples of in-house and commercially designed PCR platforms for qualitative and/or quantitative *in vitro* detection of *Candida* species-specific DNA. Other than identification of infecting species, the scope of PCR based methodologies in diagnostic mycology is manifold [12,13]. These techniques can be employed for detection of mutations associated with antifungal resistance, quantification of fungal load in clinical specimen, antifungal therapy monitoring and pathogenesis of *Candida* infection. The yeast Traffic Light peptide nucleic acid fluorescence in situ hybridization assay (PNA FISH) and pyrolysis and matrix-assisted laser desorption-time of flight mass spectrometry (MALDI-TOF MS) are example of non-PCR based molecular methods for identification of *Candida* spp. MALDI-TOF MS can also be used for detection of resistance and molecular characterization of *Candida* spp. [12].

Conventional methods for identification of *Candida* isolates: As compared to rapid commercial kit based system and precise molecular techniques most of diagnostic laboratory still rely on conventional or traditional methods for identification of *Candida* species. Conventional methods include germ tube test, morphology study and carbohydrate fermentation and assimilation tests [12,13].

Most of conventional methods require isolation of *Candida* on suitable laboratory media. Being non-fastidious in nature, *Candida* luxuriantly grows on common laboratory media used for isolation of pathogenic bacteria and fungi [12]. Sabouraud Dextrose Agar (SDA) is widely used media for primary isolation of *Candida* spp. from clinical specimens [14]. *Candida* produces creamy, smooth, pasty and convex colonies which may become wrinkled on further incubation [15].

Potato Dextrose Agar (PDA) aids in differentiating between colonies of different yeasts species from the same clinical specimen. 16 Variety of media like Malt Peptone Agar (MPA), Pagano-Levin agar, phosphomolydate agar and Nickerson's medium can be utilized for isolating and differentiating *Candida* spp. from clinical specimens harboring a mixture of yeasts [12]. *Candida* grows well on plain blood agar plates. It is frequently isolated in bacterial culture and may be referred to the mycologist for species differentiation. Combination of SDA and brain heart infusion can be also for isolation of *Candida* spp.

A wet mount preparation should always be performed first when growth yeast like organism is seen on culture medium. This is essential to differentiate between yeast and bacterial growth. Examination of wet preparation also reveals size, shape, number of buds and pattern of attachment of bud to the yeast cell. Gram stain used for bacteria also demonstrates *Candida*. *Candida* appears as gram positive. Large size, presence of bud and pseudohyphae makes yeast cell very distinctive in gram stained smear. Gram staining is most useful staining technique for demonstration of yeast cells in vaginal secretion, sputum, purulent discharges, gastric washing, lung aspirates and urine.

Germ tube production test: Germ tube test is the most widely used conventional technique for identification of *Candida* spp. The basic mycological workup for species identification of *Candida* isolates starts with screening its ability to produce germ tube in serum

Table 2: Carbohydrate assimilation pattern of *Candida* spp. [21-23].

Organisms	Assimilation											
	Dextrose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol
C. albicans	+	+	+	-	+	-	-	-	+	-	+	-
C. glabrata	+	-	-	-	-	-	-	-	-	-	+	-
C. parapsilosis	+	+	+	-	+	-	-	-	+	-	+	-
C. tropicalis	+	+	+	-	+	-	+	-	+	-	+	-
C. kefyr	+	-	+	+	+	-	+	-	+	+	-	-
C. krusei	+	-	-	-	-	-	-	-	-	-	-	-
C. lipolytica	+	-	-	-	-	-	-	-	-	-	-	-
C. guilliermondii	+	+	+	-	+	+	+	-	+	+	+	+
C. rugosa	+	-	-	-	-	-	-	-	+	-	-	-
C. viswanatii	+	+	V	-	+	-	+	-	+	-	+	-
C. dubliniensis	+	+	+	V	+	-	-	-	-	-	+	-

† '+' positive, '-'negative, 'V' variable.

or other proteinaceous medium [16,17]. Germ tube is filamentous overgrowth that arises from the blastoconidium and has parallel walls without any constriction at their point of origin [17]. It is very essential to differentiate germ tube from pseudohyphae [18]. In contrast to germ tube, pseudohyphae are constricted at the point of emergence from the blastoconidium [18].

Germ tube test is known as "Reynolds-Braude phenomenon". Among *Candida* spp. of medical importance, *C. albicans* and *C. dubliniensis* produce germ tubes. In addition to these species, *C. africana* is also germ tube positive isolate [17]. As 90% of the *Candida* spp. isolated from clinical specimens that have a germ tube test positive are *C. albicans*, most of laboratories report germ tube positive yeasts as *C. albicans* without further testing.

Although, human serum is conventionally used for germ tube test, many laboratories have shifted to alternative proteinaceous media like egg white, saliva, sheep serum, peptone water and trypticase soya broth due to inherent safety problems associated with the use of human serum [17]. In one of our study, trypticase soya broth was found to be the most efficient media for germ tube production [17]. Trypticase soya broth is readily available in most clinical microbiology laboratories. Use of this media also eliminates time required to prepare, freeze and subsequently thaw the individual vials required for use with human serum [17].

Germ tube formation within 2 h of incubation is considered significant. At least five germ tubes should be observed in entire wet mount preparation to label isolate as germ tube producer [17]. Negative results are confirmed by examining minimum 10 high power fields.

Chlamydospore formation: Certain *Candida* spp. like *C. albicans*, *C. dubliniensis* and *C. tropicalis* (few strains) produce chlamydospores on nutritionally deficient media [19]. In addition, few saprophytic, non pathogenic *Candida* spp. like *C. australis* and *C. clausenii* also produce chlamydospores [19]. Chlamydospore formation test is less subjective but more time consuming than germ tube technique.

Chlamydospores are round, highly refractile and resistant asexual spores [20]. Chlamydospore formation and its relationship to hyphae, pseudohyphae and other fungal structure can be studied by inoculating *Candida* isolates from primary culture on Corn Meal Agar (CMA) [21]. As CMA is clear media, the pattern of yeast growth can be examined directly by placing media plate on the stage of a bright field microscope. The pattern of growth on CMA can be used for speciation of *Candida* isolates. Morphological features of some medically important *Candida* spp. on CMA is shown in table 1.

CMA plate technique is also known as 'Dalmau plate' method [22]. Addition of tween-80 (polysorbate) to corn meal agar enhances the chlamydospore formation. It also favors the development of pseudohyphae, hyphae and blastoconidia [22]. Dalmau plate method can also be used to detect ascospores by prolonging incubation for up to 1 month. In addition to corn meal agar, other media like rice extract agar, casein agar, sunflower seed agar, tobacco agar and Staib agar can be utilized for chlamydospore formation [22,23].

Chlamydospore formation in *C. dubliniensis* differs from that of *C. albicans*. In *C. dubliniensis* chlamydospores are often attached in pairs, triplets, or larger clusters to the same suspensor cell rather than singly at the hyphal (or pseudohyphal) ends in *C. albicans* [24]. However, the pattern may vary strain to strain and therefore, additional test may be required to differentiate these two species.

Phenotypic tests to differentiate between *C. albicans* and *C. dubliniensis*:

A) Growth on Staib agar: 25 Staib agar is basically a niger seed agar without antibiotics. On this media, *C. albicans* produces white colonies with smooth edges whereas; colonies of *C. dubliniensis* are white with a fringe. 25 On Staib agar, *C. albicans* does not produce chlamydospores whereas, *C. dubliniensis* does [25].

B) Growth on methyl blue SDA: On this medium, *C. albicans* isolates fluoresce with a yellow color on exposure to long wave UV light, while *C. dubliniensis* isolates fail to fluoresce under this condition [26]. The property of fluoresce may be lost in isolates subjected to storage and repeated subculture.

C) Growth at 45°C: Pinjon et al. [27] have described simple, inexpensive and reliable method for differentiation of *C. albicans* from *C. dubliniensis*. In this method, all germ tube and chlamydospore producing isolates are subcultured on potato dextrose agar (PDA) in

Table 3: Fermentation reactions of frequently isolated *Candida* spp. [21,22].

Organism	Fermentation of Carbohydrates					
	Dextrose	Maltose	Sucrose	Lactose	Galactose	Trehalose
C. albicans	F	F	-	-	F	F
C. glabrata	F	-	-	-	-	F
C. parapsilosis	F	-	-	-	F	F
C. tropicalis	F	F	F/V	-	F	F
C. kefyr	F	-	F	F	F	F
C. krusei	F	-	-	-	-	-
C. lipolytica	F	-	-	-	-	-
C. guilliermondii	F	-	F/W	-	F	F
C. rugosa						
C. viswanatii	F	F	-	-	F/W	F

† 'F' acid or gas, 'V' variable, 'W' weak.

duplicate. One plate is incubated at 45°C while the other 37°C [27]. *C. albicans* isolate grow well at both temperature whereas the growth of *C. dubliniensis* is inhibited at 45°C [27].

D) Casein agar: Mosca et al. [28] described casein agar as a useful media for differentiation of *C. albicans* and *C. dubliniensis*. *C. dubliniensis* isolates produces abundant chlamydospores compared to *C. albicans* on this media [28].

E) β-glucosidase activity: *C. albicans* generates fluorescence in the presence of methyl umbelliferyl labeled glucosidase whereas *C. dubliniensis* does not [29]. This test indicates the secretion of intracellular β-glucosidase by *C. albicans*. 29

F) Coaggregation with *Fusobacterium nucleatum*: It is a rapid, specific and cost effective test to differentiate *C. dubliniensis* from *C. albicans* isolates in the laboratory. *C. dubliniensis* have ability to coaggregate *in vitro* with the oral bacterial spp. *F. nucleatum* while *C. albicans* lack this property [30].

Carbon and nitrogen assimilation: The ability of *Candida* spp. to assimilate a particular carbohydrate as the sole carbon source has been used identification [13]. Assimilation is defined as the process of glucose metabolism via the hexose monophosphate pathway under aerobic condition [13]. As any microorganism that can ferment a carbohydrate can also assimilate it therefore most laboratories use only assimilation tests. Carbohydrate assimilation test is simple and cost effective conventional method for speciation of *Candida* isolate.

Carbohydrate assimilation test is based on the use of carbohydrate-free yeast nitrogen base agar and observing for the presence of growth on carbohydrate containing media after an appropriate period of incubation. 31 A positive test is indicated by the presence of growth on the media and not merely a change in the color of an indicator.

The technique for carbohydrate assimilation of yeasts was first developed by Wickerham and Burton in 1948 [31]. As most Wickerham media are not commercially available, they are prepared in laboratory [31]. Wickerham and Burton assimilation method, the yeast isolate to be identified was grown in a set of defined liquid media containing an indicator supplemented with different carbohydrates. A change in color of indicator was used to indicate assimilation [31]. This method was precise but laborious and time consuming and therefore not routinely used [31]. Liquid media was further replaced by slants which yielded reproducible and easier to read results.

Wickerham and Burton assimilation method was further replaced by auxanographic technique (disc diffusion) [19]. Since many yeasts can 'carry-over' nutrients from the initial isolation medium, the negative control should be set-up for each test type and isolate. In auxanographic technique, the carbohydrate discs are placed on agar medium on which the isolate is inoculated. Yeast growth around the individual disc can be detected visually. The growth of yeast around a specific carbohydrate disc indicates assimilation of that carbohydrate [19].

Dye Pour-Plate Auxanogram (DPPA) is a more practical approach for comprehensive phenotypic identification of *Candida* spp. [13]. This method allows testing of multiple substrates on the same agar plate. Bromocresol purple (pH indicator) in the media allows easy interpretation of results. Auxanotrophic method being time consuming, are replaced by commercial kits like Analytical Profile Index (API) 20C and API 32C and many others. These commercial kits are modification of the auxanographic assimilation techniques [13].

The ability of *Candida* spp. to assimilate nitrate can be tested in broth containing α-naphthylamine and sulfanilic acid reagents. Nitrate assimilation is highly sensitive and specific method [13].

There are a number of problems associated with the interpretation of carbohydrate assimilation test. 21, 22 Particular assimilation test results may not be consistent because the same *Candida* isolate may yield a positive test result on one occasion and a negative on another [21,22]. This may be due to phenotypic switching or the existence of a mixture of metabolic variants.

Furthermore, genetically diverse *Candida* spp. can yield similar carbohydrate utilization profiles, resulting in poor discrimination between unrelated species. When these results are taken together with morphological and serological test results, the likelihood of an accurate identification is increased greatly. Carbohydrate assimilation pattern of some medically important *Candida* spp. is shown in table 2.

Carbohydrate fermentation: Fermentation is a process of an enzymatic oxidation-reduction in which organic substrates serve both as the electron donor and receptor. In *Candida* spp., carbohydrate fermentation tests supplement carbohydrate assimilation test's results when there is difficulty in making the definitive identification of an isolate [21,22].

Carbohydrate fermentation tests are performed in liquid media and are based on demonstration of acid and/or carbon dioxide production. Positive fermentation is indicated by the turbidity and accumulation of gas (CO2) in the Durham tube or underneath Vaspar seal [21,22]. As change in the color of indicator (bromothymol blue) from blue to yellow signifies carbohydrate assimilation, the production of gas is absolutely necessary to indicate fermentation. If a carbohydrate is fermented, it is also assimilated but the converse is not always true [21,22].

As compared to assimilation tests, carbohydrate fermentation tests are more difficult to perform and are considered less sensitive and reliable. This method for identification of *Candida* spp. is time consuming and laborious and therefore it is not routinely performed [21,22]. Most of the commercial kit systems do not use fermentation assays, but rely on assimilation tests. Table 3, shows fermentation

reactions of frequently isolated *Candida* spp.

Urease test: Urease test can be used for identification of *Candida* spp. like *C. krusei*, *C. lipolytica* and *C. humicola*. Enzyme urease spilts urea to ammonia and carbon dioxide, which raises the pH and causes a color shift in the phenol red indicator from amber to pinkish red [32]. A positive urease test indicates conversion of yellow color to pink or red. A test is considered negative when there is no color change. The Christensen's urea agar slant is inoculated with the *Candida* spp. to be identified and incubated at 25°C for 2-5 days.

Chromogenic media: A variety of chromogenic media are available for speciation of *Candida* isolates. Improved isolation rate, rapid identification and differentiation of poly-fungal populations in clinical samples are prominent advantages of chromogenic media. Some of these media can be inoculated directly with clinical samples whereas others require prior isolation of pathogen. Few chromogenic media used for species identification of *Candida* isolates are discussed below.

CHROMagar *Candida* system: CHROMagar *Candida* (CHROMagar Company, Paris, France) is a commercially available selective and differential media for isolation and identification of *Candida* spp. This medium contains chromogenic (hexosaminidase) substrates that react with species-specific enzymes secreted by yeast cells, resulting in development contrasting colored colonies [13].

On CHROMagar, *Candida* spp. like *C. albicans*, *C. tropicalis* and *C. krusei* can be easily differentiated on the basis of colony morphology and color [13]. *C. albicans* produce leaf-green colored colonies, *C. tropicalis* colonies are dark blue-grey with a purple halo and *C. krusei* forms pink colonies with whitish border. Colonies of other species are entire and smooth and colony color ranges from white to dark pink [13].

CHROMagar can be reliably used for differentiation of *C. dubliniensis* and *C. albicans*. *C. dubliniensis* produces dark green colored colonies. However, the ability of *C. dubliniensis* to form characteristic dark green colored colony may be lost on storage at -70°C and after repeated subcultures probably due phenotypic switching [13]. CHROMagar supplemented with Pal's medium (powdered sunflower seed) can be used for differentiation between *C. albicans* and *C. dubliniensis* [33]. On this medium, *C. albicans* produces smooth colonies with light green color whereas, *C. dubliniensis* produces bluish green and rough colonies [33].

On CHROMagar, NAC spp. like *C. famata*, *C. firmeteria*, *C. guilliermondii*, *C. kefyr*, *C. lusitaniae*, *C. norvegenesis* and *C. parapsilosis* produce colonies of variable shades of ivory, lavender and pink indistinguishable from each other [13]. *C. glabrata* colonies appear as a dark violet colored and can be differentiated from the pink and white colors produced by other species. *C. rugosa* produces distinct small, dry colonies of a brilliant blue color with distinctive pale or white border [34].

The ability to detect mixed yeast infections directly from clinical specimens is an added advantage of this medium. CHROMagar is reported to have better performance with specimens obtained from sterile sites compared to that with non-sterile ones. Fluconazole susceptibility of *C. albicans* and other *Candida* spp. can be successfully predicted by CHROMagar.

Fluorogenic membrane filtration method: This method is a sensitive and rapid method for identification of *Candida* isolates like *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* [13]. It is an unusual two-step method consisting of microcolony formation on a nylon membrane followed by an enzymatic assay using fluorogenic substrates in the presence of a membrane permeabilizer [13].

***Candida* ID system:** *Candida* ID system (bioMérieux, Marcy l'Etiole, France) is recently developed medium for identification of *C. albicans* and other medically important NAC spp like *C. tropicalis*, *C. lusitaniae* and *C. guilliermondii* [13]. *Candida* ID system contains a chromogenic indolyl glucosaminide substrate, which is hydrolyzed by *C. albicans* isolates to form a turquoise or blue insoluble product.13 *C. tropicalis*, *C. lusitaniae*, *C. guilliermondii*, and *C. kefyr* form pink color colonies on this medium after incubation at 48 h whereas, other *Candida* spp. produce white color colonies [13]. However this medium is not completely selective and hence blue stained bacterial colonies may be confused with *C. albicans* .

CandiSelect 4 medium (CS4): CS4 is a new chromogenic medium for the isolation and identification of *C. albicans* and medically important NAC spp. [13]. Typically on CS4, colonies produced by *C. albicans* are pink to purple, with a purple pigmentation which diffuses out around the colonies [13]. Intense turquoise pigmented colonies, with a mat, uniformly colored, convex, smooth morphotype, are suggestive of *C. glabrata* [13]. Turquoise-blue colonies, with a characteristically rough morphotype, a dry appearance and irregular outline, are typical of *C. krusei* [13].

***Candida* Diagnostic Agar (CDA):** CDA contains a novel chromogenic substrate of β-N-acetylhexosaminidase [13]. On CDA, *C. albicans* and *C. dubliniensis* produce white colonies with deep-red spots on a yellow transparent background whereas *C. tropicalis* and *C. kefyr* forms uniformly pink colonies [13]. Other NAC spp., produce white color colonies on this medium [13].

BiGGYagar system: On this media, *C. albicans* and *C. tropicalis* colonies appear as light and dark brown in color, respectively [13]. *C. krusei* produces typical large, rough, dark brown colonies with surrounding yellow zone [13]. *C. parapsilosis* produce light brown-greenish, grey-cream-colored colonies on BiGGY agar.

Conclusion

Emergence of Non *albicans Candida* species as predominant cause of infections has highlighted importance of species identification. Accurate identification of infecting strain of *Candida* is essential for selection of appropriate prophylactic and therapeutic antifungal drug. Although commercial systems and molecular diagnostic methods are rapid and reliable, high cost limits their use. Conventional techniques remain the mainstay of species identification of *Candida* isolates in most clinical microbiology laboratories.

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