

Research Article

Isolation, speciation and antifungal susceptibility patterns of candida isolated from cases of chronic balanoposthitis

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ABSTRACT

Background: Balanoposthitis is a common condition affecting 11% of male genitourinary clinic attendees and it can be a recurrent or persistent condition. Various predisposing factors like diabetes mellitus, sexual intercourse and usage of oral antibiotics can cause chronic balanoposthitis. The Objective of the study was to isolation and speciation of candida and their antifungal susceptibility patterns from the cases of chronic balanoposthitis.

Methods: The study group comprised of swabs collected from 62 male patients with chronic balanoposthitis attending sexually transmitted diseases (STD) outpatient department (OPD), King George Hospital (KGH), Visakhapatnam. Standard mycological tests for the candida isolation, speciation and antifungal susceptibility were done.

Results: Out of 62 samples, (85%) were culture positive for candida. The most common species isolated was *C. parapsilosis* (37.7%), followed by *C. glabrata* (28.3%), *C. albicans* (15.09%), *C. dubliniensis* (9.4%), *C. krusei* (7.5%) and *C. tropicalis* (1.88%). Most of the candida species showed sensitivity to amphotericin B, Nystatin, clotrimazole and ketoconazole. A relative resistance to fluconazole and itraconazole was observed.

Conclusions: Chronic balanoposthitis is the most common infection in men attending STD, OPD. In the present study, diabetes is main predisposing factor than sexual intercourse and *candida non albicans* predominated over *C. albicans*. Resistance of candida species to azoles is on rise. This establishes the importance of determination of antifungal susceptibility patterns to prevent the emergence of drug resistance, prior to initiation of therapy.

Keywords: Chronic balanoposthitis, Diabetes mellitus, *Candida parapsilosis*, Antifungal susceptibility, Amphotericin B

INTRODUCTION

Balanoposthitis is defined as inflammation of the glans penis and prepuce. It is a common condition affecting 11% of male genitourinary clinic attendees and it can be a recurrent or persistent condition.¹

There are various predisposing factors; balanitis is more common among uncircumcised men possibly as a result of poorer hygiene and aeration or because of irritation by smegma.² underlying medical conditions can also predispose to balanitis, such as a source of fever and bacteremia in neutropenic men, and *Candidal balanitis*

may be especially severe in patients with diabetes mellitus.^{3,4}

Infection with candida species is the most common cause of balanitis, usually with *Candida albicans*.

It is generally sexually acquired although carriage of yeasts on the penis is common, being 14-18%, with no significant differences between carriage rate in circumcised and uncircumcised men. Symptomatic infection is more common in the uncircumcised male.^{5,6} Significantly more of the female partners of men carrying yeasts were found to have candidal infection.⁵

Infection may occur without sexual contact, usually in the presence of diabetes or after the use of oral antibiotics.⁴ Symptoms are burning and itching of the penis with generalized erythema of the glans and prepuce which may have a dry glazed appearance, with eroded white papules and white discharge.^{7,8}

In diabetic patients the presentation may be more severe with oedema and fissuring of the foreskin, which may become non-retractile.⁴ Aim of the study was to isolate and speciate candida from the cases of chronic balanoposthitis and to determine their antifungal susceptibility patterns.

METHODS

The present study was carried out in the department of microbiology, Andhra medical college, Visakhapatnam, during the period of January 2015 to June 2015. The study group comprised of 62 male patients with complaints of excessive white discharge, pruritus and fissuring of glans attending sexually transmitted diseases, outpatient department (OPD), King George Hospital, Visakhapatnam, India.

Inclusion criteria

- Patients on oral hypoglycemic agents
- Patients who had multiple sexual partners
- Patients with recurrent balanoposthitis

Exclusion criteria

- Patients already on anti-fungal agents

Under strict aseptic conditions, the thick white curdy discharge which accumulated over the glans penis and coronal sulcus was collected onto two swabs, after retracting the prepuce.

One was used for direct microscopy and the other for culture. Direct microscopic examination of KOH mount (Figure 1) and gram's stained smear (Figure 2) was done and examined for the presence of budding yeast cells and pseudohyphae.

Samples were inoculated on to sabouraud's dextrose agar (SDA) with gentamycin and incubated at 25°C for up to 48-72 hours. Within 1-3 days, creamy white, smooth, pasty colonies with a yeasty odour were noted (Figure 3). Standard mycological tests were used to identify the isolates.⁹

Candida isolates were identified by microscopic examination of gram's stained smear (Figure 4) and germ tube formation. Rapid method of identifying *C. albicans* is by its ability to form germ tubes within two hours when incubated in human serum at 37°C (Reynolds-braude phenomenon) was done (Figure 5).¹⁰

Strains of candida isolated were inoculated on corn meal agar (CMA) by Dalmau culture plate (Figure 6) technique and incubated at 25°C and observed for chlamydospore production (Figure 7).

For species identification, the isolates from SDA were inoculated on chrom agar medium and incubated overnight at 37°C in dark for 48 hours. Only pigmented colonies were considered for species identification (Figure 8,9).

C. albicans - light green ; *C. dubliniensis* - dark green; *C. tropicalis* – blue; *C. glabrata* - pink to purple; *C. krusei* - pink; *C. parapsilosis* - cream to pale pink.

Antifungal susceptibility testing was done by modified kirby-bauer disk diffusion method as per CLSI guidelines M44-A.¹¹

Inoculum was prepared from the yeast grown on SDA for 24 hours and was adjusted to match the turbidity of 0.5 mc farland standards. Sterile applicator swab was moistened in that cell suspension and inoculated on the surface of mueller-hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue.

Antifungal discs were placed and incubated in biological oxygen demand (BOD) for 24 hours and the zones of inhibition were observed (Figure 10).

The following antifungal discs were placed:

Amphotericin-B- 20µg; Itraconazole - 10µg; Fluconazole - 10µg; Ketoconazole - 10µg; Clotrimazole - 10µg; Nystatin - 100 units/disc

Standard zone size interpretation

For azoles

- Susceptible: ≥17mm diameter
- Intermediate: 14mm-16mm
- Resistant: ≤13mm

For amphotericin b

- Susceptible: ≥15 mm
- Intermediate: 13 mm-14 mm

RESULTS

Table 1: Age wise distribution of cases.

Age group (years)	No	Percentage (%)
20 – 30	8	12.9
31-40	13	20.9
41-50	36	58
51-60	5	8
Total	62	100

Table 2: Predisposing factors.

Factors	No	Percentage (%)
Diabetes	40	64.5
Multiple sex partners	20	32.2
Antibiotic usage	2	3.2
Total	62	100

Table 3: Culture positivity from total no of cases =62.

Total no	62 (100%)
Culture positive For <i>candida</i>	53 (85%)
Culture negative For <i>candida</i>	9 (15%)

Table 4: Distribution of *Candida albicans* and *Candida non albicans* from total isolates.

<i>C. albicans</i> (%)	<i>C. non albicans</i> (%)
8 (15.09%)	45 (84.9%)

Table 5: Speciation of candida isolates.

Species	No (%)
<i>C. Parapsilosis</i>	20 (37.7%)
<i>C. glabrata</i>	15 (28.30%)
<i>C. albicans</i>	8 (15.09%)
<i>C. Dublinensis</i>	5 (9.4%)
<i>C. krusei</i>	4 (7.5%)
<i>C. tropicalis</i>	1 (1.88%)

Table 6: Antifungal susceptibility candida albicans and non albicans species.

Antifungal drugs	<i>C. albicans</i> (sensitivity %)	<i>C. non-albicans</i> (sensitivity%)
Amphotercin B	82	86
Itraconazole	64	52
Fluconazole	58	70
Ketotconazole	84	72
Clotrimazole	88	64
Nystatin	80	74

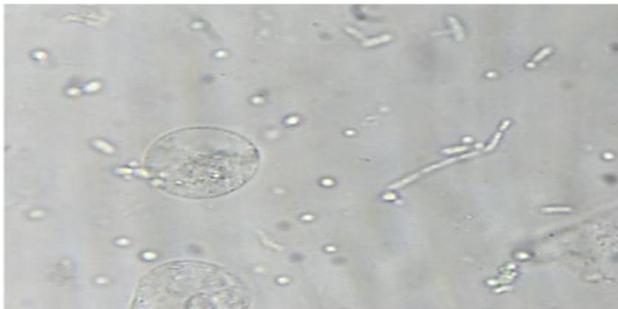


Figure 1: KOH mount showing pseudohyphae and budding yeast cells.

During the study period, total 62 male patients with complaints of excessive white discharge, pruritus and fissuring of glans were included in the study.

In the study group, majority of patients were in 41-50 years (58%), followed by 31-40 years (20.9%), 20-30 years (12.9%) and 51-60 years (8%) (Table 1). Out of 62 cases, 40 (64.5%) were diabetes, 20 (32.2%) have multiple sex partners, and 2 (3.2%) patients were on antibiotics (Table 2).

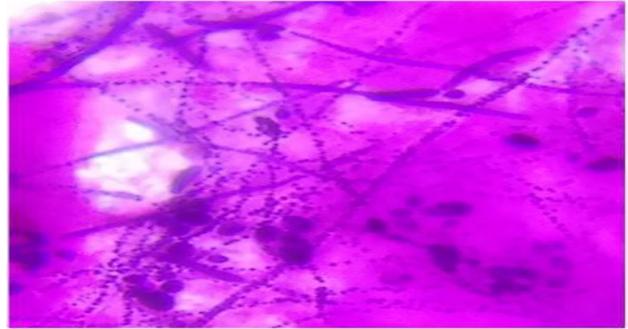


Figure 2: Direct gram's stained smear showing pseudohyphae and budding yeast cells.

Out of 62 samples, 53 (85%) were culture positive for *candida*, 9 (15%) were culture negative for *Candida spp* (Table 3). Among all *candida* isolates, 8 (15.09%) were *Candida albicans*, 45 (84.9%) were *Candida non albicans* (Table 4).



Figure 3: SDA showing smooth, creamy white pasty colonies.

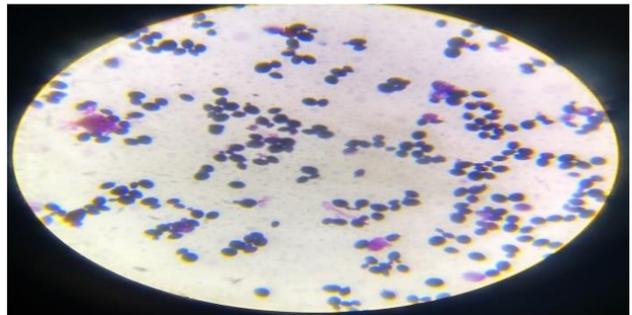


Figure 4: Gram's stained smear from growth on SDA.

The most common species isolated was *C. parapsilosis* 20 (37.7%) followed by, *C. glabrata* 15 (28.3%), *C. albicans* 8 (15.09%), *C. dubliniensis* 5 (9.4%), *C. krusei* 4 (7.5%) and *C. tropicalis* 1 (1.88%) (Table 5). Most of the candida species showed sensitivity to amphotericin B, nystatin, clotrimazole and ketoconazole with zones ≥ 13 mm. A relative resistance to fluconazole and itraconazole with zones ≤ 12 mm was observed.



Figure 5: Germ tube test in *C. albicans* (Reynolds-Braude phenomenon).



Figure 6: Dalmau plate culture for chlamyospore formation.



Figure 7: Chlamyospores of *C. parapsilosis*.

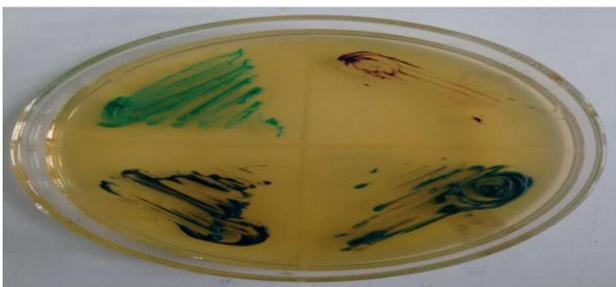


Figure 8: Candida species on chrom agar.



Figure 9: Candida species on chrom agar.

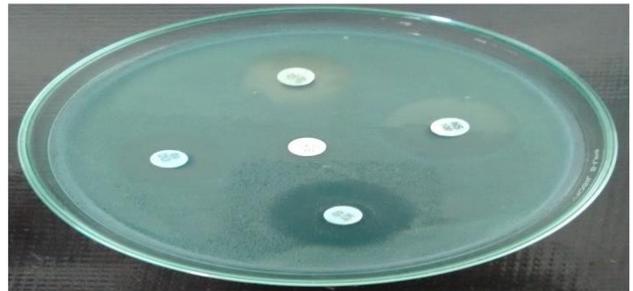


Figure 10: Antifungal susceptibility test for candida species.

DISCUSSION

Candidal balanoposthitis is well recognized condition in males who are attending the STD clinics, first described by Engman in 1920.¹² Most patients present with penile irritation associated with mild penile erythema and small irregular eroded papules. An acute fulminating oedematous balanoposthitis is commonly associated with diabetes because diabetes interferes with both facets of host defence against infection, the innate as well as adaptive immunity.^{4,13} Fissuring along with balanoposthitis was found to be more common in sexually active males.

In the present study majority of patients were in 41-50 years (58%) followed by 31-40 years (20.9%). Similar findings were noted by Liboa C et al.¹⁴

In our study, major predisposing factor was diabetes 64.5% followed by multiple sex partners 32.2% and usage of antibiotics 3.2%, which coincides with the studies of M .A. Waugh et al, Verma Sb et al and Liboa C et al.^{4,14,15}

Culture positivity for candida was 85% in our study, whereas wg dockerty et al showed 35% culture positivity, Philip Rodin et al reported 40% positivity and Liboa C et al showed 18%.^{6,14,16}

Among all candida isolates in our study, increased prevalence of candida non albicans 84.9% was observed which coincides with the studies of s shivaprakasha et al

with predominance of candida non-albicans species in 83% of cases.¹⁷ Whereas Waugh MA et al, S Abdennader et al and Fornasa CV et al reported candida albicans as predominant agent.^{4,18,19}

Predominant isolate in our study was *C. Parapsilosis* 37.7%, followed by *C. glabrata* 28.3%, *C. albicans* 15.09%, *C. dubliniensis* 9.4%, *C. krusei* 7.5% and *C. tropicalis* 1.88% , whereas Philip Rodin et al isolated *C. parapsilosis* was the second commonest isolate.⁶ In this study, most of the candida species showed sensitivity to amphotericin B, nystatin, clotrimazole and ketoconazole and were relatively resistant to fluconazole and itraconazole which coincides with the studies of Jithendra K et al, Velvizhi et al and Pahwa N et al.²⁰⁻²²

CONCLUSION

Chronic balanoposthitis is the most common infection in men attending sexually transmitted diseases, OPD. In the present study, diabetes is main predisposing factor for balanoposthitis than sexual intercourse and candida non albicans predominated over *C. albicans*. Resistance of candida species to azoles is on rise. This establishes the importance of determination of antifungal susceptibility patterns to prevent the emergence of drug resistance, prior to initiation of therapy.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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