

FREQUENCY OF BACTERIURIA AND CANDIDURIA IN CHILDREN ATTENDING PRIMARY SCHOOLS IN AMASSOMA, NIGER DELTA REGION, NIGERIA

¹OLUWAYEMISI* A. Olorode ²OFONIME M. OGBA AND ³EGEIN B. BEINMOIPRE

¹Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

²Department of Medical and Allied Sciences, University of Calabar, Nigeria

³Department of Pharmaceutical Microbiology and Biotechnology, Niger Delta University, Nigeria

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Abstract – Candida co-infections with bacteria have become a public health interest. Can this occur in Children? This study was conducted between March and November, 2017 to determine the frequency of bacteriuria and candiduria of apparently healthy children of different age group and sex attending primary schools in Amasoma, Bayelsa State, Nigeria. A total of 142 primary school children Mid Stream Urine (MSU) were collected in sterile bottles and immediately taken to the laboratory in ice packed for culture using selective media, Sabouraud Dextrose Agar (SDA), Mannitol Salt Agar (MSA) and the pure isolate characterized and identified on the basis of their cultural and biochemical properties was standardized using 0.5 Mc Farland standard of Turbidity. Antibiotics susceptibility of pure standardized culture was evaluated by Kirby Buer method. The findings showed asymptomatic Bacteriuria and Candiduria in 91 (64.1%) and 97 (68.3%) samples respectively; the subjects who had Staphylococcal co-infection with Candidiasis were 75 cases (52.8%), with female (ages 5 to 12) preponderance over males. The total number microorganisms isolated were two hundred and seventy-eight (278) these include *Staphylococcus aureus* (58, 20.8%), *Candida glabrata* (39, 14%), *Staphylococcus epidermidis* (37, 13.3%), *Candida albicans* (19, 6.8%); *Candida tropicalis* (14, 5%); *Bacillus* species (12, 4.3%); *Candida parapsilosis* (9, 3.2%); *Klebsiella* species (4, 1.4%), other *Staphylococcus* species was (27, 9.7%) and unidentified *Candida* sp. (53, 18.9%). All the *Staphylococcus* species isolated were highly susceptible to Levofloxacin (97.4%); Gentamycin (93.2%); Ciprofloxacin (93.1%); Streptomycin (92.6%); moderately susceptible to Rifampicin (71.2%); least susceptible to Erythromycin (58.9%), but resistant to Chloramphenicol (14.6%). *Bacillus* sp. and *Enterococcus* sp. were susceptible to Levofloxacin (100%); Streptomycin (100%); Ciprofloxacin (97%); Gentamycin (93%); Rifampicin (91.7%), but resistant to Chloramphenicol (16.7%). All *Candida* isolates were most susceptible to Fluconazole (87.9%) followed by Nystatin (71%) and moderately susceptible to Ketoconazole (68%), but resistant to Itraconazole (8.1%). There is no significance difference between the male and female infected at $p = 0.05$. This study has shown that co-infection of Staphylococcal and *Candida* is prevalent in Amassoma Community primary schools Bayelsa State.

INTRODUCTION

Candida species especially *Candida albicans* are normal flora of human's body, but when there is a depletion in the hormones, changes of diet or an impact on the immune machineries, the organisms can cause disease which is commonly known as yeast or fungi infections. Among these infections is thrush caused by *Candida*, which affects warm,

moist regions like mouths, genitals and occasionally the skin surface. *Candida* infections have become a public health interest due to its spread among the people and the medical researchers are considerably looking for a strategic technique of combating the disease Bamford *et al.*, 2009. *Candida* species have the ability to infect the blood and organs causing Candidemia, which is common among the older and weakened immune people Abad, 2009. In

apparently healthy adults, less than 1% of yeast can be detected in their urine samples and 5% to 10% among the hospitalized patients especially those with indwelling catheters insertion due to bladder dysfunction and diabetes people. New born babies are also prone to candida infections. Candida species can co-infect with different bacteria in the human body Jenkinson, 2011. Majority of the infected people are asymptomatic, while the organisms are detected in their urine sample, but people expressing the candida infections find it difficult to distinguish this infection from bacterial infection. *Staphylococcus aureus* known as golden Staph is a facultative anaerobic, gram positive coccid bacterium, a member of firmicutes normally inhabit the respiratory tract and the human skin. *Staphylococcus aureus* is not always pathogenic, but it can cause skin infections of the respiratory tract like sinusitis and food poisoning by producing potent protein toxins and expressing cell surface proteins that bind and inactivate antibodies. *Staph aureus* has the ability to cause many infections ranging from tissues when the skin or mucosal barriers have been breached. This eventually leads to boils and carbuncles (a collection of boils). The infection of *Staph aureus* can proliferate when in close association with pus from an infected person or her formites, and result into severe infections. Strains of *Staph aureus* can host phages such as Q-PVL producing (panton- valentine leucocidin) that increase virulence Bays and Anadeto, 2010. The ability of *Staph aureus* to cause disease lies in its virulence factors which include – enzymes (coagulase, hyaluronidase, deoxyribonuclease, lipase, staphylokinase, beta-lactamase Toxins depending on the strain; secretion of several exotoxins which are associated with specific diseases which include superantigens, exfoliative toxins, alpha toxins, beta toxin, delta toxins, panton-valentine leucocidin (PVL)- component toxin, this latter is associated with severe necrotizing pneumonia in children; and production of some pigment known as Staphyloxanthin- a golden coloured carotenoid pigment Talha, 2013).

Candida infection of the prostate gland, the cause of candiduria, normally expresses with the discomfort of the lower abdomen and sexual dysfunction. There is a shift in the study of candiduria from *Candida albicans* being the sole causative agent to non – *Candida albicans* taking about 50% or more of urinary isolates. Their presence in the urine samples can lead to morbidity

and mortality especially in patients in intensive care units (ICU) Abad, 2009. Though candiduria may not directly associate with mortality, it can be a marker for serious infection. Candida species have the ability to adhere to the uroepithelium of both the upper and lower urinary tract especially *Candida albicans* with four recognition systems: cell surface manno-proteins which recognize and adhere to fucosyl; glycosaminylglycosides on the epithelial cells; additional mannoproteins (integrin –like receptors); and mannan, a complex polymer of mannose which binds to certain cellular surface proteins. Some candida cell walls proteins such ALs, Eapl and Hwpl, also extracellular matrix proteins such as collagen are used in attachment. Among the non- specific attachment mechanisms are surface hydrophobicity, electrostatic charge and Vander Waals forces help in docking with target cells in urine environment. *Candida glabrata* has been discovered to be the most common cause of fungal Urinary Tract Infection. Most human are colonized with candida species co-infection with *Staphylococcus aureus* and develop diseases due to these among which genito- urinary manifestations are extremely common. The forms of genitourinary candidiasis are distinct from each other and affect different populations which include neonates, immunocompromised patients, elderly and hospitalized. Despite its high incidence and clinical importance, genitourinary candidiasis is understudied Chuan *et al.*, 2014. This study is then undertaken to evaluate the prevalence of Candida species co-infected with Staphylococcus species in apparently healthy children attending elementary schools in Amassoma community.

MATERIALS AND METHODS

Study Location

This study was carried out at Niger Delta University, Amassoma Community Elementary Schools. Two (2) schools (Government and Private) were studied with the population of about 500 and 1000 pupils respectively. Amassoma community is an Island called Wilberforce Island with population of about 500,000, it is located in Southern Izon Local Government area of Bayelsa State, Niger Delta region of Nigeria. The major occupation of the inhabitants is farming.

Study Design

This study ran for 9 months from March to

November 2017. One hundred and forty two (142) urine samples were collected from apparently healthy pupils and their demographic data was obtained through their informed consent. Ethical approval was obtained from the pupil's parents and the head teachers.

Sample Collection

There was provision of questionnaire for the subjects to fill in their age and gender. The samples were collected at random into commercially available universal bottles. Only one bottle per subject and the samples were immediately taken to the laboratory for analysis using both Sabouraud Dextrose Agar (SDA), Mannitol Salt Agar (MSA) and Chromogenic agar. Pure microbial isolates were characterized and identified on the basis of their colonial morphology, gram staining, germ tube and biochemical tests. Antibiotic susceptibility testing was carried out using Kirby- Bauer disc diffusion method on Muller Hinton agar and interpreted accordingly based on National Committee for Clinical Laboratory Standard. The class of antibiotics used include Fluoroquinolone (Ciprofloxacin), Macrolides (Gentamycin), Miscellaneous group (Rifampicin), Chloramphenicol and Ampicillin. Antifungal include Ketoconazole, Itraconazole, Fluconazole and Nystatin.

RESULTS

Figure 3.0, 4.0 and 5.0 depicted the Pie Chart showing frequency distribution of Staphylococci species against Candida species; the frequency of various candida species; and frequency distribution of Staphylococcus species respectively. Figure 6.0, 8.0 and 9.0 show the Bar Chart frequency of infected

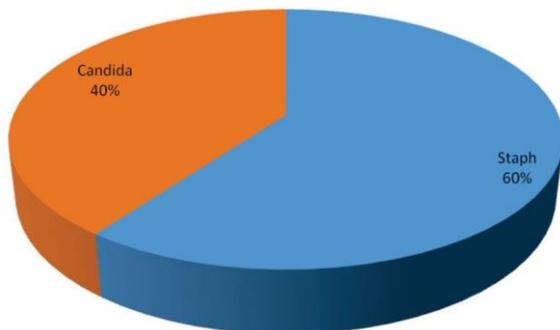


Fig. 3. Pie-chart showing frequency distribution of Staphylococci species against Candida species

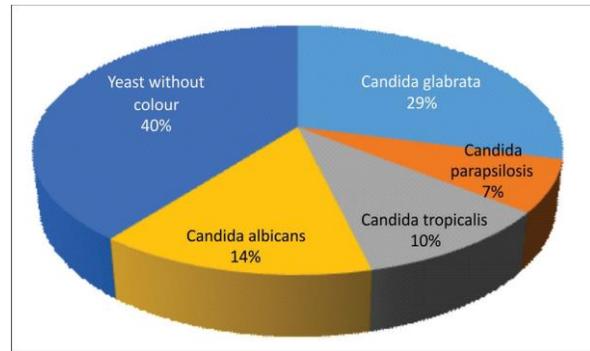


Fig. 4. Pie-chart showing the frequency of various candida species

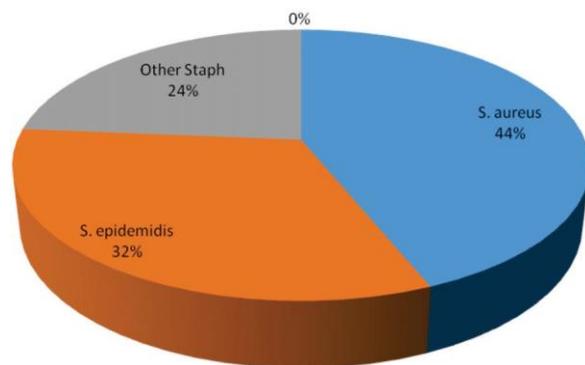


Fig. 5. Pie-chart showing the frequency distribution of Staphylococcus species Age (years)

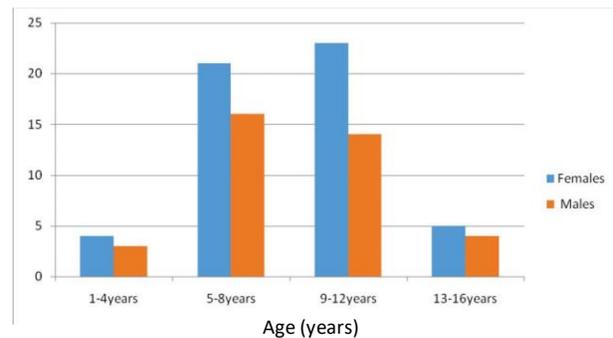


Fig. 6. Bar-chart showing frequency distribution of infected Females against Males subjects

females against male subjects; the percentage susceptibility test result of the various antifungal agents; and the bar chart showing the percentage Antibacteria susceptibility test result.

DISCUSSION

Candiduria and bacteriuria infections can be severe and it can lead to morbidity if it is not treated and if their balance with other organisms is distorted as in

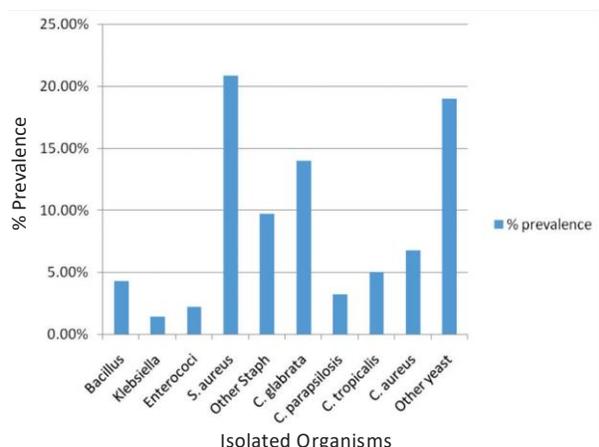


Fig. 7. Bar-chart showing percentage prevalence of the microbial isolates

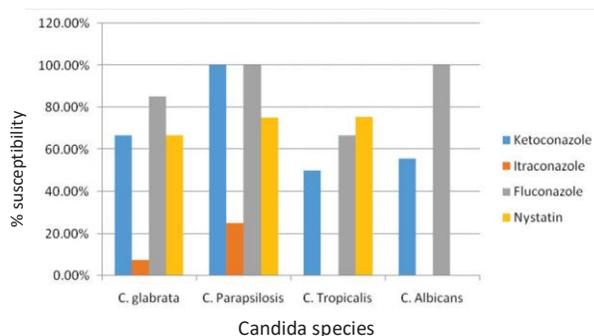


Fig. 8. Bar chart showing percentage susceptibility test result for various antifungal agents

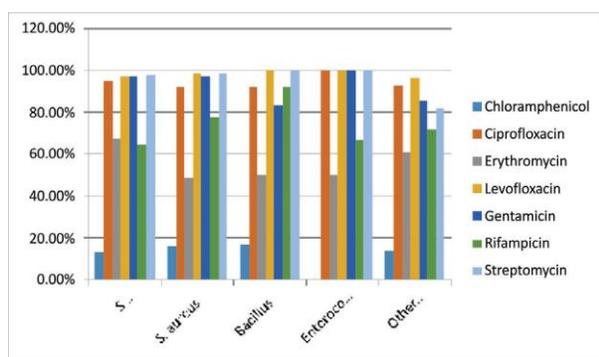


Fig. 9. Bar chart showing percentage Antibiotic susceptibility test result to Bacteria isolates

the case of *Candida albicans*. Out of 142 urine samples investigated for *Candiduria* and *Bacteriuria*, 91(64.1%) and 97 (68.3%) subjects were infected respectively. *Staphylococcal* and *Candida* coinfection occurred in 75 (52.8%) subjects with female having the dominating value of 53 (37.3%) over the males with incidence of 37 (26.1%), this finding is in accordance with the

work done by Bink *et al.* (2011). Out of Two hundred and Seventy- eight (278) microorganisms isolated from the test urine samples, *Candida* species were 81 (29.1%), Non- *Candida* species were 53 (19.1%), *Staphylococcus* species were 122 (43.9%), *Bacillus* species were 12 (4.3%), *Enterococcus* species were 6 (2.2%), *Klebsiella* species were 4 (1.4%). The higher percentage demonstrated by the female subjects could be due to anatomical proximity of anal and vagina openings with short urethra. The investigated pupils' standard of living could also be a contributing factor, the higher percentage of 51.4% use the public latrine while 48.6% use private convenience. The findings also revealed that *Staphylococcus aureus* had the highest prevalence of 43.9%, followed by *Candida* species 19.1%; *Bacillus* 4.3%; *Enterococcus* 2.2% and the least was *Klebsiella* with 1.4%. Out of the total number of *Bacteria* isolated (144), *Staphylococcus aureus* had the highest number of 58 (43.7%), followed by *Staphylococcus epidermidis* 37 (25.5%), *Bacillus* 12 (8.3%), *Enterococci* 6 (4.1%) and other *Staphylococcus* species 28 (19.3%). This shows that *Staphylococcus aureus* is more prevalent in primary schools in Amassoma community, Nigeria, this is in concordance with the work done by Bays and Anadeto (2010); Bink *et al.* (2011) which stated that the frequency of *Staphylococcus* in apparently healthy elementary school pupils is quite alarming, because about 30% of human beings harbor staphylococcus on their skin and this organism is a good indicator of the standard of hygiene during handling. The presence of *Bacillus* species could be due to the organism natural habitat which is soil and the study group is a play group, in which *Bacillus* could one way or the other get attached to their skin. The isolation of *Enterococcus* in these urine samples could be due to vancomycin resistant strains which can eventually lead to kidney failure.

All the microbial isolates were susceptible to all the test antibiotics except Chloramphenicol with cumulative percentage of 2.3%, this observation could be due to its misuse being the oldest antibiotic that most parents are exposed to and this may have led to organisms developing resistance to this drug. The percentage efficacy of the test antibacterial agents on the microbial isolates in this study is as follows: Chloramphenicol 16.7%, Ciprofloxacin 94.2%, Erythromycin 55.5%, Levofloxacin 98.4%, Gentamycin 92.6%, Rifampicin 74.4% and Streptomycin 95.6%. These findings show the most effective antibiotic to be Levofloxacin (98.4%)

followed by Streptomycin (95.6%), Ciprofloxacin 94.2%; Gentamycin 92.6%; Rifampicin 74.4%; Erythromycin 55.5% and the least is Chloramphenicol 16.7%, this is in correlation with the work done by Olorode *et al.* (2014). A total of 81 (60.4%) *Candida* species were isolated and 53 (39.5%) non-*Candida* species, giving an overall number of 134 species. Out of 81 (60.4%) *Candida* species isolated, *Candida glabrata* had the highest prevalence with 39 (29.1%), followed by *Candida albicans* 18 (14.2%), then by *Candida tropicalis* 14.0 (10.4%) and the least is *Candida parapsilosis* 9 (6.7%). The 53 (39.5%) of the isolates showed non-*Candida* fungi which include *Cryptococcus sp.*, *Aspergillus sp.* and *Penicillin sp.*, but less attention was paid on this since the scope of this study was on *Candida albicans* and non-*Candida albicans*. Among the four different antifungi agents used for susceptibility testing, Fluconazole was effective on *Candida albicans* and *Candida parapsilosis* with 100% efficacy, followed by *Candida glabrata* and *Candida tropicalis* of 55.2% and 66.7% respectively showing less potency of Fluconazole to non-*Candida albicans*. This is in concordance with Chuan *et al.* (2014) work which stated that fluconazole was the first-line therapy for candiduria in many hospitals, caused by *Candida albicans*, but non-*albicans* prevail in the urine and showed resistance to this first line drug. This means most candiduria infections are caused by non-*albicans* nowadays. He further stated that in Malaysia, there are several reports of non-*albicans* *Candida* overtaking *Candida albicans* as leading cause of Candiduria. Also in India Medical College of Vellore non-*albicans* candiduria was recorded as high as 81%. The second prevalent was *Candida albicans* 18 (14.2%), followed by *Candida tropicalis* 14.0 (10.4%) and the least is *Candida parapsilosis* 9 (6.7%). This is in concordance with Chuan *et al.* (2014) work which stated that a high prevalence of non-*albicans* species in urine is a concern for areas that employ fluconazole as first line therapy, because some species are intrinsically resistant to azoles and only susceptible to high doses such as *Candida tropicalis* and *Candida glabrata*. They stated further that persistent candiduria is also significantly higher with non-*albicans* *Candida*. Ketoconazole had 100% potency on *Candida parapsilosis* while Nystatin was more potent on *Candida tropicalis* with 75.6% efficacy, followed by *Candida parapsilosis* 75%; *Candida glabrata* and *Candida albicans* showed susceptibility of 66.7% to Nystatin. Itraconazole was not effective against all

the *Candida* species isolated in this study.

CONCLUSION

This study has revealed the prevalence of Staphylococcus co-infection with candidiasis in children attending elementary school in Amassoma Community. The frequency is not encouraging considering the fact that apparently healthy subjects were used in the study rather than those with clinical manifestations. The pupils between the ages of 5 and 12 had Staphylococcal co-infected with *Candida* species and can lead to Urinary dysfunction, infertility, septicaemia even meningitis in female if left untreated in future. *Candida glabrata* and Staphylococcus species were frequently isolated. Fluconazole was generally potent except to *Candida tropicalis*. Levofloxacin (fluoroquinolone) class had the highest efficacy on the isolated bacteria.

RECOMMENDATION

One of the leading causes of Candidiasis is over use of antibiotics which can lead to eradication of friendly bacteria that keep the balance between them and *Candida* species, therefore parents/wards should see health professional before administration of antibiotics. Balanced diet can prevent candidiasis therefore parents/wards should provide for their children good diet that will boost the immune system. Proper hygiene practice should be incorporated into the curriculum of primary schools since most of the isolated organisms in this study are abundant in the environment. Effective awareness should be conveyed to the students through Parent/Teachers Association meeting and Health Sciences should be taken seriously in primary schools. This will enlighten the pupils on how to guide themselves against these infections. Research work on candiduria in primary school pupils are still few in this part of the world; hence researchers should be encouraged to research in this area to safeguard the health of the children.

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