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Mycological Findings of Sputum Samples from Pulmonary Tuberculosis Patients Attending TB Clinic in Nairobi, Kenya

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Abstract

Objective: To determine the prevalence of fungal pathogens in patients with pulmonary tuberculosis at Mbagathi District Hospital TB clinic.

Design: One hundred and seventy two sputum samples were collected from patients who were confirmed to have pulmonary tuberculosis at Mbagathi District Hospital TB clinic. These samples were subjected to mycological investigation using microscopy and culture.

Results: Pulmonary fungal pathogens were isolated as co-pathogens with *Mycobacterium tuberculosis* in 76 (44.18%). Yeasts accounted for 46/172 (26.7%), with 33/172 (19%) being *Candida albicans*, 3/172 (1.7%) were identified as *Candida dubliniensis,* 1/172 (0.6%) was *Candida guilliermondii,* while 3/172(1.7%) were *Candida tropicalis. Cryptococcus laurentii* was isolated in 2/172 (1.2%). Colonization of *Mycobacterium tuberculosis* with moulds was as follows: 2/172 (1.2%) *Aspergillus flavus,* 3/172 (1.7%) *Aspergillus fumigatus* 4/172 (2.3%) *Aspergillus niger,* 2/172 (1.2%) *Scytalidium hyalinum* and 4/172 (2.3%) *Trichosporon asahii. Pneumocystis jirovencii* oocysts were positive in 19/172 (11.0%) on Toluidine O blue. Gram stain of the sputum samples yielded: 4/172 (2.3%) Gram negative rods, 10/172 (5.8%) Gram positive cocci and 6/172(3.5%) Gram positive rods.

Conclusion: Pathogenic fungi and other bacterial pathogens may be significant co-infecting pathogens complicating the management of TB. Clinicians in Kenya should be aware of co-infection of *Mycobacterium tuberculosis* with opportunistic pulmonary fungal and bacterial pathogens. HIV infection is a significant pre-disposition to pulmonary tuberculosis. The two conditions present severe immunosuppression. Confounded by prolonged TB treatment, this group represents a high risk for acquiring opportunistic fungal pathogens.

Keywords: Co-infection; *Mycobacterium tuberculosis;* Opportunistic; Fungal; Bacteria; Pathogens

Introduction

The incidence of fungal pneumonias has increased significantly since the 1960s, particularly due to increasing number of immunocompromised patients [1,2]. The opportunistic fungi, which are either ubiquitous or part of normal flora and infections, are acquired by inhalation of contaminated soil [3]. For instance, *Cryptococcus neoformans* can infect people with intact immune systems at a rate of 0.2 cases per million populations per year. However, HIV/AIDS patients are more at risk, with approximately 80-90% of patients with HIV/AIDS developing cryptococcosis [4]. Cryptococcosis is caused by members of the *Cryptococcus neoformans* species complex, comprising the three variants *C. neoformans v. gattii (Cryptococcus gattii)*, C. *neoformans v. neoformans* and *C. neoformans v. Grubii* [5]. Cryptococcosis is believed to be acquired by inhalation of the infectious propagule from the environment. The prevalence of cryptococcosis has been increasing over the past 20 years for many reasons, including the HIV pandemic and the expanded use of immunosuppressive drugs [6]. In people with a normal immune system, the lung (pulmonary) form of cryptococcoses may be asymptomatic, but with impaired immune systems, the *Cryptococcus* spp. may disseminate to the meninges causing life-threatening meningoencephalitis [7].

Aspergillosis is a term referring to a wide variety of diseases caused by the genus *Aspergillus*. The most common forms are allergic bronchopulmonary aspergillosis, pulmonary aspergilloma and invasive aspergillosis [8]. Aspergillosis develops mainly in individuals who are immunocompromised, either from disease or from immunosuppressive drugs. It is a leading cause of death in acute leukemia and hematopoietic stem cell transplantation. *Aspergillus fumigatus* is a fungus of the genus *Aspergillus*, and is one of the most common opportunistic pathogen in immuno-compromised individuals. The spores are ubiquitous in the atmosphere, and it is estimated that everybody inhales several hundred spores each day; typically these are quickly eliminated by the immune system in healthy individuals [8].

In immuno-compromised individuals, such as organ transplant recipients and people with AIDS or leukaemia, the fungus is capable of becoming pathogenic; over-running the host's weakened defenses and causing a range of diseases generally termed aspergillosis [9].

Aspergillus flavus is a common environmental mold and can cause storage problems in stored grains. However, it can also be a human pathogen, associated with aspergillosis, corneal, otomycotic and nasoorbital infections. Many strains produce significant quantities of aflatoxin, which is a carcinogenic and acutely toxic compound or allergenic spores [10]. Aspergillosis is mainly caused by *Aspergillus fumigatus*, an organism found in hay and grains. In histology, it appears as a mixture of coarse, fragmented hyphae and fungalball [11].

Candidiasis or 'thrush' is a fungal infection caused by any of the *Candida* species, of which *Candida albicans* is the most common. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-

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threatening diseases [12]. *Candida* infections of the latter category are also referred to as candidemia, and are usually confined to severely immunocompromised persons, such as cancer, transplant and AIDS patients. Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are, however, common in many human populations [13].

Candida species of medical importance includes; *Candida albicans* (50-60%), *C. glabrata* (15-20%), *C. parapsilosis* (10-20%), *C. tropicalis* (6-12%), *C. krusei* (1-3%), *C. kefyr* (<5%), *C. lusitaniae* (<5%), *C. quilliermondii* (<5%), *Candida dubliniensis* (<5%). The risk factors for candidiasis are: granulocytopenia, bone marrow transplantation, solid organ transplantation (liver, kidney), hematologic malignancies, foley catheters, recent chemotherapy or radiation therapy, corticosteroids, broad-spectrum antibiotics, burns, prolonged hospitalization, severe trauma, recent bacterial infection, recent surgery, gastrointestinal tract surgery, central intravascular access devices, premature birth, hemodialysis [13,14].

Most tuberculosis patients are HIV positive, and are likely to develop Candidiasis that can disseminate to respiratory cavities, which if left untreated can be fatal [3,15].

Pneumocystis jirovecii remains a major pulmonary pathogen in immunocompromised patien,t and the most commonly diagnosed in HIV/AIDS [16]. *Pneumocystis jirovecii* cannot be propagated in cultured and specific identification relies on microscopic examination of respiratory specimens, such as expectorated sputum and bronchoalveolar lavage (BAL) [17]. Before PCP was thought to be absent in Africa and in 1995, only two cases of *Pneumocystis pneumonia* were reported in Kenya. However, local studies recently confirmed that 37.2% of adult patients with clinical and radiological features compatible with *Pneumocystis* pneumonia were actually infected with *Pneumocystis jirovecii* [18]. *Pneumocystis* pneumonia is more common in children than in adults infected with HIV, with an estimated risk of 7-20% in the first year in those perinatally infected with HIV. In Kenya, it has been found to be a significant co- pathogen with other respiratory infections in children with severe pneumonia, with or without HIV infection [19]. However, locally no studies have been done to determine the significance of co-infection of *Pneumocystis* pneumonia with tuberculosis.

Methods

Between July 2009 and August 2009, 172 sputum samples were collected from Mbagathi District Hospital TB clinic and trasfered to Mycology Laboratory, Centre for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI). The specimen were from both adults and children who were diagnosed to be tuberculosis positive, as defined the World Health Organization (WHO) clinical criteria of persistent cough, loss of weight and night sweats and confirmed by ZN smear. After written informed consent was obtained from patients, all the patients were requested to produce first morning sputum samples. The samples were subjected to mycological investigation using microscopy and culture. The study protocol was approved by the KEMRI Scientific Steering Committee and Ethical Review Committee (ERC). All ethical issues were adhered to in all the stages of the protocol implementation.

Experimental Procedures

All the sputum specimens were subjected to Gram staining (bacteria), Ziehl-Neelsen (TB), Toluidine O Blue for detection of *Pneumocystis jirovecii and* potassium hydroxide (KOH) preparation for fungi.

Mycological culture was done on Sabourauds dextrose agar and Brain Heart Infusion Agar, and incubated at 30°C and 37°C ambient air, respectively, for up to four weeks. Detection of mixed yeast infestation and preliminary identification was done on CHROMagar Candida (Oxoid Company).

Casein agar was used for differentiation of *Candida albicans* and *Candida dubliniensis.* Preliminary identification of *Cryptococcus* spp. was done using Urea Hydrolysis.

Analytical profile Index (API 20 Caux) (Biomeriux) was used as a confirmatory test for all the yeasts isolates.

Identification of all the moulds was done using macro and micro-morphological features on Lactophenol cotton stains. All the procedures were done under level two containment facilities at Mycology laboratory using KEMRI biosafety guidelines.

All the sputum samples were also subjected to Gram staining procedures to detect the presence of bacteria, yeasts or fungal elements in sputum samples. Gram staining was also used to differentiate bacterial species into Gram-positive and Gram-negative based on the chemical and physical properties of their walls.

Heat fixed smears were prepared on glass slides. The slides were put on a staining rack, and then flooded will crystal violet stain for one minute.

The excess dye were poured off and washed gently in tap water. The smears were exposed to Grams iodine for one minute. Then the iodine was washed carefully with tap water. The smears were washed with acetone as a decolorizer for 30 seconds. Smears were washed with tap water to stop the decolorization. The smears were counter stained with neutral red for 30 seconds.

The smears were scored for the presence or absence of bacterial, fungal elements and yeast cells [20]. The Ziehl-Neelsen stain also known as the acid-fast stain was used for detection of *Mycobacterium tuberculosis.* Briefly**,** the smears were prepared and heat fixed and flooded with Carbol Fuchsin stain. Using a Bunsen burner, the smears were heated slowly until they steamed.

The steaming was maintained for five minutes by using low heat, i.e. the flame from the Bunsen burner were passed occasionally beneath the slides.

The slides were then rinsed with water and then flooded with acid alcohol, and were allowed to decolorize for five minutes. The slides were then rinsed thoroughly with water, and then the excess water was drained from the slides.

The sputum smears were made and air dried then flooded with counterstain Methylene Blue and kept on the slides for one minute. The slides were then rinsed thoroughly with clean tap water, and then were allowed to air dry. The smears were observed under light microscope using oil immersion for the presence of *Mycobacterium tuberculosis.* The slides containing *Mycobacterium tuberculosis* bacilli were scored positive.

A modified Toluidine blue O staining technique was used for the detection of *Pneumocystis jirovecii* [21,22].

Sputum smears were made on glass slides and allowed to air dry. It was heat fixed and then placed in sulfation reagent (45 ml of glycial acetic acid mixed with 15 ml of concentrated sulfuric acid) for 10 minutes. The smears were then rinsed in cold water for 5 minutes and excess water drained. The smears were then placed in Toluidine Blue O (0.3 g of dye in 6 ml of water) for 3 minutes.

The smears were then rinsed with 95% ethanol, followed by absolute ethanol and then xylene.

The smears were then air dried and examined at ×100 for the presence of *Pneumocystis jirovecii* oocysts, and the slides with *Pneumocystis jirovecii* scored positive.

The toluidine positive samples were confirmed by immunoflorescence (IF) staining using IF staining kit (Shield Diagnostics, Dundee, UK)

All sputum samples were also subjected to Potassium hydroxide preparations for the detection of fungal elements. Briefly, purulent portion of the specimen was mixed with 10% KOH left to stand for 20 minutes, and examined at X40 magnification for fungal elements [23]. The purpose was to provide descriptive morphological information of these pathogens to aid in the identification.

Mycological culture was done on Sabourauds dextrose agar and Brain Heart Infusion Agar media supplemented with chloramphenicol. Briefly, purulent portion of the sample was inoculated onto the media and incubated at 30°C and37°C, respectively for up to four weeks, before the culture was reported negative.

Fungal identification

CHROMagar was used for preliminary identification of yeast and to detect mixed infections [24].

Casein agar was used to distinguish *Candida dubliniensis* from *Candida albican. Candida dubliniensis,* which produce abundant chlamydospores in triplets, while *C. albicans* does not.

Analytical Profile Index (API) 20 Caux (BioMerieux® SA) was used as a confirmatory test for all the yeasts. The procedure and results were done and interpreted according to the manufacturer's instruction.

Yeast populations were differentiated by colonial morphology and colors, which were generated by a chromogenic in the agar, as described by Wortman et al. [25]. This was based on different colors given by different Candida species on CHROMagar, which was able to identify yeasts from 46 samples. The entire control strains exhibited typical colony morphology and color on CHROMagar. Only two strain which gave the characteristic matt, blue-colored (blue grey) colonies, and was identified as *C. tropicalis*, two gave characteristic pink color, which were identified as *C. parapsilosis,* 36 identifications were not very specific which gave varying shades of leading to difficulties in the identification, thus API 20 C AUX was done. C. *albicans* strains produce ß-N-acetylgalactosamini-dase, which interacts with the chromo-genic hexosaminidase substrate incorporated into the agar, and after incubation for 48 h, green colonies were produced, characteristic of all C. *albicans* isolates. *Candida dubliniensis* gave a green color almost like *C. albicans,* and hence, the technique could not differentiate the 2 isolates, which gave the characteristic green colonies between *C. albicans* and *C. dubliniensis.* Further test of Casein agar was done to distinguish between the two, as described by Sogaard et al. [26]. The 36 yeast isolates which gave light green-colored colonies on CHROMagar were inoculated onto Casein agar. The culture growth was stained with lactophenol cotton blue, and was examined for chlamydospore production by light microscopy. All *isolates* tested did not produce chlamydospores on casein agar after 48 h of incubation, but there was pseudomycelial growth and were confirmed to be *C. albicans,* and those with abundant chlamydospores in triplets were scored as *C. dubliniensis*.

Positive mould cultures were identified morphologically using macro and micromorphological features on Lactophenol cotton Blue stain.

Results

The demographics of the study participants were 164/172 (95.3%) adults and 8/172 (4.7%) children (>18 years). The gender participation were, 69/172 (40.1%) females and 103/172 (59.9%) males.

Among the 172 sputum samples analyzed 14/172(8.1%) were positive for fungal elements, 50/172(29.1%) were positive for yeast cells, 1/172(0.6%) of the speciments were positive for both yeast and gram positive rods. One (0.6%) was positive for yeast cells together with fungal elements, and 3/172(1.7%) of the samples were positive for Gram negative rods and 10/172 (5.8%) were positive for gram positive cocci, 8/172(4.7%) were Gram positive rods, while 85/172 49.4% of samples were scored negative for potential pathogens on Gram reaction (Table 1).

Pulmonary fungal pathogens isolation in *Mycobacterium tuberculosis* patients were: 46/172 (26.7%). Yeasts were isolated in 46/172 (26.7%) samples, in which 33/172 (19.2%) were *Candida albicans*, 3/172 (1.7%) were *Candida dubliensis,* 1/172 (0.6%) was *Candida guilliermondii,* 3/172 (1.7%) were *Candida tropicalis. Cryptococcus lauretii* was isolated in 2/172 (1.2%) of the samples.

Filamentous fungal colonization with *Mycobacterium tuberculosis* was as follows: *Aspergillus flavus* 2/172 (1.2%), *A. fumigatus* 3/172 (1.7%), *A. niger* 4/172 (2.3%), *Scytalidium hyalinum* 2/172(1.2%) and 4/172(2.3%) *Trichosporon asahii* (Table 2).

Among the pathogens isolated; 46/172 (26.74%) were yeasts and 33/172 (19.2%) were *Candida albicans*, 3/172 (1.7%), *Candida dublieniensis* and *C. tropicalis* each, 1/172 (0.6%) were *Candida guilliermondii,* 2/172 (1.2%) were *Cryptococcus lauretii.* The samples negative for yeast were 126/172 (73.3%) (Table 3).

Pneumocystis jirovecii

Toluidine O Blue staining for *Pneumocystis jirovecii* detected 19/172 (11.0%) samples positive for *Pneumocystis jirovecii* oocysts.

Discussion

Opportunistic fungal infections are emerging significant

Category	Frequency (%)
Fungal elements	14 (8.1)
Fungal elements and yeasts	1 (0.6)
Gram negative rods	3 (1.7)
Gram positive cocci	10 (5.8)
Gram positive rods	8 (4.7)
Yeast cells	50 (29.1)
Yeast cells and gram positive rods	1 (0.6)
Gram reaction negative samples	85 (49.4)

Table 1: Results of Gram stain of sputum samples from *Mycobacterium tuberculosis* positive patients.

Name of fungal pathogen	Frequency (%)
Aspergillus flavus	2 (1.2)
Aspergillus fumigatus	3 (1.7)
Scytalidium hyalinum	2 (1.2)
Trichosporon asahii	4 (2.3)
Negative	157 (91.3)

Table 2: Filamentous fungal isolation in *Mycobacterium tuberculosis* positive samples.

Species name	Frequency (%)
Candida albicans	33 (19.2)
Candida dubliensis	3 (1.7)
Candida quilliermondii	1 (0.6)
Candida parapsilosis	2 (1.2)
Candida tropicalis	3 (1.7)
Cryptococcus laurentii	2 (1.2)
Actinomycetes	2 (1.2)
Negative	126 (73.3)

Table 3: Species diversity of yeasts isolated from sputum of *Mycobacterium* positive patients.

pathogens, especially in the context of expanding population of immunocompromised people with HIV/AIDS and pulmonary tuberculosis. The frequency of invasive mycoses due to opportunistic fungal pathogens has increased significantly over the past two decades. This increase in infections is associated with excessive morbidity and mortality. This is directly related to increasing patient populations at risk for the development of serious fungal infections. Individuals undergoing solid-organ transplantation, blood and marrow transplantation (BMT), and major surgery, and those with AIDS, neoplastic disease, immunosuppressive therapy, advanced age and premature birth are most at risk [27].

Opportunistic fungal organisms, such as *Candida* species, *Aspergillus* species, *Mucor* species and *Cryptococcus neoformans* tend to cause diseases in patients who are immuno-compromised [3,28]. In a total of 172 samples analyzed for fungal pathogens, 80/172 (46.5%) were positive for fungi, in which 46/172(26.74%) isolates were yeasts, 15/172 (8.72%) were molds and 19/172 (11.04%) were *Pneumocystis jirovecii*. The immunosupression due HIV/AIDS pandemic confounded by TB co-infections is a severe immunosupressive combined predisposition to serious opportunistic fungal infections. The reasons for increased prevalence are lowering of immune system due to tuberculosis, and the prolonged use of anti-tuberculosis drugs which promote the growth and reproduction of the fungus flora, and in turn, aggravate the course of underlying process in the lung tissues [29].

The study showed that *Candida albicans* is the most common fungal co-infection with *Mycobacterium tuberculosis, with* a prevalence of 33/172 (19.18%). These opportunistic fungi are potential pathogen in the immunocompromised individuals, patients with some pre-existing disease and patients with a long history of antibiotics use [3,30]. Besides, a syntropic relationship between *C. albicans* and *Mycobacterium tuberculosis* has also been reported in a number of studies, where tubercle bacilli were found to enable *C. albicans* to grow on Lowenstein Jensen's medium. Studies have confirmed that the polysaccharide fraction of *C. albicans* enhance the growth and reduction of the generation time of tubercle bacilli [31]. This is in accord with other studies, indicating that *C. albicans* is the most common opportunistic yeast, accounting for up to 19.18% of the oral infection in HIV/AIDS/TB patients [32]. It is also notable that *Candida albicans* is a recognized opportunistic pathogen in HIV/AIDS, and is a WHO AIDS defining illness [3]. Co-infection of TB with HIV are two severe immune debilitating conditions, and patients with the two conditions are more susceptible to acquiring opportunistic fungal infection, unfortunately we did not determine the HIV status of the study participants due to consenting and ethical reasons. *Candida albicans,* being a normal flora in the mouth can be easily disseminated to cause infection, when host defenses are compromised [33].

Majority of samples were obtained from adults (95.3%). There were more samples from males (59.9%) than females (40.1%), probably because of the health seeking behavior or probably males are at more risk of TB infection than females. Only 8/172 (4.7%) samples were from children (4.7%). These could be associated with the difficulty in obtaining good quality specimens from the minors.

Gram staining was to determine and differentiate bacterial species into two large groups of Gram-positive and Gram-negative was carried out. Based on the chemical and physical properties of their walls, Gram stain is also important in mycology because the stain showed morphological structures of the fungal pathogens especially yeasts [24,34]. On Gram stain procedure, 15/172 (8.1%) samples were positive for fungal hyphae. The rest were yeasts 50/172 (29.1%), 3/172 (1.7%) were Gram negative rods, 10/172 (5.8%) Gram positive cocci, 8/172 (4.7%) were Gram positive rods. Gram stain is a useful indicator of possible pathogens whenever culture is not feasible, and has been used for preliminary diagnosis in clinical practice. Based on this technique, there was an indication of bacterial and fungal co-infection with pulmonary tuberculosis [32].

Examination of the sputum specimens using 10% KOH revealed that 65/172 (37.8%) samples were positive for fungal elements, while 107/172 (62.2%) samples were negative. Those fungal positive were lower than findings by Fraser et al. [3] of 68%. This could be attributed organism load or population differences, but needs further investigations. Based on culture, macro and micro morphological features on Lactophenol Cotton Blue Stain 15/172 fungal pathogens were identified, of which 14/172 (8.1%) were filamentours, with 1/172) (0.6%) having both fungal elemens and yeast cells. 50/172, which is 29.1% were positive for yeast cells, while 107/172 (62.2%) of the samples were negative. Althought majority of the samples were negative for fungal pathogens, there was a significant portion with fungal co-infection with pulmonary tuberculosis, which has also been documented elswhere [13,32].

Aspergillosis of the lungs is a disease caused by an exceedingly common mold, *Aspergillus fumigatus*. The fungus of the genus *Aspergillus*, is one of the most common *Aspergillus* species to cause disease in immuno-compromised individuals. Aspergillosis is a serious pathologic condition caused by *Aspergillus* organisms, and is frequently seen in immunocompromised patients. In recent years, it has been shown that *Aspergillus* infection can result in a broad range of airway complications with radiological and pathologic features mimicking those of TB [35]. *Aspergillus fumigatus* is a saprotrophic fungus that is widespread in nature, typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling [36]. Other reported pathogenic species are *Aspergillus flavus. Aspergillus niger* and *Aspergillus parasiticus.* It is a disease which clinically and pathologically so closely resembles tuberculosis that it usually escapes recognition and is misdiagnosed as tuberculosis. The true incidence of aspergilloma is not known but presents as pulmonary cavity secondary to tuberculosis in 11% patients with pulmonary cavities. The most common predisposing factor is the presence of a preexisting lung cavity formed secondary to tuberculosis [36]. There is a common belief that this mold is purely saprophytic; that it can live only on preexisting lesions in the lungs; that it cannot attack living tissues because it has no primary pathogenic powers. However, studies have shown that a prerequisite for the pleural aspergillosis is that the lung or pleura is previously damaged previously by active tuberculosis causing destruction of the lung [37].

Aspergillus niger is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. Aspergillosis is, in particular,

frequent among horticultural workers that inhale peat dust, which can be rich in *Aspergillus* spores [36,37]. *Aspergillus flavus* is a common mold in the environment, and can cause storage problems in grains [38]. It can also be a human pathogen, associated with aspergillosis of the lungs, and sometimes causing corneal, otomycotic and nasoorbital infections. *A. flavus* is the second most common agent of aspergillosis after *Aspergillus fumigatus*. *A. flavus* may invade arteries of the lung or brain and cause infarction [39,40]. Neutropenia predisposes to aspergillus infection [35], Chronic obstructive pulmonary disease due to coexistence of *Mycobacterium* and aspergillus infection is a fatal combination; resulting to progressive lung destruction, leading to early death despite prolonged antimycobacterial chemotherapy [40-43].

Toulidine Blue 0 stainining for the detection of the *Pneumocystis jirovecii* yielded 19/172 (11.0%) positive for *P. jirovencii* cysts. This is slightly lower than that detetcted on Bronchioalveolar Lavage (BAL) of children with severe pneumonia [19]. Previously PCP was thought to be absent in Kenya, however studies by Haque [18] confirmed a prevalence of 37% PCP in HIV/AIDS patients, with PCP compatible symptom in Mbagath District Hospital. The current figure is lower than that reported in the two studies, probably because of the degree of immunosuppression and the sensitivity of sputum varses BAL and Toluidine O blue compared to Immunoflourescent staining. The BAL and IF has higher sensitivity and specificity than Toluidine O Blue.

Analytical Profile Index (API) 20C AUX confirmed various species of *Candida,* including *C. parapsilosis, C. albicans, C. tropicalis, and C. guilliermondii. C. albicans* was the most predominant accounting 33/172 (19.2%), followed by *C. tropicalis* and *C. dubliniensis* (1.7%), each and *C. guilliermondii* (0.6%). Occurrence of the rest was 1.2%. According to this study, *Candida albicans* is a significant co-infection with pulmonary tuberculosis, as reported in another study by Mårdh et al. [32]. It is also good to note that *Candida dublinensis* has not been previously reported in Kenya

Conclusion

According to the study, it is confirmed that fungi are significant co-pathogens in TB patients. The study has also shown that among the most predominant yeasts that co-infect with pulmonary tuberculosis are the *Candida albicans*. As reported elsewhere [44], the study showed that pulmonary tuberculosis patients can be co-infected with *Cryptococcus laurentii, Pneumocystis jirovecii, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Trichosporon asahii,* other *Candida* spp like *C. parapsilosis, C. guilliermondii, C. tropicalis* and *C. dubliniensis* [45-47].

The study shows that there is significant co-infection of fungal and bacterial pathogens with *Mycobacterium tuberculosis.* HIV/AIDS-Tuberculosis patients are severely immune-suppressed, hence at a risk of acquiring opportunistic fungal infection. Clinical and radiological presentations are inconclusive [42]. There is need, therefore, for mycological and bacteriological investigations of pulmonary tuberculosis patients for any secondary fungal or bacterial infections for better management of this high risk population. It is possible that the high relapse cases, treatment failures, resistance and high mortality associated with TB infection is partly attributed to co-infection with opportunistic fungal pathogens and drug resistant non TB bacteria [46].

Recommendations

- Pulmonary tuberculosis patients, particularly retreatment cases, relapse and treatment failures, should be subjected to bacterial and fungal investigation to reduce the disease burden, and for better clinical management of tuberculosis
- Fungi isolated from tuberculosis media should be considered to be of clinical significance, and not merely as contaminants.
- Laboratory personnel should be trained on mycological procedures of isolation and identification fungal pathogens of clinical significance.
- Awareness ought to be made to clinicians and pulmonary tuberculosis patients on fungal infection as possible pathogen which can contribute to the complication of pulmonary tuberculosis.
- Further studies should be done using more sensitive methods, such as polymerase chain reaction (PCR), to compare and make a logical conclusion in the diagnosis of the fungal infection in pulmonary tuberculosis patients.
- For patient management purposes, understanding and treatment of fungal co-infection with *Mycobacterium tuberculosis* is important for good prognosis.

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