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A Rising Profile of Non Albican Candida in Vulvovaginal Candidiasis Among Symptomatic women in Portharcout

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Abstract
Background; *Non albican candidas had for a long time been neglected because of the assumption that candida albican causes all or almost all case of Vulvovaginal candidiasis.*
Aim; *To determine the proportion of non albican Candida (NAC) causing Vulvovaginitis in Port Harcourt using mycology culture and germ tube test.*
Material and Method*; High vaginal swab (HVS), demographic data and epidemiological risk factors were collected from 247 respondents with symptomatic vulvovaginal candidiasis. While germ tube test was used to differentiate between albican and non albican candida. The data was analyzed using the SPSS version 20. Association between variables was compared by using the Chi -square (χ2) test and level of significant was set at P < 0.05.*
Results*; The prevalence of NAC among symptomatic women with VVC was 76.68%, with modal age range of respondents and prevalence as 24 -29years and 32.79% respectively this was however not statistically P<0.05.Also,the mean and median age of the respondents were respectively 37.50±13.69 and 37.50.*
Conclusion *;The high prevalence of non albican candida in this study may necessitate routine species identification of all isolates of candida, to prevent cases of inadequate treatment occasioned by intrinsic resistant of some non albican candida to the common azoles group of antifungal.*

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I. Introduction

Vulvovaginal candidiasis (VVC) is an inflammation of the vulva and the vagina secondary to candida infections [1],and manifest clinically as severe genital itching, abnormal vaginal discharge which is usually fowl smelling, burning sensation in the vaginal[2]. About 75% of all women have been documented to have one or more episodes of VVC at some point in their life and 5% have ongoing infection [3]and another 5% have recurrent VVC, the etiology of this candida infections is still erroneously attributed to only Candida albicans and treated empirically as such, because several studies supported it. As a result, clinicians treat all VVC empirically with focus on candida albican as the sole etiology. In same vein, most laboratories do not do speciation of isolated candida nor conduct antifungal susceptibility testing for candida isolate from VVC because of similar assumptions. Consequently, inappropriate treatments are administered to patient, which may result in recurrent or complicated VVC because, most commonly isolated species of NAC have been noted have intrinsic or acquired resistant to the common drugs use for empirical treatment of candida infections[4].

A worldwide review studies show that the incidence of Candida albicans has declined from 70% to 50% over the years[5]. In North America and many European countries, there have been gradual decrease in C. albicans infections and increase of NAC species, notably C. glabrata infections[6]. Also, obvious increase in the incidence of C. parapsilosis and C. tropicalis infections have also been noted in other regions such as Australia, China and Thailand with prevalence of 52.7%,58.2% and 64,4% respectively for NAC species[6'7'8]

54.1% of NAC species was isolated in multicenter study in South Africa[9] another multicenter done in Lagos, Nigeria, NAC accounted for 79.9% of all isolates of Candida isolated from VVC[10]. Other studies have shown almost an inverse incidence of both candida albican and NAC species in VVC as there has been obvious decrease in the incidence of C. albicans and a corresponding increase of the non-albicans Candida species in cases of VVC[11'12]

II. Objective

To determine the proportion of non albican Candida (NAC) causing Vulvovaginitis in Port Harcourt using mycology culture and germ tube test

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Study Design

This is a descriptive cross-sectional study and was conducted in the Department of Medical Microbiology and Parasitology, University of Port Harcourt Teaching Hospital. Rivers state, Nigeria.

Study Population

Participants included symptomatic women with clinical presentation suggestive of Vulvovaginitis sent from the hospital out patients' clinics (STI, family medicine clinic, Obstetrics and Gynecology clinics) to the laboratory of medical microbiology for high vaginal swab (HVS).

Inclusion Criteria includes all symptomatic women of reproductive age from the outpatient clinic and who are sexually active while Exclusion Criteria are women on their monthly menstruation, women who are virgins and patients who decline consent

The minimum sample size required for the study was 247 (given a prevalence of 79.9%)[10] based on sample size calculation given below[13], $N=Z^2 (p)(q)/d^2$

Patients were recruited consecutively excerpt for those who did not meet the inclusion criteria

Study Procedure

This study was carried out in the department of medical microbiology and parasitology of the University of Port Harcourt Teaching Hospital, involving two hundred and forty seven (247) symptomatic women. Appropriate samples were collected by high vaginal swab (HVS). HVS collection was done according to the recommended standard protocol (BSOP 28, 2005)[14] by first explaining the procedure and then obtaining a verbal consent from each of the respondents. The procedure was done with aid of sterile speculum and swab stick, all samples collected were immediately sent for analysis which included microscopy, culture and germ tube test. The HVS samples were inoculated on Sabouraud dextrose agar (SDA) plate and incubated aerobically at 35-37° C for 24-48hours.

The agar plates were examined for visible growth after the incubation period, however, any plate that yielded no growth after this incubation period, was incubated for additional 2-3 days before discarding as negative[15]. Identification of yeast was done by regarding the colonial morphology of cultures isolates on sabouraud dextrose agar, Gram staining and Germ tube for categorization of candida species into candida albican and non albican candida.

Germ Tube Test

The procedure of is as follows. Some colonies of the isolated yeast cell was inoculated in human serum in a test tube and incubated at 35-37°C for about 3 hours. A drop of the incubated serum was placed on a grease free microscope slide and covered with a cover slip and examined under the microscope with x40 objective magnifications for the presence of germ tube[16] Candida albican is germ tube test positive. From the study, all gem tube negative candida species were regard as NAC.

Method of Data analysis

Information extracted from the questionnaires and the results of the laboratory test were entered into Microsoft excel and then imported into SPSS version 20 for data analysis. Data was displayed using tables and graph.

Inferential analysis such as chi square with 95%confidence interval was used to determine the association between variables. Level significance was set at p=0.5

Ethical considerations

Approval for the study was sought from the ethical committee of the University of Port Harcourt and the head of department of medical microbiology and parasitology, University of Port Harcourt teaching hospital. Also, informed consent was obtained from the individual respondent.

Results

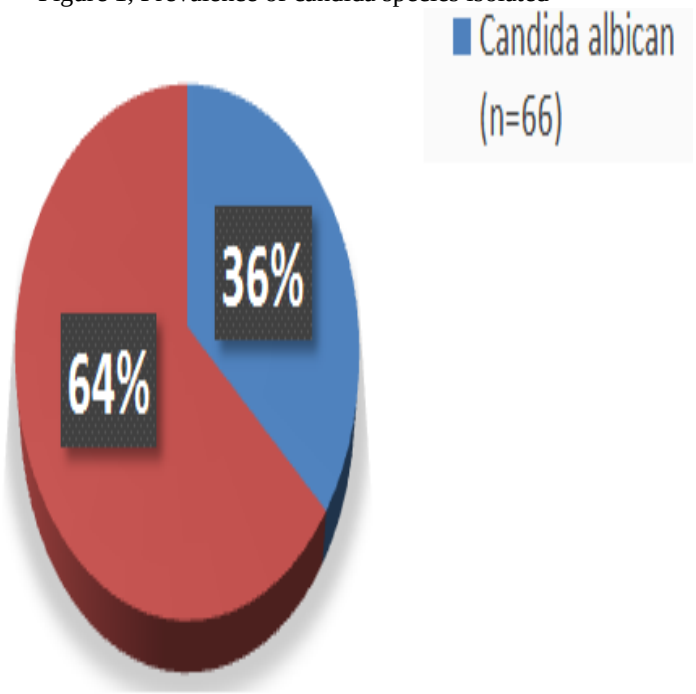
Age range (years)					□□□ (p-value)
	Total respondents	**Positive for candida spp**	**NAC**	**Candida albicans**	
15-19	20 (8.5)	15 (8.3)	9 (8.2)	6 (8.6)	0.004 (0.9490)**
20-24	53 (21.5)	36 (19.8)	23 (19.7)	13 (20.0)	0.012 (0.9690)**

25-29	65 (26.2)	55 (30.2)	38 (32.8)	17 (25.7)	0.527 (0.4675)**
30-34	42 (16.9)	38 (20.8)	29 (24.6)	9 (14.3)	1.431 (0.2314)**
35-39	34 (13.8)	22 (12.5)	11 (9.8)	11 (17.1)	1.085 (0.2975)**
40-44	17 (6.9)	6 (3.1)	2 (1.6)	4 (5.7)	1.101 (0.2941)**
45-49	6(2.3)	6 (3.1)	2 (1.6)	4 (5.7)	1.101 (0.2941)**
50-54	4 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	-
55-59	6 (2.3)	4 (2.1)	2 (1.6)	2 (2.9)	0.161 (0.6876)**
Total	**247(100.0)**	**182 (100.0)**	**116(100.0)**	**66(100.0)**	

Table 1: Age Related Prevalence Of The Isolated Candida Species From Vvc

□□□ : Chi-square statistic **difference is not statistically significant (p > 0.05) mean age was 37.50±13.69. median age =37.50 Of the 247 respondents for the study, 182 were positive for candida species representing a prevalence of 73.68% of candida. Analysis of age related prevalence showed a pyramidal distribution of both respondents and candida incidence as 4 of the 9 age groups involved in the study contributed as much as 78.4%,83.3%and 86.9% of the total respondents, candida species and non albican candida respectively. While the extreme of ages in the study comprising five(5) ages groups contributed only 21 .6%,16.7% and 13.1% of respondents, total candida species and Non albican candida respectively. Similarly, 25-29years age range has the highest incidence of both the respondents, NAC and candida species each respectively representing 26.2%, 3 0.2% and 32.8%.Table 1.

Figure 1, Prevalence of candida species isolated



A total of 182 candida species were isolated in the study, 64% of this was non albican candida and 36% belong to the albican candida specie which represented 116 and 66 of the total respondents. **Figure 1**

Age range (years)	NAC	Percentage (%)
15-19	9	8.20
20-24	23	19.67
25-29	38	32.79
30-34	29	24.59
35-39	11	9.83
40-44	2	1.64
45-49	2	1.64
50-54	0	0.00
55-59	2	1.64
Total	**116**	**100**

Table 2, Age group /NAC prevalence in study subjects

III. Discussions

The well-established nature and characteristics of infectious diseases have undergone significant changes especially with regard to the virulence of the organisms .This has become very obvious during the past few decades. Such that the previously nonpathogenic or saprophytic organisms are acquiring pathogenic qualities, causing significant morbidity and mortality worldwide. In present study, a total of 182 candida species were isolated which represents 73.85% prevalence of candida species from Vulvovaginitis. Of this, 63.74% of the total candida species belong to NAC species, while the other 36.26% was candida albican. This finding is in accordance with the studies done by Deorukhkar et al (2014)[4] where a prevalence of 66.3% of NAC species was recorded. Also, in studies by Mohanty et al (2007)[17] and Kumari et al (2013)[18], prevalence of 64.8% and 67.6% of NAC species were found respectively. This finding corroborated the assertions that there is a shift from predominantly albican candida as the sole pathogenic candida species to non albican candida species, as this is now more frequently isolated from candida infections[10].This observations may be due to increasing use of over the canter antimycotic agents, suboptimal dosages and dissemination of the resistance strains of candida species and cross resistant among candida species as well as to newer antifungal agents[19].

On age related prevalence of NAC species; the finding from this study, put the modal age group of prevalence at 20-24years representing 32.79%of NAC species<0.05, this was closely followed by age range 24-29years with 24.59% P<0.05.both were not statistically significant. This observations was similar to the finding of sehgel et al[20], who reported incidence of 55% for age range of 20- 30years, this high distribution of NAC species within this age group however also reflect the distribution of candida albican, which is probably due to the fact that women in this group are more sexually active, may abuse antibiotics, self-medicate antifungal and use contraceptives to prevent pregnancy thereby reducing their vaginal immunity[1]. In addition, there was notable observation of reduction in the incidence of candida Species with age advancement, such that as low as 14.4% of NAC was found in four age bracket which includes ages ranges 35-39years,40-44years,45-49years and 50-54years P<0.05 ,this result is also in accordance with the observations of Okungbowa et al 2003[10].This finding is most probably due to pre-menopausal or menopausal changes with attendant less sexual activities and reduction in the use of contraceptives for pregnancy prevention as such, there will be decrease estrogen and corticoids hence increased vaginal immunity[1].

IV. Conclusion

The erroneous assumption that candida albican causes almost all cases of vulvovaginal candidiasis, had over the years made NAC neglected pathogens of man. This study has to a great deal awaken the conscious of physician and other health care providers to the high prevalence of these group of organism in our setting and by extension Nigeria.

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