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Saumya Nanda
Department of Obstetrics and Gynaecology, SCB Medical College, Odisha, India

Soumyadarshan Nayak
Department of Neurology, SCB Medical College, Odisha, India

Corresponding Author:
Soumyadarshan Nayak
Department of Neurology, SCB Medical College, Odisha, India

Antifungal sensitivity of *Candida Sp.* isolated from gynaecology and medicine patients attending at a tertiary care hospital

Saumya Nanda and Soumyadarshan Nayak

Abstract
Objective: In this study we have found the prevalence of Candidal Sp. in vulvo-vaginal and skin infections of the patients attending the OPD of medicine and Gynaecology. We have additionally evaluated the drug sensitivity patterns of recognized Candida sp.
Methods: This observational study was conducted in IMS and SUM hospital for 14 months from January 2015 to February 2016, and the purpose was to find the causative organisms for infections. Species differentiation was confirmed via particular tradition medium methods. Antifungal susceptibility of isolated Candida species have been evaluated with disc diffusion methods.
Results: Of the 219 Candida isolates, majority of them have been isolated from the branch of Gynaecology 160(73.1%) and Medicine department 59(26.9%). Moreover, 144(65.8%) samples have been of unmarried girls and 75(34.2%) were of married women. Candida albicans 128 (58.45%) was the most principal species followed by Candida glabrata 30(13.69%), Candida tropicalis 26(11.87%), Candida krusei 17(7.76%), Candida parapsilosis 12(5.47%), Candida dubliniensis 3(1.37%) and Candida lusitaniae 3(1.37). All isolates have been most susceptible to Itraconazole with a susceptibility charge of 213(97.26%). The highest resistance become found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%).
Conclusion: Candida species has been the most common cause of vulvo-vaginitis in both married and unmarried women and many antifungals are effective against it but in this observational study we found out that Itraconazole was the most effective antifungal for vulvo-vaginal and skin infections.

Keywords: Candida albicans, Non-albicans (NAC), Antifungal sensitivity test, CHROMagar Candida.

Introduction
Fungal infections, are a serious clinical condition and causes substantial morbidity and mortality amongst the patients globally [1]. *Candida species* are the normal commensal of the oral cavity, GIT, vulvo-vaginal and other mucosal surfaces in the body as well as the pathogens leading to colonization and infection. Vulvovaginal candidiasis (VVC) is frequent in women worldwide and usually responds rapidly to topical or oral antifungal therapy.
C albicans is responsible for the majority and several factors have been associated to infections in women with recurrent vulvovaginal candidiasis (RVVC): treatment of RVVC mandates species determination confirmed by laboratory findings and effective treatment. Factors associated with infections in women with RVVC include genetic (polymorphism, familial, ethnicity), immune mechanisms (HIV, uncontrolled diabetes, steroids, antibiotics, hormone replacement therapy), behavioural (oral sex, oral contraceptive, intercourse frequency) and idiopathic [2].
In the recent times incidence of fungal infections has increased with the increased incidence of immunocompromised patients [3]. *Candida* species are frequently isolated from such patients as well as those who are diabetic [4], on immune-suppressants or neutropenic[5] with malignancy undergoing chemotherapy/ radiotherapy [6], long term steroid therapy, long term antibiotic therapy as well as pregnancy.[7] It has been implicated as a cause of UTI, vulvo-vaginitis, respiratory infections, septicaemia as well as other cutaneous and mucocutaneous infections. More than 17 different *Candida species* are known to be aetiological agents of human infections. Though *Candida albicans*is the most commonly isolated fungal pathogen from clinical samples, gradually *non-albicans Candida species* are becoming predominant pathogens[7]. Moreover, the increased use of anti-fungal agents for treatment haslead to

development of resistance against commonly used anti-fungal agents in the treatment [3, 5, 7]. However the *Candida species* have variable resistance towards various antifungal agents. The objectives of this study were to isolate and identify the species of *Candida* from vulvo-vaginal (vulval in children, unmarried women) & skin infection samples and to determine the susceptibility pattern of the *Candida species* isolates.

Materials and Methods

In this prospective study all the patients attending at the department of medicine and the department of Gynaecology were participated for a period of 1 year. Thus the specimens whose Gram stained smears showed presence of any yeast cells or yeast-like cells with budding and with or without pseudohyphae were processed for fungal culture and inoculated on Sabourad's Dextrose Agar (SDA). Plates were incubated aerobically at 37^0C for 24 hours. The colonies of *Candida species* were obtained after overnight incubation. The colonies were identified by colony morphology on SDA, colony colour on Candidal differential agar Media, germ tube test and chlamydospore formation as follows. The colonies were identified according to colour. In addition to the colour of the colonies on HiCrome, a germ tube test and observation of chlamydospore formation on cornmeal agar were carried out for identification of *Candida albicans*. For germ tube test, a well isolated colony from SDA was emulsified in 0.5 ml of human serum using sterile straight wire. The test tubes were incubated at 35^0C and no longer than 2 hours. A drop of serum sample was placed on a clean, grease free slide and a cover slip was placed over it. This slide was then observed first under 10X and then under 40X objective lens of microscope for the presence of germ tubes. Germ tube is a filamentous extension from yeast cell without constriction at the neck (true germ tube) and is seen in *C. albicans*. Antifungal Susceptibility test was carried out for *Candida species* according to CLSI guidelines for testing anti-fungal agents for yeasts

Results

Of all, 219 samples were positive for candida infections, including 160(73.1%) from the Gynaecology department, and rest from the department of Medicine. All these positive samples produced cream to white, smooth and glossy colonies - characteristic of Candida species on the SDA. These Candida-positive colonies were gram stained and only those which were round to oval with purple-coloured budding yeast cells were further processed for germ tube (GT) test. A total of 131(59.82%) strains produced germ tubes, hence were categorised as either *C. albicans* or *C. dubliniensis*, while 88(40.18%) strains which were GT negative and were designated as *Candida* species. Species level identification was performed by using CHROM agar Candida and corn meal agar. On the basis of growth on both the media, out of all the positive isolates *C. albicans* 128(58.45%) was the most predominant species followed by *C. glabrata* 30(13.69%), *C. tropicalis* 26(11.87%), *C. krusei* 17(7.76%), *C. parapsilosis* 12(5.47%), *C. dubliniensis* 3(1.37%) and C. lusitaniae 3(1.37). Among NACs, C. glabrata was the most abundant species. The Candida species were also identified through various biochemical tests and the results confirmed microscopic and morphological observations. Moreover, 139(63.5%) of the infections were acquired in hospitals compared to 80(36.5%) community-acquired infections. *C. albicans* was the most abundant species in both the OPD and IPD, followed by *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*. *C. krusei* was more prevalent in OPD, while other species were abundant in IPD. Highest prevalence of Candida species was in Gynaecology (Table-1). It was observed that the number of *C. albicans* and all the NAC species was high in Unmarried girlss as compared to married women. Among the NACs, *C. tropicalis*, *C. glabrata* and *C. krusei* were the predominant species in Unmarried girlss. In case of married women, *C. tropicalis*, *C. glabrata* were high in number after *C. albicans* (Table-2). Patients were divided into six age groups. The highest rate of Candida species was obtained from the patients aged above 60 years with highest prevalence of *C. albicans* followed by *C. glabrata*, *C. tropicalis* and *C. krusei*. In the age group 26-40 and 41-60 years, *C. glabrata*, and *C. tropicalis* were prevalent. *C. krusei* was most abundant within the middle-aged group, i.e. 41-60 years (Table-3). In our study, Itraconazole was the most effective antifungal against all the Candida species with a susceptibility rate of 213(97.26%). Resistance towards Itraconazole was noted for 3(2.34%) *C. albicans*, 1(3.33%) *C. glabrata* and 1(5.88*%) C. krusei* species. Interestingly, the highest resistance was found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%). *C. krusei* 4(23.5%) were the most resistant Candida species to fluconazole followed by *C. albicans* 24(18.75%), *C. glabrata* 3(10%) and *C. parapsilosis* 1(8.3%). However, *C. parapsilosis* was the most resistant to voriconazole 4(33.3%), followed by *C. krusei* 4(23.5%), *C. albicans* 26(20.3%), *C. glabrata* 4(13.3%) and *C. tropicalis* 2(7.7%). A 100% susceptibility rate was noted in *C. dubliniensis* and *C. lusitaniae* for both the azole antifungals (Table-4). According to the antifungal resistance data of this study, cross-resistance between fluconazole and voriconazole was found among 18(8.2%) of the isolates. Of them, 16(88.9%) were *C. albicans* while 2(11.1%) were *C. glabrata*. Both the *C. glabrata* isolates were cross-resistant to fluconazole and voriconazole. Among *C. albicans*, 14(87.5%) isolates were cross-resistant to fluconazole and voriconazole, 1(6.25%) isolate was resistant against Itraconazole and voriconazole while 1(6.25%) C. albicans isolate was resistant to all the three antifungals i.e., Itraconazole, fluconazole and voriconazole.

Table 1: Candida Species isolated from the study

C. albicans	*C. glabrata*	*C. tropicals*	*C. krusel*	*C. parapsllosis*	*C. dubllnieniensis*	*C. lusitaniae*	**Total**
47	9	10	5	7	7	0	78 (35.6%)
31	9	6	7	3	3	1	59(26.9%)
20	6	4	1	1	1	0	32(14.6%)
13	1	3	2	0	0	0	20(9.1%)
5	2	0	2	0	0	1	10(4.6%)
5	1	3	0	0	0	1	10(4.6%)
7	2	0	0	1	1	0	10(4.6%)
43	11	11	10	4	4	0	80(63.5%)
85	19	15	7	8	8	3	139(14.6%
128	30	26	17	12	12	3	219(100%)

Table 2: Marital status distribution of Candida albicans and Non albicans (Nacs) species

Gender	C. Albicans (128)	C. Glabrata (30)	C. tropicals (26)	C. krusel (17)	C. parapsllosis (12)	C. dubllnieniensis (3)	C. lusitaniae (3)	Total	Total isolates
Unmarried girls	81	21	17	12	8	3	2	63	144(65)%
Married women	47	9	9	5	4	0	1	28	75(34.2%)

Table 3: Age wise Candida species distribution

Gender	Children (0-1)	Teenagers (12-18)	Young adults (19-25)	Age group Adults (26-40)	Middle aged (41-60)	Senior citizen (>60)	Total
Unmarried girls	4	2	13	42	35	48	144(65.8%)
Married women	3	2	3	9	20	38	75(34.2%)
Total	7	4	16	51	55	86	219(100%)

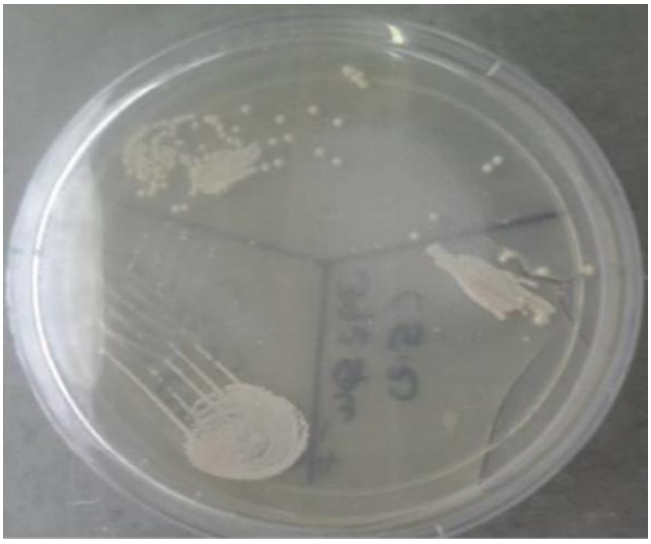

Fig 1: Colony of Candida Species on SDA

Fig 2: Anti-fungal susceptible of Candida species

Discussion

The virulence factors and antifungal susceptibility profile of *C. albicans* and NACs vary which has necessitated correct and rapid species identification as this has a direct impact on the choice of treatment [8]. In our study, *C. albicans* (58.4%) was the leading pathogen as compared to NACs similar to earlier reports [9-12]. Nucci *et al.* [13] also reported *C. albicans* (37.6%) as major contributor of Candida infection followed by *C. parapsilosis* and *C. tropicalis*. The order of prevalence of NACs in our study was *C. glabrata* (13.7%), *C. tropicalis* (11.9%), *C. krusei* (7.8%), *C. parapsilosis* (5.5%), *C. dubliniensis* (1.4%) and *C. usitaniae* (1.4%). A significant finding of our study is *C. glabrata* among NACs being the most common species in clinical samples. This could be a perturbing threat due to high incidence of increased resistance of this species to the routinely used antifungal agents. Patel *et al.* [11] isolated highest number of Candida isolates from urine and sputum, which is similar to our work where urine 78(35.6%), vagina 59(26.9%) and sputum 32(14.6%) had predominant Candida species. Farooqi *et al.*

[13] reported a different epidemiological trend where C. tropicalis was the most common organism followed by *C. parapsilosis* and *C. glabrata*. Candida infection was higher in Unmarried girls 144(65.8%) as compared to married women (30.9%) in our study, which is in accordance with findings of Nardin *et al* [15]. The reason of high distribution and virulence of Candida species in Unmarried girls is that it has a receptor for Unmarried girls reproductive hormones. Rashwas *et al.* [16] observed candiduria in 34.4% Unmarried girls and 14.9% in married women. Aslam *et al.* [17] also reported nosocomial candidiasis more frequent in Unmarried girls patients (56%) as compared to married women patients (44%). In our results, high percentage of Unmarried girls patients visiting the QIH may be due to problem in personal hygienic conditions. In this study, Candida infection was most prevalent within the age group of >60 years and middle aged-group, which is in accordance with studies of Furnaleto *et al.* [18] and Al- Hussaini *et al.* [19] In the present study, Candida infection rate was high in Gynaecology wards. However, other studies reported that Candida infection was more common in ICU and surgical ward [20]. Itraconazole was found to be highly effective against all tested species except for *C. albicans*, *C. glabrata* and *C. krusei*, which is similar to report of De Almeida *et al* [21]. Antifungal susceptibility data of this study also observed marked rise in azole resistance in NACs as compared to *C. albicans*. *C. krusei*was the most resistant species among all the isolates followed by *C. albicans*, *C. glabrata* and *C. parapsilosis*. Oberoi *et al.* [22]. reported high fluconazole sensitivity in *C. tropicalis*, high resistance in *C. glabrata* and less resistance in *C. parapsilosis*. All tested *C. tropicalis* local isolates were fluconazole sensitivity in contrast to *C. parapsilosis* and *C. glabrata*. Badiee and Alborzi[23] report 89.5% susceptibility of *C. albicans* to fluconazole; which is quite similar to our results. Fluconazole resistance was 18.8% similar to the Sojakova *et al.,* [24] which reported 13% fluconazole resistance in 227 Candida isolates. Kaya *et al.* reported an alarming increased fluconazole resistance in *C. albicans* (68.7%) and NACs (63.2%) [25].

Conflict of Interest: Nil

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Ethical Clearance: This study was approved from the competent authority of our Institutional ethics committee.

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