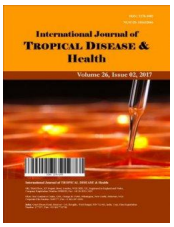

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Tuberculosis-*Candida* Co-Infection in Patients having Pulmonary Tuberculosis Attending DOTs Clinic in Rumuigbo Model Primary Health Centre in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASE and GNW managed the analyses of the study. Author GNW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Some pulmonary tuberculosis subjects were some co-infected with *Candida* such as *C. albicans*, *C. tropicalis*, *C. kruezi* and *C. paraspsilosis* which were initially thought to be normal floras of the oral cavity. The percentage of tuberculosis patients co-infected with *Candida* is becoming a concern and might complicate the treatment of tuberculosis.
Materials and Methods: A total of 400 sputum samples were collected and subjected to Ziehl-Neelsen staining technique and Genexpert system, Gram's stain, Germ tube test and examination in KOH preparation were conducted. Culture on Sabouraud Dextrose Agar with gentamicin, and cultured on CHROMagar *Candida* and sugar fermentation were carried out for *Candida*.
Results: Out of the 400 sputum samples examined 93(23.3%) had TB and 32(8.0%) were positive for *Candida*. By gender the prevalence of tuberculosis were females 51(22.4%), males 42(24.4%) while the prevalence of *Candida* were females 18(35.3%) and males 14(33.3%). The percentage

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occurrences of *Candida* sp. isolated were *C. albicans* which was the predominant species 10(21.5%), *C. tropicals* 5(5.4%), *C. krusei* 4(4.3%) and *C. Parapsilosis* 3(3.2%) respectively.
Conclusion: *Candida albicans* remains the most predominant species of *Candida* in patients suffering from pulmonary tuberculosis and colonization by *Candida* sp should not be ignored. The presence of *Candida sp.* might contribute to in one way to the progression of the disease.

Keywords: Tuberculosis; Candida; co-infection; Port Harcourt.

1. INTRODUCTION

Co-infection is the simultaneous infection of a host by more than one disease causing agent. The incidence of co-infection in human was unknown but it has been reasoned to be common [1] and even more common than single infection. Co-infection is of particular human health significance because the disease causing agents can interact within the host. The overall effect of it in humans is of course negative [2] but interactions may either have negative or positive effects on other parasites. In a positive parasite interaction, the spread and progression of disease are made stronger (enhanced) and this is also called synergism [3]. A globally common co-infection is that of tuberculosis and Human Immunodeficiency Virus (HIV) and of recent, tuberculosis and *Candida* co-infection. In some countries about 80% of tuberculosis patients are co-infected with HIV [4] and about 15-32% of tuberculosis patients are also co-infected with *Candida* sp. [5].

Other examples of co-infections apart from tuberculosis and candidiasis are acquired immunodeficiency syndrome (AIDS) which involves co-infection of end-stage HIV with opportunistic parasites and poly-microbial infections like lyme disease with other diseases [6]. Candidiasis can also spread to other parts of the body [7]. In spite of vaccination with BCG and the provision of highly potent drugs for the treatment of tuberculosis, there has been no constant reduction in the global burden. This is due to the fact that about 90-95% of new tuberculosis infections could become dormant and this explains the immunological poise between the pathogen and the host [8]. The combined action of *Candida* and tuberculosis have been studied, the altered respiratory commensals and its importance in pulmonary tuberculosis patients have been on the increase [9]. Though *Candida albicans* remains the most prominent organism in pulmonary candidiasis, other species of *Candida* have also been incriminated in pulmonary candidiasis and the rate of occurrence is on the increase [10].

One-third of the world population came down with infection due to *Mycobacterium tuberculosis* and about two million people died as a result of infection with this organism causing tuberculosis [4]. Tuberculosis is treated with antibiotics for months, this may enhance fungal growth. Immune-suppression by tuberculosis might cause the patients to be immune-compromised and as such become susceptible to fungal infections. *C. albicans* is found everywhere such as the hospital environment, in the air, on surfaces (floors and roofs) as well as in foods [10]. The burden of the tuberculosis in the form of active tuberculosis still continues at an alarming rate especially amongst the low and middle income countries with an estimated 580,000 new cases arising as a result of multiple drug resistance TB (MDR-TB) worldwide [11]. MDR-TB might aid colonization by *Candida* species.

Mycobacterium tuberculosis (Mtb) in the host arises as a result of its very powerful immune evading capability which permits it to persist indefinitely. In order to maintain its dormancy phase, Mtb manipulates toll-like receptors (TLR), cytokines and immune cell function [12]. Tuberculosis is an infection of lungs, but other parts of the body can also be affected. Latent tuberculosis in most cases are asymptomatic, becoming active with accompanying signs which includes chronic cough (not less than three (3) weeks) with blood containing sputum, sweating at night, fever, loss of appetite and weight loss. About 10% of the latent tuberculosis can progress to the active form of the disease if left untreated [11]. *Mycobacterium tuberculosis* (Mtb) in the host arises as a result of its very powerful immune evading capability which permits it to persist indefinitely. In order to maintain its dormancy phase, Mtb manipulates toll-like receptors (TLR), cytokines and immune cell function [12]. In spite of vaccination with BCG and the provision of highly potent drugs for the treatment of tuberculosis, there had been no minimal reduction in the global burden. The aim of this work is to determine the prevalence of *Candida* co-infection with tuberculosis in

pulmonary disease patients, isolate and identify the *Candida* sp. associated.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was conducted in Model Primary Health Centre, Rumuigbo in Obio/Akpor Local Government Area of Rivers State. The rationale behind choosing this health facility is because it serves as the focal point for all other health centre in the area for GeneXpert Rifampicin assay.

2.2 Study Population

The study population include pulmonary tuberculosis patients (male and females) attending Rumuigbo Model Primary Health Centre that were not HIV/AIDS positive. Samples of non-tuberculosis patients were used as negative control.

2.3 Ethical Consideration

Ethical approval was sought and obtained from Rivers State Hospitals Management Board. Participants were briefed on the objectives and procedure of the study and were assured of confidentiality. Consent forms were also given to participants for their consent and for patients below 18 yrs consent were sought from parent or guardian.

2.4 Sample Collection

A total of 400 sputum samples were obtained from patients attending Rumuigbo Model Primary Health Centre, Port Harcourt and analyzed for tuberculosis and candidiasis. Out of 400 samples 90% were diagnosed of TB before administration of drug.

2.5 Sampling Techniques

Clinical specimens comprising sputum samples were obtained from the Rumuigbo Model Primary Health Centre, Port Harcourt adopting the method used by Ndukwu et al. [12]. The samples were transported in cool box with ice parks to the laboratory and kept in the refrigerator at 4°C in case of delay. Statistical analyses were done using percentages and t-Student test with GraphPad Prism 5.03.

2.6 Sampling Analysis

2.6.1 Direct smear examination (Microscopy)

The criteria for candidiasis diagnosis is based on the presence of pus cells with budding yeast cells and pseudohyphae in direct Gram stained smear using the xl00 objective. The smear was fixed with alcohol to avoid over absorption of stains by *Candida* hyphae [13]. Colonies on Sabouraud agar plates were examined microscopically (wet mount preparation) using Xl0 and X40 objectives.

2.6.2 Germ tube test

A small portion of an 18-72 Hrs old culture of the yeast was suspended in 0.5 mL of human serum in a test-tube. Positive and negative control was set. All the test-tubes were incubated at (37°C) for 2-3 Hrs. A drop of the yeast suspension was placed on a clean glass slide and covered with a coverslipe and examined microscopically for presence or absence of germ tubes.

2.6.3 KOH Mount (KOH Preparation)

A drop of 20% w/v KOH solution was placed on a clean, grease free glass slide. Sputum sample was transferred to the drop of 20% w/v KOH solution and covered with a coverslipe. This was placed in a petridish with a damp piece of filter paper underneath to prevent the preparation from drying out. The preparation was examined microscopically using the X10 and X40 objectives with the condenser iris diaphragm closed sufficiently to give contrast as soon as the sputum sample was cleared.

2.6.4 Ziehl-Neelsen technique

A thin smear of the sputum sample was made on a clean grease free slide, allowed to air dry and heat- fixed by passing the slide 3 to 4 times through Bunsen burner flame. The slides were stained with strong Carbol Fuchsin on a staining rack. The underside was gently heated by passing flame under the staining rack until it fumed without allowing it to boil. Care was taken not to overheat. The slides were allowed to stand for 5 minutes, rinsed with water until no colour appeared in the effluent. Decolourization with 3% acid alcohol (HCl) was done until the slide appeared light pink colour, rinsed in water and then counterstained with methylene blue (0.5%) for 1-2 minutes. Slides were rinsed with water and allowed to air dry after wiping the back of the

slide and viewed with the xl00 objective microscopically.

2.7 Cultural Methods

These include streaking of specimens (sputa) on modified Sabouraud agar medium with gentamicin and incubating at 37°C for up to 72 hours. The Gentamicin inhibits bacterial growth. Colonies of *Candida* species isolated were cultured on CHROMagar *Candida* for identification.

2.7.1 CHROMagar *Candida* culture method

This medium is used to identify *Candida* organisms to species level. Colonies formed on the Sabouraud-dextrose agar were cultured on the medium and incubated at 37°C for 48 hours. Identification of colonies was based on the colour produced by the *Candida* sp. Light green colonies *Candida albicans*; *Candida tropicalis* blue; *Candida krusei* pink; and *Candida parapsilosis* cream. CHROMagar *candida* is very useful in the rapid identification of *Candida* sp. It is also useful in identifying dual infections and makes identification less-cumbersome than the conventional cultural methods. Significant counts were based on Kahampaa's criteria [14].

2.8 GeneXpert Test (Molecular Assay)

Sputum samples were collected from suspected TB patients. Sputum was liquefied and inactivated with 2:1 sample reagent. 2 ml of material was transferred into test cartridge. Cartridge inserted into MTB/RIF test platform (end of hand on work). Sample automatically filtered and washed. Ultrasonic lysis of filter captured organisms to release DNA. DNA molecules mixed with dry PCR reagents. Semi-nested real-time amplification and detection in integrated reaction tube Printable result (Shear and Perween, 2004).

2.9 Sugar Fermentation Tests

Basal medium (peptone water) containing a single carbohydrate source such as glucose, lactose, sucrose and the other carbohydrates were used. A pH indicator (phenol red) was also present in the medium. It detects the lowering of the pH as a result of acid production. Durham's tubes were also immersed in the medium to detect the production of gas.

3. RESULTS

3.1 Prevalence of TB and *Candida* Co-Infection among Male and Female Patients

The overall prevalence of tuberculosis and *Candida* co-infection among the 400 patients attending directly observed treatment centre were; tuberculosis 93 (23.3%) and *Candida* 32 (8.0%) respectively.

Out of 288 female examined, 51 (22.4%) had tuberculosis and 18 (7.9%) had TB/*Candida* co-infection, while out of 172 males examined, 42 (24.4%) had TB and 14 (8.1%) had TB/*Candida* co-infection.

3.2 Prevalence of *Candida* among TB Patients by Gender

The prevalence of *Candida* among pulmonary TB patients by gender showed that out of 51 patients that had TB, 18 (35.3%) had *Candida* co-infection, while among 42 males that had TB, 14 (33.3%) had *Candida* co-infection. The overall TB/*Candida* co-infections from 93 TB subjects were 32 (34.4%) respectively.

3.3 The Prevalence of *Candida* Species among TB Patients

The prevalence of *Candida* sp. Isolated from TB patients showed that *Candida albicans* were 20 (21.5%), *Candida tropicalis* 5 (5.4%), *Candida krusei* 4 (4.3%) and *Candida parapsilosis* 3 (3.2%) respectively. *Candida albicans* were the most prominent species associated with TB/*Candida* co-infections.

3.4 The Prevalence of *Candida* sp. by Gender

Sex prevalence of *Candida* among TB patients showed female had prevalence of 12 (12.9%) for

Table 1. Prevalence of TB and *Candida* co-infection among patients

Gender	Number examined	Number positive TB	Number positive *Candida*
Female	228	51 (22.4)	18 (7.9)
Male	172	42 (24.4)	14 (8.1)
Total	400	93 (23.3)	32 (8.0)

Numbers in parenthesis = percentages

Table 2. Gender prevalence of *Candida* co-infection among TB patients

Gender	Number examined	Number positive for *Candida*	Percentage
Female	51	18	35.3
Male	42	14	33.3
Total	93	32	34.4

Table 3. Prevalence of *Candida* sp. isolated from TB patients

Candida sp.	Number. Isolated	Percentage
Candida albicans	20	21.5
Candida tropicalis	5	5.4
Candida krusei	4	4.3
Candida parapsilosis	3	3.2
Total	32	34.5

Table 4. Prevalence of *Candida* species among TB patients

Candida species	Male (N=42)	Female (N=53)	Total
Candida albicans	8 (8.6)	12 (12.9)	20 (21.5)
Candida tropicalis	2 (2.2)	3 (3.2)	5 (5.4)
Candida krusei	2 (2.2)	2 (2.2)	4 (4.3)
Candida parapsilosis	2 (2.2)	1 (1.1)	3 (3.2)
Total	14 (8.1)	18 (7.9)	32 (34.5)

Numbers in parenthesis = percentages

Table 5. Identification of isolated *Candida* species

Gram's RXN	KOH	Germ tube	Colour on chrome agar	Glucose	Maltose	Sucrose	Lactose	Galactose	Xylose	Inositol	*Candida* sp.
+	+	+	Light green	+	+	+	-	+	+	-	***Candida albicans***
+	-	-	Blue	+	+	+	-	+	+	-	***Candida tropicalis***
+	-	-	Pink	+	-	-	-	-	-	-	***Candida krusei***
+	-	-	Cream	+	+	+	-	+	+	-	***Candida parapsilosis***

Candida albicans, 3 (3.2%) of *Candida tropicalis,* 2 (2.2%) of *Candida kruei* and 1 (1.1%) of *Candida parapsilosis* respectively. Males had *Candida albicans* of 8 (8.6%), 2 (2.2%) of *Candida tropicalis,* 2 (2.2%) of *Candida krusei* and 2 (2.2%) of *Candida parapsilosis* respectively.

4. DISCUSSION

Globally pulmonary tuberculosis infectious disease remains a threat. Respiratory fungal infection is among the emerging cases complicating tuberculosis which has been recognized for its wide range of clinical spectrum and unremitting nature. Among respiratory fungal infections, infections caused by *Candida* species are the commonest fungal agents isolated from sputa specimens of pulmonary tuberculosis patients. In this study, 34.5% of pulmonary tuberculosis patients were co-infected with *Candida* species in a similar study by [13] they had a prevalence of 25.3% tuberculosis co-infection with *Candida* species. The increase in prevalence 10.2% may suggest increase in rate of TB/*Candida* co-infection which should not be overlooked in the treatment of tuberculosis. Although some consider *Candida* as part of the normal microbial flora of the throats in about 32.5% of healthy individuals, the sputum produced may have been contaminated with throat the *Candida* species [15]. The Kahampaa criteria suggested 3 or more reported isolates of *Candida* or more than 30 colonies of *Candida* on

sabouraud dextrose agar should be considered pathogenic [13] and pseudo-mycelial forms in sputum microscopy were more suggestive of infections rather than colonization [16].

The prevalence of *Candida* co-infection in pulmonary tuberculosis subjects as observed in this study was also in accordance with the research of [12] that had a prevalence of 40% TB/*Candida* co-infection. In this study *Candida* infections were higher in females 19.4% than in males 15.1%. There might have been vaginal oral transmission in females with vulvovagninal candidiasis which is caused by *Candida albicans* and the fungus may establish easily due to the prevailing disease conditions and effects of administered drug or drugs for the treatment of tuberculosis. The result was in agreement with the finding by [12] who observed that *Candida* co-infection was significantly higher among female patients 62.5% compared to male patients 29.4%. This result is contrary to that of Mathavi [17] which showed colonization rates of *Candida* sp. to be equal in males and females. Latha [18] found *Candida* infection to be more in males than in females. Similarly Kahampaa [13] observed that *Candida* infections were significantly higher in males 26.1% than in females 23.1%. The high prevalence in the females may be attributed to the fact that more women seek for medical attention than males and the increase in outdoor and social activities observed in among females in recent times. Using Chi-square for comparison of males and females, there was no significant difference (p=0.7457000).

Among the *Candida* species isolated *C. albicans* was the predominant species with a prevalence of 21.5%. In a similar research Ndukwu [12] and [14] reported *Candida albicans* as most predominant species with percentage occurrences as 50% and 66% respectively. It was noted that the prevalence of TB-*Candida* co-infection ranges from 45 – 92% in several studies in India [19]. In the above study the prevalence of *Candida* species isolated were 61.8%, 14.7%, 14.7% and 8.8% for *C. albicans, C. tropicalis, C. krusei* and *C. parapsilosis* respectively [19]. In Port Harcourt Nigeria [12] reported *C. albicans* 15.1%, and *C. Tropicalis* 8.4%. Similar studies [20] observed *C. tropicalis* 3.25%, *C. parapsilosis* 3.25%. Baradkar [19] documented the prevalence of *C. tropicalis* 19.95%, *C. glabrata* 16.54%, *C. parapsilosis* 13.14% and *C. krusei* 5.10%. The variations observed might be attributed to differences in local prevalence of different species due to different environmental conditions and detection methods employed. Aside *C. albicans* species, the prevalence of *Candida tropical* also correlates with findings of a other study by Ija [21]. The Comparing of the different species of *Candida* using t-Student test showed that there was significant difference at (p=0.0001) among the different species of *Candida* isolated.

5. CONCLUSION

Candida albicans is the most predominant species of *Candida* co-infecting patients with pulmonary tuberculosis as compared to other *Candida* sp. Their presence might be encouraged by reduced immune status in the patients caused by *Mycobacterium tuberculosis* and or the effects of chemotherapeutic agents administered.

CONSENT

Consent forms were also given to participants for their consent and for patients below 18 yrs consent were sought from parent or guardian.

ETHICAL APPROVAL

Ethical approval was sought and obtained from Rivers State Hospitals Management Board. Participants were briefed on the objectives and procedure of the study and were assured of confidentiality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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