ORIGINAL ARTICLE

Molecular Identification of Candida Species Isolated from Different Clinical Specimens at Al Jouf Area, Saudi Arabia

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ABSTRACT

The existence of Candida species in different clinical specimens has increased significantly over the few last years due to various predisposing factors including prior antibiotic use, prolonged hospital stay, extremes of age, diabetes mellitus and the use of immunosuppressive therapy. The purpose of this study was to apply different diagnostic techniques for characterization of Candida species in different clinical samples at Aljouf area Saudi Arabia. Two hundred and fifty (n=250) specimens were collected from various body sites (115 samples of urine, 84 stools and 51 oral swabs). Yeast isolates were purified on Sabouraud dextrose agar and then heavy suspensions were stored at -20° C in 10% glycerol peptone water until use. To identify the isolates API 20 CAUX was used according to the manufacturer instructions. Forty Candida isolates were selected for DNA extraction, PCR and sequencing using ITS1 and ITS4 primers. The sequences of well identified Candida species were compared with data in the National Center for Biotechnology Information database using the basic local alignment search tool (BLASTn). 97 Yeast isolates were recovered, 62 strains (54%) in urine, 23 (19.6%) in stool and 12 (6.1%) in oral swabs. Out of the 97 yeast, only 38 Candida isolates were recovered using API 20 CAUX. The phylogenetic relationships tree of the tested isolates showed the possibility of new C. tropicalis clade compared to the C. tropicalis type strain.

Keywords: Candida, ITS, molecular characterization, sequence.

INTRODUCTION

In recent years the incidence of infections caused by fungi increased dramatically, and the rate of morbidity and mortality increased in patients with deficiencies in immune response in addition to cancers and AIDS patients.[1] Candida infection becomes a serious risk factor for immune suppressive people.[2] Candida infections was considered as opportunistic infection leading to the impairment of the host's defense system.[3] On the other hand, Candida infections manifest in a different forms such as superficial, cutaneous, subcutaneous and systemic invasive fatal illnesses which spread to different body organs.[2,3] More than 100 yeast species have been identified as human pathogens and have been isolated from all body sites. Identification of the high diversity yeast species by conventional mycological methods is difficult and sometimes inconclusive, especially for unusual yeast species.[4] Regarding the routine conventional traditional it is less costing and simple, but it is time-consuming and with less reliable results compared to DNA based techniques. Relatively sugar assimilation reactions can be used alternatively for conventional methods when DNA based methods are not available.[5] This study aimed to isolate biotype and molecularly characterize Candida species by API 20 CAUX, molecular identification and sequencing technique to detect the common yeasts among the study population in Al Jouf Area.

MATERIAL AND METHODS

All specimens were inoculated on Sabouraud dextrose agar and incubated for 48 hrs at 37° C. Cultures were observed to detect any colonies of Yeast. Smears from colonies resembling yeast growth were examined by gram stain using oil immersion lens for the characteristic gram positive yeast cells. Characteristic Yeast colonies were purified on Sabouraud dextrose agar and a heavy suspension was made in 10% glycerol peptone water and stored at -20° for other use.

API 20 CAUX typing: Stored yeast suspensions were inoculated on Sabouraud dextrose agar and incubated at 30° C for overnight then a suspension was made into 2 ml sterile saline solution (0.85%) to achieve a turbidity equivalent to 2 McFarland standard turbidity. According to the instructions of the manufacturer (bioMérieux, Hazelwood, France), 100 µl of inoculum suspension was added to the API basal medium. The API trays were inoculated with inoculum suspension and incubated for 72 h at 30° C. The reactions were read visually by comparing the turbidity of growth controls and turbidity of each cupule. The pseudohyphae results were score, and the Identifications was obtained by using API Web software. API results were ranked as excellent, very good, good, acceptable and low discrimination.

DNA Extraction, PCR and sequencing technique: DNA was extracted from 40 Candida isolates and PCR was carried out using ITS1 and ITS4 primers. Then the internal Transcribed 1 and 2 including the 5.8 S rDNA was sequenced using ITS1, ITS2 primers commercially in Macrogen, Seoul, South Korea. The sequences generated were compared with data in the National Center for Biotechnology Information database using the basic local alignment search tool (BLASTn; http://www.ncbi.nlm.nih.gov).

RESULTS

Ninety seven yeast species were isolated using Sabouraud Dextrose Agar. Yeasts colonies appeared as smooth, glabrous, white to creamy colored. Microscopically, they

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show Gram positive spherical to oval large yeast-like cells with budding blastoconidia and pseudohyphae. The distribution of Yeasts isolates in urine and stools specimens according to sex was found as; 32 isolates (36.5%) in males and 54 (63.5%) in females.

Forty (n=40) yeast isolates were identified by API20 CAUX with different identification percentages (ID %) and identification levels (i.e. excellent, very good, good, acceptable and low discrimination). The majority of isolates were identified either as very good identification (n=24) or good identification (n=6). Six (n=6) strains were ranked as excellent identification, while two (n=2) as acceptable identifications and two (n=2) were considered of low discrimination. The total percentages of Candida isolates using API20CAUX were 19 (51.4 %) in urine, 12 (32.43%) in stool and 6 isolates (16.27%) in oral swabs.The percentages of species according to API identification was C. glabrata (23.4%), Candida famata (6.7%), Step.ciferrii (6.7%) and (3.3%) for each of Candida dublinesis, Cryp. laurienti and Candida lipolytica (Fig 1).

Using PCR and DNA sequence technique 21 isolates out of 35 were found as Candida species. The percentages of isolates; C. glabrata (25.7%), C. albicans (20%), Clavispora lusitaniae (14.3%), Pichia kudriavzevii (14.3%), C. tropicalis (8.6%), K. marxianus (8.6%), C. dublinesis (5.7%) and S. cervisiae (2.8%) (Fig.2). The sequence-based identification was matching with the original API identification in only 6 out of 35 yeasts isolates (17.1% of cases). The phylogenetic relationships tree of the tested isolates showed the possibility of new C. tropicalis clade compared to the C. tropicalis type strain. The ATCC C. glabrata positive control was correctly identified as C. glabrata with 100% sequences similarity, while three C. glabrata were far distance from the control positive (Fig.3). The ATCC C. albicans and C. dublinesis were identified with 95% and 99% respectively (Fig.4).

Figure 1 Percentages of different Yeasts isolates according to API Identification

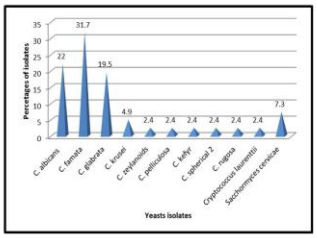


Fig 2: Percentages of different Candida species and other Yeasts isolates according to DNA based sequence identification.

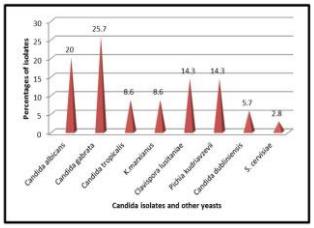


Figure 3: Phylogenetic tree with ITS sequence of Candida isolates compared to ATCC Candida species was constructed using neighbor-joining algorithm using CLC sequence viewer program version 7.6, showing the similarity of Candida isolates with ATCC Candida species.

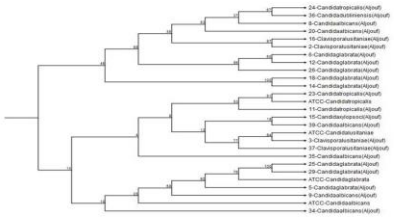
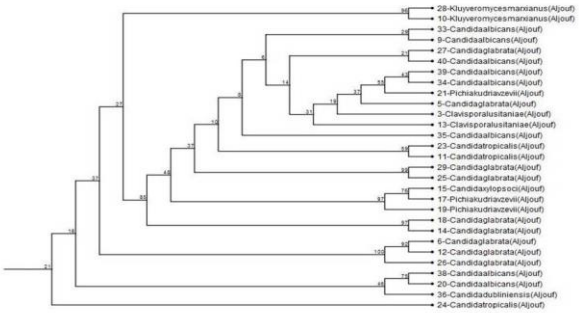


Figure 4. Phylogenetic tree with ITS sequence of Candida isolates and other yeasts was constructed using neighbor-joining algorithm using CLC sequence viewer program version 7.6, showing high similarity of Candida isolates with global strains in Genebank database.



DISCUSSION

Candida species were successfully isolated from different specimens in Al Jouf Area. The study showed that Candida infection was common in females (63.5%) than males (36.5%). Urine specimens was found as common source of Candida infections and this fact was agreeing with previous studies in Saudi Arabia [6] and disagreed with the fact which state that the High vaginal swab was the main source of Candida[7]. We attributed the difference to the fact that here in Saudi Arabia it is too difficult to ask for vaginal swap according to the tradition and culture of the people. Candida species which isolated in this study were *Candida glabrata*, *Candida albicans*, *Candida tropicalis*, *Candida lusitaniae*, *K. marxianus* and *P. kudriavzevii*. *Candida glabrata* was the most frequent isolated species in this study, and this agreed with the results of previous workds which showed an increase in the rate of *Candida glabrata* infection which might be due to the long-term utilization of antifungal drugs such as azoles[8-13]. In this study, *Candida albicans* was second isolated species, and this disagreed with the previous studies found that Candida albicans was the most isolated species[14-16]. Thus, the increasing prevalence of non albicans species especially Candida glabrata seems to be due to the increase of antifungal drug resistance.[17] API 20 CAUX is very simple and easy for the identification of the common yeast species but can miss-

identify the less common ones. In this study, the API results were matched to DNA sequence results in only 6 Candida species (C. albicans and C. glabarata) and differ in other yeasts isolates such as C. dublinesis, C. tropicalis and C. lusitaniae. For this reason, it is better to use molecular identification methods such as PCR and sequencing technique for the identification of less commonly yeasts species. DNA sequencing of all yeasts isolates will provide the better understanding of the yeast population genetics and Candida species, and this was expressed in the phylogenetic tree Fig 3 and 4.

In conclusion, many Candida species including: C. albicans, C. famata**,** C. glabrata**,** C. krusei**,** C. krusei**,** C. zeylanoids**,** C. pelliculosa**,** C. kefyr**,** C. spherical 2**,** C. rugosa and other unusual yeasts species mainly isolated from stool such as Cryptococcus laurenttii**,** Sacchormyces cervicae have been isolated from different samples collected from patients in different hospitals at Al Jouf region and healthy subjects**.** The detection of common non-albicans Candida species in this study, including C. glabrata, C. dublinesis, and C. tropicalis, and the increasing trend of unusual yeast species, such as C. lusitaniae, K. marxianus and P. kudriavzevii should be considered. Future study would be done to detect the risk factors for non-albicans Candida species infections in this area.

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