Isolation and Identification of *Candida* Species from the Oral Cavity Using CHROMagar Candida

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ABSTRACT

CHROMagar Candida is a new chromogenic culture medium that is used for the isolation and direct identification of some of the most clinically important yeast pathogens on the basis of colony colour. The aim of the study was to isolate and identify *Candida* species from the oral cavity with CHROMagar Candida. Samples (158) from 68 orthodontic patients wearing upper removable orthodontic appliances and 51 oral medicine patients were cultured on CHROMagar Candida at 37' C for 48 h. Isolates with green colonies in colour were all confirmed as *C. albicans* (53) by germ tube test and others were confirmed as *C. parapsilosis* (10) and *C. glabrata* (3) by Rapid ID 32 C kits. There were 11 cases of mixed yeast cultures in this study that were easily recognised using the medium. In conclusion, *C. albicans* is the most frequently isolated yeast from the oral cavity of both orthodontic and oral medicine patients. Moreover CHROMagar Candida is a useful culture medium for the isolation and direct identification of *Candida* species, especially mixed cultures. *Iran. Biomed. J. 4 (2 & 3): 57-61, 2000*

INTRODUCTION

Yeasts, especially *Candida* species, are part of the normal flora of humans. For example, *Candida albicans* is especially common in the human oral cavity, gastro-intestinal tract and female genital mucosa. *C. albicans* is the most important yeast pathogen in humans and is the species most frequently isolated from patients with oral candidiasis. Studies show that denture, and orthodontic appliance, wearers have more complex yeast flora than other patient groups or healthy controls [1, 2]. Long-term wearing of orthodontic appliances, or prostheses, is a major risk factor for colonisation by *Candida* of oral mucosal surfaces [3] and result may in chronic oral candidiasis.

Sabouraud's dextrose agar is widely used as a selective medium for the isolation of *Candida* and other yeast species from clinical specimens. However, it is not a differential medium so that colonies of different pathogenic yeast species grown on the medium cannot be distinguished easily from one another.

CHROMagar Candida is a new selective and an effective culture medium. This medium is used for the isolation, direct identification and differentiation of some of the clinically important *Candida* species [4, 5]. Using CHROMagar Candida, speciation of *Candida* can be performed on the grounds of colony colour. A major advantage of CHROMagar Candida is its ability to detect mixed cultures of yeasts in clinical samples. In addition, CHROMagar Candida is selective for yeasts and facilitates growth of *Candida* [4]. The medium has not previously been used for culture of orthodontic samples, although it has been used for oral medicine specimens.

In this study, we evaluated the usefulness of CHROMagar Candida for the isolation and the identification of oral yeast species from orthodontic and oral medicine patients.

MATERIALS AND METHODS

Patients. One hundred and nineteen subjects were involved in this study, after gaining their written informed consent. The orthodontic (n = 68) and oral medicine patients (51) were drawn from patients attending the Dental Hospital, University of Manchester, Manchester, UK. The medical history

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of each individual was checked for factors known to affect oral candidal carriage e.g. diabetes, anaemia, immunosuppression. Similarly individuals who smoked or who had received or currently receiving treatment with antibiotics, antifungals or steroids in the previous 3 months were excluded from the study.

Preparation of CHROMagar Candida. CHROMagar Candida (CHROMagar Candida Company, Paris, France) was prepared according to the manufacturer's instructions. CHROMagar Candida is composed of (per litre): peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g) and "chromogenic mix" (2 g) [4]. Twelve g of CHROMagar Candida powder (one vial) which was added to 250 ml of sterile distilled water in a sterile Erlenmeyer. The suspension was completely dissolved by boiling (<100°C) and mixing. The medium dose not require sterilisation by autoclave, therefore after cooling in a water bath to 45°C the agar was poured into sterile petri dishes [4]. After allowing to cool, the plates were stored at 4°C prior to use.

Collection and culture of samples. The imprint culture technique was used for sampling upper removable appliances from orthodontic patients [6, 7]. Briefly, a sterile plastic foam pad (1×1×1 cm) was soaked in sterile phosphate buffered saline (PBS) (pH 7.4, 0.01 M) (Sigma, Poole, Dorset, UK) and then placed on the centre of the contact surface of the upper removable appliance. After 30 s, the foam pad was removed and placed on the surface of a plate of CHROMagar Candida for 30 min at ambient temperature.

Unstimulated saliva samples (2 ml) were collected from both orthodontic and oral medicine patients in sterile universal bottles and rapidly transferred to the laboratory. Fifty µl aliquots of undiluted saliva were inoculated on to CHROMagar Candida plates. Swabs were collected from oral medicine patients and control patients into the transport medium (Starplex, Scientific, Canada) and all samples were immediately transferred to the oral microbiology laboratory. All plates were incubated at 37°C for 48 h aerobically, as recommended by the manufacturer.

Identification of oral yeast species. Growth colonies were microscopically examined as yeast colonies. Yeast colonies were initially identified by colonial colour when compared with standard colour photographs supplied by the manufacturer and also presented by Odds and Bernaerts [4].

Colonies that were yellow-green to blue-green in colour were confirmed as *C. albicans* by the germ tube test [8]. The germ tube test was performed using 0.5 ml horse serum (Oxoid, England) at 37°C for 3 h. Other colonies were cream, pinkish, white and dark pink. The identities of other yeast species were confirmed using Rapid ID 32 C (BioMerieux SA, Marcy-L'Etoile, France). Viability of each strain was confirmed by culture on the plates of Sabouraud's Dextrose Agar (BBL, Becton Dickinson, USA) incubated at 37°C for 48 h.

Statistical work. In this study, a chi-squared test (χ^2) was employed to determine the statistical significance of data.

RESULTS

A total of 158 saliva samples and imprints were collected from 119 patients attending the University of Manchester, Dental Hospital. Of these samples, 52.9% yielded oral yeast after primary culture on CHROMagar Candida. After 48-h incubation at 37°C, positive cultures produced colonies of 1 to 5 mm in diameter.
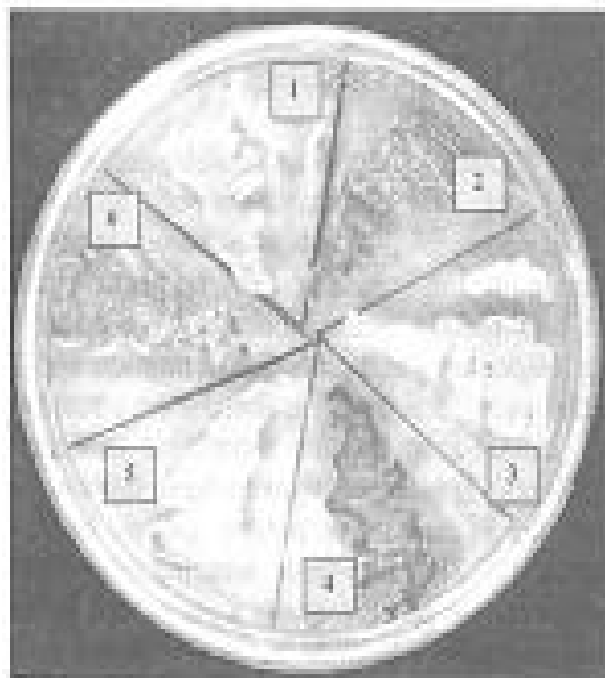
Fig. 1. Colonies plated of six different yeast species (incubated at 37°C for 48 h on CHROMagar Candida). 1, *Candida sake;* 2, *Saccharomyces cerevisiae;* 3, *Pichia etchellsii;* 4, *Candida glabrata;* 5, *Candida parapsilosis* and 6, *Candida albicans.*

Table 1. A comparison of the imprint culture technique (foam pad) and saliva sampling in detecting oral carriage of yeast flora in the patients with dental removable appliances. (Sensitivity = Total positive results / Total positive results + False negative results).

Technique	Total positive	Total negative	False negative	Sensitivity
Foam	17 (50.0%)	17 (50.0%)	9 (34.6%)	65.4%
Saliva	24 (70.6%)	10 (29.4%)	2 (7.7%)	92.3%

**Table 2.** Details of identified oral yeasts by CHROMagar Candida, germ tube test and ID 32C.

Yeast species	Green	Cream	Dark pink	Pinkish	White	Germ tube	ID 32C
C. albicans	53	0	0	0	0	53	0*
C. parapsilosis	0	9	0	1	0	0	10
C. glabrata	0	0	3	0	0	0	3
C. sake	0	1	0	0	0	0	1
S. cerevisiae	0	1	0	0	0	0	1
P. etchellsii	0	0	0	1	0	0	1
Unidentified yeast	0	3	3	0	1	0	0
Total	53	14	6	2	1	53	16

* Not tested

All *C. albicans* isolates formed yellow-green to blue-green colonies (vide supra); whereas *C. parapsilosis* produced cream or pale pink coloured colonies. Other colonies observed were dark-pink (*C. glabrata*), purple (*P. etchellsii*) and pink (*S. cerevisiae*) (Fig.1). In this study, isolation of oral yeasts from orthodontic patients was more likely to be achieved from saliva samples than from foam imprints. The sensitivity of the imprint culture technique (65.4%) was significantly lower than saliva sampling (92.3%) (χ^2 test, $p<0.01$) (Table 1).

C. albicans was the predominant oral yeast isolated from saliva in this study from both orthodontic (29) and oral medicine patients (24) (69.7%) *C. parapsilosis* was the next most frequent isolate (13.2%). Other oral yeasts less commonly detected were *C. glabrata* (3.9%), *C. sake* (1.3%), *S. cerevisiae* (1.3%), *P. etchellsii* (1.3%) and unidentified yeast (9.2%). Eleven samples yielded mixed cultures namely, *C. albicans* with *C. parapsilosis* (5 cases), *C. sake* with *S. cerevisiae* (1 case), *C. albicans* with unidentified yeast (2 cases), *C. albicans* with *C. glabrata* (1 case), *C. albicans* with two different unidentified yeasts (1 case), and *P. etchellsii*, *C. parapsilosis* and an unidentified yeast (1 case). The distribution of oral yeasts is shown in Figure 2 for each group of patients.

All 76 yeast isolates initially cultured on CHROMagar Candida were subcultured on to Sabouraud's Dextrose Agar. After 48 h of
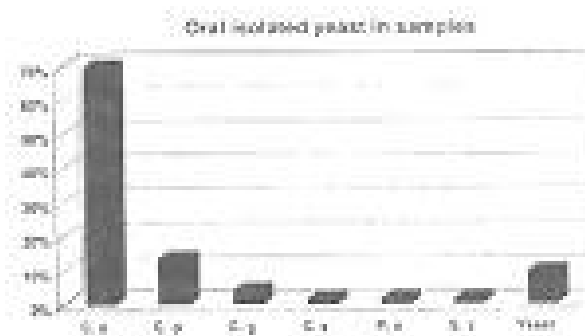
Fig. 2. Distribution of oral yeast isolates from samples (foam, swabs and saliva samples). C. a, *Candida albicans;* C. p, *Candida parapsilosis;* C. g, *Candida glabrata;* C. s, *Candida sake;* P. e, *Pichia etchellsii;* S. c, *Saccharomyces cerevisiae.*

incubation at 37°C, all of the isolates grew well on Sabouraud's Dextrose Agar. From 158 different samples (swabs, imprint cultures and saliva samples) from removable denture, saliva and swabs, inoculated on to CHROMagar Candida, no bacteria grew.

The germ tube test was used for the confirmation of *C. albicans*. Other species of oral yeast were identified by ID 32C kit. Table 2 shows the results of identification tests (CHROMagar Candida, germ tube test and ID 2C) applied to test strains.

DISCUSSION

CHROMagar Candida is a new chromogenic differential culture medium that is used for the isolation and identification of some of the most clinically important yeast pathogens on the basis of colony colour. The "chromogenic mix" employed in the medium is responsible for the differential colour reactions [9] but the details of the mixture have not been released by the manufacturer. CHROMagar Candida has two important properties: 1) direct and rapid identification of yeasts, 2) the differentiation of species in mixed cultures [4, 5, 10].

CHROMagar Candida has previously been shown to be an effective and selective medium for the direct identification of *Candida* species from clinical materials [4, 5]. This medium has previously also been used for the isolation and identification of yeasts from dental samples [2] and from swabs of soft tissues in oral cavity [4]. According to Odds and Bernaert [4], only 1.2% of CHROMagar Candida had bacterial growth after 72 h of incubation at 37°C and in this study bacterial colonies were never seen despite careful inspection of culture plates.

In our study, 11 subjects had a mixed yeast culture. Nine subjects had two yeasts and two subjects had 3 yeasts species. *C. albicans* was mixed with *C. parapsilosis* (5 cases), *C. glabrata* (1 case) and unidentified yeasts (3 cases); the latter were easily recognised as being dissimilar from *C. albicans*, *C. parapsilosis* or *C. glabrata* using CHROMagar Candida. Also, 1 case had *C. sake* with *S. cerevisiae* and 1 case had *C. parapsilosis*, *P. etchellsii* and unidentified yeast. Recognising such mixed yeast cultures was greatly assisted by use of CHROMagar Candida to detect mixed cultures of yeast in clinical materials; this confirms the finding of others [4, 5, 10]. *C. albicans* is the most frequently isolated yeast species from oral cavity, furthermore it is often recovered with other yeasts [2]. For example Beighton *et al*. [2] have reported the occurrence of *C. albicans* with *C. glabrata*, and of *C. parapsilosis* with *S. cerevisiae*, in dental samples. The present findings are similar although, in the above studies, patients studied were actually wearing full dentures [2] and hospitalised either in surgical ICU or in neonatal ICU [5].

All strains identified tentatively as *C. albicans* in this study were confirmed by the germ tube test (Table 2.) which showed that CHROMagar Candida was able to identify *C. albicans* with 100% accuracy. The sensitivity of the medium for the identification of *Candida* species has been reported previously; Pfaller *et al*. [5], Houang *et al*. [9], Baumgartner *et al*. [10] and Casal *et al*. [11] have all reported the sensitivity to be 100% for *C. albicans*. In contrast, Odds and Bernaerts [4] found a value of 99% for both specificity and sensitivity of CHROMagar Candida for the identification of *C. albicans*.

In this study, 23 out of 76 (30.3%) isolated yeasts were not *C. albicans*. They were tested by Rapid ID 32C, and while sixteen (69.6%) were identified, seven (30.4%) remained unidentified (table 2.). The sixteen strains of yeast species (other than *C. albicans*) that were identified by ID 32 C proved to be: *C. parapsilosis* (10), *C. glabrata* (3), *C. sake* (1), *S. cerevisiae* (1) and *P. etchellsii* (1). ID 32C has previously been shown to be effective system for the identification of yeast, and yeast-like, pathogens [12]. The rate of correct identification with ID 32C, reported in the literature, has varied slightly from 91% [13] and 92% [12, 14] to 97% [15] for common yeasts but 85% [13] for rare yeasts. Alternatively, Gutierrez *et al*. [15] have reported the rate to be 100% for *C. parapsilosis*. The presence of 32 substrates in ID 32C offers potential for the identification of a more diverse of clinically important yeasts [16] than earlier system using fewer tests. However the visual interpretation of test results with ID 32C systems is difficult and requires experience [14]. Low reliability of the ID 32C system for the identification of rare yeasts can be ascribed to the incomplete nature of the profile index [13].

In conclusion, *C. albicans* is the most frequently isolated yeast from the oral cavity of both orthodontic and oral medicine patients. CHROMagar Candida is a useful culture medium for the isolation and direct identification of *Candida* species (especially *C. albicans*, *C. parapsilosis* and *C. glabrata*) and mixed cultures from samples taken from either orthodontic or oral medicine patients. Plates of CHROMagar Candida cost about five times the cost of more traditional agar media. However, the medium permits recognition of mixed yeast cultures which might otherwise not noticed. Also, the identification of *C. albicans*, *C. parapsilosis* and *C. glabrata* is much cheaper using this agar than testing in any commercially available kits.

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