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Direct impression on agar surface as a diagnostic sampling procedure for candida balanitis

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ABSTRACT

Background The diagnosis of candida balanitis should be based upon both clinical and mycological data. The procedure of material collection is a critical issue to confirm or rule out the clinical diagnosis of candida balanitis.

Objective To compare direct impression of the glans on the agar surface of solid culture media with the collection of genital exudates with cotton swab for the diagnosis of candida balanitis.

Methods A prospective cross-sectional study was carried out during a 36-month period. Sexually transmitted disease clinic attendees with balanitis and asymptomatic men were included. Specimens for yeast culture were collected from the glans penis and inner preputial layer using the direct impression on CHROMagar candida medium and by swabbing with a sterile cotton swab.

Results Among 478 men enrolled, 189 had balanitis. The prevalence of candida balanitis was 17.8% (85/478) confirmed after culture by direct impression; the swab method detected only 54/85 (63.5%) of these men. Of the 289 asymptomatic men, 36 (12.5%) yielded *Candida* spp; the swab method detected only 38.9% of these men. The risk of having candida balanitis is 8.9 (IC 95% 2.48 to 32.04) whenever the number of candida colonies recovered by direct impression was greater than 10.

Conclusions Direct impression on CHROMagar candida medium resulted in the highest *Candida* spp recovery rate. More than 10 colonies yielded by impression culture were statistically associated with candida balanitis. This method shows the ideal profile for sampling the male genital area for yeasts and should be included in the management of balanitis.

Balanitis (balanoposthitis) is a common condition affecting up to 11% of men attending a sexually transmitted disease (STD) clinic[1 2] and is due to a variety of unrelated causes.[2–4] *Candida* spp are the most frequent cause of infectious balanitis. European studies have demonstrated that 30–35% of all patients with balanitis had candidosis,[2 5 6] most being sexually acquired.[7] The patient with candida balanitis usually has an erythematous rash with soreness and/or pruritus accompanied by pinpoint papules and pustules and occasionally abnormal subpreputial exudates. However, these symptoms and signs are non-specific and can be the result of a variety of infectious and non-infectious aetiologies.[3 8 9] The lack of specificity of signs and symptoms makes the confirmation of the clinical diagnosis of genital candidosis by laboratory tests mandatory. The diagnosis of candida balanitis should be based upon both clinical and mycological data.[6 8 10] However, reports on the incidence of male candidosis with diagnosis based upon clinical and mycological findings are exceptional. It is believed that there is overdiagnosis of candida balanitis, which leads to the inappropriate use of antifungal drugs as well as an over-the-counter use of antifungal agents, as a result of the absence of laboratory confirmation. However, the sampling method for culture seriously determines the laboratory results. Dockerty and Sonnex[6] evaluated the sensitivity of different diagnostic methods of candida balanoposthitis and found that the procedure of material collection is a critical issue to confirm or rule out the clinical diagnosis of candida balanitis. The recovery of specimens for mycological study is often difficult because of the scarcity of material to be collected. The aim of this study was to evaluate the direct impression of the glans and inner preputial layer on an agar surface of solid culture media and compare it with the collection of genital exudates with cotton-tipped swabs, for the diagnosis of candida balanitis.

MATERIALS AND SUBJECTS

Study design and participants

A prospective cross-sectional study was designed and local ethics committee approval was granted. The study enrolled men aged between 16 and 80 years, attending the STD clinic of Hospital S João, Porto, between January 2005 and December 2007. All consecutive patients with balanitis (balanoposthitis) and asymptomatic men without genital lesions, attending for sexually transmitted infection screening (involving tests for syphilis, HIV, hepatitis B and C, gonorrhoea and chlamydia) were included. Patients with genital ulcers, urethral discharge and condylomata acuminatus were excluded. Men who had applied antibacterial and antifungal therapy or taken antifungal agents within 6 weeks before enrolment were invariably excluded. Balanitis was diagnosed when patients had inflammation of the glans penis, which often involves the prepuce (balanoposthitis). Age, sexual behaviour, underlying diseases, history of sexual partner with genital candidosis, clinical presentation and data from clinical examination were recorded.

Genital sample collection

Specimens for yeast culture were collected from the glans penis, the coronal sulcus and inner preputial layer using two distinct procedures: a direct impression on the culture medium plate and swabbing with a sterile cotton tipped swab (VWR International, Lisbon, Portugal) a similar surface

area of the glans and inner preputial layer with immediate inoculation on the same culture medium. To facilitate the contact of the genital mucous membranes with the culture medium surface a contact dish (65.0 mm diameter inverted Petri dish, the culture medium surface protruding above the borders of the plate; Greiner Bio-One, Germany) was used. CHROMagar candida (CHROMagar Company, Paris, France) was the culture medium used. Specimen collection was always performed by the same clinical investigator.

Yeast isolation and speciation

The agar plates were incubated at 37°C for 48 h. Positive cultures were evaluated for the number, colour and other characteristics of yeast colonies, according to Odds and Bernaerts.[11] A second reading for colony appearance was performed after an additional 24 h of incubation at 30°C because some isolates just form pinpoint colonies at 48 h of incubation, thus being identified more accurately following an additional 24 h of incubation at 30°C.[11 12]

Candida albicans was also confirmed by germ tube formation in bovine serum after 2 h at 37°C. Vitek YBC identification cards (BioMérieux, Paris, France) and API 20C AUX galleries (BioMérieux) were used to characterise non-albicans and non-candida yeast isolates.

Data analysis

The study population was separated into three categories: men with balanitis and with candida-positive culture (candida balanitis); asymptomatic men with candida-positive culture (genital candida 'colonisation'); men with balanitis but with candida-negative culture. The sensitivity of the two sampling methods was compared between these categories.

Categorical variables were described as absolute frequencies (n) and relative frequencies (%). Logistic regression models were constructed to assess the predictive effect of the 'the number of candida colonies' being asymptomatic compared with those with balanitis. Data analysis was performed using the statistical software program SPSS V.16.1 for Windows.

RESULTS

During a 36-month period, of 2147 men attending the STD clinic, 552 men were recruited, but just 478 were enrolled in the final study (figure 1).

The mean age of the 478 participants was 41 years, ranging from 16 to 80 years, and the majority (470; 98.3%) were heterosexual; 372 (78%) reported a single sexual partner in the previous 6 months, 36 (7.5%) reported genital candidosis in a regular sexual partner, 13 (2.7%) were HIV positive, 16 (3.3%) had an immunosuppressive condition (other than HIV) and 38 (7.9%) had diabetes mellitus. Most of the participants (465; 97.3%) were uncircumcised.

The prevalence of candida balanitis was 17.8% (85/478); the swab method detected only 54/85 (63.5%) of these men (table 1). Among the 289 asymptomatic men, 36 (36/289; 12.5%) yielded *Candida* spp; again, the swab method detected *Candida* spp in only 38.9% (14/36) of these men (see table 1).

A total of 189 yeast isolates was recovered (from 158 of the 478 enrolled men) either by contact impression or the swab method (table 2). The most common isolate was *C albicans*, accounting for 64 (33.9%) of the total of isolates followed by *Candida parapsilosis*, accounting for 41 (21.7%). Forty-one (21.8%) yeast isolates corresponded to non-candida yeasts, 33 being identified as *Rhodotorula mucilaginosa*. Direct impression allowed the recovery of 171 of the isolates (90.5%) compared

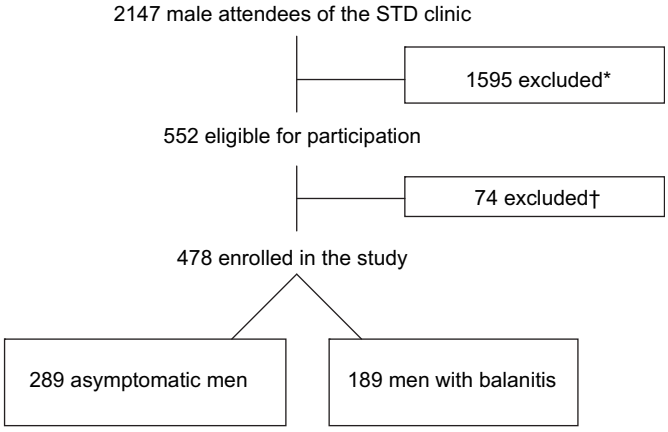

Figure 1 Subject participation in screening, January 2005—December 2007. *Patients attending the sexually transmitted disease (STD) clinic with genital ulcers, urethral discharge or genital warts and men aged under 16 years were excluded. †Attendees reporting antibiotic or antifungal use within 6 weeks before enrolment and attendees not willing to participate were also excluded.

with 82 (43.4%) by swabbing. Some yeast species were only recovered when using the impression method (see table 2).

The prevalence of mixed isolates among the 158 patients yielding a positive yeast culture was 24.7% (39/158). All the 39 mixed cultures were identified by direct impression; swabbing failed to recover 10 of 39 (25.6%) of such mixed cultures; in addition, the swab method also failed to detect any isolate from these 10 mixed culture cases. The most common associations involved *C albicans* plus *C parapsilosis*, *C albicans* plus *Candida guilliermondii*, *C albicans* plus *R mucilaginosa* and *C guilliermondii* plus *R mucilaginosa* in five cases each; in four cases *C parapsilosis* plus *R mucilaginosa*. Eight of the 39 mixed culture cases yielded more than two yeast species.

A semiquantitative evaluation of positive cultures for *Candida* spp recovered by direct impression was performed and its relationship to signs and symptoms was analysed (table 3). The prevalence of patients with balanitis increased as the number of colonies increased. Logistic regression analysis concluded there was a significant risk of candida balanitis (odds ratio 8.9; 95% CI 2.48 to 32.04) whenever the number of candida colonies recovered by direct impression was greater than 10 (see table 3).

DISCUSSION

Several studies stressed the importance of the method of sample collection for the recovery of yeasts.[6 8 10] Nevertheless, no established guidelines are yet available for obtaining a microbiological sample for the culture of candida balanitis. The swab method of sampling the genital area is the procedure currently used by most clinicians to perform microscopy or culture.[6]

There is previous reference in the literature to the use of impression culture as a method of genital specimen collection.[10 13 14] However, direct impression by contact has not become a popular method in clinical practice. Interestingly, the most commonly used collection procedure, the swab technique, has never been compared with contact impression for mycological investigation of the male genital area. Our study shows a greater ability of direct impression to recover yeasts from penile mucous membranes. Swabbing as a method of specimen collection detected only 63.5% of men with candida balanitis and failed to identify *Candida* spp in 61.1% of asymptomatic men. Considering the group of men with balanitis but with a candida-negative culture (which may mean that the laboratory methods

Table 1 Comparison between sampling procedures for recovery of *Candida* spp

Characteristics	Direct impression	Swabbing method	Both methods*
Men with balanitis + positive candida culture	85	54	86
Asymptomatic men + positive candida culture	36	14	39

Of 189 men with balanitis, a candida-negative culture was found in 102 (54%) using direct impression versus 135 (71.4%) by the swabbing procedure.
*Any candida-positive culture using either direct impression or the swab method.

were not sensitive enough to detect *Candida* spp or that another aetiological cause may be admissible), the swab failed to culture in 71.4% of such cases; in contrast, contact impression decreased this figure to just 54%. Direct impression was shown to be a method of high sensibility and a reliable sampling technique to be used in clinical practice as well as for epidemiological studies, namely the evaluation of *Candida* spp prevalence in the male genital region. Candida balanitis accounts for 30–35% of all patients with balanitis.[2 5 6] We found 45% using direct impression as a sampling method; conversely, the swab sampling procedure found just 28.5%, as shown in previous studies.

Although PCR assays are increasingly being developed, they still lack standardisation and validation in clinical settings, and thus have a very restricted value for the diagnosis of genital candida infections.[15] In our study we did not perform direct microscopy of smears routinely because of its previously confirmed low sensitivity as a candida balanitis diagnostic method.[6] Culture methods are more sensitive than direct microscopy of smears.[6] Although Sabouraud agar is considered the standard culture medium for fungal culture, we decided to use CHROMagar candida as it is a selective and differential culture medium. It allows the selective isolation of yeasts and identifies colonies of *C albicans*, *Candida tropicalis, Candida krusei, Trichosporon* spp[11] and *Candida glabrata*[16] on the basis of contrasted colony colours as a result of species-specific enzymes reacting with a proprietary chromogenic substrate.[11] It has been shown to be useful in the rapid presumptive identification of clinically important yeast species from clinical samples.[16 17] This identification is not difficult to learn by inexperienced observers, reaching 100% accuracy for *C albicans*.[17] We found that *C parapsilosis* and *C guilliermondii* were the second and the third species most commonly isolated after *C albicans*. In fact, *C glabrata* is frequently described as the second most common *Candida* spp.[18 19]

Table 2 Identification of the 189 isolates recovered either by contact impression or the swab method and distribution of the total yeasts recovered by each of the sampling methods used (impression and swab)

Yeast species	Total n=189 (%)	Impression n=171	Swab n=82
Candida spp			
Candida albicans	64 (33.9)	60	39
Candida parapsilosis	41 (21.7)	36	17
Candida guilliermondii	24 (12.7)	22	4
Candida utilis	6 (3.2)	4	2
Candida glabrata	4 (2.1)	4	4
Candida tropicalis	3 (1.6)	3	1
Candida pelliculosa	2 (1.1)	2	1
Candida famata	1 (0.5)	1	0
Candida lusitanea	1 (0.5)	1	0
Candida krusei	1 (0.5)	1	0
Candida rugosa	1 (0.5)	1	0
Non-candida yeasts			
Rhodotorula mucilaginosa	33 (17.5)	28	11
Rhodotorula glutinis	2 (1.1)	1	1
Trichosporon inkin	3 (1.6)	4	1
Trichosporon asahii	2 (1.1)	2	1
Trichosporon beigelii	1 (0.5)	1	0

Table 3 Candida colony counts and its relation to clinical data

No of colonies	Asymptomatic men n (%)	Men with balanitis n (%)	OR	95% CI
<5	26 (74.3)	36 (43.4)	1.00	
5–10	6 (17.1)	10 (12)	1.20	0.39 to 3.73
>10	3 (8.6)	37 (44.6)	8.90	2.48 to 32.04

Logistic regression analysis values indicating the probability of having candida balanitis.
OR, odds ratio.

An additional advantage of such a chromogenic medium is its ability to detect mixed cultures of yeasts.[11 12 16 17] Different species cultured were easily recognisable by the different colony colours. The male genital area is peculiar in its microbiological complexity and mixed cultures had never been documented in the clinical setting. We found 24.7% of mixed yeast cultures from male genital specimens on CHROMagar candida. The most frequent yeast associations involved the four most common yeast isolates, namely *C albicans, C parapsilosis, R mucilaginosa* and *C guilliermondii*. Additional studies are needed to assess the real significance of this result. We should stress that the swab method of specimen collection failed to recover one third of the mixed cultures and additionally failed to recover any isolate from these mixed cases.

It is generally accepted that the most rational approach to the diagnosis of candida balanitis is to consider both clinical and mycological evidence for the infection. However, the concentration value of candida organisms that should be regarded as pathological rather than representing the indigenous genital microbial population has not yet been established. Several investigations in which semiquantitative culture methods were used have shown that the higher the amount of yeast recovered the greater the likelihood that the patient will show symptoms and clinical signs of vaginal candidosis.[20–22] However, similar studies in male genital candidosis are still lacking. Our results support a cut-off value of 10 candida colonies cultured by direct impression to correlate with signs and symptoms of balanitis. Concerning the swab procedure, a similar statistical analysis was not possible because of the much smaller number of positive cases. Using the same logistic regression analysis aimed at establishing a relationship between the number of candida colonies independent of the sampling procedure and asymptomatic patients compared with patients with balanitis, a number of candida colonies greater than 10 was again statistically associated with balanitis (data not shown).

Further studies should evaluate the correlation, if any, between the number of candida organisms and the severity of candidosis and the hypothetical differences between distinct species of candida.

In conclusion, direct impression on CHROMagar candida medium as a sampling method of the male genital area resulted in the highest yield of *Candida* spp and the greatest ability to detect mixed fungal cultures. More than 10 colonies of *Candida* spp recovered by impression culture were statistically associated with candida balanitis. The impression culture on CHROMagar candida is rapid to perform, convenient to the patient and to his doctor and is cost effective,[23 24] while also avoiding the cost of €0.13 per swab. This method proved to be a useful topic for clinical audit and should be included in the management of balanitis.

Key messages

- Direct impression on CHROMagar candida medium as a sampling method resulted in the highest yield of *Candida* spp compared with the swabbing procedure.
- We found a prevalence of candida balanitis of 17.8%; the swab method detected only 63.5% of these men.
- More than 10 colonies of *Candida* spp recovered by direct impression culture were statistically associated with candida balanitis.
- The impression culture on CHROMagar candida medium proved to be useful in the management of balanitis.

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Competing interests None.

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