

**PULMONARY MYCOSES AMONG HIV/AIDS PATIENTS IN KIRINYA PRISONS
COMPLEX, JINJA**

**KISAKYE MOSES
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DECEMBER, 2015

DECLARATION

I, **KISAKYE MOSES** declare that this research report is my original work and has never been submitted to any institution for an academic award.

Signature.....

Date.....

APPROVAL

This work was supervised and submitted with approval of the following supervisor.

Signature.....

MWAMBI BASHIR

(MLS, MSc.)

Date.....

DEDICATION

This report is dedicated to my beloved wife Mrs. Gladys Kisakye and my mother Alice Kisolo in appreciation of their love, care, support, encouragement and financial commitment over the years.

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I am grateful to my supervisor Mr. Mwambi Bashir for the guidance, moral and technical support he offered during the study period and preparation of this report. His tireless efforts have enabled the success of this study.

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Finally I appreciate the management of Kirinya Prisons Complex (KPC) for allowing me to carry out this study in their institution.

‘MAY THE GRACE AND LOVE OF GOD BE WITH YOU’

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LIST OF ABBREVIATIONS

| | | |
|------------|---|--|
| AIDS | : | Acquired Immune-deficiency Syndrome |
| HIV | : | Human Immune-deficiency Virus |
| PLWHIV | : | People Living With HIV |
| HAART | : | Highly Active Anti-retro Viral Treatment |
| KPC | : | Kirinya Prisons Complex |
| MOH | : | Ministry of Health |
| OIs | : | Opportunistic Infections |
| P. mycoses | : | Pulmonary mycoses |
| TB | : | Tuberculosis |
| PTB | : | Pulmonary Tuberculosis |
| ZN | : | Ziehl Neelsen |
| MTB | : | Mycobacterium Tuberculosis |
| SOPs | : | Standard Operating Procedures |
| WHO | : | World Health Organization |
| SDA | : | Sabouraud Dextrose Agar |
| KOH | : | Potassium Hydroxide |
| HCIII | : | Health Centre iii |
| UDHS | : | Uganda Demographic Health Survey |

DEFINITION OF TERMS

Mycoses: These are human or animal diseases (infections) caused by fungus.

Opportunistic infections: These are infections that occur when the immune system is not functioning properly or it has been lowered. Bacteria (fungus) that are usually harmless can overwhelm the body and cause disease.

Opportunistic mycoses: These are fungi which take advantage of the lowered immunity of the host to cause disease.

Pulmonary mycoses: These are systemic infections caused by; yeasts, moulds and dimorphic fungi which are commonly opportunistic infections especially among People Living with Human Immune deficiency Virus (PLWHIV) and those who have been on strong antibiotics for a long time but can also be true or primary infections.

Suspected pulmonary Mycoses: These are cases treated for pulmonary mycoses without laboratory confirmation.

Tuberculosis (TB): This is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). These bacteria cause infection after a given period of latency. They attack the lungs and other organs of the body like the kidneys, spine, and the brain among others.

Pulmonary tuberculosis: This occurs when the TB microbes have attacked and damaged the lungs.

Clients: These are known PLWHIV and on HAART.

ABSTRACT

Pulmonary mycoses (P. mycoses) are a group of fungal infections commonly occurring as opportunistic infections (OIs) among individuals who are immune suppressed more especially those with human Immuno-deficiency virus (HIV) and others who have been on antibiotics for a long time, though during routine practice are misdiagnosed for Tuberculosis (TB) leading to mismanagement with associated toxicities and drug resistance among the patients.

This study was carried out at Kirinya Prisons Complex found in Jinja district with the major aim of determining the prevalence and factors associated with pulmonary mycoses among PLWHIV.

Two sputum samples were collected from 127 participants. The samples were processed systematically by; KOH mount, ZN staining and culturing on SDA microscopy with Lactophenol cotton blue staining. The cultures were identified using the morphological characteristics and stained slides read by microscopy.

Amongst the clients recruited for the study, 31/127 (24.41%, 95% CI= 17.92-33.67) were found to have pulmonary mycoses. Out of the 31 patients who were found to have pulmonary mycoses, 15 (27.78%) were females while 16 (21.92%) were males. Still we found that, 3 (2.36%) clients had TB and 1 (3.23%) had a co-infection with P. mycosis. Of the 31 fungal organisms, majority were *Candida* species, 11 (35.48%, 95% CI =21.1-56.31), followed by *C. neoformans* 8 (25.81%, 95% CI=11.46-43.4), *Aspergillus* species 6 (19.35%, 95% CI=7.21-36.44), and *Zygomycetes* 6 (19.35%, 95% CI=7.21-36.44). Independently the factors; weight loss, chest pain and night sweats were not associated with P. mycoses (OR= 1.11, 1.15 and 2.51; p. Values= 0.82, 0.82 and 0.11 respectively). Also P. mycoses presented similarly in both acute (≤ 2 weeks) and chronic (>2 weeks) coughs. A decline CD4 cell count ≤ 250 was a risk factor associated with the development of P. mycoses (OR = 9.7, p. Value < 0.001) and duration of stay in Prison was not associated with the development of P. mycoses (OR = 1.44, p. Value = 0.38).

In conclusion, the overall prevalence of pulmonary mycoses in KPC was relatively high (24.41%). *Candida* species were the most prevalent followed by *C. neoformans*, *Aspergillus* species and *Zygomycetes*. Weight loss, chest pain and night sweats were not associated with P. mycoses. A decline CD4 cell count $\leq 250\mu\text{l}$ was a risk factor associated with the development of P. mycoses and duration of stay in Prison was not associated with the development of P. mycoses.

CHAPTER ONE: INTRODUCTION

1.0 Background

P. mycoses are a group of fungal infections commonly occurring as opportunistic infections (OIs) among individuals who are immune suppressed more especially those with human Immuno-deficiency virus (HIV) and others who have been on antibiotics for a long time, though during routine practice are misdiagnosed for Tuberculosis (TB) leading to mismanagement with associated toxicities and drug resistance among the patients (Monica, 2010).

Globally, P. mycoses are increasingly being reported as the causes of morbidity & mortality among people living with HIV (UNAIDS, 2011). Many of the cases occur in tropical Africa and other developing countries. This is because of the poor health infrastructure coupled with escalating poverty levels (WHO, 2014).

P. mycoses may present as acute and chronic cough and often with, fever, weight loss, chest pain, night sweats, haemoptysis among other signs and symptoms similar to TB and or other diseases of obscure etiology, which unless detected and treated early may result into poor management with serious consequences (Adams *et al.*, 1997). Medically important species causing P. mycoses include; *Candida* spp, *Cryptococcus* spp, *Aspergillus* species, *Pneumocystis* spp, *Histoplasma* species, among others (Monica, 2010).

In Uganda, several studies on the disease burden of P. mycoses among PLWHIV have been done but little is documented and the rate of information uptake is low.

In KPC, there are no diagnostic strategies to screen for P. mycoses yet anecdotal data obtained from monthly reports indicate blind treatment of pulmonary syndromes including use of antifungals, antibacterials and anti TB (if laboratory tests are negative but client has cardinal TB signs and symptoms) therapy among PLWHIV. This predisposes individuals to toxicities as WHO warns (WHO, 2014).

1.1 Statement of the Problem

P. mycoses are on the increase among PLWHIV in KPC, many of whom show no improvement to common antibiotics used.

World Health Organization (2014) reported increasing cases of P. mycoses but warned against blind treatment of mycoses to avoid toxicities and drug resistance which comes along with it. This was contrary to the routine management of pulmonary syndromes with obscure etiology where patients were blindly treated with antifungals, antibiotics and anti TB drugs. Many of the patients received the above treatment with negative laboratory tests results, majorly TB but treatment would be given concurrently (MOH, 2013).

P. mycoses are associated with radiologic patterns including upper-lobe cavitory disease, nodules, pleural-based lesions, and diffuse infiltrates, usually of the lower lobe (David *et al.*, 1991). These features were routinely used to make diagnostic conclusions of TB amongst sputum smear negative patients. In this case individuals are empirically treated for TB.

Thus this study was carried out to determine the burden of pulmonary mycoses among PLWHIV in KPC, Jinja.

1.2 Study Objectives

1.2.1 Main Objective

To determine the prevalence and factors associated with P. mycoses among PLWHIV at KPC, Jinja.

1.2.2 Specific Objectives

1. To determine the proportion of PLWHIV at KPC infected with P. mycoses.
2. To determine the common fungal species causing pulmonary mycoses among PLWHIV at KPC.
3. (a) To establish the risk factors associated with P. mycoses among PLWHIV.
(b) To determine the signs and symptoms associated with P. mycoses.

1.3 Research Questions

1. What is the proportion of PLWHIV at KPC infected with pulmonary mycoses?
2. What are the common fungal species causing pulmonary mycoses among HIV/AIDS patients in KPC?

3. (a) What are the risk factors associated with P. mycoses among PLWHIV?
(b) What are the common signs and symptoms of P. mycoses?

1.4 Justification of the Study

This study sought to provide the necessary and relevant information to the planning unit of the ministry of health (MOH), prison authorities and other stakeholders regarding the management of cough amongst PLWHIV.

The study results would in addition be used for the partial fulfilment of the requirements for the award of a degree of Medical Laboratory Sciences and will also be vital to future scholars.

1.5 Conceptual Frame work

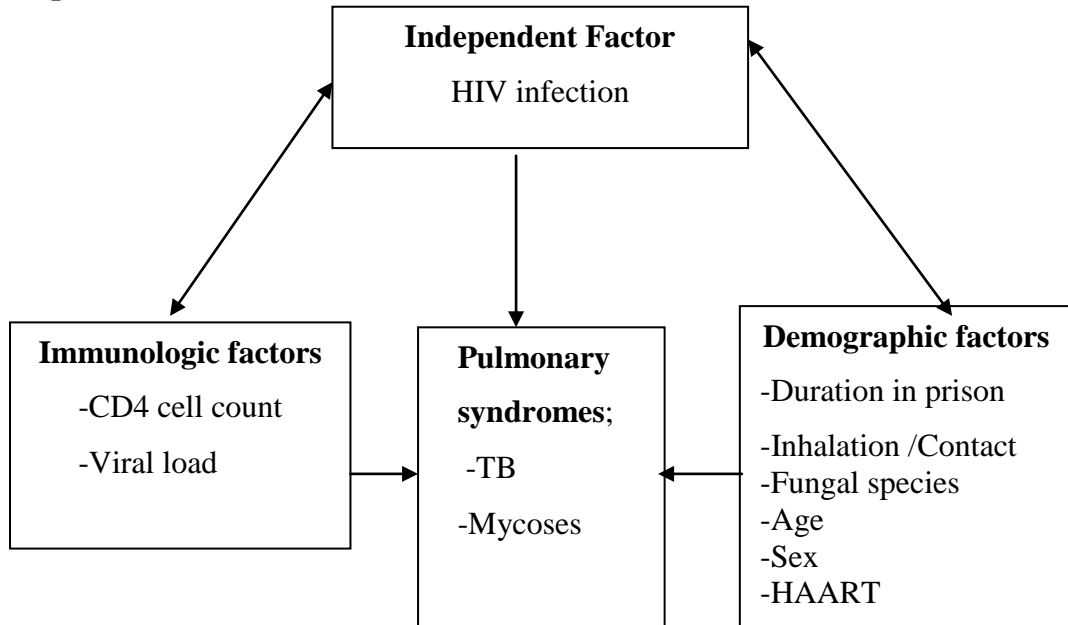


Figure 1: The association of variables that cause pulmonary mycoses

In the conceptual framework, the independent variable is HIV infection. HIV results in immunological suppression by lowering CD4 cell count especially when viral load is high. Socio-demographic factors, contribute to the immunological status among PLWHIV. These include; duration in prison, inhalation /contact, fungal species, age, sex and HAART naïve. These factors combined can result in opportunistic mycoses including PTB and P. mycoses (see figure 1).

1.6 Scope

The study was cross sectional carried out in March 2015 among PLWHIV in KPC. A study population of 127 participants who met the selection criteria were randomly sampled. Sputum was the specimen of choice. Diagnostic tests performed included; sputum culture on SDA, lactophenol cotton blue staining, ZN staining, and KOH mount. Biochemical tests performed were Germ tube and urease.

CHAPTER TWO: LITERATURE REVIEW

2.1 Mycoses

Mycoses are infections caused by fungi. Several fungi cause different infections which can be true infections or opportunistic infections, acute or chronic, mild or severe and range from superficial (including *Tinea versicolor*), cutaneous (including *Taenia oncomycoses*), sub-cutaneous (including; *mycetomas*, *chromoblastomycoses*), and systemic (including; *otomycoses*, *fungemia*, *meningitis*) (Monica, 2010).

2.2 Pulmonary mycoses

Pulmonary mycoses are systemic infections caused by; yeasts, moulds and dimorphic fungi which are commonly opportunistic infections especially among PLWHIV (Khan and Chugh, 2000). Risk factors include; the use of strong and broad spectrum antibiotics for a long time, steroid use, neutropenia, pneumonia due to other pathogens, marijuana smoking but can also be true or primary infections (Khan *et al.*, 2000).

2.2.1 Fungal Species Causing Pulmonary mycoses

Common fungal opportunists causing pulmonary mycoses include; *Cryptococcus neoformans* (*C. neoformans*) causing Cryptococcosis, *Aspergillus* species causing Aspergillosis, *Pneumocystis jiroveci* causing Pneumocystis pneumonia, *Histoplasma* species causing Histoplasmosis, *Penicillium marneffeii* causing penicilliosis, *Coccidioides immitis* causing coccidioidomycosis, *Blastomyces dermatitidis* causing blastomycosis, *Paracoccidioides brasiliensis* causing paracoccidioidomycosis, among others (Monica, 2010). These mycoses are acquired through inhalation of infectious propagule from the environment.

2.2.2 Signs and Symptoms of Pulmonary mycoses

Most P. mycoses present with signs and symptoms similar to PTB including; Fever, Weight loss, Cough, chest pain, dyspnea, loss of appetite, night sweats and bloody sputum (Adams *et al.*, 1997). David *et al.*, (1991) adds that pulmonary mycotic infections are associated with radiologic patterns including; upper lobe cavitory disease, nodules, pleural based lesions and diffuse infiltrates usually of the lower lobe.

2.3 Epidemiology of Pulmonary mycoses

Pulmonary mycoses are a worldwide problem especially among those living with HIV and others using immunosuppressive drugs.

2.3.1 Pulmonary mycoses in Europe

One study in Europe reported that fungal infections are common amongst immunosuppressed patients (Cornelia, 2009). They reported that the major species causing invasive fungal diseases were; *Candida albicans* (*C. albicans*) which constituted over 50% of total fungal infections. However, they further report that the infection rate with *C. albicans* for over time has been reducing due to an increase in the use of antifungal prophylaxis. The other half is constituted by *Zygomycosis*, *Aspergillosis* and *Fusariosis* which in contrast to *C. albicans* have been increasing. They further emphasise that, early initiation of antifungal therapy is critical for improving outcomes though this is hampered by failure to diagnose the infections early and accurately. Therefore there is need to routinely carry out fungal diagnostic tests especially among PLWHIV for the right therapeutic management options (Cornelia, 2009).

2.3.2 Pulmonary mycoses in South America

In South America, decreasing rates of *Candida* species and the existence of other non albicans species such as *C. glabrata* among AIDS patients were reported (Clark *et al.*, 2002). The study however reported an increase in *Aspergillosis*. These infections present a high disease burden. Despite the successful efforts to reduce the trends of fungal infections in PLWHIV in developed countries like USA, the burden of these mycoses in developing countries is large and increasing (Clark *et al.*, 2002).

2.3.3 Pulmonary mycoses in Asia

A Study in China reported an increase in the incidence (11.3%) of P. mycoses among immunocompromised patients. This was attributed to the wide usage of broad spectrum antibiotics. The major etiological agents of P. mycotic infections were; pulmonary *Aspergillus* (55.9%) followed by pulmonary *Cryptococcosis*, pulmonary *candidiasis* (7.4%), pulmonary *histoplasmosis* (5.8%), pulmonary *Sporotrichosis* (1.5%) and *Actinomyces pneumonia* (1.5%). The methods used in their study included; clinical manifestations, radiographic characterization and diagnostic methods like; open lung biopsy, transbronchial biopsy and computerized tomography. The study also shows the main symptoms to be; cough (75.0%), haemoptysis (37.8%), fever (29.4%), and (11.1%) asymptomatic cases (Bai-ling *et*

al., 2011). Therefore there's an urgent need to restrict the use of antibiotics and other immunosuppressive drugs.

Another study by Hidalgo & Vazquez, (2004) reported that the distribution of pulmonary mycotic infections was distributed equally amongst males and females.

2.3.4 Pulmonary mycoses in Africa

A study done in Nigeria found the overall prevalence of pulmonary mycoses to be 36.0%, with *Candida albicans* being the most prevalent (11.8%). This was followed by *Pneumocystis jirovecii* at (7.4%). The study further shows that patients aged 25-34 years were at the highest risk of pulmonary mycoses (43.9%). PLWHIV co-infected with mycoses had lower mean CD4 counts than those without mycoses. In their study the test panel used included; direct microscopy, culture and serology (Ogba *et al.*, 2010). The study further indicates that decline in CD4 cell counts among PLWHIV is a risk factor to P. mycoses. In a later and similar study by the same author (Ogba *et al.*, 2013), reported an overall prevalence of 31.6%, with *Candida albicans* still being the most prevalent (11.8%). They further reported that P. mycoses and TB co infection rates were low (7.7%). In the latter study, lowering of P. mycoses from 36.0% to 31.6% was attributed to by the increased enrolment of PLWHIV on HAART. The prophylaxis treatment they receive helps maintain their immune statuses high thus clearing most of the opportunistic fungal pathogens.

Another study by (Aluyi *et al.*, 2010) reported an overall prevalence of 71.8%. The study was carried out among PLWHIV with Cd4 counts lower than 200/ μ l. The tests performed included culture, KOH mount and lactophenol cotton blue staining. The study reports that *Candida albicans* (19.0%) was the most prevalent, *Candida stellatoidea* (9.7%), *Cryptococcus neoformans* (9.7%), *Candida parapsilosis* (9.7%), *Torulopsis glabrata* (5.6%), *Mucor* spp (7.2%), *Penicillium marneffeii* (4.1%), *Rhodotorula rubra* (3.6%) and *Fusarium* spp (3.1%) respectively. All participants were grouped into <20 (3.1%), 20-30 (32.3%), 31-40 (30.3%), 41-50 (17.4%), \geq 51 (2.1%) age groups. All (9) organisms were isolated from participants within 21-30 and 31-40 age groups; 8 organisms from 41-50 age group and 3 organisms each from \leq 20 and \geq 50 age brackets. *Candida albicans* and *Cryptococcus neoformans* occurred in all age groups.

Another study in South Africa found out that oral candidiasis was the only opportunistic fungal infection that occurred among PLWHIV with CD4 cell counts below 200 counts/ μ l

(David T, 2006). However prevalence rates were not given and comments on the burden could not be revealed.

2.4 Laboratory Diagnosis of Pulmonary mycoses

Because signs and symptoms of pulmonary mycoses are similar to PTB, diagnosing PTB was paramount.

Sputum was the specimen of choice used.

Diagnostic tests included; sputum culture on SDA, lactophenol cotton staining, ZN staining and KOH mount (Monica, 2010).

2.5 Treatment of Pulmonary mycoses

Several drugs are available on market, amongst which include; Itraconazole, Voriconazole, Posaconazole, Fluconazole, Caspofungin, Micafungin, Flucytosine, Amphotericin B, Nystatin, among others (MOH, 2012)

CHAPTER THREE: METHODOLOGY

3.1 Study Design

A Cross sectional study design was carried out to determine the disease burden and factors associated with pulmonary mycoses among PLWHIV in the three prison units of Jinja main, Jinja Remand and Jinja Women. The study used both quantitative and qualitative techniques of data collection. The quantitative technique helped in the analysis of data while the qualitative helped in defining themes and concepts used in the study.

3.2 Study Site

The study was carried out in KPC, found in Jinja district, about 80km from Kampala, Uganda's capital city. It comprises of three prison units; Jinja main prison, Jinja remand prison and Jinja women prison. Jinja main prison had a population of about 1000 inmates and about 3000 staffs and their families. It was followed by Jinja remand prison with a population of about 800 inmates and 2000 staffs and their families. Jinja women were the least with around 80 inmates, 20 children and about 400 staffs and their families (UDHS, 2011). KPC has a government aided Health Centre III (HCIII) which provided most of the clinical services. The prison units were enclosed having poor sanitary facilities and poor health infrastructure.

3.3 Study Population

The study population comprised of all adults (18 years and above) both males and females in KPC living with HIV and were coughing at the time the study was carried out. Consent was sought from each individual before being enrolled.

3.4 Sample Size Estimation

A total of 127 clients who met the selection criteria were sampled using the formula by Martin *et al.*, (1997), $n = \frac{Z^2 pq}{d^2}$

Where, n is the sample size and d is the allowable error (5%).

Z is the standard normal deviation corresponding to 95% which is 1.96

P is the prevalence (9.3%)

Q is 1-p

$$n = \frac{1.96 \times 1.96 \times 0.093 \times 0.892}{0.05 \times 0.05}$$

= 127 patients.

3.5 Sampling Technique

A simple random sampling technique was used during data collection. Participants were organized into a cluster during a clinic day and each had an equal opportunity of being sampled. They were given questionnaires and asked to complete them (appendix 1). Sputum mugs were given to them and asked to collect sputum, one on spot and the other early morning which was analysed systematically. Clinic days were conducted differently in each prison unit. Jinja main prison was Monday, Jinja remand prison was on a Wednesday and Jinja women on a Friday.

3.6 Selection Criteria

3.6.1 Inclusion Criteria

All adult individuals (18 years and above) in KPC, living with HIV and were coughing (both acute and chronic cough) and agreed to sign the consent form were recruited in the study.

3.6.2 Exclusion Criteria

All those already diagnosed with pulmonary mycoses and were on treatment and those diagnosed with TB and on anti TB drugs.

3.7 Data Collection

Using the ZN technique to analyze sputum samples, we determined individuals infected with PTB. Using sputum culture and morphological characteristics, lactophenol cotton blue staining, and KOH mount, we determined the common fungal species causing pulmonary mycoses among PLWHIV. Clients CD4 cell counts were tested using the PIMA counter. They were categorized into ranges of ≤ 250 and >250 cells/ μ l against which the rate of developing P. mycoses was assessed.

By analyzing the information given in questionnaires, we determined the common signs and symptoms associated with P. mycoses including; cough, chest pain, night sweats and weight

loss. Also questionnaires were used to assess the duration of stay of prisoners and its effect on developing *P. mycoses*. They were also used to categorize cough as acute (those who had coughed for less than 2 weeks) and chronic (those who had coughed for more than 2 weeks). Still using questionnaires, participants' ages were grouped into 18-35 years (youth), 36-60 years (adults) and above 60 years (elderly). The effect of age to developing *P. mycoses* was then assessed.

3.8 Laboratory diagnosis

Diagnostic tests performed included; sputum culture on SDA, lactophenol cotton blue staining, ZN staining, KOH mount and CD4 cell count. Biochemical tests performed were Germ tube and urease.

3.9 Data Management

Data was entered and organized into Ms Excel which was later imported to STATA Ver13 statistical tool for analysis. Results were interpreted with Odds ratios and chi square at a *p. Value* of 0.05 and presented in graphs and tables.

3.10 Dissemination of Results

Copies were submitted to the Institute of Allied Health Sciences and the University library.

3.11 Ethical Consideration

An introductory letter from the Dean, institute of Allied Health Sciences, IHSU was sought after the ethical committee had approved this study (Appendix ii) and presented to the Regional Prisons Commander, South eastern region Jinja seeking permission to carry out the study which was granted (Appendix iii). Consent was sought from every respondent and confidentiality highly observed.

3.12 Quality Control

Standard Operating Procedures (SOPs) were adhered to.

All positive specimens and some negatives were examined by another technician for confirmation.

Every new batch of stains was quality controlled by staining known positive and negative slides.

CHAPTER FOUR: RESULTS

4.0 Introduction

The study was conducted in March 2015 with a major aim of determining the prevalence and factors associated with pulmonary mycoses among PLWHIV. We recruited 127 participants, 54 (42.52%) females who were from Jinja women prison and 73(57.48%) males who were from Jinja remand prison and Jinja main prison. Two sputum samples were collected from each participant from the three prison units. The tests that were performed on these samples were; culture on SDA, lactophenol cotton blue staining, KOH mount, ZN staining, and CD4 cell count.

4.1 Proportion of PLWHIV Infected with *P. mycoses*

Amongst the clients recruited for the study, 31/127 (24.41%, 95% CI= 17.92-33.67) were found to have pulmonary mycoses. Out of the 31 patients who were found to have pulmonary mycoses, 15 (27.78%) were females while 16 (21.92%) were males. There was no statistical difference of the infection rate between males and females (see table 1).

Still we found that, 3 (2.36%) clients had TB and 1 (3.23%) had a co-infection with *P. mycosis* (Aspergillosis).

Table 1; Number of Males and Females infected with *P. mycoses*

| Sex | Pulmonary mycoses | | Total | OR | p. Value |
|--------|-------------------|--------------|-------|------|----------|
| | Positive (%) | Negative (%) | | | |
| Female | 15 (27.78) | 39 (72.22) | 54 | 1.37 | 0.45 |
| Male | 16 (21.92) | 57 (78.08) | 73 | | |
| Total | 31(24.41) | 96 (75.59) | 127 | | |

Among those sampled, the youth (aged 18-35) were the majority [74/127 (58.27%)]. This was followed by the adults (ages 36-70) who were 51/127 (40.16%) and finally the elderly (> 60 years of age) who were 2/127(1.57%). *P. mycoses* was diagnosed in 19 (25.68%) of the youth, 11 (21.57%) adults and 1 (50%) among the elderly. However, there was no statistical significance of the infection rates with *P. mycoses* amongst the different age groups ($X^2 = 0.99$; *p. Value* = 0.61).

4.2 Common Fungal species Causing Pulmonary mycoses among PLWHIV

Of the 31 fungal organisms majority were *Candida* species, 11 (35.48% 95% CI =21.1-56.31), followed by *C. neoformans* 8 (25.81%, 95% CI=11.46-43.4), *Aspergillus* species 6 (19.35%, 95% CI=7.21-36.44), and *Zygomycetes* 6 (19.35%, 95% CI=7.21-36.44). See figure 2 below;

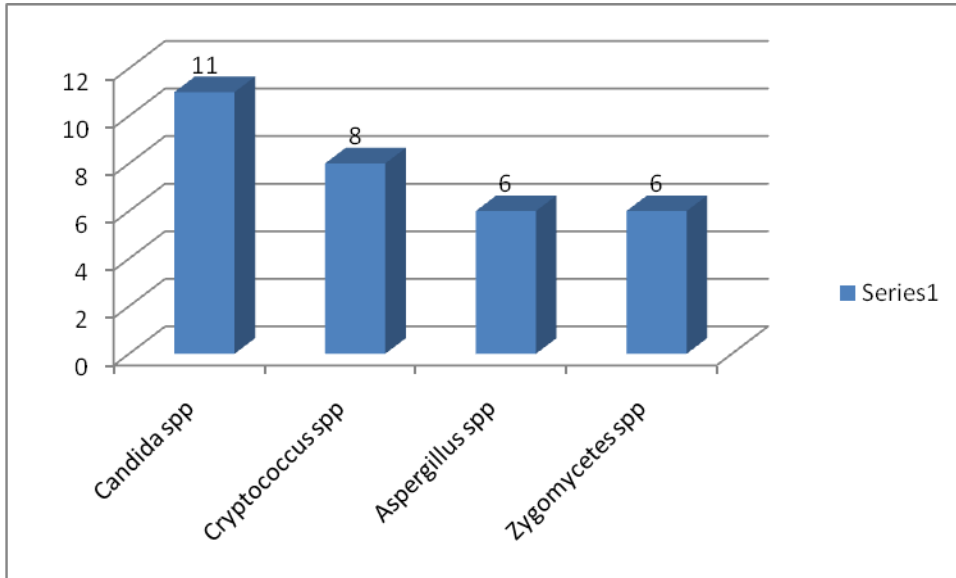


Figure 2; Distribution of fungal spp causing P. mycoses.

4.3 Risk factors associated with P. mycoses

The mean CD4 count was 447.28 cells/ μ l (95% CI= 391.77-5022.78). Individuals who had CD4 cell count \leq 250 were 38 (29.92%) and those who had CD4 cell count $>$ 250 were 89 (70.08%). In the study, we found that individuals with a CD4 cell count \leq 250 presented more with P. mycoses. (OR = 9.7, p. Value < 0.001). See table 2.

Table 2; CD4 cell count as a risk factor to P. mycoses

| CD4 count | Pulmonary mycoses | | Total | OR | p. Value |
|---------------------------|-------------------|--------------|------------|------------|------------------|
| | Positive (%) | Negative (%) | | | |
| \leq 250 cells/ μ l | 21(67.74) | 17(17.71) | 38 | 9.7 | <0.001 |
| $>$ 250 cells/ μ l | 10 (32.26) | 79(82.29) | 89 | | |
| Total | 31 | 96 | 127 | | |

The study reported that duration of stay in Prison was not associated with the development of P. mycoses (OR = 1.44, p. Value = 0.38).

4.4 Common Signs and Symptoms indicating Pulmonary mycoses in PLWHIV

This study found out that independently the factors; weight loss, chest pain and night sweats were not associated with P. mycoses (OR= 1.11, 1.15 and 2.51; p. Values= 0.82, 0.82 and 0.11 respectively). Also the study reported that P. mycoses could similarly present in both acute (≤ 2 weeks) and chronic (>2 weeks) coughs (See table 3).

Table 3; The occurrence of P. mycoses by duration of cough

| Duration with cough | Pulmonary mycoses | | Total | OR | p. Value |
|---------------------|-------------------|--------------|------------|-------------|-------------|
| | Positive (%) | Negative (%) | | | |
| Acute | 16 (25.81) | 46 (74.19) | 62 | 1.16 | 0.72 |
| Chronic | 15 (23.08) | 50 (76.92) | 65 | | |
| Total | 31 | 96 | 127 | | |

By duration of coughing, *Candida* infections presented more in the acute phase 8/11 (72.73%) while *Cryptococcus* and *Zygomycetes* presented more in the chronic phase [5/8 (62.50%) and 4/6 (66.67%) respectively]. However *Aspergillus* infections could occur equally in both acute and chronic phases of cough. See table 4.

Table 4; Fungal species as distributed by cough duration

| Species | Duration with cough | | Total |
|---------------------|---------------------|-------------|-----------|
| | Acute (%) | Chronic (%) | |
| <i>Aspergillus</i> | 3 (50.00) | 3 (50.00) | 6 |
| <i>Candida</i> | 8 (72.73) | 3 (27.27) | 11 |
| <i>Cryptococcus</i> | 3 (37.50) | 5 (62.50) | 8 |
| <i>Zygomycetes</i> | 2 (33.33) | 4 (66.67) | 6 |

CHAPTER FIVE: DISCUSSION OF RESULTS

P. mycoses present important issues of public health concern in the management of PLWHIV in the world (David *et al.*, 2011). They are a major cause of disease in these people because their immunity is compromised. Despite government improvement in the provision of medical care to the inmates, little is documented on the management of these opportunistic fungal infections. This study aimed to determine the prevalence and factors associated with *P. mycoses* among PLWHIV.

The study showed an overall prevalence of pulmonary mycoses among PLWHIV in KPC to be 24.41% (95% CI= 17.92-33.67). This was lower than the prevalence of a similar study in Nigeria by Ogba *et al.*, (2010) which was 36.0%. In their study the test panel used included; direct microscopy, culture and serology. In our study, serology was not used and this could explain the low case detection rate as compared to their study. Therefore the prevalence of *P. mycoses* among these individuals could be higher than what we obtained. Still in Nigeria, the prevalence of 71.8% was reported amongst individuals with CD4 cell counts <200 cells/ μ l (Aluyi *et al.*, 2010). This rate was far higher than what we obtained in our study yet similar methods were used. However, we observed that lowering Cd4 below 250 predisposes individuals to developing *P. mycoses* (OR = 9.7, p. Value < 0.001). Therefore the explanation for the high prevalence in a study by Aluyi *et al.*, (2010) was because participants recruited for their study had CD4 counts (<200) yet in our study the average CD4 cell count (447.28 cells/ μ l (95% CI= 391.77-5022.78).

There was no statistical difference in the rates of infection amongst males and females. This was similar to a study by Hildago & Vazquez., (2004). The similarity in the rates of infection is attributed to the fact that both male and female prisoners in KPC are exposed to the same conditions (they eat the same food, live in a similar environment, same work, same medical conditions, among others). However, a study by Bai-ling *et al.*, (2011) reports that males are more infected (63.0%) than females (37.0%). It goes further to attribute this to the fact that males are more exposed to addictive behaviours like smoking, drug and substance abuse, among others which in turn compromise their immunity paving way for these opportunistic mycotic infections. This was not the case for the population we studied.

Infection rates amongst the youth were 25.68%, adults with 21.57% and the elderly with 50.00% but the differences in the infection rates amongst the age groups were not statistically significant. This concurs with Aluyi *et al.*, (2010) who reported an even distribution of P. mycoses across all ages. Therefore while planning for screening of P. mycoses; similar screening tests should be used for all ages.

We obtained low TB-P. mycoses co infection (3.23%) compared to Ogba *et al.*, (2013) (7.7%). Ogba *et al.*, (2013) further reported a statistically significant relationship ($X^2=4.4$, p. Value=0.03) between P. mycoses and TB. In our study, having TB could not predict having P. mycoses ($X^2=0.13$, p. Value=0.72). This indicates that each infection independently causes the disease (cough) amongst PLWHIV with similar signs and symptoms (Monica, 2010) and (Adams *et al.*, 1997). Therefore we differ from MOH guideline of treating ZN sputum smear negative for TB using signs and symptoms such as chest pain, night sweats, haemoptysis, weight loss and cavities in the lungs which are similar to P. mycoses. Treating individuals for TB empirically predisposes them to drug toxicities and promotes drug resistance which hampers TB control programmes. Therefore routine screening for P. mycoses would improve care.

In the current study, *Candida* species, 11 (35.48%) were the majority isolated, followed by *Cryptococcus neoformans* 8 (25.81%), *Aspergillus* species 6 (19.35%) and *Zygomycetes* 6 (19.35%) (See figure 2). Cornelia, (2009), Clark *et al.*, (2002) and Aluyi *et al.*, (2010) also reported that *Candida* spp were the most prevalent which was in agreement with our finding. Contrary, Bai-ling *et al.*, (2011), reported that *Aspergillus* species (55.9%) was the most prevalent followed by *Cryptococcus* species (27.9%), *Candida* species (7.8%). Still, Bai-ling *et al.*, (2011) isolated *Histoplasma* species (5.8%), *Sporotrichosis* species (1.5%) and *Actinomyces* species (1.5%) which we never did in our study. The reason for not isolating the latter species in our study stems from the diagnostic tests used. Bai-ling *et al.*, (2011) included serology in their panel of tests which we never did. The frequency of the three common species was (from highest to lowest) *Candida* species, *C. neoformans* and *Aspergillus* species in our study. It was the vice versa with Bai-ling *et al.*, (2011). In their study, Bai-ling *et al.*, (2011) used open lung biopsy and transbronchial biopsy in addition to sputum (the only test specimen used in our study). *Aspergillus* and *C. neoformans* have high dissemination rate compared to *Candida* species which normally colonize the epithelium

(Monica, 2010). Thus including biopsies would not only improve case detection rate but also increase chances of isolating *Aspergillus* and *C. neoformans*.

It is therefore important that a panel of tests is developed to include species specific tests for fungi that cannot easily be diagnosed easily with sputum. Suggested tests include; serology, x-ray, CT scan, complement fixation test, histochemical staining, and bronchoscopy (Monica, 2010).

This study found out that independently the factors; weight loss, chest pain and night sweats were not associated with P. mycoses (OR= 1.11, 1.15 and 2.51; p. Values= 0.82, 0.82 and 0.11 respectively). Also the study reported that P. mycoses present similarly in both acute (≤ 2 weeks) and chronic (>2 weeks) coughs. This is contrary to studies by (Monica., 2010 and Mc Adams *et al.*, 1997), who reported that weight loss, chest pain, cough, night sweats and dyspnea were cardinal features of P. mycoses. Therefore treatment for P. mycoses should not be given on basis of signs and symptoms but rather after laboratory confirmation.

Our study reported that duration of stay in Prison was not associated with the development of P. mycoses (OR = 1.44, p. Value = 0.38). Therefore, prisoners can be convicted for as many years as the magnitude of their crime deserves. However, the study found out that individuals with a CD4 cell count ≤ 250 presented more with P. mycoses (OR = 9.7, p. Value < 0.001). Therefore a decline in CD4 cell counts was a risk factor associated with the development of P. mycoses. This was true in a sense that these mycotic infections are opportunistic which take advantage of the lowered immunity. This was in agreement with another study in South Africa which showed that P. mycoses occurred among PLWHIV with CD4 cell counts < 200 cells/ μ l (David, 2006).

CHAPTER SIX: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

The overall prevalence of P. mycoses in KPC was relatively high (24.41%). *Candida* species were the most prevalent followed by *C. neoformans*, *Aspergillus* species and *Zygomycetes*. Weight loss, chest pain and night sweats were not associated with P. mycoses. We observed that a decline in CD4 cell count $\leq 250\mu\text{l}$ is a risk factor associated with the development of P. mycoses and duration of stay in Prison has no effect with the development of P. mycoses.

6.2 Limitations

Loss to follow up. Some participants were released and others transferred to other prison units for security reasons before the study was completed.

6.3 Recommendations

A more comprehensive study covering antifungal susceptibility testing should be done.

Treatment for P. mycoses should not be given on basis of signs and symptoms but rather after laboratory confirmation.

A panel of tests (serology, x-ray, CT scan, complement fixation test, histochemical staining, and bronchoscopy) should be developed to include species specific tests for fungi that cannot easily be diagnosed easily with sputum.

Routine screening for P. mycoses among PLWHIV should be incorporated in their care package.

REFERENCES

- Aluyi, H., Otajevwo, F., Lweriebor, O. (2010). Incidence of Pulmonary mycoses in Patients with Acquired Immuno-deficiency Syndromes. Nigerian journal of Clinical Practice. Volume 13, issue 1. Pages 78-83.(Retrieved on 23rd.09.2015).
- Bai-ling, L., Le-meng, Z., Cheng-ping, H., and Zeng X. (2011). Clinical analysis of 68 patients with Pulmonary Mycosis in China. Research Gate. Volume 6, issue 5. PubMed. (Retrieved on 13th.01.2015).
- Clark, A. and Rana, A. (2002). Recent trends in the epidemiology of invasive mycoses. Volume 15 - Issue 6, pages 569-574. (Retrieved on 19th.09.2015).
- Cornelia Lass-florl. (2009), The changing face of epidemiology of invasive fungal disease in Europe. Volume 52; Issue 3; pages 197–205. (Retrieved on 19th.09.2015).
- David, T., Fisk, A., Steven, M., and Powel, H. (2006). Pneumocystis carinii Pneumonia in Patients in the Developing World Who Have Acquired Immunodeficiency Syndrome. Journal of Acquired Immune Deficiency Syndromes. Volume 42 - Issue 4, pp 464-469
- David, D., Stephen, F., Michael, S., Stephen, N., Howard, E., and David, A. (1991) Pulmonary Aspergillosis in the Acquired Immunodeficiency Syndrome. The New England Journal of Medicine. Volume 324: Issue 24: pages 654-66. (Retrieved on 18th.09.2015).
- Hidalgo, A. and Vazquez, A. (2004). Candidiasis. Medicine Journal. Volume 5, issue 3. (Retrieved on 7th.10. 2014).
- Khan, U. and Chugh, D. (2000). Invasive fungal Infections in Kuwait. Indian Journal of Chest Disease and Allied Sciences. Volume 42, issue 4. Pages 279-289. (retrieved on 11.11.2014).
- Mc Adams, P., Rosado de Christenson, M., Stroll., and Patz, F. (1997). Pulmonary mucormycosis; Radiologic findings in 32 cases. American Journal of Roentgenology. Volume 168, issue 6. Pages 123-129. (Retrieved on 19th.09.2015).

Monica Cheesbrough. (2010). *District Laboratory Practice in Tropical Countries, Part 2*, (third edition), pages 243-247, Cambridge university press, Cambridge.

Ministry of Health (2013), *Treatment Guidelines*. Kampala, Uganda.

Ministry of Health (2012), *Treatment Guidelines*. Kampala.

Ogba, M., Abia-Bassey, N., and Epoke J. (2010). Prevalence of Symptomatic Opportunistic Respiratory mycoses and Mycobacterium Tuberculosis among HIV Sero-positive Patients in Calabar, Nigeria. *Journal of Fungal infections*, volume 1. (Retrieved on 7th. 09. 2015).

Ogba, M., Abia-Bassey., N., and Epoke, J., (2013). The Relationship between Opportunistic Pulmonary Fungal Infections and CD4 count levels among HIV Sero-positive Patients in Calabar, Nigeria. *Journal of Fungal infections*, volume 1. (Retrieved on 7th. 09. 2015).

Uganda Demographic and Health Survey. (2011). pages 423-424, Kampala.

UNAIDS (2009), *Reports Increasing Cases of HIV Related Opportunistic Infections*. Geneva
World Health Organization. (2014). *Report on Pulmonary mycoses and their Management*.
Geneva.

APPENDIX I: QUESTIONNAIRE

Number

Date

Instructions

1. Do not write your name.
2. Put a tick in the box adjacent to the answer of your choice.
3. Where applicable, write your answers in the spaces provided.

Demographic information

1. Age; >18 years 18-25 years 25-30 years 30 years and above
2. Sex; Male Female
3. Highest level of education attained
Primary level O-level A-level Diploma holder
Degree holder others (specify)

Environmental factors

4. Do you ventilators and windows on your ward
YES NO
5. Approximately, how many do you sleep in a ward.....
6. Have you ever been in close proximity with someone having a bad cough?
YES NO
7. If yes to (6) above, what advice did give that person.....
.....
8. Do you rare birds at home?
YES NO

History of Previous Sickness

9. In the last six months, did you ever suffer from chronic cough?
YES NO
10. If YES to (9) above, for how long did you fall sick?
2 weeks 3 weeks 4 weeks more
11. Where did you seek medical services?
Private clinic public health facility prisons health facility
Others
(specify).....

12. What was the diagnosis?

.....
.....

13. What drugs were you given?

.....
.....
.....

Current Patient's Complaint

Chest pain Cough Loss of weight Loss of appetite
Night sweats
Others

(specify).....

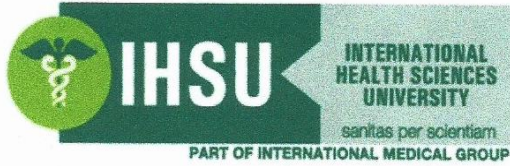
Prevention

14. Suggest ways we prevent the spread of opportunistic infections like chronic cough?

.....
.....
.....

Thank you so for your cooperation

APPENDIX II: INTRODUCTORY LETTER



making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, 10th March 2015

The Regional Prisons Commander;

South – Eastern Region,
JINJA.

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Kisakye Moses**, Reg. No. **2012-BMLS-PT-023** who is a student of our University. As part of the requirements for the award of a Bachelors of Medical Laboratory Science of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **Pulmonary Mycoses among people Living with HIV in Kirinya Prisons Complex.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to our student.

Sincerely Yours,


Okiria John Charles
Senior Lecturer/ Dean, Institute of Allied Health Sciences

The International Health Sciences University
P.O. Box 7782 Kampala – Uganda
(+256) 0312 307400 email: deanahs@ihsu.ac.ug
web: www.ihsu.ac.ug

APPENDIX III: ACCEPTANCE LETTER

TELEGRAMS: "REGPRISONS"
TELEPHONE: +256-43-4-123077



Regional Prisons Headquarters,
South Eastern Region
P.O Box 189,
JINJA.

A REPLY TO THIS LETTER SHOULD BE
ADDRESSED TO THE REGIONAL PRISONS
COMMANDER AND THE FOLLOWING
REFERENCE NO QUOTED **P/K/527**

16th February, 2015.

The Officer in Charge
U.G Prison Jinja (M)
U.G Prison Jinja Remand
U.G Prison Jinja (W)

RE: MR. KISAKYE MOSES

This is to introduce to you Mr. Kisakye Moses Medical staff of Jinja remand prison who is a student of International Health Sciences University persueing bachelors in medical labarotory science in his final year of study. He is undertaking a research study titled pulmonary mycoses among people living with HIV in Kirinya Prisons complex.

The purpose of this communication is to request you allow him collect the necessary data for writing his dissertation.

Please accord him the necessary assistance needed.


(VINCENT .B. KUBOMU) SSP
REGIONAL PRISONS COMMANDER,
SOUTH EASTERN REGION, JINJA.

cc. The Commissioner General of Prisons,
Prisons Headquarters, KAMPALA.