Candidemia Among Mycobacterium Tuberculosis Patient Attending Aminu Kano Teaching Hospital

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ABSTRACT
Candidemia is one of the most serious forms of fungal infections, its incidence has been increasing because of the development of immunosuppressive conditions and therapies that encourages proliferation of opportunistic pathogens, as well as aging of population. The study included 165 participants and their blood samples were aseptically collected and inoculated in thioglycolate agar before subculture onto Sabouaud Dextrose Agar (SDA). Gram staining technique, Germ tube test and KOH test were done and later, subcultured onto CHROM agar for phenotypic speciation. From the 165 samples examined, 26 representing a prevalence of 15.8% were found to be positive for candidemia. Out of the total participants 92 were males while 73 were females. The Candida species isolated were C. albican 46.1%, C. krusei 26.9%, C. glabrata 23.2% and C. tropicalis 3.8%. The results showed that, Candida albican was the most common isolated specie 12(46.1%). Based on the results also, age range of 31-40 had the highest isolation frequency 9(34.6%) among all the age groups. According to the sex, male 15(57.7%) were more infected than female 11(42.3%). The findings showed that candida species are found to be among the compounders of health-related problems among TB patients in the study area.

Keywords
Candidamia, TB Patients, Fungal infections, Candida species.

Introduction
As a disease, invasive candidiasis is a clinical condition that mostly occurs in immunosuppressed individuals and those with general defects in their immune system. Among the species, Candida albicans is the most common pathogen capable of causing infections, ranging from slight mucocutaneous disorders to invasive diseases which affect multiple organs [1]. The disease tuberculosis (TB), is infectious in nature and still a serious threat to many populations especially in resource poor settings despite all efforts taken to contain its negative effects, this is so, because, according to the World Health Organization, up to 9.6 million people were detected as new cases of tuberculosis, and up to 1.5 million deaths associated with it, in 2014 alone [2].

There exits many types of pulmonary and extra-pulmonary diseases related to tuberculosis that leads to serious difficulties in differential and therapeutical diagnosis, other compounders include diabetes, old age, immunodeficiency, lung cancer and fungal lung infections, the diagnosis of which gain strong interest from researchers due to their frequency of association with TB and other low immunity related diseases [1,3].

Generally, invasive candidiasis which is normally caused by a yeast genus called Candida are serious in nature than vaginal yeast infections and Candida infections in the mouth and throat (the oral thrush) which are localized to their respective parts of the body, the invasive candidiasis is a serious condition that can affect many organs and systems like heart, the brain and many other body parts via the blood stream [4].

In nature, Candida normally lives in some body parts such as the gut, the mouth, and the vagina. Other areas affected are the throat and the skin where they live as normal flora without causing harm [5]. However, in people who are immunocompromised which tend to be prone to many opportunistic infections, Candida can enter...
the bloodstream resulting in candidamia (the most common form of invasive candidiasis) [4]. In the US, for instance, candidemia is one of the most common bloodstream infections associated with hospitalization and in most cases causes prolonged hospital stays and eventual death of the patients involved [6,7]. Fungi are widespread, mostly in the environment. Some are associated with animals and humans as commensals, but turn pathogenic or opportunistic after alteration of the host immune system. Usually due to extensive use of antifungals for prophylaxis in patients leads to colonization of body by the non-albicans Candida (NAC) species with increased antifungal drugs resistance [8,9].

Among the non-albican species, it was found in some places that Candida tropicalis is the most common cause of nosocomial candidamia because some epidemiological data obtained from Indian showed that a range of 67-90% of Nosocomial candidamia cases were found to be as a result of (NAC) species in which C. tropicalis appeared as the most prevalent [10,11]. It was also documented that Candida species are believed to be the major healthcare-associated bloodstream infection (BSI) globally [12,13]. The same species are also linked to great morbidity and mortality rates, particularly among intensive care units (ICU) patients in many hospitals where at least 50% episodes of candidamia do occur [14].

According to some records, apart from C. albicans as the most common specie frequently isolated from bloodstream, there exist now, a gradual shift of infection towards non-albicans species such as C. parapsilosis, C. glabrata, and C. tropicalis, which together account for more than 50% of candidamia in some cases [13,15]. But there exist some significant variations in the infection rates due to individual non albicans Candida species depending on the geographic location, patient population, age involved and prior exposure to antifungal drugs [16]. However irrespective of the geographical location, C. albicans is ranked the commonness isolates among patients of age up to 18 years, but the frequency of C. parapsilosis decreases with patient age while C. glabrata is mostly associated with elderly patients, as part of the variations, researchers found in Northern Europe and the USA [15], and also a relatively higher infection rates due to C. glabrata as against C. parapsilosis in Brazil and Spain [12]. But it was however found that, C. tropicalis were found as the major isolate of non-albicans Candida species in Asia [17].

Materials and Methods

Study area
The study was conducted in Aminu Kano Teaching Hospital (AKTH), situated in Kano metropolis which is a referral center for both private and public health institutions in and around the neighboring states. Kano lies between latitude 11°30’N and longitude 8°30’E. Kano state borders Katsina to the north-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west. The total land area of Kano state is 20,760 square kilometers with a population of 9,383,682 based on the official 2006 National Population and Housing Census [18].

Study design
The research was prospective cross-sectional study.

Study population
The study population included patients enrolled in DOTS (Directly Observed Treatment Short course) clinic of Aminu Kano Teaching Hospital.

Sample size determination
The sample size was calculated using the following formula [19].

\[ n = \frac{Z^2 \times P \times (1-P)}{d^2} \]

Where:
- \( n \) = sample size
- \( Z \) = statistic for level of confidence at 95% (1.96)
- \( P \) = the prevalence =11% (Yahaya et al., 2014)
- \( d \) = allowable error of 5% (0.05)

\[ n = (1.96)^2 \times 0.11 \times (1-0.11)/0.0025 = 150.437 \]

\[ n = 150 \]

Plus attrition rate=10%

\[ 10/100 (150) = 15 \]

\[ 150 + 15 = 165 \]

\[ n = 165 \]

Inclusion and exclusion criteria

Inclusion criteria
Suspect and follow up patients of all age ranges with tuberculosis attending DOTS Clinic, AKTH and referred to the laboratory were included in the study area.

Exclusion criteria
All non-tuberculosis patients, attending Aminu Kano Teaching Hospital, Kano and those TB patients on antifungal therapy were excluded from the study.

Ethical consideration
Ethical approval to conduct the research was collected from the research ethics committee of Aminu Kano Teaching hospital. The participant (subject) consent was also sought for prior to the administration of questionairs.

Sample collection and processing
Blood samples were collected from the participants and processed immediately, but where delay was anticipated, the samples were stored in the fridge at 2-8°C before use.

Inoculation onto culture media

Culture into thioglycolate
The samples were introduced into thioglycolate agar and incubated at 37°C for 72 hours. Isolates obtained from thioglycolate agar incubation were subcultured onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48 hours to obtained pure isolates.

Microscopy

Gram's staining technique
Smear of the colonies obtained from purity plates were prepared...
by emulsifying few colonies in a drop of water on a clean grease free glass slide, air dried and heat fixed by quickly passage over flame three (3) times. The primary stain (crystal violet) was applied for 1 minute and rinsed with water, Gram’s iodine was applied for 1 minute and rinsed with water then decolorized with acetone for few seconds and rinsed with water immediately, the smears were finally counterstained with neutral red for 1 minute and rinsed with water then allowed to air dry and examined microscopically using 100x objective [20].

Germ tube test
About 0.5ml of serum was dispensed into small test tubes, the colonies from pure culture was picked and gently emulsified in the serum and incubated at 37°C for 2-3 hours. A drop of the preparation was transferred onto a clean grease-free glass slide and covered with cover slip and focused using 10x and 40× objectives [20].

Inoculation on chromogenic agar for phenotypic speciation
The isolates obtained from pure culture were subcultured onto the chromogenic agar and incubated at 37°C 24- 48 hours, after the incubation period, the colonies appeared with different colours depending on the species types [21].

Statistical analysis
The data collected and results obtained were analysed using statistical package for social sciences (SPSS) version 20.0 and presented in form of tables.

Result
The study includes 165 participants, out of which 26 with prevalence (15.8%) were found to be positive for candidemia while the other 139 (84.2%) patients were found to be negative as shown in table 1. Out of the 165 participants recruited 92 (55.8%) were males and 73 (44.2%) were females with 15 (57.7%) and 12 (46.1%) as their respective positivity rates as seen in table 2.

Based on the results obtained, Candida albicans was the specie that had the highest prevalence of 11 (42.3%) followed by C. krusei 7 (26.9%), C. glabrata 6 (23.1%), and Candida tropicalis 1 (3.8%) table 3. With respect to age and gender of the participants, higher percentage was recorded in 31-40 age group in both males and females, with 5 (3.0%) and 4 (2.4%) isolation frequencies respectively, this was followed by age groups of 21-30 and 41-50 which had 2 and 3 as isolation rates for males and females respectively, table 4.

Discussion
The total prevalence obtained in this study was 26 (15.8%), this was however lower than the findings of Astekar et al. [22], in a study titled Prevalence and characterization of opportunistic candidal infections among patients with pulmonary tuberculosis.

Infection rate according to gender showed that males were more infected 55.8% than females 44.2%. however, Arunava et al. [23] had in a study, the Candida co-infection rate of 62.5% in female subjects, while it was observed in only 29.4% of the males counter parts. Also, according to Astekar et al. [22], males had the predominant rate of infection than females with the prevalence of Candida in males and females been 20 (60.7%) and 13 (39.3%), respectively.

Species distribution revealed Candida albican as the most abundant isolate in the study area followed by Candida kruzie, Candida glabrata and Candida tropicalis with 11, 7, 6 and 1 number of isolates, representing 42.3%, 26.9%, 23.1% and 3.8% respectively.

According to Khan et al. [24], in one study, observed C. albicans as the most frequently isolated species accounting for 539 (37.22%) of the cases closely followed by 502 (34.67%) cases due to C. parapsilosis, while the remaining cases were due to C. tropicalis 210 (14.5%), C. glabrata 148 (10.22%), C. krusei 27 (1.81%) and C. dubliniensis 22 (1.5%).

Based on a 2013 study, Arunava et al. [23], it was found that, Candida albicans was the most common isolate observed in 50% of the patients with Candida-TB co-infection, followed by C. tropicalis (20%) and C. glabrata (20%). In the study, age group distribution of the infections showed that, the age limit of 31-40 had the most frequent isolation rate 9 (34.6) followed by 21-30 and 41-50 with 5 (19.2%) each while the age bracket of 71-80 had the least isolation rate of 1 (3.8%).

Table 1: The Candida species isolated in the study

| Candida Species | Frequency | Percentage (%) |
|----------------|-----------|----------------|
| C. Albican     | 12        | 46.1           |
| C. Krusie      | 7         | 26.9           |
| C. Glabrata    | 6         | 23.2           |
| C. Tropicalis  | 1         | 3.8            |
| Total          | 26        | 100            |

Table 2: Gender distribution of candidamia in the study area

| Subjects status | Frequency | Percentage (%) |
|----------------|-----------|----------------|
| Positive       | 26        | 15.8           |
| Negative       | 139       | 84.2           |
| Total          | 165       | 100            |

Table 3: The Candida species isolated in the study

| Age (Years) | Frequency | Male Infected Positive (%) | Female Infected Positive (%) | Total Infected Positive (%) | P value |
|-------------|-----------|----------------------------|------------------------------|-----------------------------|---------|
| 11 – 20     | 15        | 2 (13.3)                   | 0 (0.0)                      | 2 (7.7)                     | 0.282   |
| 21 – 30     | 42        | 2 (13.3)                   | 3 (27.3)                     | 5 (19.2)                    |         |
| 31 – 40     | 49        | 5 (33.3)                   | 4 (36.4)                     | 9 (34.6)                    |         |
| 41 – 50     | 31        | 2 (13.3)                   | 3 (27.3)                     | 5 (19.2)                    |         |
| 51 – 60     | 16        | 2 (13.3)                   | 0 (0.0)                      | 2 (11.5)                    |         |
| 61 – 70     | 8         | 1 (6.7)                    | 1 (9.1)                      | 2 (7.7)                     |         |
| 71 – 80     | 4         | 1 (6.7)                    | 0 (0.0)                      | 1 (3.8)                     |         |
| Total       | 165       | 15 (100)                   | 11 (100)                     | 26 (100)                    |         |
This is not in agreement with Astekar et al. [22], which had in his results, 60–80 years as the age range with predominant cases 18 (54.54%) followed by 40–60 years 10 (30.30%) and the least was 20–40 years 5 (15.16%) according to their study.

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