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ORIGINAL RESEARCH

Antifungal Susceptibility testing of Candida Species Isolates from vulvo vaginal Candiasis cases from a Tertiary Care Hospital in Central India

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ABSTRACT

Background: Drug resistance among Candida species is a serious concern. The present study is undertaken to perform the antifungal susceptibility of the Candida species isolated from the cases of vulvo vaginitis from a tertiary care centre. **Methods:** High vaginal swabs from 350 patients with symptoms of vulvovaginitis cases were collected and organisms were identified by gram stain, KOH wet mount and culture on Sabourauds Dextrose agar. Species differentiation was done by standard methods. Antifungal susceptibility was performed by disc diffusion methods. **Result:** A total number of 116 (33.1%) subjects showed pure growth of Candida species by conventional standard methods. Isolation of NAC was higher (26.7%) than C.albicans. The most common species isolated by conventional methods was C. albicans (36.3%) followed by C. glabrata (24.1%), C. tropicalis (22.5%), C. krusei (10.3%) and C.parapsilosis (7.7%). Disc diffusion results showed maximum resistance to ketoconazole (20.6%) followed by that to fluconazole and nystatin (15.5%), amphotericin B (8.6%) and voriconazole (7.7%). **Conclusion:** Identification of the species and antifungal susceptibility testing is necessary to prevent the spread of drug resistance candida species in the management of vulvovaginal candidiasis.

Key words: Vulvo vaginal Candidiasis, Candida albicans,Non albicans candida ,disc diffusion test.

INTRODUCTION

In women of reproductive age, Candida species are the primary cause of vulvovaginal infections. Previously, Candida spp. was infrequently documented for antifungal resistance; however, this has now become a significant global health care concern. The sensitivity patterns of NAC spp. (Non albicans Candida) are more unpredictable and may be influenced by factors such as patient age and geographic location,1 despite the fact that the majority of C. albicans strains are innately susceptible to commonly used antifungal medications. Choice of an antifungal agent for candidiasis treatment has been confounded by the gradual increase in the number of new and broad-spectrum antifungal drugs and the emergence of antifungal resistance 2,3. Consequently, the selection of a therapeutic agent that is both accurate and appropriate necessitates the antifungal susceptibility testing of Candida spp. that are infected. Antifungal susceptibility testing has emerged as a critical tool in the development of drugs and the monitoring of antifungal resistance in epidemiologic studies in recent years.4 Susceptibility testing of fungi was uncommon until recently.5 This was the result of a combination of factors, including the scarcity of antifungal medications, the scarcity of clinical cases that required systemic antimycotic therapy, and the unreliability of the correlation between in vivo and in vitro results. The current situation has undergone a significant transformation as a result of the

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proliferation of disseminated mycotic infections, the expansion of the number of antifungal agents available, and, most significantly, the development of inherent and acquired immunity in numerous yeasts to antifungal agents that are frequently employed.6,7 The objective of the current investigation is to evaluate the antifungal susceptibility pattern of the Candida species that were isolated from the vulvovaginal Candidiasis cases at a tertiary care centre in India.

MATERIALS AND METHODS

This is a cross-sectional study was conducted in the Department of Microbiology, tertiary care hospital, central India. A total number of 350 patients with complain of vaginal discharge attending department of Obstetrics & Gynaecology are included in the study.

High vaginal swabs were collected from the patients with symptoms of vulvovaginitis. The organisms were preliminarily identified by Grams staining and potassium hydroxide wet mounts The culture was done on the Sabouraud's dextrose agar (SDA) and the organisms were further confirmed by Gram staining in which gram positive budding fungal yeast cells were observed. The growth of Candida on SDA was confirmed based on colony morphology and Gram stain examination. Species identification of Candida isolates was done by following standard mycological methods including germ tube test, sugar fermentation and sugar assimilation, colour of colony on Hi Chrome Candida agar and chlamydospores formation on Corn meal agar.

Antifungal susceptibility testing: Antifungal susceptibility testing was performed by disk diffusion method using Mueller-Hinton Agar, 2% Glucose with Methylene Blue Dye Medium as per CLSI guidelines (C.L.S.I. document M44-A2, 2009.). The Inoculum was prepared by taking five distinct colonies of approximately 1 mm in diameter from at least 24 h old culture of Candida species. Colonies were suspended in 5 ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5McFarland standard was used to standardize the inoculums density. Antifungal susceptibility testing was undertaken by the disk diffusion method. Using disk dispenser (Oxoid™), fluconazole disk (10 µg), itraconazole (10 µg), voriconazole (10 µg), clotrimazole (10 µg) and nystatin (100 IU) antifungal discs (Thermo Scientific™ Oxoid™) were applied on MHA (ThermoScientific™ Oxoid™) as recommended by the Clinical Laboratory Standard Institute (CLSI) M44A document.The plates were incubated in ambient air at 37°C and read at 24 hours. The diameters of zones of inhibition were measured in millimetres using a ruler for each antifungal disk. Interpretation of all antifungal susceptibility (susceptible S, susceptible dose dependent [SDD], and resistant R) was done according to CLSI standards. Quality control was undertaken by using quality control trains, C.albicans ATCC 90028 and C. parapsilosis ATCC 22019. All the culture media, Antifungal disk, and control strains were obtained from Himedia Laboratories, India.

RESULT

Totally 116 Candida species were isolated from 350 high vaginal swabs. Out of 116 Candida isolates,74/116 (63.7%) were Non-albicans Candida (NAC) and 42/116(36.2%) were C.albicans. Among NAC, 26/116(22.4%) were C. glabrata, followed by 24/116(20.6%) C. tropicalis, 16/116(13.7%) C.parapsilosis and 10/112(8%) were C. krusei. The speciation of Candida species done by Candida HiChrom agar color of the colony and Germ tube test. Candida albicans was showing green color colonies & germ tube positive, Candida glabrata shows purple color colonies and germ tube negative, Candida krusei shows Pink color colonies & germ tube negative, Candida tropicalis shows Blue color colonies & germ tube negative and Candida parapsilosis shows cream color colonies and germ tube negative.

Out of the 42 Candida albicans isolated (35 isolates,83.3%) were susceptible to Fluconazole, (34 isolates,80.9 %) were susceptible to Voriconazole, (32isolates, 76.1%) susceptible to Ketoconazole, (38isolate 90.4%) was susceptible to Nystatin and (40isolates 95.2%) was susceptible to Amphotericin B. For 76 isolates of Non albicans candida the 26 isolates of Candida glabrata, (21 isolates, 80.7%) were susceptible to Fluconazole, (24 isolates, 92.3%) were susceptible to Voriconazole, (19 isolates, 73.0%) susceptible to Ketoconazole, (22 isolate 84.6%) was susceptible to Nystatin and (24isolates 92.3%) was susceptible to Amphotericin B. Of the 24 isolates of Candida Tropicalis, (18 isolates, 75%) were susceptible to Fluconazole, (23 isolates, 95.8%) were susceptible to Voriconazole, (17 isolates,70.8%) susceptible to Ketoconazole, (18 isolate 75%) was susceptible to Nystatin and (23 isolates 95.8%) was susceptible to Amphotericin B. Of the 16 isolates of Candida Parapsilosis, (13 isolates, 81.2%) were susceptible to Fluconazole, (16 isolates, 100%) were susceptible to Voriconazole, (13 isolates, 81.2%) susceptible to Ketoconazole, (11 isolate 68.7%) was susceptible to Nystatin and (14 isolates 87.5%) was susceptible to Amphotericin B.Of the 8 isolates of Candida krusei, (4 isolates, 50%) were susceptible to Fluconazole, (7 isolates, 87.5%) were susceptible to Voriconazole, (5 isolates, 62.5%) susceptible to Ketoconazole, (6 isolate 75%) was susceptible to Nystatin and (6 isolates 75%) was susceptible to Amphotericin B.

DISCUSSION

The rate of isolation of NAC was 63.7% higher in our study than that of C. albicans, which was 36.2%. Additionally, Kikani B et al[8]. have reported that NAC is isolated at a higher rate than C. albicans (55.6% vs

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44.4%), Deepa Babin et al.[9] (64.5% vs 35.5%), and Namrata et al.[10] (53% vs 47%). Nevertheless, Tehran [11](65.1% vs 34.9%), Sudan [12](92% vs 8%), Egypt[13] (60.3% vs 39.7%), Turkey[14] (59.9% vs 40.1%), and India[15] (66% vs 34%) have reported higher isolation rates of the most prevalent species, C. albicans, than NAC. C.glabrata (24.1 %) was the second most prevalent isolate in the current study, following C. albicans. It has been reported to be the second most prevalent isolate in cases of VVC from Saudi Arabia [16] (31%), Turkey [17] (34.5%), Australia[18] (20%), Egypt[19] (12.7%), and India [20](11%). C.tropicalis was the third most prevalent isolate in the current study, following C. albicans and C. glabrata.[20,21,22] The rates of C. tropicalis isolation in VVC cases ranged from 4% to 26.4%. Candida isolates that were resistant to fluconazole by the disk diffusion method were identified in our study (15.5%). This discovery is comparable to the resistance reports of Lee et al [23](17.1%) and Kustimur et al [24](16%). Nevertheless, Ooga et al.[25] (25%), Negri et al.[26] (27%), and Zomorodian et al.[27] (3.4%) reported a higher rate of resistance, compared to Colombo et al.[28] (6%), Kikani et al. [29] (8.2%), and Pfaller et al.[30] (9.9%), a lower rate than that of our investigation. In our investigation, fluconazole resistance was observed in 7.1% of C. albicans. Our results are comparable to those of Capoor et al [31](21.8%). However, Doddaiah V et al.[32] reported that it was present in 8.6% of their C.albicans isolates. Despite the fact that none of our isolates were resistant, several researchers [33,34,35] have reported fluconazole resistance in C. tropicalis (10-11%) and C. glabrata (31-33%). 7.7% of our isolates exhibited resistance to voriconazole. This is in close agreement with the results of Babin et al[37] (14%), Dalia Saad El Feky et al (7.9%), and Das P P et al [36](6.45%). Voriconazole resistance was observed in 21.1% of the C. albicans isolates in our study and in 50% of the C.parapsilosis isolates. It is possible that the higher level of resistance to Ketoconazole (20.6%) than to voriconazole (9.1%) observed in this study is due to the fact that Ketoconazole is used more frequently than voriconazole. The resistance to ketoconazole is a cause for concern, as it is the most frequently used azole for the treatment of candidiasis and is also cost-effective. Therefore, it is imperative to exercise caution when prescribing and/or using Ketoconazole. Voriconazole, on the other hand, appears to be a superior option due to its more effective binding to the cytochrome P-450 isoenzyme of Candida[38] species and the lower resistance it has encountered. In the current study, 8.6% of Candida species exhibited resistance to Amphotericin B, a figure that has been reported as 1.37% by Kashid et al [39] and zero percent by Negri et al [40] and Dota et al.[41] The resistance to amphotericin B in C. albicans of our study was 4.7%, which is comparable to the rates reported by Capoor et al [42] and Badiee et al [43] (4.3% and 7%, respectively).

CONCLUSION

The present study showed the distribution of various candida species in the initiation of vulvovaginitis and the results of antifungal susceptibility by disc diffusion method showed maximum resistance to ketoconazole (20.6%) followed by that to fluconazole and nystatin (15.5%), amphotericin B (8.6%) and voriconazole (7.7%). Identification of the species and antifungal susceptibility testing is necessary to prevent the spread of drug resistance candida in the management of vulvovaginal candidiasis.

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