Clinical study

YEAST COLONIZATION OF MUCOUS MEMBRANES: PREVALENCE AND IDENTIFICATION OF DIFFERENT SPECIES

A. Stary, A. Kuchinka-Koch and A. Schiller

ABSTRACT

The prevalence of different *Candida species* on mucous membranes of the genital, perigenital, anal, or pharyngeal region was evaluated in 451 male and female patients. Furthermore, the reliability of CHROMagar™ Candida for the identification of certain yeast species, such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *Saccharomyces cerevisiae* was determined. The most prevalent yeast isolated from the genital tract was *C. albicans* (86.1%) followed by *C. glabrata* (8.5%), *C. parapsilosis* (3.7%), and *Saccharomyces cerevisiae* (4.2%). The perigenital and anal regions were colonized most frequently with *C. albicans* (87.5%) only. In contrast to these samples a large variety of different *Candida spp.* was cultured from the pharyngeal region. Mixed yeast cultures mainly with *C. albicans* together with *C. glabrata* or *S. cerevisiae* were observed more frequently with pharyngeal (11.3%) than with vulvovaginal samples (4.6%). All 408 presumptive *C. albicans* strains could be identified on CHROMagar™ Candida and were confirmed either by rice agar, germ-tube test, or API resulting in a sensitivity and specificity of 100%, respectively. The same performance characteristics could be observed for *C. glabrata, C. krusei*, and *C. tropicalis*. The comparison of different methods demonstrate that CHROMagar™ Candida permits a reliable and highly sensitive as well as specific differentiation of *C. albicans*, *C. glabrata, C. tropicalis*, and *C. krusei* from other *Candida spp.* by characteristic appearance of colonies within few days.

KEY WORDS

yeasts, prevalence, mucous membranes, CHROMagar™ Candida, genital tract

INTRODUCTION

In the past few years increasing rates of colonization of mucous membranes with different yeast species have been observed. This fact may be due to debilitating diseases such as AIDS, diabetes mellitus, and cancer, as well as to the increasing use of broad-spectrum antibiotics or corticosteriods (1,2). Furthermore, high-estrogen-content contraceptives, pregnancy, and tight-fitting clothes are reported risk factors especially for increased vaginal *Candida* colonization whereas sexual intercourse alone has no influence on the vaginal colonization rate with yeasts (3,4).

Candida albicans is the most frequently isolated yeast associated with mucocutaneous colonization (5). This yeast is responsible for up to 90 per cent of recurrent vulvovaginal candidiasis (VVC) (3,6).

Table 1. Mixed yeast cultures isolated from the female genital tract listed according to yeasts found.

Number of samples	*C. albicans*	*C. glabrata*	*S. cerevisiae*	*C. parapsilosis*
8	+	+		
3	+		+	
1		+	+	
1	+			+
Σ 13	12	9	4	1

However, elevated isolation rates of other yeast species such as *C. glabrata* or *C. tropicalis* from genital mucous membranes have been reported (7, 8).

Primary as well as acquired resistance of certain yeast pathogens to antifungal drugs requires rapid and reliable identification of a broad spectrum of yeast species (9). Traditionally, methods for the identification of yeasts rely on a combination of morphological and biochemical characteristics. Potassium hydroxide microscopy is a rapid test for diagnosis of yeasts in different specimen types with a sensitivity of approximately 70% but the lack of identification poses limits (10). A rapid and cost-effective technique for the identification of *C. albicans* is the germ-tube test which has been reported to be negative in up to 5% of *C. albicans* isolates (11, 12). Since the differentiation of other *Candida spp.* is not possible by the germ-tube test, cultivation is necessary for further identification. There are different media available for the detection of chlamydospores and the production of hyphae or pseudohyphae permitting the identification of diverse *Candida spp.* as well as the differentiation of the genera *Cryptococcus*, *Saccharomyces*, *Geotrichum*, and *Trichosporon* based on microscopic morphological features. The principal biochemical criteria for the identification of different species are assimilation of carbohydrates or nitrate, and fermentation of sugars. Since some of these methods are expensive and time-consuming, evaluations of newly developed, rapid, and accurate identification systems were performed (13).

Recently, the potentially useful, rapid, and cost-effective Microbial Identification System (MIS) for aerobic gram-positive, gram-negative bacterial species, and yeasts has been scrutinized for accuracy in identification of diverse yeast species with the result that it cannot be taken into consideration as alternative identification system for clinical microbiology laboratories at the moment (14, 15).

For routine laboratory use exact and time-saving identification systems are of great importance. Recent reports described the chromogenic isolation medium CHROMagar™ Candida for the presumptive identification of *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis* based on species specific enzyme reactions (16, 17, 18, and 19). The results reveal CHROMagar™ Candida superior to Sabouraud glucose agar in its ability to detect mixed yeast cultures and to suppress bacterial growth.

In the present study, the reliability of the chromogenic differential culture medium for the identification of different yeast species as well as for the detection of mixed yeast cultures isolated from clinical samples was determined. Furthermore, the prevalence of various *Candida species* on mucous membranes of the genital, perigenital, anal, and pharyngeal region was evaluated.

PATIENTS AND METHODS

During a period of two months 474 samples positive for yeasts were collected from 451 female and male patients attending the Outpatients' Centre for STD. Most of the specimens were collected from the genital mucous membranes, namely 283 vulvovaginal specimens and 70 samples from the urethra and/or penis. Furthermore, 97 pharyngeal, 11 anal, and 13 perigenital specimens were collected. Sampling was carried out with a sterile cotton-swab or wire-loop followed by immediate inoculation in Sabouraud broth (Oxoid, Unipath LTD., Hampshire, England) independent of specimen type. Yeasts were cultivated in Sabouraud broth at 37°C for two days before inoculating BBL® CHROMagar™ Candida (Becton Dickinson, Microbiology Europe, 38240 Meylan Cedex, France) and rice extract agar plates (Becton Dickinson, microbiology Systems, Cockeysville, MD 21030 USA), respectively. Identification of different yeast species was performed by distinctive colour and colony characteristics on BBL® CHROMagar™ Candida after incubation for two days at 37°C. Confirmation of yeasts identified by BBL® CHROMa-

gar™ Candida, was assessed by microscopy of rice extract agar plates incubated for two days at room temperature, germ-tube test, API20C AUX (Bio-Mérieux™, Marcy l'Etoile, France), and Krusei-Color® Fumouze agglutination kit (Fumouze Diagnostics, 92600 Asnières, France).

RESULTS

PREVALENCE OF YEASTS ON MUCOUS MEMBRANES

The prevalence of various yeast species isolated from different mucous membranes in a total of 451 patients was 86.1% for *C. albicans*, 7.6% for *C. glabrata*, 5.1% for *S. cerevisiae*, 3.2% for *C. parapsilosis*, 1.7% for *C. krusei*, 1.3% for *C. tropicalis*, and 0.6% for *C. guilliermondii*. Twenty-six (5.5%) samples positive for yeasts could not be further identified and are given as *Candida spp.*

The distribution of different yeasts cultivated from the genital tract according to gender is shown in Fig. 1. Mixed yeast cultures were detected in 13 (4.6%) out of 283 vulvovaginal samples but not in specimens obtained from the male genital tract (Tab. 1.).

C. albicans was isolated from 11 out of 13 perigenital samples (84.6%) but was not present in the only mixed yeast culture obtained from the perigenital region containing *C. krusei*, *S. cerevisiae*, and *Candida spp.* In 11 specimens collected from the anal region only *C. albicans* (90.9%) and *C. krusei* (9.1%) were identified.

A large variety of different *Candida spp.* was cultivated from the pharyngeal region with mixed yeast cultures observed in 11 (11.3%) out of 97 samples (Tab. 2.).

RESULTS OF BBL CHROMAGAR™ CANDIDA

A total of 528 yeasts out of 474 positive cultures could be diagnosed with CHROMagar™ Candida. All 408 presumptive *C. albicans* strains could be identified on CHROMagar™ Candida and were confirmed either by microscopy of rice agar plates, germ-tube test, or API20C AUX resulting in a sensitivity and specificity of 100%, respectively (Tab. 3.). A total of 36 *C. glabrata*, 6 *C. tropicalis*, and 8 *C. krusei* isolates could be distinguished from other *Candida spp.* by the characteristics of their colonies on the chromogenic medium. Confirmation of these non-albicans strains was performed by morphological criteria on rice agar plates (all 50 isolates), and additionally by biochemical characteristics in API20C AUX (5 *C. glabrata*, 6 *C. tropicalis* isolates) as well as by agglutination test specific for *C. krusei* (8 *C. krusei* isolates) resulting also in a sensitivity and specificity of 100%, respectively. Species, such as *C. parapsilosis*, *C. guilliermondii*, and *S. cerevisiae* could

Table 2. Yeasts isolated from the pharyngeal region.

No. of samples	*C. albicans*	*C. glabrata*	*S. cerevisiae*	*C. krusei*	*C. tropicalis*	*C. guilliermondii*	*C. parapsilosis*	*C. kefyr*	*Candida spp*
73	+								
1		+							
3			+						
1				+					
2					+				
2						+			
2							+		
2									+
3	+	+							
1	+	+			+				
4	+		+						
1	+			+					
1		+		+					+
1	+							+	
Σ 97	83	6	7	3	3	2	2	1	3

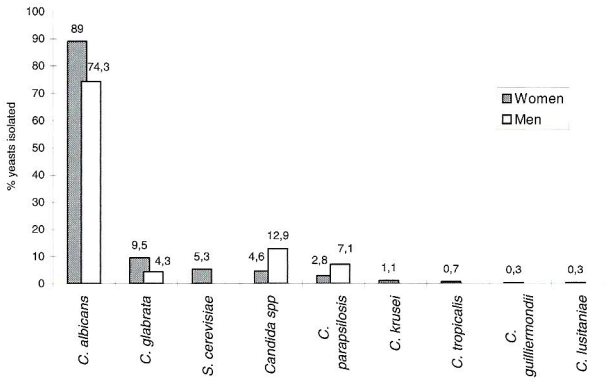

Fig. 1. Frequencies of yeasts isolated from genital mucous membranes. The different yeast species are given in per cent for female ■ and male □ patients.

be identified by microscopy and API20C AUX only, although these isolates formed colonies with characteristic surfaces creme to pink coloured on CHROMagar™ Candida.

DISCUSSION

In this study 451 patients colonized with yeasts on different mucous membranes were included showing *C. albicans* (86.1%) as the most prevalent yeast followed by *C. glabrata* (7.6%) and *S. cerevisiae* (5.1%). Samples collected from the genital tract showed that both, men and women are most frequently colonized with *C. albicans* (86.1%) which is in concordance with earlier reports (6, 20). While in women *C. glabrata* and *S. cerevisiae* were isolated frequently, males showed colonization with *C. parapsilosis* more often (Fig. 1). In 12.9% of positive specimens from the male genital tract the yeast species could not be further identified. This may be partly due to the fact that men are often pretreated with topical antimycotics before culture is obtained resulting in poor growth and diminished development of characteristic morphological features such as formation of chlamydospores. *C. albicans* is published to be the most prevalent yeast in men with balanitis but little is reported about the prevalence of other yeasts than *C. albicans* colonizing the male genital tract (20). Genital candidiasis in men is frequently associated with the presence of vaginal yeast colonization in a sexual partner (21). Since the prevalence of symptomatic and asymptomatic vulvovaginal candidiasis (VVC) has increased, epidemiologic studies were carried out to investigate variation in the yeast flora. After all, observations of changed occurrence of vaginal yeast pathogens are controversial (22,23,24,25). A possible change in the prevalence of yeast species may be associated with the type of antimycotic therapy as well as the selection of more resistant *Candida* species as a result of inappropriate or incomplete course of therapy (3,4). However, information on the prevalence of diverse yeast species

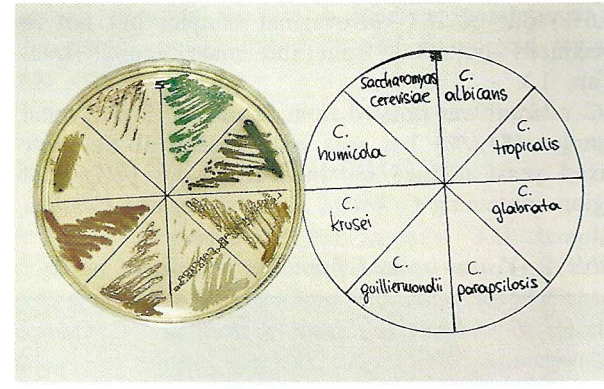

Fig. 2. Coloured colonies of different Candida species on CHROMagar™ Candida incubated for 72 h at 37 C.

Table 3. Comparison of different methods for the identification of Candida albicans.

Number of isolates	CHROMagar Candida	Formation of chlamydospores	Germ-tube test	API20C AUX
342	+	+	nd[1]	nd
30	+	-	+	nd
8	+	-	+	+
1	+	-	-	+
27	+	-	nd	+
S 408	408	342	38	36

[1] not done

on different mucocutaneous membranes is rare. Thus additional studies involving a broad spectrum of specimen types are certainly of great importance and interest.

Results of this study revealed CHROMagar™ Candida as a reliable differentiation medium for *C. albicans* and *C. glabrata*, the most frequently cultivated yeasts from mucous membranes (3,5,7). For the identification of *C. albicans* this medium showed a sensitivity as well as specificity superior to traditional methods such as the tests for the production of germ-tubes or chlamydoconidia techniques reported to give negative results with certain *C. albicans* strains (Tab. 3) (11,12,26). Thus the particular value of CHROMagar™ Candida is obvious considering that confirmatory tests may no longer be indicated (16). Nevertheless, it has to be mentioned that clinical isolates of the less common yeast *C. dubliniensis*, a species closely related to *C. albicans,* have been misidentified as atypical strains of *C. albicans* with CHROMagar™ Candida (18,27).

Beside *C. albicans*, the identification of the predominant non-albicans *Candida* species *C. glabrata* is of importance for patients with recurrent VVC especially in immunosuppressed patients since it may cause clinical therapy failures (3,4,28,29). In the present study identification of *C. glabrata* isolates with CHROMagar™ Candida was performed with the same accuracy as for *C. albicans*, *C. tropicalis*, and *C. krusei*. These observations are in agreement with the findings of Pfaller and co-workers (19). Although the prevalence of *C. tropicalis* and *C. krusei* is rather low (0.6% and 1.3%, respectively), identification of these species is necessary for treatment management. Resistance to antimycotic drugs is seldom but occurs with strains of *C. albicans* in AIDS patients, *C. glabrata*, *C. krusei*, and *C. tropicalis* rendering these yeast species as medically important (3,9,30).

A further advantage of CHROMagar™ Candida is the detection of mixed yeast cultures isolated from clinical samples on the basis of the distinctive and strongly differentiated colony colours of different yeast species growing. The results observed with mixed yeast cultures mainly containing *C. albicans* together with *C. glabrata* or *S. cerevisiae* in the present study are in accordance with earlier reports but could be detected more frequently with pharyngeal (11.3%) samples than with vulvovaginal specimens where 4.6% contained more than one yeast species (Tab. 1 and 2) (16,19).

In sum, this report presents additional evidence for the usefulness of CHROMagar™ Candida for medical mycological laboratories. We showed that the most prevalent as well as medically important yeast species can be reliably differentiated from other yeast species and that the detection of mixtures of yeasts isolated from clinical specimens is facilitated with CHROMagar™ Candida.

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