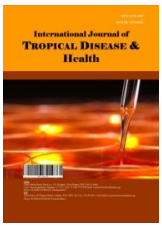

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The Influence of Age, Health Care and Hygienic Habits on *Candida* Species Prevalence in the Human Oral Cavity and Genitourinary Tract

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Authors' contributions

This work was carried out in collaboration between both authors. Author ENA designed the study, performed the statistical analysis, managed the literature searches and wrote the protocol and the first draft of the manuscript. Author JIO supervised the study and managed the analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study examined the influence of age, health care and hygienic habits on the prevalence of *Candida* species in the human oral cavity and genitourinary tract.
Study Design: The study was a cross sectional study.
Place and Duration of Study: Department of Microbiology, University of Nigeria, Nsukka and Bishop Shanahan Hospital, Nsukka, between March 2006 and February 2007.
Methodology: Oral and genitourinary samples were collected from 218 individuals (45 males, 173 females) within the ages of 12 and 67 years. Ninety-four of these volunteers responded to the questionnaire on health care and hygienic habits. The clinical specimen collected were cultured for the presence of *Candida* species. The data obtained were statistically presented as means and percentages.
Results: Out of 298 samples collected, 61/154 oral (19 males, 42 females) and 53/144 genitourinary (0 male, 53 females) samples yielded growth of *Candida* species. There was no

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significant difference in the prevalence of *Candida* species between subjects who use toothpaste and those who use chewing stick for oral hygiene (*P*=0.93). Respondents who douched were more colonized with *Candida* 26(39.39%) than those who did not (0%). Species of *Candida* were significantly associated with the textile material of the undergarment (p = 0.044). Age significantly influenced the prevalence of *Candida* species in the oral cavity (p < 0.05) but not in the genitourinary tract (p = 0.612).

Conclusion: The study recommends good personal hygiene and health care habits to reduce proliferation of *Candida* species.

Keywords: Age; Candida; candidiasis; genitourinary; hygienic habits; oral; prevalence.

1. INTRODUCTION

Candida species belonging to the class Ascomycetes, are aerobic, thin-walled small yeast measuring 4 to 6 micrometers and which reproduce asexually by budding [1,2]. They are normal commensal of the mucosal surfaces of the oral cavity, the gastrointestinal tracts and the vagina of humans [3,4,5,6,7]. Alteration in the host's normal flora or defect in the host immune defenses due to old age, disease or iatrogenic intervention [4,8,9], may transform a benign *Candida* species colonization into opportunistic pathogen, leading to host tissue invasion and causing superficial to life-threatening systemic candidiasis [10,11,7].

A review of the international literature clearly showed that in recent decades, fungal infections have increased dramatically in all hospital sectors and in all classes of age, with the emergence of new, potentially pathogenic *Candida* species [12,13,14,15,16,17]. Infections due to *Candida* species account for approximately 80% of all fungal infections of the immunocompromised [12,9,11,18,19,20,21]. *Candida* species are also the second most common cause of urinary tract infections, and other gynecological problems among women of child bearing age as well as the fourth most common cause of nosocomial blood stream infections, which in turn are associated with considerable mortality [12,22,23,14,24]. *Candida* species have been implicated in cases of abortion, arthritis, osteomyelitis, endophthalmitis, endocarditis, myocarditis, fungemia, meningitis and peritonitis which are major causes of mortality in both tertiary care centers and community hospitals [25,22,23,26,14]. The increasing incidence of candidiasis has been attributed to compromised immunity in patients. Thus, the asymptomatic presence of *Candida* species in the oral cavity or genitourinary tract increases the likelihood of developing clinical diseases especially following immune suppression [27,19,28,17].

A number of factors has been associated with increased rate of *Candida* colonization in the oral or genitourinary tracts. These factors include age, pregnancy, use of contraceptives, uncontrolled diabetes mellitus, use of broad spectrum antibiotics, poor dietary habits and poor personal hygiene [29,30,31,32,33,34,35,19,36,37,38]. It has also been reported that dressing pattern influences the prevalence or colonization of *Candida* species [39]. However, similarities and differences have been observed in reports from different study areas on the influence of the aforementioned factors on *Candida* species prevalence. In Nigeria, there is also sparse report on the frequency of *Candida* species as well as factors influencing their colonization in the oral cavities and genitourinary tracts of humans. Thus, the present study is aimed at assessing the influence of age, health care and hygienic habits on the prevalence of *Candida* species in the human oral and genitourinary tracts, in Nsukka, Southeastern Nigeria.

2. MATERIALS AND METHODS

2.1 Population and Design for the Study

This cross sectional study was conducted over a one year period (March 2006 to February 2007) in Nsukka, Enugu State Nigeria which is located on latitude 6°51′24″N and longitude 7°23′45″E. The population for the study was 218, made up of patients with cases of oral or genitourinary infection and apparently healthy humans within the ages of 12 and 67 years. All participants (218) submitted either oral or genitourinary samples or both for culture. Participants or volunteers who were currently on antifungal medication were excluded. Participants who used antifungal drugs within the past 2-3 months were included in the study.

2.2 Administration of Questionnaire

A questionnaire was administered to assess the influence of health care and hygienic habits on the prevalence of oral and genitourinary *Candida* species in Nsukka and environs. Information on previous treatment of oral or genital infection, drug used for treatment, health care provider, douching, use of tight fitting underwear, stuff of undergarment (pant), as well as care of the genital and oral cavity were assessed. Explanation was given on how to fill the questionnaire and the benefit of the study. Participants who were literate filled the questionnaire whereas an interpreter helped those who were not literate to fill their questionnaire. Ninety-four out of the 218 participants completed the questionnaire. Participants who were currently on antifungal medication were excluded as well as those who completed the questionnaire but failed to submit their oral and/ or genitourinary sample.

2.3 Clinical Sample Collection and Culturing

Oral and genital samples were collected with sterile swab sticks (Evepon sterile swab stick $^{(R)}$). Oral sample was collected from the dorsum of the tongue. Urine samples were collected with sterile sample bottles by participants after instructions on how to collect mid-stream urine. Oral and genital swabs were collected with the help of the physician or medical laboratory scientist in-charge, for culture. Information on participant's age, sex, oral and genital health status, were recorded at the time of sample collection. Each swab was rolled onto Sabouraud dextrose agar (SDA) plate supplemented with chloramphenical (50 µg/mL) and incubated at 37^{o}C for 2 days. Five milliliters of urine were transferred from the sample bottle to sterile centrifuge tube and centrifuged at 2000 rpm for 10 minutes. The supernatant was decanted and a loopful of the sediment was spread on SDA plates and incubated at 37^{o}C for 2 days.

2.4 Identification of *Candida* Isolates

2.4.1 Identification on chromogenic agar

Distinct yeast colonies from each culture plate were subcultured on two differential media plates for the identification of *Candida* species: Fluka *Candida* Ident agar (Sigma Aldrich Chemie GmbH, India) and Oxoid Chromogenic *Candida* agar (Oxoid, Basingstoke, UK) plates. These plates were incubated at 37^{o}C for 2 days. Identification was done by the specific colour of colonies on the media as described by the manufacturers. *Candida albicans* ATCC 90028 and *C. krusei* ATCC 6258, respectively served as positive controls.

2.4.2 Germ tube test

A light suspension of the 48 h old yeast isolate was made in 0.5 mL of sheep serum contained in clean sterile test tube and incubated at 37^{o}C for 2 – 3 h. A known germ tube positive organism, *C. albicans* ATCC 90028 was used as a positive control while an uninoculated serum sample served as negative control. A loopful of each yeast suspension was placed on a clean glass slide; a coverslip was applied and examined under X40 objective lens for germ tube production.

2.4.3 Chlamydospore production

A small inoculum of the 48 h old culture was inoculated onto rice meal agar plates containing 1% Tween 80 and incubated at 25^{o}C for 2 – 4 days. The inoculation was made by the cut-streak method. A mount was made from deep cut where the organism grew with reduced oxygen tension, then placed on a clean microscopic slide and stained with a drop of lactophenol cotton blue. The stained slide was examined under X40 objective lens for chlamydospore production.

2.4.4 Carbohydrate assimilation test

Due to the non-availability of API 20C AUX yeast identification system (bioMerieux), an alternative method was used. The method used was the auxanographic method for carbon assimilation studies developed by Haley and Standard (H – S) [40]. The H-S Yeast Nitrogen base was prepared with the following: Yeast Nitrogen base (0.67 g); Agar (20.0 g); Distilled water (1000 mL). Aliquots (20 mL) were dispensed in screw capped bottles and sterilized at 121^{o}C for 15 minutes and allowed to solidify. Eleven sugars which served as carbon sources were impregnated singly in disks and they were: dextrose, glucose, maltose, sucrose, lactose, galactose, raffinose, inositol, xylose, trehalose and melibiose. Below is a brief description of how the test was carried out. Approximately, 4 grams of each sugar was dissolved in 100 mL of distilled water to make a concentration of 40 mg/mL. Each sugar solution was sterilized by seitz filtration and stored in screw capped bottle.

Labeled sterile paper disks, impregnated with each sugar were prepared using 10 µL of the sugar solution. Each sugar disk has label representing the sugar. Example: 1 for Glucose; 2 for dextrose, etc. The yeast colony was then standardized by preparing a suspension of the 24 – 48 h old yeast culture in 5 mL of sterile distilled water. The turbidity of the yeast suspension was adjusted by comparison with 0.5 McFarland turbidity standards. The inoculum size was 1 x 10^5 – 2.5 x 10^5 CFU/mL. The standardized yeast suspension (0.2 mL) was poured into the tube of molten yeast nitrogen base agar (cooled to about 45^{o}C) and gently mixed by inverting the tube twice or thrice. The yeast-medium mixture was poured into a sterile Petri-dish and allowed to solidify at room temperature. Using tweezers, the sugar disks were placed on the plate and incubated at room temperature (25^{o}C – 30^{o}C) for 18 to 48 h. At the end of the incubation time, the plates were examined for presence or absence of growth of the yeast around each disk. Disks that do not have a zone of growth around them indicate that these sugars were not assimilated while those sugar disks that have cloudy zone of growth around them were assimilated. An uninoculated plate with sugar disks placed on it served as negative control while two plates (with sugar disks) inoculated with *Candida albicans* ATCC 90028 and *C. krusei* ATCC 6258, respectively served as positive controls.

2.5 Statistical Analysis

The data obtained was expressed as mean and percentages. Using the Statistical package for Social Sciences (SPSS 15.0), chi-square (χ^2) test was used to determine whether the differences observed in the prevalence of *Candida* species among the different groups studied were statistically significant or not. Differences were recorded as significant whenever the probability was less than or equal to 0.05.

3. RESULTS

Of the 298 samples collected from the oral cavities (144) and genitourinary tracts (154) of 218 participants, 114 (38.26%) participants were colonized by *Candida* species in the oral cavities (39.61%) and genitourinary tracts (36.81%). None (0%) of the 14 samples from male genitourinary tracts yielded growth of *Candida* species while 53 (40.77%) of the 130 samples from female genitourinary tracts were positive for *Candida* species. Of the 154 oral samples, 40 were from males while 114 were from females. Nineteen (47.50%) of the 40 samples from male oral route yielded growth of *Candida* species while 42 (36.84%) of the 114 samples from female oral route were positive for *Candida* species. A total of 132 *Candida* isolates were obtained (Table 1).

The *Candida* isolates were identified to the species level (Table 2). The two chromogenic media gave green colonies of *C. albicans* and blue colonies of *C. tropicalis*. Oxoid Chromogenic *Candida* agar gave a more precise identification for *C. glabrata, C. krusei* and *C. parapsilosis* than Fluka *Candida* Ident agar. *Candida albicans* had the highest frequency of isolation in the oral cavities while the non-albicans *Candida* species predominated in the genitourinary tracts (Fig. 1).

Candida species co-colonization or occurrence of more than one species of *Candida* (mixed culture of *Candida* species) in a clinical sample was detected in 18(15.79%) of the *Candida* positive samples. *C*andida *krusei* (11) had the highest occurrence as mixed culture followed by *C. tropicalis* (7) in samples from the genitourinary tracts (Table 3).

Table 1. Frequency of *Candida* species in oral Cavities and genitourinary tracts

Source of sample	No. samples	No. with disease symptoms (%)	*Candida* positive n (%)	Number of *Candida* isolates
Genitourinary tract	**144**	**97 (67.36)**	**53 (36.81)**	**66**
Male	14	7(50.00)	0	0
Females	130	90(69.23)	53 (40.77)	66
Oral cavity	**154**	**65 (42.21)**	**61 (39.61)**	**66**
Male	40	27(67.50)	19 (47.50)	21
Female	114	38(33.33)	42 (36.84)	45
Grand Total	**298**	**162(54.36)**	**114 (38.26)**	**132**

Table 2. Identification of *Candida* species Based on Chromogenic Agar and other Characteristics

Candida species	Colour on Chromogenic agar		Germ tube	Chlamydospore	Sugar assimilation[#]
	OCCA	FCIA			
C. albicans	Green	Green	Present	Present	G, D, M, S, Ga, X, T
C. glabrata	Beige yellow	White	Absent	Absent	G, D, T
C. krusei	Brown pink	White	Absent	Absent	G, D
C. parapsilosis	Brown	White	Absent	Absent	G, D, M, S, Ga, X, T
C. tropicalis	Blue	Blue	Absent	Absent	G, D, M, S, Ga, X, T
Ck	Brown pink	White	Absent	Absent	G, D
Ca	Green	Green	Present	Present	G, D, M, S, Ga, X, T

Key: OCCA = Oxoid Chromogenic Candida agar; FCIA = Fluka Candida Ident agar. # = Sugar used for carbon assimilation test: G = glucose; D = dextrose; M = maltose; S = sucrose; L = lactose. CK ATCC 6258 = Typed culture of C. krusei; Ca ATCC 90028 = Typed culture of C. albicans

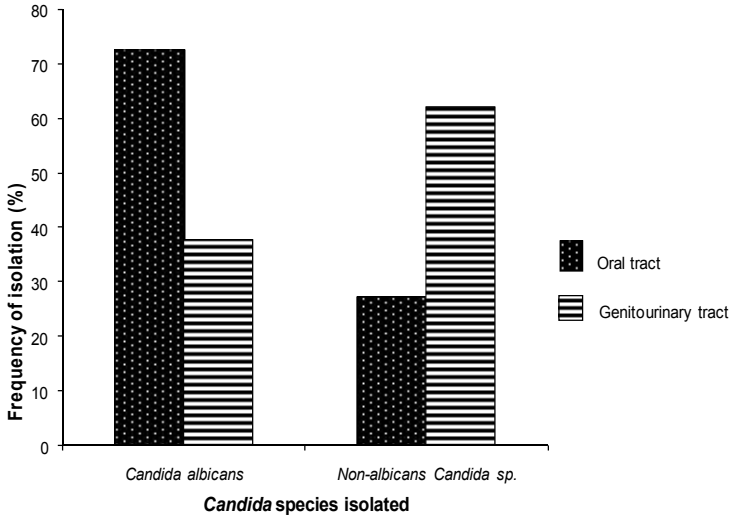

Fig. 1. Frequency of occurrence of *Candida albicans* and non-*albicans Candida* species in Oral Cavities and Genitourinary Tracts

The relationship between age and prevalence of *Candida* species in oral cavity and genitourinary tract is shown (Fig. 2). *Candida* species were more prevalent in genitourinary tract of middle age group (20 – 39 years) than the younger (10 – 19 years) and older (30 years and above) subjects while the prevalence of *Candida* species in oral tract increased with age. Age significantly influenced the prevalence of *Candida* species in the oral cavity (p=0.00) but not in the genitourinary tract (p = 0.612).

The *Candida* species distribution in the oral tract shows that *C. albicans* was the most prevalent species both within and across the age groups (Table 4). *C. krusei* occurred at low frequency in middle age group (20-39 years) but was not isolated in younger (10-19 years) and older age groups (50 and above). *C. parapsilosis* was not isolated in younger age group (10 -19 years).

The distribution of *Candida* species in the genitourinary tracts shows that the rate of isolation of *C. tropicalis* in the genitourinary tract increased with age (Table 5). *Candida albicans* and *C. glabrata* also were found in all age groups except 40 years and above. *Candida krusei* occurred among all the age groups while *C. parapsilosis* occurred only within age groups 10 – 19 and 20 – 29 years. *Candida krusei* had the highest frequency of occurrence within age group 10 – 19 years (44.44%) while *C. albicans* was highest within age group 20 – 29 years (55.56%), and 30 – 39 years (36.84%).

Table 3. Rate of Detection of *Candida* species Co-colonization in Clinical Samples

Sample source	No. sampled	C+ (%)	Co-colonization (%)	*Candida* species combinations (n)				
Oral cavity	154	61(39.61)	5(8.20)	Ca, Cp (2)	Cg. Ck (1)	Ct, Ck (2)		
Genitourinary tract	144	53(36.81)	13(24.53)	Ca, Cp (1)	Ca, Ct (1)	Cg, Ck (2)	Ck, Cp(3)	Ck, Ct (6)
Total	298	114(38.26)	18(15.79)					

Key: C+ = number of Candida positive sample; Ca = Candida albicans; Ct = C. tropicalis; Cp = C. parapsilosis; Ck = C. krusei; Cg = C. glabrata

Table 4. Age and distribution of *Candida* species in the oral cavities

Age (Years)	No. sampled	No. colonized (%)	*Candida* species isolated (%)			
			C. albicans	***C. tropicalis***	***C. parapsilosis***	***C. krusei***
10 – 19	42	4(9.52)	3(75.00)	1(25.00)	**0**	**0**
20 – 29	29	12(41.38)	11(78.57)	1(7.14)	1(7.14)	**1 (7.14)**
30 – 39	52	26(50.00)	21(75.00)	2(7.14)	2(7.14)	3(10.71)
40 – 49	22	11(50.00)	7(58.33)	2(16.67)	3(25.00)	**0**
50 & above	9	8(88.89)	6(75.00)	0	2(25.00)	**0**
Total	**154**	**61(39.61)**	**48(72.72)**	**6(9.09)**	**8(12.12)**	**4(6.06)**

$\chi 2 = 24.543$; degree of freedom (df) = 4; p =.00.

Table 5. Age and distribution of *Candida* species in the genitourinary tracts

Age (Years)	No. sampled	No. colonized (%)	*Candida* species isolated (%)				
			C. albicans	***C. tropicalis***	***C. parapsilosis***	***C. krusei***	***C. glabrata***
10 – 19	39	14(35.90)	3(16.67)	1(5.56)	3(16.67)	8(44.44)	3(16.67)
20 – 29	48	22(45.83)	15(55.56)	5(18.52)	1(3.70)	5(18.52)	1(3.70)
30 – 39	38	16(42.11)	7(36.84)	5(26.32)	0	6(31.58)	1(5.26)
40 & above	5	1(20.00)	0	1(50.00)	0	1(50.00)	0
Total	**130**	**53(40.77)**	**25(37.88)**	**12(18.18)**	**4(6.06)**	**20(30.30)**	**5(7.58)**

$\chi 2 = 1.814$; df = 3; p = 0.612

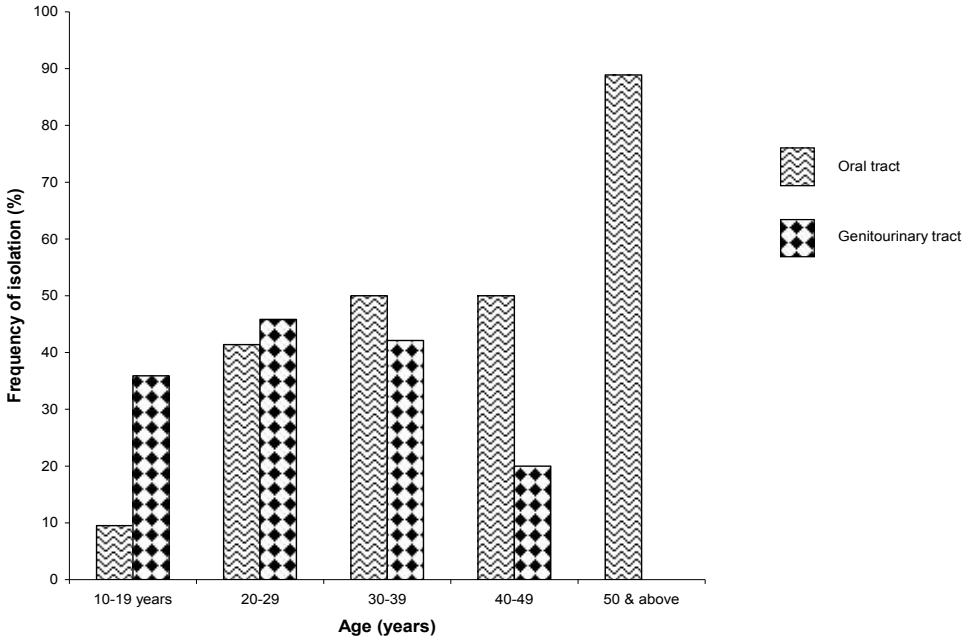

Fig. 2. Prevalence of *Candida* species in the oral cavities and genitourinary tracts among different age group

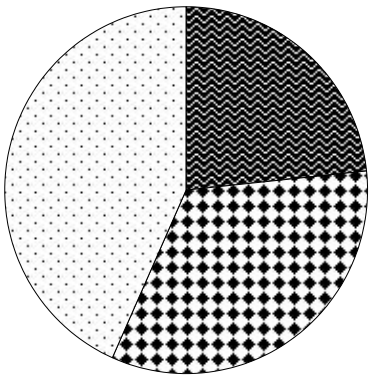

Fig. 3. Prevalence of *Candida* species based on stuff of undergarment
Key: *Cotton* *Nylon* *Stuff unknown*

Table 6 and also Fig. 3 show the results of the questionnaire on health care and hygiene habits and association to the prevalence of *Candida* species. Among the 94 subjects who responded to the questionnaire, 46 (48.94%) had symptoms of genital infection, 11 (23.91%) had never used any drugs for treatment of genital infection while 35 (76.09%) had used drugs for treatment of genital infection (at most, for the past three months). Species of *Candida* were more frequently isolated from genital tract of respondents who had never used drugs for treatment of genital infection 6 (54.55%) than those who used drugs for treatment 11 (31.43%) (Table 6). Increase in the occurrence of *Candida* species was observed among 8 (36.36%) respondents that used clotrimazole cream, but none among users of ketoconazole. Twenty-three (65.71%) respondents indulged in self-medication or use of doctor's previous prescription and had *Candida* prevalence of 26.09%.

Table 6. Health care and hygienic habits versus *Candida* species prevalence

Health care & Hygienic Habit	Respondent (%)	*Candida* Prevalence (%)	P*
Treatment of genital infection (n=46)			**0.166**
Yes	35(76.09)	11(31.43)	
No	11(23.91)	6(54.55)	
Drugs used for treatment of genital infection (n = 35)**			
Nystatin tablet (vaginal)	9(25.71)	3(33.33)	
Canesten cream	22(62.86)	8(36.36)	
Canesten tablet (vaginal)	9(25.71)	2(22.22)	
Ketoconazole	2(5.71)	0	
Antibiotics	14(40.00)	4(28.57)	
Herbs	4(11.43)	1(25.00)	
Health care provider (n = 35)*			
Self	23(65.71)	6(26.09)	
Medical doctor	17(48.57)	6(35.29)	
Nurse	11(31.43)	3(27.27)	
Patent medicine dealer	6(17.14)	0	
Care of the genital (n = 82)			**0.410**
Clean genital with water only	13(15.85)	3(23.08)	
Clean genital with soap and water	69(84.15)	24(34.78)	
Douching (n = 69)			**0.168**
Yes	66(95.65)	26(39.39)	
No	3(4.35)	0	
Wear tight-fitting underwear (n = 70)			**0.354**
Yes	34(48.57)	15(44.12)	
No	36(51.43)	12(33.33)	
Frequency of wearing "tight" (n = 34)			**0.213**
Whenever away from home	26(76.47)	10(38.46)	
Every time	5(14.71)	4(80.00)	
Afternoon only	3(8.82)	1(33.33)	
Oral hygiene (n = 88)			**0.934**
Use chewing stick	11(12.50)	2(18.18)	
Use tooth brush and paste	22(25.00)	3(13.64)	
Use both	55(62.50)	9(16.36)	

** Significance set at P <0.05 **Respondents were allowed to choose more than one treatment option*

Prevalence of *Candida* species and hygienic habits indicated that respondents who cleaned their genital parts with soap and water had a higher *Candida* prevalence 24 (34.78%) than those who cleaned with water only 3 (23.08%). Respondents who douched were colonized with *Candida* 26 (39.39%) while those who did not were not colonized. Species of *Candida* were significantly associated with textile material of the undergarment (P = 0.044). A high *Candida* prevalence 11 (52.38%) was observed among respondents who did not know the stuff or material of their undergarment, followed by those who wore nylon undergarments 4 (40%) but was low among respondents who wear cotton pants 11 (28.21%) and those who did not wear undergarment (Fig. 3). Respondents who use either chewing stick, tooth brush, or both were similarly colonized by *Candida* species in their oral cavity (Table 6).

4. DISCUSSION

In this study, *Candida* species was found only in female genitourinary tracts and oral cavities of both males and females. *Candida* species has been described as normal commensals of oral cavities of both males and females. Colonization or infection of the genitourinary tracts by *Candida* species is a common problem among females. A study in Nigeria also failed to isolate *Candida* species from urethral swab of 47 male participants but reported its occurrence in genital samples from females [41]. The presence of *Candida* species in male genitourinary tract has been regarded as sexually transmitted, as in balanitis [6]. In this study, the disparity observed in the ratio of male to female participants is a reflection of men's poor attitude toward visiting their doctor for oral or genitourinary health. They hide their illness and only visit the doctor when

their life is in danger. The observed 7(50.0%) and 27(67.50%) of male participants with symptoms of genital and oral infection respectively when compared to that of females {symptom of genital infection 90(69.23%); symptom of oral infection 38(33.33%)} showed that both sexes had need for oral and genitourinary healthcare. However, the non-isolation of *Candida* species from men with complaint of genital infection is an indication that other microorganisms might be responsible for the infection.

C. albicans was the most common *Candida* species from the oral route (72.72%) while the non-*albicans Candida* species dominated in the genitourinary route (62.12%). The report that *Candida albicans* predominated in oral cavities in this study agreed with the 45% report from Nigeria [18] and 59% from other parts of the world [14]. The low prevalence of *C. albicans* (37.88%) in the genitourinary tract observed in this study (Fig. 1) has also been reported by Okungbowa [34] who indicated a prevalence of 20.1%. Mohanty [42] also reported a high prevalence of non-*albicans Candida* species from genital tract (64.8%) while Chong [43] and Lopes-Consolaro [44] reported a prevalence of 21.9% and 40% respectively. On the contrary, Alli [41] and Akortha [45] reported a high prevalence of *C. albicans* (60%) and (63.9%) respectively in Nigeria. The increasing detection of non-*albicans Candida* species could be related to the widespread and inappropriate use of antimycotic drugs especially in treatment of presumed genital candidiasis. This present result indicates the emergence of *Candida* species other than *C. albicans* in the genitourinary tract and reinforces the need for appropriate diagnosis in cases of oral or genitourinary infection.

The result of this study revealed that age influenced the prevalence of *Candida* species in the oral cavity but not in the genitourinary tract. The age group "50 years and above" had the highest frequency of occurrence of *Candida* species (88.89%) while the age group "10-19 years" had the least (9.52%). Lockhart [46] reported that oral candidal colonization in healthy human increases as a function of age especially in elderly subjects who were over 60 years of age. It is generally known that prevalence of *Candida* species in adults' mouths increases with age but it is not clear that old age alone is a predisposing cause of *Candida* infection. Odds [47] suggested that increased frequency of illness and medical therapies associated with senescence were more likely predisposing factors than old age itself. Moreover, [35] reported that no significant differences were noted in the incidence of *Candida* species between middle aged subjects (35-44 years) and the elderly (56-70 years) but a significant difference was observed between elderly subgroup aged 56-70 years (35%) and advanced age subgroup 71-92 years (74%).

Age did not influence the prevalence of *Candida* species in the genitourinary tract. The highest frequency of isolation of *Candida* species from genitourinary tracts occurred among individuals within "20-29 years" (45.83%), closely followed by individuals within "30-39 years" (42.11%); "10-19 years" (35.90%) and ''40 years and above'' (20.00%) but the observed differences were not statistically significant (p=0.612). Sexual activity, drug abuse, and child bearing have been suggested to influence occurrence of *Candida* species [29,34]. Mathema [30] did not find any significant relationship between age and colonization by *Candida* species among college age women with previous exposure to over-the-counter azole antifungals. Parveen [48] also did not find significant relationship between age and genital candidal colonization among pregnant women in their study. However, the result of this present study is different from that of Enweani [29] and Okungbowa [34] who reported that age has a significant relationship with the prevalence of *Candida* in the genitourinary tract. Enweani [29] reported that highest incidence of *Candida* colonization in the genitourinary tract occurred among age group less than 20 while Okungbowa [34] detected highest occurrence among age group 20 – 30 years. The age group 20-49 years was reported by Jombo [49], an age group that coincided with the period of highest sexual activity. Dou [36] identified age as a factor in genital *Candida* infection, but the present study focused on colonization.

In this study, 65.75% of respondents indulged in self-medication and use of doctor's previous drug prescription. This practice has been reported in Finland [50] where women also use antifungal drugs without having a physician-diagnosed *Candida* infection. The availability of drugs as over-the-counter (OTC) has promoted self-medication in Nigeria and other countries. Self-diagnosis and self-medication may result in delayed proper treatment. Infections due to non-*Candida albicans* are increasing. Thus, OTC antifungal drug like imidazole known to suppress *C. albicans* may facilitate the overgrowth of non-

albicans *Candida* species against which imidazole is ineffective [50]. Signs and symptoms have limited value in diagnosis of vaginal infection [51]. There is need to educate women especially on the dangers of self-medication, and benefits of prompt diagnosis and treatment of genitourinary infections.

This study also showed that the textile material of which the undergarment is made of, influenced genital *Candida* colonization. Subjects who wear nylon pants had higher prevalence rate than subjects who wear cotton pants (Fig. 3). This finding has also been reported by Elegbe [39] whose report indicated that nylon underwear create an environment favorable to *Candida* species colonization. Furthermore, wearing of tight-fitting underwear did not influence colonization by *Candida* species. This finding disagrees with that of Elegbe [39] who also reported that *Candida* colony counts were reported to be far higher in patients with vaginitis wearing tight-fitting clothing than in patients wearing loose fitting clothing. In this present study, the result is for both asymptomatic individuals and symptomatic patients.

Candida species prevalence among subjects who douche (39.39%) and those who did not douche was statistically not different which suggests that douching did not influence *Candida* prevalence in the female genitourinary tracts. A study conducted by Shaaban [52] also showed that the vaginal douching did not significantly influence the possibility of having candidal growth. However, Corsello [53] and Heng [54] indicated that douching increases the risk of vulvovaginal candidiasis. Ugwa [55] also reported that douching was the most common risk factor responsible for vulvovaginal prevalence occurring in 42.6% of patient. The observed differences in report might be due to disparity in douching frequency and use of douching products which might exhibit varying effect on C*andida* species in women [56].

The use of toothbrush or chewing stick in cleaning the mouth did not influence *Candida* species occurrence in the oral tract. This showed that the use of toothpaste or chewing stick effectively keeps the mouth clean. The present report is consistent with the findings of Darwazeh [57] who recommended the use of chewing stick as an oral hygiene tool for health promotion in developing countries. Chewing stick is used in Asia, Africa and Middle East for dental hygiene with strong antimicrobial properties [58,59]. However, the research by Ogundiya [60] showed that the extracts of some chewing stick had no activity against *Candida albicans*.

5. CONCLUSION

Age of the subjects was found to influence prevalence of *Candida* species in the oral route but not in the genitourinary route. The textile material of the undergarment influenced the prevalence of *Candida* species in the genitourinary tract. As such, the public should be educated on the factors that influence *Candida* species occurrence in the oral cavity and genitourinary tracts as well as the clinical implications of improper use of antibiotics/drugs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All participants in the study gave verbal consent after explaining the benefits of the study. The management of the hospitals or laboratories where clinical samples were collected and cultured also gave their verbal approval after receiving a letter of introduction for the study. They include: Bishop Shanahan Hospital; Akulue Memorial Hospital; Saint Anthony Hospital; All Saints Medical Center; University Medical Center; Safety Medical Diagnostic Laboratory; Saint Anthony Medical Diagnostic Laboratory; Kenol Medical Diagnostic Laboratory. All in Nsukka, Enugu State, Nigeria. Participation was voluntary and informed consent was obtained from the participants or their parents (in case of children).

ETHICAL APPROVAL

The study was approved by the ethics and biosafety committee of the Faculty of Biological Sciences, University of Nigeria Nsukka with reference number unn/fbs/ec/1030. Verbal approval was also obtained from the hospital/clinic management after consideration of the proposal for the study.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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