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Prevalence of Pulmonary Tuberculosis and Candidiasis among those Living with HIV Positive Patients in Two Medical Centres in Benin City, Edo State, Nigeria

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ABSTRACT: People living with human immunodeficiency virus (HIV) are predisposed to tuberculosis and co-infections from many pathogens. Hence, the objective of this paper was to investigate the prevalence of pulmonary tuberculosis and candidiasis among those living with HIV in Two Medical Centres in Benin City, Edo State, Nigeria using Standard methods. The results obtained showed that 136 (64.76 %) of the HIV patients also had fungal infection, 36 (17.14 %) had co-infection with *Mycobacterium tuberculosis*, 37 (17.62 %) were neither infected with *M. tuberculosis* nor *Candida* spp. and 1 (0.48 %) had tuberculosis infection. Prevalence of resistant rifampicin *M. tuberculosis* among HIV patients was 6 (2.85 %) while candidiasis and *M. tuberculosis* co-infection among the patients was 30 (83.33 %). That which involved *Candida albicans* and *M. tuberculosis* had the highest percentage of occurrence 25 (83.33 %). HIV patients between 28 - 36 years had the highest 15 (50 %) number of cases of co-infection. Among the study population, the prevalence of co-infection in males and females was 11 (15.27 %) and 19 (13.76 %), respectively. Anaemia was observed in highly active antiretroviral therapy (HAART) and HAART-naïve patients. Due to lower prevalence of rifampicin resistance and anaemia among HARRT patients compared with HART-naïve patients, the Ministry of Health and relevant government and private agencies should intensive efforts to provide affordable/free antiretroviral treatment to HIV patients.

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Human Immunodeficiency Virus (HIV) is a pathogen that weakens the immune system by damaging CD_4 cells and open doors to many infections by bacterial and fungal pathogens which manifest several diseases leading to full-blown Acquired Immunodeficiency Syndrome (AIDS) (Favour *et al*., 2017). The global fight against HIV infections has lasted for four decades and still on (Salako *et al*., 2022). People living with human immunodeficiency virus (PLWHIV) are likely to experience pulmonary tuberculosis, candidiasis, and chronic diarrhea, among other opportunistic infections. A survey carried out in 2018 by the Nigeria AIDS Indicator and Impact Survey (NAIIS) estimated national prevalence of HIV to be 1.4 % (Saha *et al*., 2011; Adeoye *et al*., 2021). According to Saha *et al*. (2011), death among those

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living with HIV is mainly attributed to co-infections and opportunistic infections (OIs). An infectious disease known as tuberculosis is caused by *Mycobacterium tuberculosis* (Peña *et al*., 2021). Increasing drug resistant strains of *M. tuberculosis* in developed and developing countries has been reported in recent years. It is a public health concern (Otokunefor *et al*., 2018).There is indications that tuberculosis could quicken the progress of HIV infection (Xun *et al*., 2020). The likelihood of people living with HIV to develop tuberculosis is between 20-30 times higher than HIV negative individuals. To worsen the situation, the progress of HIV in the body is enhanced by tuberculosis and vice versa. Approximately one third of deaths associated with AIDS is caused by tuberculosis (Moreno *et al*., 2020). In 2018, it was estimated that tuberculosis caused 1.2 million deaths globally out of 10 million cases reported. According to a 2019 report, about900, 000 new cases of tuberculosis among HIV-1- positive persons occurred while 251, 000 deaths was reported (Peña *et al*., 2021). Cases of co-infection of tuberculosis and HIV infection in Sub-Saharan is higher than what is obtainable in other parts of the world (Moreno *et al.,* 2020). Co-infection of HIV and tuberculosis is making global efforts to prevent new HIV and tuberculosis infections difficult. Due to HIV-tuberculosis co-infection, there is 100 % possibility that patients involved will experience full blown AIDS (Ehondor *et al.,* 2019). According to Saha *et al.* (2011), oral candidiasis is an early manifestation of HIV infection/disease which is also associated with AIDS-related illness and morbidity. The disease is caused mainly by a fungal pathogen known as *Candida albicans* (Favour *et al*., 2017). It is estimated that more than 90 % of people living with HIV manifest candidiasis as the infection progresses (Ekwealor *et al*., 2023).

Peña *et al*., (2021) determined the prevalence of oral candidiasis among those diagnosed with human immunodeficiency virus-1 and pulmonary tuberculosis (pTB). Findings from the study indicate that HIV-1/pTB co-infection has no association with oral candidiasis. Thirty five percent (35 %) prevalence of oral candidiasis was reported in patients co-infected with HIV-1 and pulmonary TB. Data obtained from infection prevalence rate is useful to clinicians and policymakers because it helps them to develop effective and timely strategies to prevent new infections (Ozim *et al*., 2023; Onovo *et al*., 2023). In this regard, periodic study of the population is needed. Therefore, this study seeks to determine the prevalence of co-infection of pulmonary tuberculosis and candidiasis among those. Hence, the objective of this paper was to investigate the prevalence of pulmonary tuberculosis and candidiasis among those living with HIV in Two Medical Centres in Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Study location and population: The study was carried out in two (2) hospitals in Benin City which include Faith Mediplex and Central Hospital. It involved male and female human immunodeficiency virus (HIV) outpatients between the ages $1 \leq 90$ years.

Ethical Approval/Informed Consent: Ethical approval was obtained from Central Hospital and Ministry of Health, Benin City, Edo State through letters referencedA732/T/1 and HM1208/199 respectively. Informed consent form in English language was given to the participants to fill before collection of samples.

Determination of Sample Size: The sample size (N) for this study was determined using prevalence from previous studies in literatures. According to 2018 Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS), the prevalence of HIV infection in Nigeria is 1.4 %.The spectrum HIV prevalence in 2022 in Edo State is 1.9% (Onovo *et al.,* 2023). The sample size for this study was obtained using the formula described by Daniel (1999).

$$\mathrm{N} = \frac{\mathrm{Z}^2\mathrm{P}(1-\mathrm{P})}{\mathrm{D}^2} \quad (1)$$

Were N = required sample size; Z = confidence level at 95% (standard value of 1.96; P = estimated prevalence of HIV infection in Edo State (1.90%); D = margin error at 5% (standard value of 0.05)

$$N = \frac{1.96^2 \text{ x } 0.0190(1-0.0.190)}{0.05^2}$$

$$\frac{3.8416 \text{ x } 0.0190(0.9810)}{0.0025}$$

$$\frac{0.0730(0.9810)}{0.0025} = 28.6$$

N = 29 minimum sample size

Therefore a working sample size of 210 test samples was chosen for this research.

Study Population: A total of 210 participants who are HIV positive were recruited for this study. One hundred and eighty eight (188) were on highly active antiretroviral therapy (HAART) while 22 were HARRT-naïve. The number of males and females are 72 and 138, respectively.

*Collection of sputum and blood samples***:** Non-repetitive patient's samples were collected using random sampling technique. Sputum and blood samples were collected from Central Hospital and Faith Mediplex Hospital all in Benin City, Edo State. A clean-wide mouth screw-capped container was given to the patients to collect their sputum. The patients were asked to rinse their mouth with water before spitting out sputum to reduce contamination of the specimen. The patients were instructed to inhale air deeply 2-3 times with his/her mouth open, cough out deeply from the chest, open the container and spit out the sputum into the container. The cap of the container was carefully and tightly screwed. The cap was checked to ensure that it is secure. The specimen type, date and time of collection was written on the container label before it was immediately transported to the laboratory for analysis. Ten millilitre (10 ml) of blood was collected from each patient by veno-puncture into a sterile ethylenediaminetetraacetic acid (EDTA) container and labeled appropriately. The blood samples were used for retroviral screening as well as for full blood count, CD_4 count and viral load.

Processing of samples: MTB/Genexpert RIF Test: The test was carried out according to the manufacturer's instruction (Cepheid AB. Rontgenvagen5 SE-171 54 Solona, Sweden). The lid of sputum container was unscrewed. Two (2) volumes of sputum reagent (SR) was directly poured into 1 volume of sputum inside the sputum container. The lid was replaced and screwed tightly and shook vigorously 10-20 times (one back and forth movement is a single shake or vortex).The sputum mixed with sputum reagent inside the container was incubated at room temperature (28±2 ^oC) for 10 minutes. The sample was shook vigorously for another 10-20 minutes and incubated for five minutes at room temperature. Upon observing that the sample was perfectly fluid with no visible clump of sputum, it is ready for inoculation into the cartridge. The cartridge was opened and 2.4 ml of prepared sample was carefully transferred into the cartridge with the aid of a plastic transfer pipette. The lid was closed carefully. The expert was switched on and allowed to initialize. The cartridge was scanned for the system to recognize it; necessary data was fed into the computer. The cartridge was inserted into one of the free modules and the door was closed for the machine to process the sample. At the end of processing the sample, the machine opened the door of the module and the result was printed out.

Isolation of Candida spp. from sputum samples: This was done following a modified standard microbiological technique as described by Cheesbrough (2004). The sputum was first streaked unto Sabouraud dextrose agar (SDA) containing chloramphenicol (16 µg/ml) to isolate *Candida* spp. and other fungi.

Germ tube test: This test was done to differentiate *Candida albicans* from other species of yeast. Briefly, the suspected yeast was lightly inoculated into 0.5ml of screened human serum and then incubated at 37^oC for 3 hours. A wet preparation of this mixture was examined microscopically for the production of germ tubes that appear as sprouting yeast cells.

Sugar fermentation test: This test was done as described by Milne *et al*. (2013). The essence of the test is to detect the ability of an organism to utilize various sugars and generate energy. The yeast colony was inoculated into phenol red peptone water containing 1 % of glucose, lactose, maltose, sucrose, galactose and trehalose. Durham's tube was inserted only in the test tube containing 1 % glucose. The solutions were incubated at 37^oC overnight. A change in colour from red to yellow indicated acid production, while a space in the prefilled Durham's tube indicated gas production.

Identification and speciation of Candida isolates using Candida CHROM AGAR: Emergent *Candida* colonies were further identified and speciated using *Candida* CHROM agar as described by Paritpokee *et al*. (2005). CHROMagar *Candida* is a useful chromogenic method for the identification of yeasts. The CHROMagar *candida* was prepared according to manufacturer's instructions. CHROMagar has a chromogenic substance which help in the rapid identification of the *Candida* spp is based on the reactions between the specific enzymes of the different species and chromogenic substances. The yeast colony that grew on Sabouraud dextrose agar (SDA) was sub-cultured onto the chromogenic medium and incubated at 37 ^oC for 24 – 48 hours. The colour and colonial morphology of the yeast colonies were observed at 24 and 48 hours and compared with the conventional identification chart (Paripokee*et al*., 2005). The colour distribution of the yeast colonies is as follows: *Candida albicans*-green colonies, *Candida krusei*-dry flat pink colonies, *Candidia tropicalis*- metallic blue colonies, *Canidida lusitanea*-light purple colonies, *Cryptococcus neoformans*- white colonies, *Saccharomyces cerevisiae*-dark purple colonies, and *Candida parasilopsis*- light pink colonies.

Blood Specimen: Human Immunodeficiency Virus (HIV) Test: The HIV status of the recruited subjects/participants were reconfirmed. National algorithms of using three rapid test kits for detection of HIV antibodies in the blood of patients were

employed. The rapid test kits include Determine (Alere Medical Co, Ltd, Japan), Unigold (Trinity Biotech PLC, Wicklow, Ireland), and stat park (Chembio diagnostic systems Inc USA). The tests were carried out according to manufacturer's instruction.

HIV test using determine kit: The protective foil cover from each test card was momentarily removed. Exactly 50µl of patient's plasma was pipetted and dispensed on the sample pad. Two (2) drops of buffer was added to the sample pad. The preparation was allowed to stand for a minimum of 15 min (up to 60 min) and the result was read. It is a positive test result when red bars appeared in both the control window (labelled "control") and the patient's window (labelled ''patient'') of the strip. The appearance of one red bar in the control window of the strip (labelled "control"), and no red bar in the patient's window of the strip (labelled "patient") is reported as negative test result. If there is no red bar in the control window of the strip, the result is invalid and should be repeated.

HIV 1/2 STAT-PAK Assay: The Chembio HIV 1/2 STAT- PAK test device was removed from its pouch and placed on a flat surface. The test device was labelled with patient's name. Using a sample loop, 5µl of plasma was pipette and dispensed onto the centre of sample pad(s).The running buffer was inverted and held vertically over the sample well and 3 drops of buffer was slowly dropped into the sample(s) well. The result was read 10 minutes after the addition of the running buffer. The appearance of two pink/purple lines, one in the TEST (T) area and the Control (C) area indicate a reactive result. One pink/purple line in the control C area, with no line in the test T area indicates a non -reactive result. An invalid result is an indication that a problem was encountered while running the test which is either related to the specimen or the device. Consequently, the test will be repeated using a new device and /or a new sample.

Uni-Gold™HIV: At room temperature, the Uni-gold™ HIV device was removed from their pouches. The device was placed on a clean flat surface and labelled accordingly. Disposable pipette was used to pipette the plasma. Holding the pipette vertically, 2 drops of plasma were allowed to drop into the sample pad. The sample was allowed to be fully absorbed. By holding the wash bottle in a vertical position and above the sample port 2 drops of wash solution was dispensed into the sample port. The test result was read after 10 minutes. The appearance of two pink/red lines of any intensity in the device window after 10 minutes is interpreted as a reactive result. The first line is adjacent to letter 'T' (test) and the second adjacent to 'C' control. A pink/red line of any intensity which appeared adjacent to the letter 'C' control, but failed to appear adjacent to 'T' test is indicative of a non-reactive test. When there is no appearance of pink/red line in the device window adjacent to the letter 'C' (control) irrespective of whether or not a pink/red line appeared in the device window adjacent to' T '(test) is reported as invalid and must be repeated.

RESULTS AND DISCUSSION

Figure 1 shows the frequency of occurrence of bacterial and fungal pathogens among HIV positive patients in Benin City. The result obtained showed that fungi and *Mycobacterium tuberculosis* had the highest (64.76%) and lowest (0.48%) frequency of occurrence, respectively. *Candida* species were among the fungal isolates encountered among the people living with HIV. Table 1 shows the result of sugar fermentation tests carried out on the isolates which were identified as *Candida tropicalis*, C. *albicans, C. krusei* and *C. glabrata*. The percentage occurrence of the *Candida* species including other fungal isolates encountered among the people living with HIV is presented in Figure 2. The result showed that *Candida albicans* (54.65%) and *C. krusei* (4.07%) had the highest and lowest percentage occurrence, respectively.

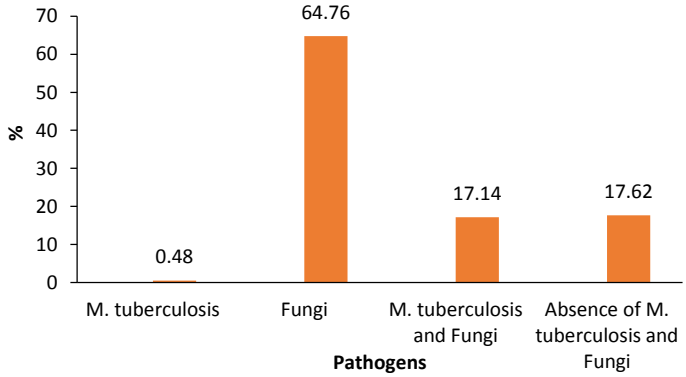

Fig 1: Frequency of occurrence of bacterial and fungal pathogens among HIV positive patients

Table I: Sugar fermentation of different *Candida* species

Carbohydrate	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. glabrata*
Glucose	+	+	+	+
Lactose	-	-	-	-
Sucrose	+	+	-	-
Maltose	+	+	-	-
Galacotose	+	+	-	-
Mellabiose	-	-	-	-
Cellabiose	-	+	-	-
Xylose	+	+	-	-
Raffinose	-	-	-	-

Key: + represent positive result; - represent negative result

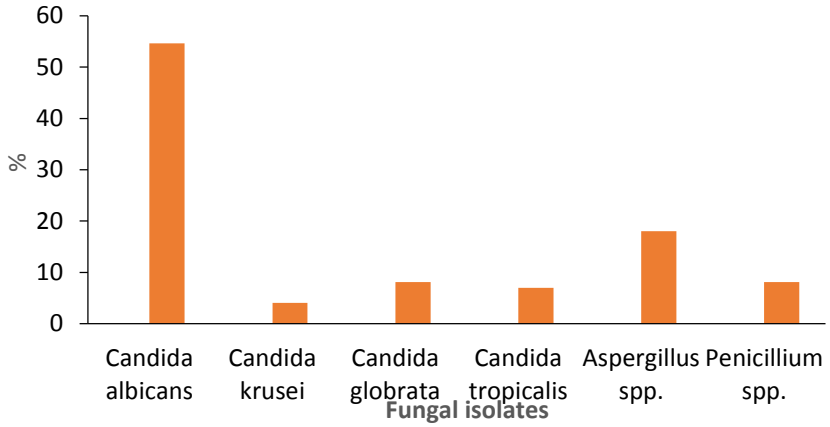

Fig 2: The percentage occurrence of fungal isolates from HIV positive patients

Table 2: Distribution of *Mycobacterium tuberculosis* and fungi co-infection among HIV positive patients in Benin City.

Type of infection	Number of patients	Percentage (%)	*Rif* resistant cases (%)
M. tuberculosis/*Candida* spp.	30	8.33	5 (13.89)
M. tuberculosis/*Penicillium* spp.	3	8.33	1 (2.78)
M. tuberculosis/*Aspergillus* spp.	3	8.33	0 (0.00)
Total	36	100	6 (16.67)

Key: Rif resistant = Rifampicin resistant

Table 2 shows the percentage occurrence of *Mycobacterium tuberculosis*co-infection with other fungi isolates from sputum of HIV positive patients. The result shows that *M. tuberculosis*/*Candida* spp. had the highest percentage occurrence (83.33 %) whereas the least (8.33 %) involved *M. tuberculosis*/*Penicillium* spp. and *M. tuberculosis*/*Aspergillus* spp., each. Similarly, *M. tuberculosis*/*Candida* spp. had the highest occurrence in terms of *Rif* resistance (13.89 %) whereas *M. tuberculosis*/*Aspergillus* spp. had the least (0.00 %).It was observed that co-infection by *Candida albicans* and *Mycobacterium tuberculosis* was the type of co-infection that dominated the rifampicin resistance in this study. The prevalence of rifampicin resistant *Mycobacterium tuberculosis* among HIV Positive patients co-infected with Candidiasis in Benin City was 16.67 %. Presented in Table 3 is the distribution of co-infection of candidiasis and *Mycobacterium tuberculosis* among HIV positive patients. The result shows that *M. tuberculosis*/*Candida albicans* co infection had the highest occurrence (83.33 %) whereas *M. tuberculosis*/*C. krusei* had the least occurrence (3.33 %). Table 4 shows the distribution of co-infection of candidiasis and tuberculosis among the age groups of the participants. The result obtained showed that highest (50 %) and lowest (0 %) number of cases involved participants within the age group 29-36 and 10-18 years, respectively. Presented in Table 5is occurrence of candidiasis and pulmonary tuberculosis co-infection among two genders who were HIV positive. The result showed that candidiasis and pulmonary tuberculosis co-infection caused by *M. tuberculosis*/*Candida albicans* affected females (68%) more than males (32 %). On the contrary, co-infection caused by *M. Tuberculosis/Candida glabrata* affected only males (100%).Table 6 shows the load of tubercle bacilli among patients co-infected with *M. tuberculosis* and *Candida* spp. relative to gender. In terms of highest load of tubercle bacilli, the result shows that females had the highest percentage occurrence (68.42%) whereas males had the lowest (54.55%). Under the category of very low load of tubercle bacilli among the patients, the result showed

that males (9.09 %) were slightly higher than the females (5.26 %).Table 7 shows the occurrence of *Candida* spp. in rifampicin resistant co-infection of *Candidiasis* and Pulmonary tuberculosis. It was observed that *Candida albicans* and *Mycobacterium tuberculosis* was the type of co-infection that dominated the rifampicin resistance in this study.

Table 3: Distribution of co-infection of candidiasis and *Mycobacterium tuberculosis* among HIVPositive patients

Type of co-infection	Number of cases (n=210)	Percentage (%)
M.Tb/*Candida albicans*	25	83.33
M.Tb/*Candida tropicalis*	2	6.67
M.Tb/*Candida krusei*	1	3.33
M.Tb/*Candida glabrata*	2	6.67
Total	30 (14.29%)	100

M. Tb -*Mycobacterium tuberculosis;* n -Total number of patients tested

Table 4: Distribution of co-infection of candidiasis and tuberculosis among the age groups of the participants

Age range (years)	N	Number of patients co-infected	Percentage (%)
10 – 18	18	0	0.00
19 – 27	26	1	3.33
28 – 36	59	15	50.00
37 – 45	57	9	30.00
46 – 54	27	3	10.00
≥55	23	2	6.67
Total	210	30	100

n =210 Total number of patients/subjects recruited in the study.

The prevalence of rifampicin resistant *Mycobacterium tuberculosis* among HIV positive patients co-infected with Candidiasis in Benin City was 16.67 %.Out of 210 blood samples tested, 188 were on HAART while 22 were HAART-naïve. Table 8 shows that out of 30 patients who were co-infected with candidiasis and pulmonary tuberculosis; 22 (73.34 %) were on HAART and 8 (26.67 %) were HAART-naive. The occurrence of rifampicin resistant *M.tuberculosis* on HAART and HAART-naive patients co-infected with candidiasis and pulmonary tuberculosis is shown in Table 8. It was observed that rifampicin resistance was detected in 40.00 % of HAART patients while the percentage observed in HAART naïve patients was 60.00 %. In this study, rifampicin resistant *Mycobacterium tuberculosis* in co-infection of Candidiasis was found to be 16.67 %. Table 9 shows the occurrence (%) of co-infection among HAART and HAART-naive patients in relation to gender. The result shows that females had higher percentage of co-infection and cases were worse in HAART-naïve patients compared to HAART patients. HAART-naïve females had higher prevalence (87.50 %) of co-infection with *M. tuberculosis* compared to males (12.50 %). A similar scenario was observed among HAART patients with co-infection which accounted for 54.54 % prevalence for females while males had 45.56 %.Co-infection with *Candida albicans* had the highest occurrence in both genders. Table 10 shows anaemic status (%) of HIV positive HAART and HAART-naïve patients co-infected with candidiasis and pulmonary tuberculosis. Anaemia was observed in both HAART and HAART-naïve patients, however, it is worthy of note that the condition was worse in HAART-naïve patients with 100.00 % of anaemic status. Only 4.55 % HAART patients were not anaemic.

Table 5 Occurrence of candidiasis and pulmonary tuberculosis co-infection in relation to gender of HIV patients.

Type of infection (*n*)	Male (%) n_1 = 72	Female (%) n_1 = 138	P value
M.Tb/*Candida albicans*(25)	8 (32.00)	17(68.00)	0.9744
M.Tb/*Candida tropicalis* (02)	1 (50.00)	1(50.00)	0.6380
M.Tb/*Candida krusei*(01)	0(0.00)	1(100.00)	0.4690
M.Tb/*Candida glabrata*(02)	2 (100.00)	0(0.00)	0.7400
Total (n_2)	11 (36.67)	19 (63.33)	0.9291

M.Tb = *Mycobacterium tuberculosis; n* - Number of *M. tuberculosis* and *Candida* cases; n_1 - Total number males/females recruited for the study is 72/138; n_2 - Total number of males and females co-infected by *Candida* spp and *Mycobacterium tuberculosis*=30

Table 6: Load of tubercle bacilli among patients co-infected with *M. tuberculosis* and *Candida* spp. relative to gender.

MTB Load	Male (%)	Female (%)
High	6 (54.55)	13 (68.42)
Medium	4 (36.36)	1 (5.26)
Low	0 (0.00)	4 (21.05)
Very low	1 (9.09)	1 (5.26)
Total	11 (100.00)	19 (100.00)

Key: MTB = *Mycobacterium tuberculosis*

Table 7: Occurrence of *Candida* spp in rifampicin resistant co-infection of candidiasis and pulmonary tuberclosis

Type of co-infection(*n*)	Rif detected (%)	Percentage
M.Tb/*Candida albicans* (25)	5 (20.00)	100.00
M.Tb/*Candida tropicalis* (02)	0 (0.00)	0.00
M.Tb/*Candida krusei* (01)	0 (0.00)	0.00
M.Tb/*Candida glabrata* (2)	0 (0.00)	0.00
Total (30)	5 (16.67)	100.00
Ptb = Pulmonary tuberculosis		

Table 8: Occurrence of rifampicin resistant *Mycobacterium tuberculosis* on HAART and HAART-naive patients co-infected with candidiasis and pulmonary tuberculosis

Rif status (*n*)	HAART	HAART-Naïve	Total (%)
Rif not detected (25)	20 (80.00)	5 (20.00)	83.33
Rif detected (05)	2(40.00)	3 (60.00)	16.67
Total (30)	22 (73.33)	8(26.67)	100.00

Rif = Rifampicin

This study showed that prevalence of *Mycobacterium tuberculosis* and fungal species co-infection among HIV patients in Benin City was 17.14 %. In previous studies, Okonkwo *et al*.(2017) and Okonko *et al.* (2018) reported that percentage prevalence of tuberculosis among HIV patients in Nnewi and Port Harcourt was 12.5 % and 14.0 % (Port Harcourt),

respectively. In Plateau State, the Federal Ministry of Health (FMH) reported a prevalence of 30.0% while in Nassarawa State it was 34.5 % (Gyar *et al.*, 2007). The variations in prevalence could be attributed to differences in method of analysis, geographical location, education and awareness of the people about predisposing factors for HIV/AIDS infection. According to Mathavi *et al.* (2015), the prevalence of *Mycobacterium tuberculosis* and fungi co-infection in India is 18%.This report is in agreement with our research findings. According to Sehar *et al*. (2004) and Baradkar *et al*. (2009), the prevalence of HIV patients co-infected with *Candida* and *M. tuberculosis* in India is 15.2and 26 %, respectively.

Table 9: Occurrence (%) of co-infection among HAART and HAART-naive patients in relation to gender.

Type of co-infection	Male (%)	Female (%)
HAART (*n*)		
M.Tb/*Candida albicans*(18)	8 (44.44)	10 (55.56)
M.Tb/*Candida tropicalis* (02)	1 (50.00)	1 (50.00)
M.Tb/*Candida krusei*(01)	0 (0.00)	1 (100.00)
M.Tb/*Candida glabrata*(01)	1 (100.00)	0 (0.00)
Sub-total	10 (45.46)	12 (54.54)
HAART Naive *(n)*		
M.Tb/*Candida albicans*(07)	0 (0.00)	7 (100.00)
M.Tb/*Candida tropicalis*(00)	0 (0.00)	0 (0.00)
M.Tb/*Candida krusei*(00)	0 (0.00)	0 (0.00)
M.Tb/*Candida glabrata*(01)	1 (100.00)	0 (0.00)
Sub-total	1 (12.50)	7 (87.50)
Total	11 (36.67)	19 (63.33)

M.Tb = *Mycobacterium tuberculosis:* HAART = Highly Active Antiretroviral therapy

Table 10: Anaemic status (%) of HIV positive HAART and HAART naïve patients co-infected with candidiasis and pulmonary tuberculosis.

Type of co-infection	Anaemic status	
	Anaemic (%) Hb<12g/dl	Not anaemic (%) Hb>12/dl
HAART (n)		
M.Tb/*Candida albicans*(18)	17(94.44)	1 (5.56)
M.Tb/*Candida tropicalis* (02)	2(100.00)	0(0.00)
M.Tb/*Candida krusei*(01)	1(100.00)	0(0.00)
M.Tb/*Candida glabrata*(01)	1(100.00)	0(0.00)
Sub-total	**21 (95.45)**	**1 (4.55)**
HAART-naive *(n)*		
M.Tb/*Candida albicans*(07)	7 (100.00)	0(0.00)
M.Tb/*Candida tropicalis*(00)	0(0.00)	0(0.00)
M.Tb/*Candida krusei*(00)	0(0.00)	0(0.00)
M.Tb/*Candida glabrata*(01)	1(100.00)	0(0.00)
Sub-Total	**8 (100.00)**	**0(0.00)**

M.Tb = *Mycobacterium tuberculosis;* Highly Active Antiretroviral therapy

Findings from this study showed that *Candida* (83.33 %), *Aspergillus* (8.33 %) and *Penicillium* (8.33 %) species were the fungal isolates co-infecting HIV patients also infected with tuberculosis. Thedominant fungi were *Candida* spp (83.33 %). This finding is in agreement with the report by Nwako *et al*. (2014) who isolated *Candida*, *Aspergillus* and *Penicillium* spp in sputum of HIV patients. They reported that *Candida* spp had the highest prevalence of 97.5% whereas *Aspergillus* and *Penicillin* species had 2%, respectively. In a related study, Justus *et al*. (2017) isolated *Candida* spp., *Aspergillus* spp. and *Penicillium* spp. from sputum samples of patients infected with HIV. According to the researchers, these fungal genera were opportunistic. The prevalence of rifampicin resistant *M. tuberculosis* in this study is alarming. In other words, 16.67 % (1 in every 6 cases of co-infection with fungi). Our result is higher than 0.7% reported by Nsikak and Frankl and (2018) in Yenegoa. In a related study, Anochie *et al*.(2013) and Rasaki *et al*. (2014) reported that prevalence of rifampicin resistance is 0.15 % in the East and 7.2 % in Ilorin (in the west), respectively. The variation in the results could be attributed to the diagnostic methods used for detection of tubercle bacilli.

The age group which had the highest number of co-infection is 28-36 years. This age bracket represent the youth population that are sexually active which account for the highest risk factor for the spread of HIV infection. In addition, individuals within this age group are inclined to engage in outdoor activities which increases the risk of being infected by airborne infectious pathogens. In a related study, Talle *et al*. (2017) reported that pulmonary infections among HIV infected people recorded highest prevalence among those between ages 31 to 35.

Our result suggests that co-infection with *M. tuberculosis* and candidiasis in patients also infected with HIV was age dependent. It is in agreement with the report by Yahaya *et al*. (2014) which stated that mycotic infections are dependent on the age of patients. The researchers reported that patients below 10 years had no sign of systemic mycoses while those above 76 years had a very low prevalence. The reason for age dependency could be a function of low environmental exposure of individuals within the age brackets to secondary infections. It is also possible that receptors for infection could be underdeveloped or absent in the individuals.

The result obtained from this study showed that co-infection of candidiasis and pulmonary tuberculosis in patients infected with HIV involved higher number of females (63.33 %) than males (36.67 %). Statistically, there is no significant difference (*p-value*>0.05) in co-infection with *Candida* species and *M. tuberculosis.* This result implies that polymicrobial infection (co-infection with *Candida* and *M. tuberculosis*) among HIV patients put males and females at equal risks. Somonis *et al*. (1994) also suggested that candidiasis and pulmonary tuberculosis co-infection is independent of gender, but dependent on possible risk factors such as colonization by *Candida* spp., integrity

of skin mucomembrane, prolong duration of antimicrobial therapy, corticosteroids therapy, diabetes mellitus, neutropenia and occupational hazards such as farming. Our result is in agreement with the report by Hidalgo and Vazquez (2004) who showed that distribution of fungal infections is independent of gender. On the contrary, Bensod and Rai (2008) reported that infection was higher in males compared to females. High vulnerability of men to fungal infection compared to females could be attributed to longer exposure of males to the environment. Kali *et al*. (2013) reported that *Candida* spp and pulmonary tuberculosis co-infection was statistically significant (*p-value* = 0.0133) among female patients compared to male patients. Higher tendency of *Candida* spp to colonize females compared to males could be responsible for increased risk of co-infection involving *Candida* spp. and pulmonary tuberculosis. The essence of determining mycobacterial burden in sputum is to identify individuals with active tuberculosis (TB) who pose the highest risk of transmitting *Mycobacterium tuberculosis* (MTB) to other people. This study showed that load or burden of tubercle bacilli amongst patients co-infected with *M. tuberculosis* and candidiasis is relative to gender. It was reported in this study that females (68.42 %) had a higher load of *M. tuberculosis* compared with their male counterparts (54.55%). In this study, the burden of tubercle bacilli was measured using the Gen Xpert PCR machine for accuracy and easy reproducibility. The Xpert MTB/RIF cycle threshold (C_T) values is a measure of sputum mycobacterial burden. Some studies used traditional method of smear microscopy as a proxy of infectiousness (Behr *et al*., 1999).The bacillary burden in sputum is lower in HIV-infected individuals which make smear microscopy to have a diminished utility in the study population (Burchfield *et al*., 2002; Chaidir *et al*., 2013). Going by the superior diagnostic accuracy of Xpert over smear microscopy, the results obtained in this study suggest that Xpert is a suitable replacement for smear microscopy not only for diagnosis of *Mycobacterium tuberculosis*, but also as a correlate of bacterial burden (Steingart *et al*., 2013). Therefore, it can be inferred that diagnosis of *M. tuberculosis* using Gene-xpert machine is essential for the purpose of precision and accuracy. The manual method of detecting *M. tuberculosis*is "very slow". It is interesting to state that "low" MTB load (tubercle burden) can be detected by Gene-xpert which is very difficult to achieve using manual method. According to Steingart *et al*. (2013), the use of traditional method over Xpert would mean losing up to 20 % of positive cases which was the scenario observed in this study.

Candida albicans (100%) dominated other *Candida* spp reported as rifampicin resistant co-infection of *Candida* spp. and pulmonary tuberculosis. There is paucity of data in this regard from previous studies. The prevalence of rifampicin resistant *Mycobacterium tuberculosis* in co-infection of *Candidiasis* is 16.67 %. This result is higher than the value reported by Rasak *et al*. (2014) in Ilorin which stated that *M. tuberculosis* (7.2 %) were rifampicin resistant whereas 31.4% were sensitive. In a related study, Olusoji *et al*. (2000) and Lawson *et al*. (2000) reported that 8.6% and 19% of *M. tuberculosis* were resistant to rifampicin, respectively. This level of resistance is contrary to the report by Akaninyene *et al*. (2013) which stated that no strain of rifampicin resistance occurred. According to Getahun *et al*. (2010), *Mycobacterium tuberculosis* is more liable to undergo mutation when it is exposed to rifampicin than a lot of second line anti-tuberculosis drugs.

Rifampicin resistance was detected in 40.00 % of highly active antiretroviral therapy (HARRT) patients while HAART-naïve patients recorded 60.00 %. Females had higher percentage of co-infection of which the number of cases is higher in HAART-naïve patients compared to HAART patients. HAART-naïve females had higher prevalence (87.50 %) of co-infection with *M. tuberculosis* compared to their male counterpart (12.50 %). A similar scenario was observed among HAART patients with 54.54 % co-infection prevalence for females and 45.56% for males of which *Candida albicans* had the highest occurrence in both genders. In a related study, Favour *et al*. (2017) reported that people living with HIV, both HAART and HAART-naïve patients are predisposed to candida infections. It has been reported that *Candida albicans* is the most prevalent *Candida*s pecies involved in co-infection with tuberculosis among patients living with human immunodeficiency virus (Talle *et al*., 2017).

Conclusion: The prevalence of *Mycobacterium tuberculosis* and candidiasis co-infection among the study population is influenced by age. Females especially highly active antiretroviral therapy (HAART)-naïve females were more vulnerable to co-infection than their male counterparts. Resistance of *M. tuberculosis* and candidiasis co-infection to rifampicin reported among the patients living with HIV affected more HAART-naïve patients compared with HAART patients.

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Data Availability Statement: Data are available upon request from the corresponding author.

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