Candida Co-Infection with *Mycobacterium tuberculosis* in Tuberculosis Patients and Antifungal Susceptibility of the Isolates

Smart Enoch Amala*, **Asikiya Hanson, Gloria N. Wokem**

Department of Medical Laboratory Science, Rivers State University Port Harcourt, Rivers State, Nigeria
Email: *amala.smart@ust.edu.ng

Abstract

It had been observed that tuberculosis (TB) subjects can be co-infected with *Candida* sp. which was previously assumed as normal flora of the oral cavity. *Candida* sp. might become an opportunistic pathogen in immune compromised individuals. *Candida* co-infection with *Mycobacterium tuberculosis* in TB patients might complicate underlying disease process in the lungs. **Materials and Methods**: A total of 400 sputum samples were collected from TB patients and examined using Ziehl-Neelsen staining technique and MDR/RIF Genexpert system for TB. Samples positive for *Mycobacterium tuberculosis* were cultured on Sabouraud Dextrose Agar with gentamicin and examined for the presence of budding yeast cells and pseudohyphae on Gram's stain. *Candida* sp. isolated from TB positive sputa were cultured on CHROMager *Candida* for identification to species level and subjected to antifungal susceptibility testing. **Results:** Out of 400 sputum samples examined for TB 93 (23.3%) were positive and 32 (34.4%) out of 93 TB positive cases were co-infected with *Candida* sp. *Candida albicans* was the most predominant species with a prevalence of 23 (67.6%), *C. tropicals* 4 (11.8%), *C. krusei* 4 (11.8%) and *C. parapsilosis* 3 (8.8%). One sample had dual infection. Female subjects had high prevalence (19.4%) than the male (15.7%). Age group 31 - 40 years had both high prevalence of TB 32.3% and *Candida* 25.0%. Antifungal susceptibility testing showed that isolated *Candida* sp. were more susceptible to vericonazole and fluconazole compared to nystatin. **Conclusions:** Tuberculosis weakens the immune systems of infected persons especially when prophylactic administration proves abortive or there is non-adherence to treatment prescriptions. This may cause the development of multidrug resistance TB. *Candida* sp. may utilize these opportunities to establish alongside *M. tuberculosis* and worsen treatment and patient condition. For good treatment of TB, *Candida* co-infection should be screened concomitantly with TB in TB suspected individuals.

1. Introduction

Co-infection is the simultaneous infection of a host with more than one disease causing agent. Worldwide, the prevalence or incidences of co-infection in the life of human were unknown but it is now known to be common [1] and even more common than single infection. A global common co-infection is that of tuberculosis and HIV and of recent, tuberculosis and *Candida*. In some countries up to about 80% of tuberculosis patients are co-infected with HIV [2] and about 15% - 32% of tuberculosis patients are also co-infected with *Candida* sp. [3]. AIDS involves co-infection of end-stage HIV with opportunistic parasites and poly-microbial infections like Lyme disease with other diseases [4]. Candidiasis is a fungal infection that can be caused by any species of *Candida*. It can spread to other parts of the body apart from the mouth and vagina which can result in fever and other symptoms depending on the site of infection [5]. Despite all efforts made globally, tuberculosis as an infectious disease remains a global threat. About 9.6 million people were diagnosed of having new cases of tuberculosis infection and about 1.5 million deaths were recorded as a result of this disease in 2014. Approximately 1.2 million (12%) of these cases were related to HIV infection [6]. A total of 1.5 million people died from TB in 2018 including 251,000 people living with HIV. TB is the major cause of death from a single infectious agent (above HIV/AIDS) and one of the top 10 causes of death worldwide [7]. The estimate in 2018 was that 10 million people were sick with tuberculosis (TB) worldwide, out of these 5.7 million men, 3.2 million women and 1.1 million children. The largest number of new TB cases occurred in the South-East Asian region, with 44% of new cases, followed by the African region, with 24% of new cases and the Western Pacific with 18%. The 30 high TB burden countries accounted for 87% of new TB cases. Eight countries account for about two thirds of the total in decreasing order, India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa [7].

Different kinds of pulmonary and extra-pulmonary infections might also aggravate factors associated with chronic obstructive pulmonary diseases such as diabetes in elderly patients, high cholesterol levels, lung cancer, immune deficiency and fungal infections of the lungs.

The rate of recurrence infections and the number of immunosuppressive disease cases are on the increase. It is of great importance that clinical research be carried out by medical health personnel into potential pathogenic secondary fungal diseases bearing in mind that it can influence the progression of tuberculosis infection which might be life threatening [8]. The occurrence of pulmonary tuberculosis co-infection cases with *Candida albicans* is about 15% - 32% in dif-

ferent studies. *C. albicans* have been isolated from different areas such as hospital environment, the air, on surfaces (floors and roofs) as well as in food [3]. The burden of active tuberculosis still continues at alarming rate especially amongst the low and middle income countries with estimated 580,000 new cases arising as a result of multiple drug resistance TB (MDR-TB) worldwide [6] and in 2018, 7.0 million new cases were notified [7]. The aim of this study were to determine the prevalence of tuberculosis, tuberculosis *Candida* co-infection, *Candida species* associated TB *Candida* co-infections and antifungal susceptibility of isolated *Candida* sp.

2. Materials and Methods

2.1. Study Area and Population

The study was conducted in the Model Primary Health Centre, Rumuigbo in Obio/Akpor Local Government Area of Rivers State. The choice of this health facility was because it is the centre for GeneXpert Rifampicin assay. The above Local Government Area covers 260 km with a population of 664,789. The target population were TB patients (male and females) attending Rumuigbo Model Primary Health Centre. Samples of non-tuberculosis patients were used as negative control. The studies were carried out from March, 2018 to May, 2019.

2.2. Sample Size

Sample size was determined using the formula of Araoye (2003)

$$n = \frac{z^2 pq}{d^2}$$

where, n = sample size minimum, z = proportion of the target population (1.96), $q = 1.0 - p$ and d = degree of accuracy (95% interval) − 0.05%, p = overall prevalence of TB in Nigeria.

$$n = 322.7 \approx 323$$

2.3. Direct Smear Examination by Microscopy

A total of 400 sputum samples were obtained from patients attending the Model Primary Health Centre and examined for TB and TB *Candida* co-infection. The presence of budding yeast cells and pseudohyphae in direct Gram's stained smear using the ×100 objective may aid diagnosis [3]. The smears were fixed with alcohol to avoid over absorption of stains by *Candida* hyphae if present [9]. *Candida* sp. grown on Sabouraud dextrose agar were examined by microscopy (wet mount) with ×10 and ×40 objectives.

2.4. KOH Mount (KOH Preparation)

A drop of 20% w/v KOH solution was placed on a clean, grease free glass slide. Sputum sample were added to the drop and covered with a cover slip. This was placed in petridish with a damp filter paper under to prevent drying. The prepa-

ration was examined microscopically using the ×10 and ×40 objectives with the condenser iris diaphragm closed sufficiently to give contrast after the digestion of sputum sample.

2.5. Ziehl-Neelsen Technique

A thin smear of each sputum sample was made on a clean grease free slide, allowed to air dry and heat-fixed by passing the slide 3 - 4 times through a Bunsen flame. The slides were stained with strong carbol-fuschsin on a staining rack. The slide was gently heated under by passing flame under the staining rack until steam rises without allowing it to boil. The slides were allowed to stand for 5 minutes, rinsed with water and decolourized with 20% H_2SO_4 until the slide appeared light pink in colour. This was 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 examined with oil immersion xl00 objective.

2.6. Germ Tube Production Test

A little portion of 18 - 72 hours old colony of yeast was suspended in 0.5mL of human serum in a test-tube. Positive and negative controls were set up. All the test-tubes were incubated at 37°C for 2 - 3 hours. A drop of the mixture of serum and yeast suspension were placed on a clean glass slide and covered with clean cover slip and examined microscopically for presence or absence of germ tube production.

2.7. Cultivation of *Candida* Sp.

The sputum specimens were cultured on modified Sabouraud-dextrose gentamicin agar medium and incubated at 37°C for up to 72 hours. Gentamicin inhibited bacterial growth [9]. Pure cultures of *Candida* sp. isolated were cultivated on CHROMagar *Candida*.

2.8. CHROMagar *Candida*

Culture on CHROMagar is used to identify *Candida* sp. Isolated yeasts sp. on Sabouraud-dextrose agar were also cultured on CHROMagar *Candida* and incubated at 37°C for 48 hours. Identification of *Candida* sp. on this agar was based on colour production. Light green colonies was *Candida albicans*, blue *Candida tropicalis*, pink *Candida krusei* and cream *Candida parapsilosis* [10], CHOR-Magar *candida* is very useful in the rapid identification of *Candida* sp.

2.9. GeneXpert Test (Molecular Assay)

Sputum samples obtained from suspected TB patients were liquefied and inactivated with 2:1 sample reagent. Two (2) mL of the liquefied sputum was transferred into test cartridge. The cartridge was inserted into MTB/RIF test plat (end of hand on work). Sample were 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 [11].

2.10. Sugar Fermentation Reactions

Peptone water containing a single carbohydrate (1.0%) such as glucose, lactose, sucrose and the other carbohydrates were used. A p^H indicator (phenol red) was added to the medium to detect change in pH as a result of acid production. Durham's tubes were also inserted to detect the production of gas. The isolated *Candida* sp. were inoculated into already prepared and sterilized set of sugars in test tubes. They were incubated at 37˚C for 18 - 48 hours and examined.

2.11. Susceptibility Testing

Each pure culture of *Candida sp*. was inoculated onto already prepared and dried iso-sensitive agar plate. The inoculated plate was spread evenly by streaking with sterile wire loop to obtain uniform seeding on the entire plate. Commercial antifungal single discs of the three (3) antifungal agents were placed on the inoculated plate with a sterile forceps. The plates were incubated at 37˚C for 18 - 48 hours and the zones of inhibitions were obtained.

2.12. Ethical Consideration and Sample Collection

Ethical approval was obtained from Rivers State Hospitals Management Board, participants were briefed on the objectives of the studies and confidentiality of each patient was assured.

2.13. Statistical Analysis

Statistical analysis was done using one-way Anova with the data generated.

3. Results

3.1. Prevalence of TB and TB *Candida* Co-Infection

A total of 400 sputum samples collected from suspected TB patients were examined for TB and candidaisis. Out of these 93 (23.3%) were positive for tuberculosis and 32 (34.4%) were positive for tuberculosis *Candida* co-infection as shown on Table 1.

3.2. The Prevalence TB and *Candida* Co-Infection by Gender

The prevalence of *Candida* among TB patients by gender showed that out of 51 females that had TB, 18 (19.4%) had TB *Candida* co-infection whereas out of 42 males with TB 14 (15.1%) had TB *Candida* co-infection respectively. The overall TB *Candida* co-infections from 93 TB subjects were 32 (34.4%). Numbers of females examined were 228 (57%) and males 172 (43%). The prevalence of TB among females was 51 (22.4%) and that of males was 42 (24.4%) as shown on Table 2.

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

Sample	No. Examined	No. Positive
Sputum for TB	400	93 (23.3)
TB *Candida* co-infection	93	32 (34.4)

Numbers in parenthesis = Percentages.

Table 2. Prevalence TB and *Candida* co-infection by gender.

Sex	Number Examined (TB)	Number Positive (TB)	Number Examined (*Candida*)	Number Positive (*Candida*)
Male	172	42 (24.4)	42	14 (15.1)
Female	228	51 (22.4)	51	18 (19.4)
Total	400	93 (23.3)	93	32 (34.5)

Numbers in parenthesis = Percentages.

3.3. The Prevalence of *Candida* sp. among TB Patients

Four species of *Candida* were isolated co-infecting with *M. tuberculosis*. Namely, *Candida albicans* 21 (65.6%), *C. tropicalis* 5 (15.6%), *C. krusei* 5 (15.6%), *C. parapsilosis* 3 (9.4%). Out of the three species, *C. parapsilosis* was isolated with *C. albicans* in particular case. *Candida albicans* was the most predominant species associated with TB *Candida* co-infection as shown on Table 3.

3.4. Prevalence of *Candida* sp. among TB Patients by Gender

The prevalence of *Candida* sp. among TB patients by gender showed that female subjects had a prevalence of 12 (66.7%) of *C. albicans*, *C. tropicalis* 3 (16.7%), *C. Krusei* 2 (11.1%), and C. *parapsilosis* 1 (5.6%) respectively, whereas among the males, *C. albicans*, 8 (57.1%), *C. tropicalis* 2 (14.3%), *C. Krusei* 2 (14.3%), and *C. parapsilosis* 2 (14.3%) as shown on Table 4 and the reactions of isolated *Candida* sp. on CHORMagar *candida* as shown in Figures 1(A)-(C) respectively.

3.5. Prevalence of *Candida* Species by Age Groups

The age groups with the high prevalence of *Candida* were between 31 - 40 yrs with a prevalence of 8 (25.0%). Age groups 41 - 50 and 51 - 60 yrs were 5 (15.5%) each and age group 61 - 70 yrs was 4 (12.5%). The other age groups were between 0.00% - 6.3% as shown on Table 5.

3.6. Antifungal Susceptibility Patterns of Isolated *Candida* sp.

Three (3) antifungal agents were used for susceptibility testing namely, voriconazole, fluconazole and nystatin. The *Candida* sp. were more susceptibility to voriconazole with 16 (80.0%), 3 (15.0%) were moderately susceptible and 1 (5.0%) was resistant. For fluconazole 14 (70.0%) were susceptible, 5 (25.0%) were moderately susceptible, while 1 (5.0%) was resistant. Nystatin 12 (60.0%) were susceptible, 6 (30.0%) were moderately sensitive and 2 (10.0%) were resistant as shown on Table 6.

Table 3. Prevalence of *Candida sp.* among TB patients.

Candida sp.	Number Isolated N = 32
Candida albicans	21 (65.6)
Candida tropicalis	5 (15.6)
Candida krusei	5 (15.6)
Candida parapsilosis	3 (9.4)

Numbers in Parenthesis = Percentages.

Table 4. Gender prevalence of *Candida* sp. among TB patients.

Candida Sp. isolated	Males	Females	Total
Candida albicans	8 (57.1)	12 (66.7)	21 (65.6)
Candida tropicalis	2 (14.3)	3 (16.7)	5 (15.6)
Candida krusei	2 (14.3)	2 (11.1)	4 (12.5)
Candida parapsilosis	2 (14.3)	1 (5.6)	3 (9.4)

Numbers in Parenthesis = Percentages.

Table 5. Prevalence of *Candida* sp. and TB by age groups.

Age groups (years)	Number of TB Positive Patients N = 93	Number infected *Candida* N = 32
1 - 10	5 (5.3)	1 (3.1)
11 - 20	17 (18.3)	2 (6.3)
21 - 30	15 (16.1)	6 (18.8)
31 - 40	30 (32.3)	8 (25.0)
41 - 50	12 (12.9)	5 (15.6)
51 - 60	8 (8.6)	5 (15.6)
61 - 70	5 (5.3)	4 (12.5)
71 - 80	0 (0.00)	0 (0.00)
81 - 90	0 (0.00)	0 (0.00)
91 - 100	1 (1.1)	1 (3.1)

Numbers in Parenthesis = Percentages.

Table 6. Antifungal susceptibility patterns of isolated *Candida* sp.

Antifungal Agent	Susceptibility Pattern	No. Susceptible	Percentages
Voriconazole	High +++	16 ± 1.00	80
	Moderate ++	3 ± 1.00	15
	Low +	1 ± 0.58	5
Fluconazole	High +++	14 ± 1.00	70
	Moderate ++	5 ± 1.00	25
	Low +	1 ± 0.58	5
Nystatin	High +++	12 ± 1.00	60
	Moderate ++	6 ± 1.00	30
	Low +	2 ± 1.00	10

(Plate A)

C. albicans and
C. parapsilosis

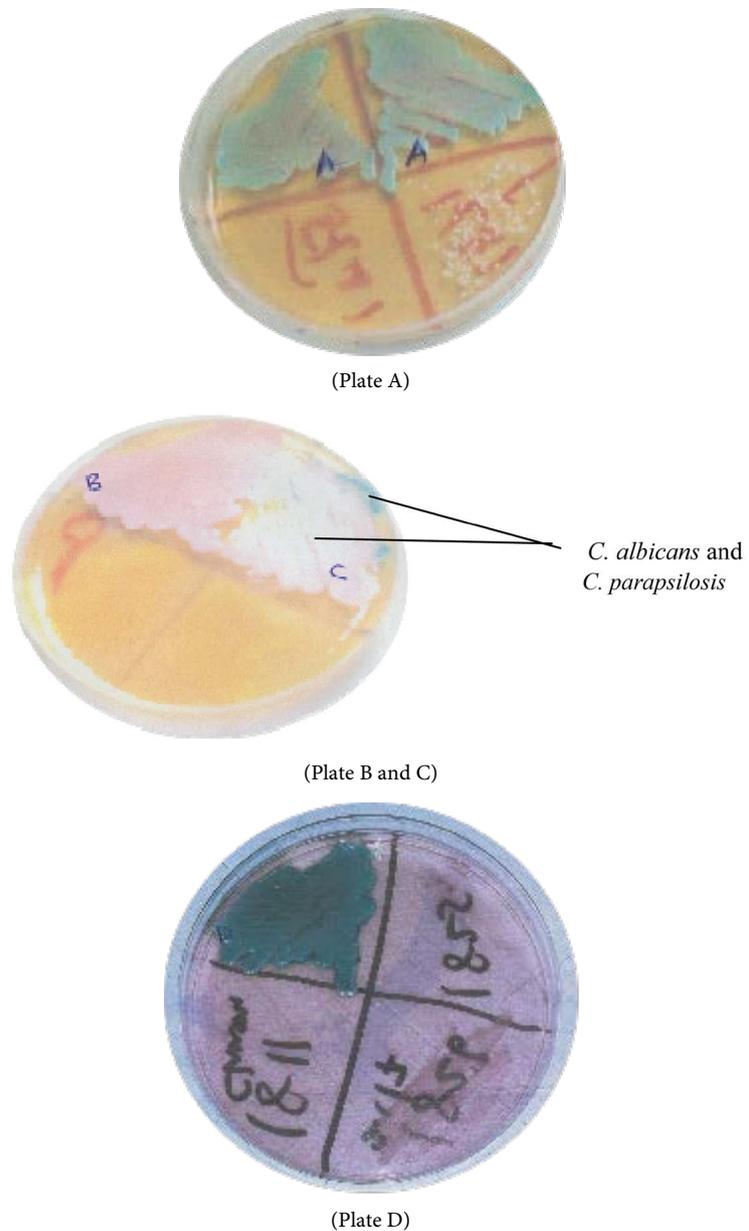(Plate B and C)

(Plate D)

Figure 1. Plate A: *Candida albican* light green on CHROMagar; Plate B and C: *Candida krusei* (Pink) and *Candida parapsilosis* (Cream); Plate D: Isolated *Candida tropicalis* (Blue).

3.7. Carbohydrates Fermentation Reactions of Isolated *Candida* sp.

Carbohydrates fermentation reactions tests showed that *C. albicans* is a non-lactose and non-inositol fermenter but ferments glucose, maltose, sucrose, galactose and xylose and was positive for germ tube test. *C. tropicalis* and *C. parapsilosis* had the same biochemical characteristics as that of *C. albicans* in utilization of carbohydrates but are negative for germ tube test. *C. Krusei* fermented only glucose and was germ tube negative. *C. parapsilosis* fermented glucose, maltose, sucrose and galactose but not lactose and inositol and was also germ tube negative as showed on Table 7 below.

Table 7. Identification of isolated *Candida* species.

Gram's Raction	KOH	Germ tube	Colour on CHROMagar	Glucose	Maltose	Sucrose	Lactose	Galactose	Xylose	Inositol	*Candida* species
+	+	+	Light green	+	+	+	−	+	+	−	*C. albicans*
+	−	−	Blue	+	+	+	−	+	+	−	*C. tropicalis*
+	−	−	Pink	+	−	−	−	−	−	−	*C. krusei*
+	−	−	Cream	+	+	+	−	+	+	−	*C. parassilosis*

KEY: + = Positive, − = Negative.

4. Discussion

Despite all global efforts to eradicate TB, tuberculosis (TB) as an infectious disease has remained a global threat. Co-infection of the respiratory tract by fungi and tuberculosis had been recognized for its wide range of clinical spectrum and chronicity. Among the respiratory fungal infections, infections by *Candida* sp. were the commonest fungal infectious agents isolated from sputa of TB patients. In this study, the prevalence of TB was 93 (23.3%) but the prevalence of TB *Candida* co-infection was 32 (34.4%). It may be assumed that some of the devastating effects of TB on infected persons might result from the synergistic actions of both organisms. A similar study [9] had a prevalence of 25.3% TB *Candida* co-infection and *Candida albicans* were the most prevalent species as also observed in this study.

Some researchers speculated that since *Candida* were part of the normal microbiota of the throat in about 32.5% of healthy individuals, the sputum produced may have been contaminated with *Candida* sp. Using Kahampaa criteria that three (3) or more repeated isolation of *Candida* or more than 30 colonies of *Candida* on sabouraud dextrose agar should be considered pathogenic and should not be treated as commensal [12]. The observations of pseudomycelial forms in sputum were also suggestive of fungal infection other than ordinary colonization. The prevalence of *Candida* co-infection among tuberculosis subjects as observed by [3] was 40%. The prevalence of TB *Candida* co-infection was high among females (19.4%) than in males (15.1%) in this study. This result however is not in agreement with the results obtained by [13], they observed that colonization rate of *Candida* sp. were equal in both males and females, [14] in their study found *Candida* infection to be more in males than in females.

Similarly, [9] observed that *Candida* infections were significantly high in males (26.1%) than in females (23.1%). In this study, the prevalence of TB *Candida* co-infection was high among females (19.4%) compared to males (15.1%) and this agrees with the study conducted by [3] who observed that TB *Candida* co-infections were significantly high among female patients (62.5%) compared to male patients (29.4%). The prevalence rates of *Candida* sp. among both sexes correlates with that of *Mycobacterium tuberculosis* in males and females. The high prevalence in the females could be attributed to the fact that more women seek for medical attention and treatment than males and due to increased out-

door and social activities observed among females. One-way Anova comparison of males and females did not show significant difference.

Among the *Candida* sp. isolated, *C. albicans* were the predominant species with a prevalence of 21 (61.8%). This was in line with the studies carried out by [3] [10] both reported similar prevalence of 50% and 66% respectively. The prevalence of TB/*Candida* co-infection ranged from 45% - 92% in several Indian studies [15]. In this present study, the prevalence of *Candida* sp. isolated were 61.8%, 14.7%, 14.7% and 8.8% for *C. albicans*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* respectively. On the contrary, [9] reported lower rates of *C. albicans* 15.1%, and *C. tropicalis* 8.4%. [15] observed *C. tropicalis* 3.25%, *C. parasilosis* 3.25%, [16] documented *Candida tropicalis* 19.95%, *C. glabrata* 16.54% *C. parapsilosis* 13.14% and *C. krusei* 5.10%. These variations may be attributed to differences in local prevalence of different species of *Candida* due to different environmental conditions as well as the isolation techniques employed. It was also observed in this study that among the non *C. albicans* sp., *Candida tropicalis* was the second predominant species which correlates with findings of similar studies conducted by [16] [17] [18]. Comparison of all species of *Candida* showed that there was significant variation (p = 0.0001) between the different species of *Candida* isolated.

The prevalence of TB *Candida* co-infection by age groups was high among age group 21 - 30 years (18.8%) and 31 - 40 years (25.0%). The high prevalence among the two age groups could be as a result of increased outdoor activities as they associate with one another, overcrowding in most of the settlements and poor standard of living. It could also be attributed to increased reproductive activities within the age groups that were described as the most active segment of any economy. This observation was in compliance with the work done by [19] with a prevalence of 81.6% among the age group 21 - 40 years. The findings of this study were also in agreement with the work conducted by [20] and [21] both reported high prevalence of TB *Candida* co-infection among age group 21 - 40 years. However, the finding of this study differs with the work conducted by [22] which showed a high prevalence of TB *Candida* co-infection among age groups 40 years and above. The corresponding high prevalence of TB and *Candida* infection among the age groups may predict predisposition and synergism in the course of the co-infection. There was no statistical difference (p = 0.1287) among the age groups for TB *Candida* co-infection. Comparison of 1 - 20 years and 21 - 50 years was not statistically significant (p = 0.1705), and there was no statistical difference between 1 - 20 years and 51 - 100 (p = 0.2321). However comparison of 21 - 50 and 51 - 100 showed significant difference (p = 0.0463). It was also noted that the prevalence of Rifampicin resistance TB (MDR-TB) was 1.1%. This prevalence rate of MDR-TB was below the estimated value based on modeling for Nigeria which ranged from 3.2% - 5.4% for new cases [23]. The findings in this study (1.1%) Rifampicin resistance was not in agreement with the findings of [24] who recorded a prevalence rate of 14.7% MDR-TB in Yengeoa, Bayelsa State. This disparity could be attributed to environmental factors. Multi drug resistant

tuberculosis (MDR-TB) were TB with bacilli resistance to at least isoniazide and rifampicin which are the two main first line anti-tuberculosis drugs which have become an important concern in the control of TB in many countries, especially in the sub-Sahara Africa where the burden of other competing diseases like malaria, enteric fever, HIV, etc. were also high [25]. The discovery of Genexpert MTB/RIF testing has made possible the detection of tuberculosis bacilli resistant to rifampicin [26]. Drug resistance emanating from inadequate treatment of active pulmonary tuberculosis could result from inappropriate therapy, poor prescription practices, in-sufficient treatment duration, irregular medication intake, poor drug selection, inadequate public health resources and socio-economic determinants of health [27].

The three antifungal agents used for susceptibility testing were voriconazole, fluconazole and nystatin. Voriconazole showed high susceptibility against the *Candida* sp. closely followed by fluconazole. In a similar research carried out [28] [29] it was clearly shown that the azoles are frequently used for pulmonary fungal infections and are preferred to intravenous amphotericin B. Though *Candida albicans* was the commonest pathogen associated with pulmonary candidiasis, there is also an increasing incidence of other *Candida* sp. Among the non *C. albicans* species isolated was *Candida tropicalis* which showed increased prevalence as a new emerging opportunistic pathogen causing severe invasive pulmonary disease. It had been shown to have great capacity of invasion than *C. albicans* into deep tissues of immunocompromised patients [14] [29] One-way analysis of variance of the susceptibility patterns showed significant variation (p = 0.0001) between Voriconazole, Fluconazole and Nystatin. Student t-test comparison of Voriconazole and Fluconazole did not show significant difference (p = 0.0705). Student t-test comparison of Voriconazole and Nystatin showed significant difference (p = 0.0003). Student t-test comparison of Fluconazole and Nystatin also showed significant difference (p = 0.0006).

5. Conclusion

Tuberculosis remains a global threat despite effort to eradicate the disease and TB co-infection with *Candida* sp. may complicate infection and treatment. The most predisposed age groups to both infections were ages 20 to 40 years, the most active segment of the economy. Screening for TB should be conducted concomitantly with candidiasis and TB treatments should be combined with the administration of anti-fungal agent confirmed by susceptibility testing in cases of TB *Candida* co-infections.

Limitation of Study

The limitation of this study was the non inclusion of the molecular aspect of *Candida*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Cox, F.E. (2001) Concomitant Infections Parasites and Immune Responses. *Parasitology*, **122**, 523-538. https://doi.org/10.1017/S003118200001698X

[2] WHO Multidrug-Resistant Tuberculosis (MDR-TB) Indicators (2010) A Minimum Set of Indicators for the Programmatic Management of MDR-TB in National Tuberculosis Control Program. WHO/HTM/TB/2010.11, World Health Organization, Geneva.

[3] Kali, A., Charles, M.P., Noyal, M.J., Sivaraman, U., Kumar, S. and Easow, J.M. (2013) Prevalence of *Candida* Co-Infection in Patients with Pulmonary Tuberculosis. *Australasian Medical Journal*, **6**, 387-391. https://doi.org/10.4066/AMJ.2013.1709

[4] Mitchell, L.S. and Jeffrey, U.S. (1990) Evolution of the Protists and Protistan Parasites from the Perspective of Molecular Systematic. *International Journal for Parasitology*, **29**, 11-20.

[5] Centre for Disease Control (2014) Symptoms of Invasive Candidiasis.

[6] WHO (2015) Estimates of Tuberculosis Incidence by Country.

[7] WHO (2019) Global TB Report WHO/CDS/TB/2019.23.

[8] Chen, X.H., Gao, Y.C., Zhang, Y., Tang, Z.H., Yu, Y. and Zang, G.O. (2015) Tuberculosis Infection Might Increase the Risk of Invasive Candidiasis in an Immune Competent Patient. *The Revista do Instituto de Medicina Tropical de São Paulo*, **57**, 273-275. https://doi.org/10.1590/S0036-46652015000300016

[9] Ndukwu, C.B., Mbakwem-Aniebo, C. and Frank-Peterside, N. (2016) Prevalence of *Candida* Co-Infection among Patients with Pulmonary Tuberculosis in Emuoha, Rivers State, Nigeria. *Journal of Pharmacy and Biological Sciences*, **11**, 60-63. https://doi.org/10.9790/3008-1105036063

[10] Mathavi, S., Shankar, R., Kavitha, A. and Sasikala, G. (2014) Indra Priyadharsin. A Study on Prevalence of Pulmonary Candidiasis among Tuberculosis Patients and Use of GiRomager Identification of *Candida* Species. *Journal of Drug and Therapeutics*, **4**, 118-121. https://doi.org/10.22270/jddt.v4i3.821

[11] Fred, T. (2009) Two-Hour Detection of MTB and Resistance to Rifampicin. *In Vitro Diagnostic Medical Device*, **21**, 332-339.

[12] Kahanpaa, A. (1972) Bronchopulmonary Occurrence of Fungi in Adults, Especially According to Cultivation Material. *Acta Pathologica Microbiologica Scandinavica Section B Microbiology Immunology*, **227**, 1-147.

[13] Hidalgo, J.A. and Vazquez, J.A. (2012) Candidiasis. https://emedicine.medscape.com/article/213853-overview

[14] Shear, A.N. and Perween, T. (2004) A Study on the Trend in Prevalence of Opportunistic *Candida* Co-Infections among Patients of Pulmonary Tuberculosis. *Pakistan Journal of Biotechnology*, **4**, 857-862.

[15] Baradkar, V.P., Mathur, M., Wanjari, K. and Kumar, S. (2009) *Candida* in Pulmonary Tuberculosis. *Bombay Hospital Journal*, **51**, 52-53. https://doi.org/10.4103/0255-0857.53216

[16] Latha, R., Sasikala, R., Muruganandam, N. and Venkatesh Babu, R. (2011) Study on the Shifting Patterns of Non *Candida albicans Candida* in Lower Respiratory Tract Infections and Evaluation of the CHROM Agar in Identification of the *Candida* Species. *Journal Microbial Biotechnology Research*, **1**, 133-139.

[17] Jha, B.J., Tamang, M.D., Joshy, M.E., Shivananda, P.G. and Brahmadatan, K.N. (2006) Characterization of *Candida* Species Isolated from Cases of Lower Respiratory Tract Infection. *Kathmandu University Medical Journal*, **20**, 290-294.

[18] Sani, R.A., Garba, S.A., Oyeleke, S.B. and Abalaka, M.E. (2015) Prevalence of Pulmonary Tuberculosis (PTB) in Minna and Suleja, Niger State. *Nigeria American Journal of Medicine and Medical Sciences*, **5**, 287-291.

[19] Nwachukwu, N.C., Orji, A., Kanu, I. and Okereke, H.C. (2009) Epidemiology of Pulmonary Tuberculosis in Some Part of Abia State Federal Republic of Nigeria. *Asian Journal of Epidemiology*, **2**, 13-19. https://doi.org/10.3923/aje.2009.13.19

[20] Uzoewulu, N.G., Ibeh, I.N., Lawson, L., Goyal, M., Umenyonu, N., Ofiaeli, R.O. and Okonkwo, R. (2014) Drug Resistance *Mycobacterium tuberculosis* in Tertiary Hospital South East, Nigeria. *Journal of Medical Microbiology and Diagnosis*, **1**, 2. https://doi.org/10.4172/2161-0703.1000141

[21] Okonko, I.O., Soleye, F.A., Adeniji, F.O. and Okerentugba, P.O. (2012) HIV and TB Co-Infection among Patients on Directly Observed Treatment of Short Course in Abeokuta, Ogun State, Nigeria. *Journal of Natural Science*, **10**, 6.

[22] WHO Nigeria (2016) Tuberculosis Country Profile 2016. World Health Organization, Geneva.

[23] Peter, O.I. and Ikenna, D.E. (2010) Prevalence of Rifampicin Resistance by Automated Genexpert Rifampicin Assay in Patients with Pulmonary Tuberculosis in Yenegoa, Nigeria. *Pan African Medical Journal*, **29**, 204. https://doi.org/10.11604/pamj.2018.29.204.14579

[24] Out, A., Umoh, V., Habib, A., Ameh, S., Lawson, L. and Ansa, V. (2013) Drug Resistance among Pulmonary Tuberculosis Patients in Calabar, Nigeria. http://www.atmph.org/text.asp?2013/6/5/491/133685 https://doi.org/10.1155/2013/235190

[25] Onyedum, C.C., Alobu, I. and Ukwaja, K.N. (2017) Prevalence of Drug-Resistance Tuberculosis in Nigeria: A Systematic Review and Meta-Analysis. *PLoS ONE*, **12**, e0180996. https://doi.org/10.1371/journal.pone.0180996.g001

[26] Amala, S.E., Goodluck, S. and Nwokah, G.E. (2019) The Prevalence of Tuberculosis (TB) and Multiple Drug Resistant Tuberculosis (MDR-TB) Tuberculosis in Bayelsa State, Nigeria. *Asian Journal of Health and Medical Research*, **5**, 1-5.

[27] Pappas, P.G., Rex, J.H., Sobel, J.D., Filler, S.G., Dismukes, W.E., Walsh, T.J., *et al.* (2004) Guidelines for Treatment of and Candidiasis. *Clinical Infectious Diseases*, **38**, 161-189. https://doi.org/10.1086/380796

[28] Lovacheva, O.V., Kornienko, I. and Kul'ko, A.B. (2005) Efficiency of Treatment of Candida-Induced Lower Respiratory Tract Lesions in Patients with Pulmonary Tuberculosis. *Problemy Tuberkuleza i Bolezneĭ Legkikh*, No. 3, 14-16.

[29] Jain, S.K., Agrawal, R.L., Sharma, D.A. and Agrawal, M. (1982) *Candida* in Pulmonary Tuberculosis. *Journal of Postgraduate Medicine*, **28**, 24-29.