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Original Research Article

Species identification of Candida by Fungichrome-1 method

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Abstract

Species identification of candida isolates was carried out by a commercial fungichrome-1 kit and compared with conventional methods. 50 candida isolates from sputum, urine, oropharyngeal swabs, blood, pus, CSF from patients admitted in Kasturba Medical College, Manipal, Karnataka were tested by the conventional methods and by fungichrome-1, a commercial yeast identification system. *Candida albicans* is the most prevalent Candida species isolated from digestive tract but can be recovered from vagina or skin as well which accounted for 32% of total isolates. The next most frequent Candida species were *Candida guillermondi* and *Candida tropicalis* which accounts for 24% and 16% of the total isolates. The result obtained in the present study indicates an alternative method to identify yeast and yeast like fungi isolated from clinical specimens.

Keywords: Candida, isolates, conventional methods, fungichrome.

1.Introduction

In recent years, the increasing incidence of HIV infection and therapeutic modalities for advanced life support and surgical procedures such as organ transplantation of prosthetic devices etc., have led to an increased susceptibility of opportunistic mycotic infections of which majority are caused by yeast and yeast like fungi[1-5].

Candida albicans and *Cryptococcus neoformans* are the most important yeast pathogens but the so called albicans yeasts are being implicated with greater frequency as opportunistic pathogens in the compromised host [6-8]. Some of these species have been associated with certain diseases and devices and some species like *Candida glabrata* and *Candida krusei* have been noted to be naturally resistant to fluconazole. Rapid and accurate identification of yeasts have thus become important not only for effective management of infections as various species respond differently to various antifungals[9-11]but also to prevent emergence of drug resistance.

Aimof the present study was to compare fungichrome-1, a new yeast identification system, with the conventional methods, for the speciation of yeast and yeast like fungi isolated from clinical specimens.

In the present study, an attempt was made to identify Candida species by fungichrome-1, a new yeast identification system [12,13].

2. Materials and methods

50 yeast isolates from sputum, urine, oropharyngeal swabs, blood, pus and CSF from patients admitted in Kasturba Medical College and Hospital, Manipal were tested by conventional methods and by fungichrome1, a commercial yeast identification system after obtaining informed consent from the subjects and approval from Institutional Ethical Committee [14-16].

2.1 Conventional tests

These methods are based on the study of different phenotypic properties of the yeast that includes colony morphology and microscopic morphology on corn meal agar supplemented with tween 80, germ tube test, sugar assimilation test.

2.2 Fungichrome-1

Fungichrome-1 is polystyrene micro titre plate with 16 wells. The enzymatic activities are revealed by 3 kinds of reactions.

a. Hydrolysis of Chromogenic Substrates: Wells GAL (contains a chromogenic substrate for N-Acetyl-B-d-

Galactosaminidase), PRO (contains a chromogenic substrate for L-proline –amidase),ONPG(contains a chromogenic substrate for orthophenyl-b-d-galactosidase), EPA (contains a chromogenic substrate for a peptidase), SGL (contains a chromogenic substrate for glycine amidase): the oxidase and peptidase activities of the yeasts hydrolyse these chromogenic substrates and lead to the release of para–nitroaniline, paranitrophenol or ortho-nitrophenol, all end products giving a yellow colour.

b. The assimilation of carbohydrate substrate: wells GAL-SAC (galactose–sucrose and bromocresol purple), TRE (trehalose and bromocresol purple), MAL (maltose and bromocresol purple), CEL (cellobiose and bromocresol purple), RAF (raffinose and bromocresol purple). The use of these sugars by the yeast is revealed by the colour change of Bromo Cresol Purple (BCP) from violet to yellow or an absence of colour [17-19].
c. The resistance to cycloheximide is revealed by the same principle of substrate utilization.
d. The hydrolysis of urea releases ammonia which alkalinizes the medium and makes phenol red (PR) turn to fuchisia pink.
e. Oxidation of synthetic substrates (well POX): the activity of phenol oxidase, produced by the yeasts, in the presence of caffeic acid produces a brown colour.

Each fungichrome-1tray also includes a positive control well (c+) which reveals the assimilation of glucose.

The interpretation of reactions is performed either by a coding system or by the yeast identification table provided along with the kit.

If at the 24hrs mark, the code identified was not referenced, the incubation was continued for another 24hrs.If at 48hrs mark, the code was still not itemized, the identification chart was referred to.

For a given yeast isolate, all the predominant characteristics mentioned in the identification table should be positive except when a positivity percentage is mentioned in the chart.

When the results of fungichrome-1 did not match the conventional tests, both the test methods were repeated.

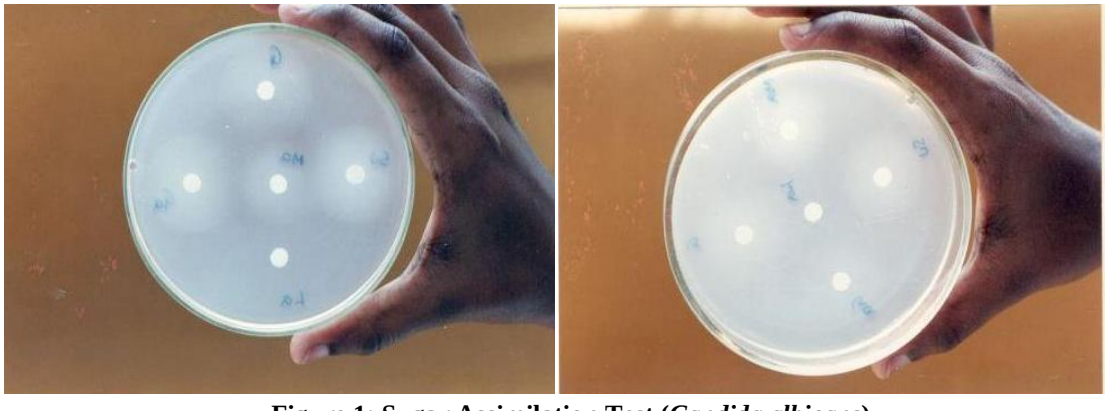

Figure 1: Sugar Assimilation Test (*Candida albicans*)

Figure 2: Species Identification of Candida by Fungichrom-1 (*Candida albicans* & *Candida guillermondii*)

3. Results

Out of 50 yeast and yeast like isolates tested, same species identification was obtained by both the test methods for 40 isolates is 80%[20].

Table 1: Tested isolates

yeast and yeast like organisms	No. of isolates similarly identified by both methods	No. of isolates identified by fungichrome-1 but not by conventional methods	No. of isolates identified by conventional methods but not by fungichrome-1
C.albicans	16	0	0
C. tropicalis	8	0	0
C.guillermondi	12	0	2
C.curvata	0	0	2
C.parapsilosis	0	4	0
Cry.neoformans	2	0	0
C.inconspicua	0	2	0
C.krusei	2	0	0
Total	40	6	4

4. Discussion

Unlike the time consuming conventional methods of microbial identification, the new commercial systems are simpler, rapid and are particularly easy to interpret. The various commercial identification systems introduced for yeast identification are based on colour changes denoting the utilization of several kinds of substrates by the metabolizing yeast. Some of disadvantages of these systems apart from the higher cost per test compared to conventional method, is that all require careful standardization of the inoculum. High density inocula often require 48hrs of subculture, which can delay identification and limit classification of the corresponding method as rapid techniques. Some of the systems also present difficulties in reading the colour reactions leading to some misidentifications.

The rate of correct identification is also based on whether strains tested were included in the manufacturer's database. Some of the widely-evaluated systems are the API-20C AVX yeast identification systems, API candida, Uni –yeast Tek identification system. API candida, Uni–yeast Tek identification system, microscan yeast identification panel, vitex yeast biochemical card, chrom agar, rapid ID yeast plus system and fungichrom-1. The results of this study show that fungichrome-1, a commercial yeast identification could identify 92% of the commonly isolated yeasts in our hospital. The sensitivity observed in the present study was comparable with those observed in the previous studies is 95.5%, 99%, 98%.

The results showed 80% correlation with the conventional methods of yeast identification, which appears to be less sensitive in our study compared to the reports made by Umabala et al as 96%. The less sensitivity of identification by fungichrome1 observed in our study may be due to less number of candida strains tested and also the isolation of the type of species of candida.

5. Conclusion

Fungichrom-1 provides a rapid, simple and accurate method of identification of medically important yeasts that can be routinely used in clinical laboratories. The time required was considerably less about 24-48hrs when compared with the time consuming conventional methods of identification. The reading is manual, expensive automation is also avoided unlike the other commercially available systems. Further evaluation of the system with large number of isolates representing additional species of yeasts need to be done. Also, the cost effective of routine use of fungichrom-1 should be assessed depending upon the type of patients being catered to by a particular laboratory.

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