ORIGINAL ARTICLE

Comparative Evaluation of CHROMagar and API 20C AUX in Isolation and Identification of *Candida Species*

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ABSTRACT

Objective: To evaluate the performance of CHROMagar and API 20C AUX for the documentation of different *Candida* species.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: The study was conducted in the Department of Microbiology at AFIP (Armed Forces Institute of Pathology), CMH Rawalpindi and Army Medical College Rawalpindi in collaboration with Departments of Pathology (Microbiology) at Pakistan Railways Teaching Hospital (PRH), Islamic International Medical College Rawalpindi from 01th April 2017 to 30th September 2017.

Materials and Methods: Collectively 100 isolates of *candida* yielded from HVS clinical samples. Phenotypic tests including growth on CHROMagar *Candida* and API 20C AUX were used for reporting different *Candida* species. Clinical *Candida* isolates along with reference institutional control strains of *Candida* species were used in the study. Data was analyzed using simple descriptive statistics (frequencies, percentages) for each categorical variable.

Results: Among 100 *candida* isolates 92 (92%) isolates of *Candida* were identified correctly to level of species by CHROMagar *Candida*, in comparison to 100% identification of *candida* species using API 20C AUX. Results of present study revealed that CHROMagar *Candida* can be used to report three species of *Candida* considering the morphology and colour of colonies of these particular species, and to distinguish them as *C. albicans, C. tropicalis, and C. glabrata*.

Conclusion: Both phenotypic tests CHROMagar plates and API 20 C AUX are effective in the documentation of *Candida species*. However API 20 C Aux is found to be more accurate than CHROMagar because less commonly isolated *Candida species* cannot be documented using CHROMagar. Moreover being less costly; use of CHROMagar *Candida* is helpful in rapid identification and constructing suitable therapeutic plan for patient's management in laboratories with limited resources.

Key Words: *Candida Species, HVS, Identification Methods.*

Introduction

Fungal infections have worldwide spread. Since 1980s, a steady rise in incidence as well as prevalence of these infections has been found contributing significantly to morbidity and mortality. Mycotic infections are frequently found in patients with depressed immunity [1,2,3] like cancer patients receiving chemotherapy, transplant patients and AIDS patients who are more prone to develop infections caused by *candida*.[4] Infections due to *Candida albicans* remains the most frequent etiology of human diseases due to genus *candida*, but the incidence of infections due to *non albicans candida* is also increasing.[5,6] There is a variety of methods for identifying *Candida species* from clinical samples, among them CHROMagar *Candida* differential medium is commonly used to isolate presumptive *C. albicans, C. dubliniensis, C. tropicalis* and *C. krusei*. Its sensitivity and specificity is considered satisfactory

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for these species.[7,8] The biochemical characterization is done using the API® 20C AUX (BioMerieux, France), which relies on variations in the assimilation of carbohydrates.[9] However, it presents limitations related to cost and to distinguish between some species.[7] Presently the emergence of *non albicans Candida* species is a major problem to be addressed in several institutions.[10,11] In 70% to 90% vulvovaginal candidiasis cases, *C. albicans* is found to be main causative agent to be followed by *C. glabrata* causing 10% - 20% of vaginal candidiasis.[5,6,12] Most species of *Candida* are involved in causing vulvovaginitis but *C. krusei, C. parapsilosis* ,and *C. tropicalis* are infrequent causative agents.[13]

Conventional methods used for *candida species* identification like assimilation and fermentation reactions are described as clumsy and beyond the range of expertise in local laboratories. Evaluation of identification methods in resource-limited settings for *candida* species such as microscopy, colonial morphology and biochemical studies require unique research studies for effective management and prompt diagnosis of fungal infections.[14]

Candida vaginitis is usually diagnosed without proper diagnostic procedures but there is possibility that women may be uninfected or may be suffering from another illness. Culture on Sabouraud's dextrose agar (SDA) for diagnosis of fungal infections is considered as gold standard, while isolation and identification using different phenotypic assays can take up to 2 – 4 days.[15,16] Rapid identification of mycotic infections is possible with use of different brands of chromogenic media. These chromogenic agars, reduces the time required for the identification of yeast by distinguishing common *Candida* species on the basis of specific color that generated because of reaction of substrate with enzymes secreted by microorganisms after incubation for 48 hours at 37 ^{0}C.[17,18]

Use of API 20C AUX kit for identification of *candida* species including *C.albicans* and *non albicans candida* is easier and has greatly reduced the laboratory time involved in the speciation of *Candida* isolates.[19,20]

Owing to limited knowledge and practice of these phenotypic methods, this study was aimed to compare and evaluate the efficacy of CHROMagar and API 20C AUX in identification of *Candida* species.

Materials and Methods

A descriptive cross-sectional study was conducted from 01st April 2017 to 30th September 2017 after approval from Research and Ethical Review Committee. Non-probability convenient sampling was done and non-parametric data were collected. The data of *Candida species* were analyzed by using frequencies distribution test on SPSS (version 20) software. A total of 100 *candida* isolates were collected from Microbiology labs of Armed Forces Institute of Pathology (AFIP), Army Medical College (AMC) and Islamic International Medical College (IIMC) in collaboration with Combined Military Hospital (CMH), Military Hospital (MH) and Railway Hospital Rawalpindi respectively. Study included HVS specimens of pregnant, nonpregnant and postmenopausal women that revealed growth of *candida* species on Sabouraud' s culture plate. Direct Gram-stained smear examination was done for all collected candida isolates after culture on Sabouraud's dextrose agar (SDA) (Oxoid, UK) incubating at 37 °C for 24 – 48 hours.

Institutional control strains were used to compare the results of present study. Conventional methods, such as germ tube test, macroscopic appearance and structural description of colonies i.e. colour, size and texture on Sabouraud's dextrose agar (SDA), and CHROMagar *Candida* were used to confirm the growth of control strains.

CHROMagar *Candida* medium in each liter contained peptone (10 g), glucose (20 g), agar (15 g), and chloramphenicol (0.5 g) and Chromogenic mixture. (2 g), while pH of the medium was maintained at 6.1 according to instructions of manufacturer. *Candida* colonies of study samples from SDA agar were inoculated onto CHROMagar. Specimens were streaked for isolation onto the surface of the medium. The plates were kept for incubation at 30°C for 48-72 hrs in an inverted position. Forty two hours incubation time is obligatory for complete color development of *Candida* colonies. The diverse species of *Candida* species revealed dissimilar colours of colonies i.e. *C. albicans* colony appeared light to medium green, *C. tropicalis* colonies gave dark blue to metallic-blue colour and *C.glabrata* colonies looked light mauve to mauve. Moreover, these colonies were flat with a whitish border. Other candida species like *C. krusei* can give rise to light to

dark mauve colour. Rest of non albicans candida species produced light creamy to light pink colour that were later on identified with API kit.

API 20 C AUX (Biomerieux, France) was used to perform Carbohydrate fermentation tests. Dehydrated substrates were added in 20 different cupules which allowed the performance of 19 assimilation tests. Semi solid minimal medium was used to inoculate the cupules and the growth of yeast was seen when utilized the added substrate as the soul carbon source. The reactions were read by linking them to growth controls and documentation was done by referring to the Analytical Profile Index.

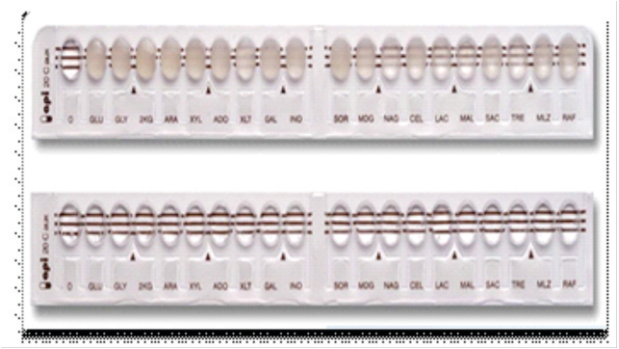

Fig 1: API 20C AUX

Results of API were finally compared with the culture results and speciation of *Candida* was also done.

Results

Results of germ tube test revealed 68 out of 100 isolates were positive for germ tube and remaining 32 were negative as shown in Fig 2.

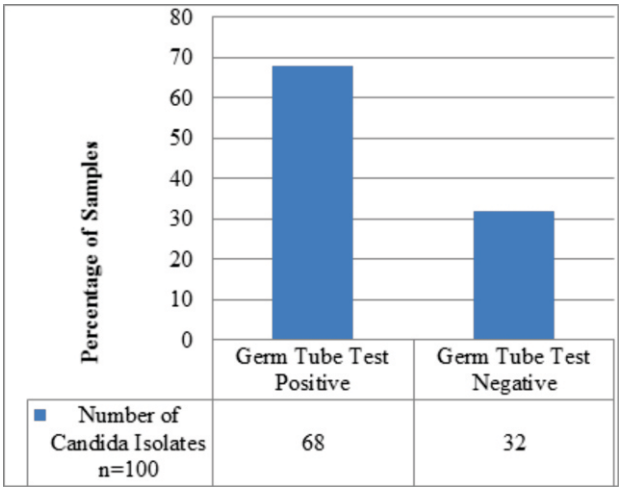

Fig 2:Frequency of Germ tube positive isolates

Among 100 samples grown on CHROMagar plates, 16 (16%) showed pinkish purple growth and were labeled as *C. glabrata*, 8 (8%) revealed blue coloured colonies and were termed as *C. tropicalis*, while 68 (68%) samples showed green coloured colonies of *candida albicans*. Among rest of 8 samples, 4 samples showed light pink colonies and 4 samples revealed blue to mauve coloured colonies which after API testing identified as *C.famata* 4(4%), *C. guilliermondii* 2(2%), *Saccharomyces cerevisiae* 1(1%) and *C. lusitaniae* 1(1%). Moreover, *Candida albicans* was found to be dominant over the *non albicans species*. The comparative identification results are shown in Table I.

Table I: Identification of Samples using CHROMagar and API 20C Aux

S.No	Candida Species	No. of Isolates (total:100)	Colony characteristics on CHROMagar Candida	Identification by API 20C AUX
1	*C. albicans*	68	Apple green colonies; consistent	*C. albicans*
2	*C. glabrata*	16	White large glossy pale pink to violet colonies	*C. glabrata*
3	*C. tropicalis*	8	Steel blue, purple diffusion into surrounding agar	*C. tropicalis*
4	*C. famata*	4	White to light pink colonies	*C. famata*
5	*C. guilliermondii*	2	Small pink to purple colonies	*C. guilliermondii*
6	*C. lusitaniae*	1	Pink gray purple	*C. lusitaniae*
7	*Saccharomyces cerevisiae*	1	Pink to purple	*Saccharomyces cerevisiae*

The API 20 C AUX tests recognized the *Candida* species on the basis of fermentation and utilization of different sugars. The confirmatory test by API yielded seven species of *Candida* in total i.e. *C. albicans, C. glabrata, C. tropicalis, C. guilliermondii, C. famata, Saccharomyces cerevisiae* and *C. lusitaniae*. API test results were matched with CHROMagar results, germ tube test results and microscopy of these selected samples. Overall CHROMagar identified strains correctly with more than 90% discrimination while comparing with API 20 C AUX results. The results of API are tabulated in Table 1. The API 20 C AUX system correctly identified about 100% of the isolates compared to 92% by CHROMagar culture technique. The CHROMagar culture plates correctly identified all organisms except 8 isolates. These 8 isolates revealed pink to

purple coloured colonies that were identified differently using API 20C AUX. The reason for this deviation is because CHROMagar has been known to identify frequently found species of *Candida i.e. C. albicans, C. glabrata, C. tropicalis*.

Discussion

In developing countries especially where resources are limited, deficiency of training skills and non-availability of proper reagents that are contributory factors in making final diagnosis of mycotic infections, identification of fungal infections to species level become quite difficult. Moreover to minimize the monetary burden on the underprivileged patients, laboratories only perform germ tube test and limit their report only to identification of *C. albicans*.[21]

In present study, all 100 candida isolates gave distinct colours on CHROMagar thus helped in the recognition of *candida species* causing vulvovaginitis in study population. This data conforms to finding of a study conducted by Horvath et al.[22] There is nonconformance of our data with a study conducted by Grace L et al who reported 78% identification of *candida species* using CHROMagar.[23] This finding might be due to direct subculture of specimen on CHROMagar plates. LYnn L et al reported that CHROMagar readily identify *C. albicans, C.glabrata, C.krusei, and C. tropicalis*.[22] Diagnosis of vaginal candidiasis is usually based on clinical symptoms and direct microscopic examination as stated by Nyirjesy et al and Faraji et al [24,25]; Although microscopic examination of clinical samples is quick, easy method and may recognize the probable causative agent, but CDC recommend that vaginal culture is mandatory to confirm the diagnosis.[26] In this study, different laboratory methods were used for prompt diagnosis of *candida species*, among these Gram staining and germ tube test were found easy and trustworthy techniques for the documentation of *Candida spp*. CHROMagar is found to be a novel medium enabling isolation and identification of different *Candida species*. This media correctly identified 92% of *Candida* strains which is in accordance with the prior study done by Ozcan et al.in 2010.[27]

Based on different colors and morphology of colonies "CHROMagar *Candida*", provided a fast and convincing recognition of frequently found yeasts species, which would ordinarily be missed during conventional plating on solid medium. According to Nejad et al the major advantage of "CHROMagar *Candida*" was its ability to detect the presence of mixed species,[28] and results of this study prove that use of CHROMagar was helpful in correct identification of *C.albicans, C.glabrata* and *C.tropicalis* depending on the colour and morphology of colonies. Chromogenic culture media are very helpful in identification of *C. albicans* [29] but its main limitation is less power of discrimination among *non albicans Candida* species.[30] In present study, 92 % of *Candida* isolates were correctly identified after growth on CHROMagar , in contrast to the study conducted by Dalia et al they stated that this medium correctly identified *C. albicans* with excellent sensitivity and specificity, but revealed lower sensitivity for *non albicans candida*.[31] The data suggested that species which were identified by CHROMagar were almost the same as were confirmed by API 20C Aux. It means that in the presence of CHROMagar, germ tube test to confirm *C.albicans* can be excluded.

On completion of study it is acknowledged that CHROMagar medium is appropriate and affordable diagnostics medium in a resource-limit setting because approximate cost per culture for complete identification of *Candida* using SDA, Corn meal agar, and API 20C Aux in Pakistan is around Rs. 1,200 while CHROMagar *Candida* is around Rs. 250 per specimen culture. Additionally, 4 - 5 samples can be inoculated on one CHROMagar plate without compromising its effectiveness that makes it more cost efficient.

Present study supports the statement that the most suitable and popular procedure for *candida* species identification is the use of commercially available kits for carbohydrate assimilation and / or enzyme detection.[31] In the present work, all *Candida* isolates were correctly identified to the species level by API 20C AUX. Results of present study about effectiveness of API 20C are in accordance with other studies.[32,33,34,35] API tests are evaluated as best methods for the final documentation of all *Candida species* but owing to the fact that in our present hospital settings where limited resources are available this technique is difficult to practice on routine basis. Comparison of CHROMagar and API 20C AUX (Biomerieux, France) reveals that use of CHROMagar is less costly while API identification

method is costly and laborious but API 20 C has an advantage that it can identify more species of *non albicans Candida* as compared to CHROMagar, which can identify only three to four commonly involved *Candida species*.

Conclusion

This study reveals CHROMagar *Candida* and API 20C AUX having good potential to rapidly identify *candida species*. In resource limit settings, CHROMagar can be used as useful adjunctive medium in the clinical laboratory but for identification of yeasts at species level, the use of API 20C Aux with a wider database is preferable. API 20C is better for the diagnosis of candidiasis especially due to less frequently found *candida* species and should be adopted as routine diagnostic procedure in the clinical microbiological laboratories.

This study has involved evaluation of two phenotypic methods in identification of *candida* species in small number of cases with vaginal *candidiasis* because of cost and availability issues. Future studies including large sample size and comparison of these two methods with other diagnostic methods used for identification of *candida* species are needed. Moreover comparison and evaluation of these two methods in identifying *candida species* in cases other than vulvovaginal *candidiasis* are also recommended.

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