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YEASTS COLONIZATION OF LOWER RESPIRATORY TRACT INFECTION IN A TERTIARY HOSPITAL IN BENIN CITY, NIGERIA

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ABSTRACT
Yeast colonization of the lower respiratory tract is on the increase. This study aimed to determine prevalence of yeast lower respiratory tract colonization, speciate the recovered yeast isolates and determine their susceptibility profile. A total of 248 sputum specimens were collected from patients with signs and symptoms of lower respiratory tract infection attending the University of Benin Teaching Hospital, Benin City. The sputum specimens were processed to recover yeast isolates. The yeast isolates were identified and speciated using morphological, biochemical techniques as well as growth on CHROMagar ^{TM} candida. Susceptibility test was performed using agar diffusion method. The prevalence of yeasts colonization was 32.66%. Age and gender did not significantly affect the prevalence (P = 0.765) and (P = 0.1478) respectively. In – patients were significantly more at risk of yeast colonization (OR 9.127, 95% CI = 2.498, 33.350; P = 0.0003). The most predominant yeast species recovered was *Candida albicans* with a prevalence of 78.31%. *Saccharomyces cerevisiae (*2.41%) was recovered from both male and female patients. The polyenes were the most active antifungal agents, while the azoles were the least active Routine surveillance for the colonization of yeast in lower respiratory tract and prudent use of antifungal agents is advocated.

KEYWORDS: Yeast, lower respiratory tract.

INTRODUCTION
Lower respiratory tract infections (LRTI) are infections that affects the trachea, bronchi and lungs (Latha *et al.*, 2006). They range from tuberculosis, whooping cough to pneumonia (Latha *et al.*, 2006). Most pneumonia infections are caused by bacteria and opportunistic fungi (Abdulla and Yehia, 2012). In time past, *Aspergillus* species and *Histoplasma* species were the leading causes of fungal pneumonia followed by *Cryptococcus neoformans* and rarely *Candida* species (Jha *et al.*, 2006). Currently, *Candida* species are the most common causes of opportunistic mycosis worldwide. *Candida* pneumonia is known to be the most challenging of all candida infections (Tamai *et al.*, 2012). *Candida* pneumonia have two classical cases, candida primary pneumonia which is restricted to the lungs and the secondary candida pneumonia that involves the movement of invasive *candida* to other areas of the body (Sol, 2002). The increase in the rate of immunocompromised persons in the last two decades and the unregulated use of antimicrobial agents has increase the number of opportunistic infections caused by candida and other yeast species (Banesh and Kalyani, 2011). Candida species are normal commensal of human. They are capable of producing a wide variety of yeast infections. The ability to distinguish between colonization and infection can sometimes be challenging (Barenfanger *et al*., 2003). *Candida* species are generally known to be the most common causes of opportunistic mycosis worldwide, is a major cause of most endogenous infections due to it commensal nature (Abdulla and Yehia, 2012). Although, candida pneumonia is rare, it is associated with high mortality rate, high morbidity and poor patient outcome (Liu *et al.*, 2003). There is lack of robust clinical studies evaluating treatments and severe underlying disease in the patients. (Romani, 2008). Identifying candida to species level from sputum specimens has become mandatory to aid the clinicians in the selection of antifungal agents in the treatment of both opportunistic and invasive fungal infections. This is because most of the non–albicans species usually exhibit reduced susceptibility to the common antifungal agents (Liu *et al.*, 2003; Elena *et al*., 2006). Due to paucity of data in our environment, this study aimed to determine

the prevalence of yeasts colonization of the lower respiratory tract, speciation of the recovered yeast isolates and their susceptibility profiles.

MATERIALS AND METHODS

Study population: This study was carried out in the University of Benin Teaching Hospital Benin City, Nigeria. A total of two hundred and forty-eight (248) patients comprising of one hundred and twelve (112) males and one hundred and thirty-six (136) females with signs and symptoms of lower respiratory tract infection were recruited for this study. Informed consent was obtained from each patient prior to specimen collection. The Ethics and Research Committee of the University of Benin Teaching Hospital, Benin City, approved the protocol for the study.

Collection and Processing of specimens

Deep coughed out early morning sputum was collected into a sterile wide mouth container. A modification of the method described by Chessbrough, (2004) was used to process the sputum specimens. Briefly, the mucopurulent parts of the sputum were obtained with the aid of a sterile wire loop and inoculated onto sabourand dextrose agar containing 20 µg/ml of gentamicin and incubated at 37^0 C for 24 – 48 hours.

Wet mount: For direct microscopic examination, the sputum specimens was placed on the glass slide and a drop of 10% (KOH) potassium hydroxide was added and the preparation was covered with a coverslip and examined under X10 and X40 objective lens for yeast cells.

Gram stain: Smears were made from the sputum specimens on a clean grease free slide and then heat fixed by just passing the glass slide over the flame. The smears were stained by Grams staining method and then examined under X100 objective lens for the presence of Gram-positive oval yeast cells.

Identification and Speciation of yeast isolates

The yeast isolates were further identified by Germ tube test, Chlamydiospore formation on corn meal agar, sugar fermentation and assimilation tests as previously described by Amar *et al.*, (2013) as well as CHROMagar ™ candida (CHROMagar Paris, France) (Paritpokee *et al.*, 2005).

Antifungal susceptibility testing

Disc antifungal testing was performed on the yeast isolates using agar diffusion method as described by the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2009). The antifungal agents used were fluconazole (25 µg), ketoconazole (10 µg), amphotericin B (25 µg) and nystatin (100 units).

Statistical analysis

The data obtained were analysed with chi square (X^2) and odd ratio analysis test using the statistical software INSTAT$^{(R)}$ (Graph pad software Inc. La Jolla Ca, USA).

RESULTS

The prevalence of yeast colonization in the lower respiratory tract in this study was 32.66% with the male and female patients having a prevalence of 33.82% and 31.25% respectively. The prevalence of yeast colonization of the lower respiratory tract between male and female did not differ significantly (P = 0.765) as well as in relation to age P = (0.1478). In- patients were more infected than out - patients with a significant difference (P = 0.0003). Among the sputum specimens, 2 (1.79%) yielded mixed growth of *Candida albicans* and *Candida lusitanea* respectively from male patients (Table1). *Candida albicans* was the most predominant species recovered generally from both male and female patients with a prevalence of 65(78.31%) *Candida parapsiplosis* and *Candida krusei* were the least recovered isolates with a prevalence of 1.2% each (Table 2). The antifungal susceptibility profile showed that the polyenes (amphotericin B and nystatin) were the most active while the azoles (fluconazole and ketoconazole) were the least active against the non-*Candida albicans* (Table 3).

Table 1: Prevalence of yeasts infection in relation to Gender, Age and source of patients.

Characteristics	No. tested	No. infected (%)	OR	95% CI	P value
Gender					
Male	112	35(31.25)	0.889	0.521, 1.518	0.7687
Female	136	46(33.82)			
Mixed infections					
Male	112	2(1.79)	6.176	0.293,130.09	0.3946
Female	136	0(0.00)			
Age (years)					
≤ 1 – 10	14	6(42.86)			0.1478
11 – 20	18	8(44.40)			
21 – 30	35	15(42.86)			
31 – 40	45	19(42.20)			
41 – 50	29	7(24.14)			
≥ 51	107	28(26.17)			
Source of patients					

In – patients	15	12(80.00)	9.127	2.498,33.350	0.0003
Out – patients	233	71(30.47)			

OR = Odd ratio; CI = confidence interval

Table 2: Distribution of yeast isolates recovered from patients.

Yeast isolates	Male n (%)	Female n (%)	Total n (%)
Candida albicans	25(73.53)	40(81.63)	65(78.31)
Candia parapsilosis	1(2.94)	0(0.00)	1(1.20)
Candida tropicalis	5(14.71)	5(10.20)	10(12.04)
Candida lusitanae	2(5.88)	1(2.04)	3(3.61)
Candida krusei	0(0)	1(2.04)	1(1.20)
Saccharomyces cerevisae	1(2.94)	1(2.04)	2(2.41)
Total	34(40.96)	49(59.04)	83(100)

Table 3: Susceptibility profiles of the yeast isolates.

Yeast isolates	FCN n (%)	KCN n (%)	AMP n (%)	NYS n (%)
Candida albicans (n=66)	28(42.42)	33(50.00)	61(92.42)	63(95.45)
Candida krusei (n=1)	0(0.00)	0(0.00)	1(100.00)	1(100.00)
Candida lusitanae (n=3)	0(0.00)	1(33.33)	3(100.00)	3(100.00)
Candia parapsilosis (n=1)	(0.00)	(0.00)	1(100.00)	1(100.00)
Candida tropicalis (n=10)	(0.00)	(0.00)	10(100.00)	10(100.00)
Saccharomyces cerevisae (n=2)	1(100.00)	1(100.00)	1(100.00)	1(100.00)

Key
FCN = Fluconazole
KCN = Ketoconazole
AMP = Amphoterin B
NYS = Nystatin

DISCUSSION

The prevalence and identification of candida in lower respiratory tract infection is rare (Romani, 2008). Due to the increase in the number of immunocompromised persons, unregulated use of antimicrobial agents, increasing population of terminally ill persons and debilitated patients, there has been phenomenal rise in the occurrence of fungal lung infection over the last two decades (Chen *et al.*, 2001). However, there is paucity of data on candida lower respiratory tract infection in our environment. Against this background this study was conducted.

The prevalence of yeast infection in this study was 32.66%. This is higher than the 14.7% and 12.1% reported by Ogba *et al.*, (2013) and Jha *et al.*, (2006) respectively. The high prevalence reported in this study could be attributed to the fact that the yeast isolates may only represent colonization and not an infection. The criteria for the diagnosis of pulmonary candidiasis are still controversial, since candida is known to be a commensal of the lower respiratory tract, the definite diagnosis of pulmonary candidiasis still rest on histological demonstration of yeast in lung tissue with associated inflammatory changes (Diaz-fuentes *et al.*, 2007). The difference may be due to geographical location. Among children with diarrhoea, prevalence of infection varies with geographical location, regions within the same countries and even overtime in the same location and population (Petri *et al.*, 2008). The study of Jha *et al.*, (2006) was carried out in India while that of Ogba *et al.*, (2013) was carried out in Calabar, Nigeria among HIV/AIDS patients. Ours was carried out in Benin City, Nigeria among patients whose HIV/AIDS status were not known.

Mixed infections were observed in two of the sputum specimens 1.79% each. It is believed that most people usually have a single strain of candida in different parts of the body for a long period of time. However, some individuals have more than one species at the same time (Klotz *et al*., 2007).

The recovery of yeast isolates from sputum specimens was found to be highest among the age group of 11 – 20 years followed by the age groups of $\leq$ 1 – 10 years and 21 – 30 years the least was among the age group of 41 – 5 years. This does not agree with Jha *et al.*, (2006) and Phino *et al.*, (2002) who found the highest isolates of *Candida* species among the age group of 70 – 80 years. The finding that yeasts colonization of the lower respiratory tract's colonization was significantly associated with in – patients (OR 9.127, 95% C.I. = 2.498, 33.350; P = 0.0003) agrees with the norm that hospitalized patients consume a lot of antibiotics which may increase the chances of opportunistic yeast infections (Panwar and Faujder, 2016).

The predominant yeast in this study isolate recovered in this study is *Candida albicans (*78.31%). The non–ablicans species of candida recovered have been previously recovered from sputum specimens (St. German *et al.*, 2001). The prevalence of *Saccharomyces cerevisae* recovered in this study was 2.41% from both male and female patients. The consideration of *Saccharomyces cerevisae* as a health related or hospital acquired infection has been reported in literature (Perapoch, *et al.*, 2000; Salonen *et al.*, 2000; Lherm *et al.*, 2002; Cassone *et al.*, 2003 and Enache - Angoulvant and Hennequin, 2005).

Generally, the yeast isolates recovered in this study were more susceptible to polyenes (amphotericin B and nystatin) while the non – albicans species of candida were resistant to azoles (fluconazole and ketoconazole). This may be due to increased antifungal resistance of the non – albicans species of candida Liu *et al.*, 2003; and Elena *et al.*, 2006; Achkar and Fries, 2010).

Conclusively, the study reveals high prevalence of yeast colonization of the lower respiratory tract. In – patients were more at risk of yeasts colonization. *Candida albicans* was the most predominant yeast isolate recovered and the polyenes were the most active antifungal agents. Routine surveillance for the colonization of yeast in lower respiratory tract infections and prudent use of antifungal agent is advocated.

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