

Pulmonary Paragonimiasis and Aspergillosis in Patients Suspected of Tuberculosis in Yaounde, Cameroon

TYPE OF ARTICLE: ORIGINAL RESEARCH PAPER

ABSTRACT

Aims: The purpose of this study was to determine the prevalence of paragonimiasis and aspergillosis in patients suspected of pulmonary tuberculosis in Yaounde.

Study design: Cross sectional study.

Place and duration of study: Participants were recruited between March and June 2014 using the tuberculosis treatment centers University Hospital Center and Jamot Hospital Center of Yaounde

Methodology: Two sputum samples were collected from participants and analysed using standard microbiological methods.

Results: A total of 260 patients were enrolled, 131 (50.4%) females and 129 (49.6%) males. Of the 260 samples collected, *Mycobacterium tuberculosis* was detected in 44 (16.92%) [CI: 12.33–21.51], *Aspergillus spp.* in 42 (16.15%) [CI: 11.65–20.66] and *Paragonimus africanus* in 7 (2.69%) [CI: 0.7 – 4.67]. Aspergillosis was more prevalent in the age group 41-60years 29 (44.62%) ($P = .000$) and among builders 19 (45%) ($P = .013$). Isolates of *Aspergillus* included *A. fumigatus* 20 (47.62%), *A. flavus* 10 (24%) and *A. niger* 10 (24%). Other pathogens isolated included *Candida albicans* 80(39.8%) and *Histoplasma capsulatum var duboisii* 21 (8.1%). Nine (3.46%) and 20 (7.69%) of patients with tuberculosis were coinfecting with *Aspergillus spp.*, and *Candida albicans* respectively. No coinfection was observed between *Mycobacterium tuberculosis* and *Paragonimus africanus*. Gram staining revealed that 20 (7.7%) were positive for fungal elements, 80 (30.8%) for yeast, 5 (1.9%) for yeast and Gram positive rods, 40 (15.4%) for Gram negative rods and 30 (11.5%) for Gram positive cocci.

Conclusion: This study demonstrates varying prevalence of *Aspergillus spp.*, *Paragonimus africanus* in patients suspected of tuberculosis in Yaounde. Other potential pathogens isolated included *Candida albicans*, and *Histoplasma capsulatum var duboisii*. Further studies will be required to shed more light on the biology and transmission of the *Paragonimus africanus* in Yaounde.

Keywords: Prevalence, Tuberculosis, Aspergillosis, Paragonimiasis, Yaounde

1. INTRODUCTION

Tuberculosis, aspergillosis and paragonimiasis are diseases caused by different classes of microorganisms which share similar preferences for the lungs and often cause lung disease with very similar symptoms including cough, fever, pain in the chest and more importantly hemoptysis (coughing up of blood or blood tinged sputum). The main causative agent of

tuberculosis is the bacterium *Mycobacterium tuberculosis* ^[1], fungi of the genus *Aspergillus* cause aspergillosis while parasitic trematodes of the genus *Paragonimus* cause paragonimiasis.

Tuberculosis has a worldwide distribution and has been estimated to have infected about one-third of the world's population ^[2]. About 8.6 million new cases and over 1.3 million deaths as a result of tuberculosis were reported in 2012, most of which occurred in developing countries ^[3]. The high incidence in developing countries could be attributed to the on-going HIV/AIDS pandemic, of which these countries are the most affected ^[4]. Like tuberculosis, aspergillosis is also widely distributed. Aspergillosis can be referred to as an opportunistic disease because majority of cases occur in people with underlying illnesses such as tuberculosis ^[5], and in immunocompromised individuals. The disease is acquired by the inhalation of *Aspergillus* spores which germinate and cause disease in the susceptible host. Contrary to tuberculosis and aspergillosis, paragonimiasis is a disease which has a predilection to certain geographical areas. The most common species, *Paragonimus westermani* is widespread across Asia ^[6]. *Paragonimus africanus* which is found in Centre Africa is the only known species in Cameroon ^[7,8]. An estimated 20 million people have been infected worldwide with paragonimiasis and more than 290 million are at risk ^[9,10]. The infection is usually acquired by eating raw or poorly cooked crustaceans including crabs and crayfish ^[11].

Since pulmonary paragonimiasis and aspergillosis usually exhibit symptoms which are very similar to tuberculosis, it is not uncommon for these diseases to be misdiagnosed and treated as tuberculosis especially in Cameroon where they are endemic. It is against this backdrop that we investigated the prevalence of paragonimiasis and aspergillosis in patients suspected of tuberculosis in Yaounde, in order to generate data of public health significance.

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2. MATERIAL AND METHODS

2.1 Study setting

This study was carried out at the University Hospital Center (UHC) and Jamot Hospital Center (JHC) in Yaounde. These are tertiary hospitals serving the population of Yaounde. Yaounde (3°52'N 11°31'E) is the political capital of Cameroon with an estimated population of 2.5million ^[12], making it the second largest city in Cameroon after Douala. Yaounde is a very diverse city with people from diverse ethnic and cultural background. The city is home to most of the administrative structures in the country.

2.2 Study design

The study was a cross sectional study performed between March and June 2014, in which participants were enrolled in the tuberculosis treatment centers of UHC and JHC.

2.3 Study population

Patients who presented with symptoms such as cough (lasting 3 weeks or more) with or without the production of purulent sputum, malaise, dyspnoea, anorexia, weight loss, fever, night sweat and chest pain were enrolled into the study. Males and females belonging to all age groups were eligible to participate in the study. Participants were not to be on any antibiotic or antifungal treatment 10 days prior to the study. Written informed consent was obtained from the patients prior to data collection. In the case of children or minor who could not provide their informed consent, their parents or guardians did on their behalf. Excluded from the study were patients that were already on antibiotic or antifungal medication within 10 days of enrollment and those who refused to give their informed consent.

The study protocol was approved by the Faculty of Health Sciences Institutional Review Board of the University of Buea, Cameroon. Administrative clearance was obtained from the

Hospital Administrations of the University Hospital Center (UHC) and Jamot Hospital Center (JHC).

2.4 Specimen collection

Two sputum samples were collected from each participant, the first at the time of enrollment and the second collected early in the following morning into sterile sputum mugs. The participants were instructed on how to collect the sputum samples. For the collection of the morning sample, participants were instructed not to brush their mouth or eat anything prior to collection and to transport it to laboratory without delay.

2.5 Laboratory analysis

2.5.1 Acid Fast Bacilli (AFB)

Specimen for AFB were first analysed using the Auramine-Phenol Fluorochrome staining technique as described elsewhere^[13].

Cultures for *Mycobacterium tuberculosis* were performed on Lowenstein-Jensen (LJ) media using the modified Petroff method^[14]. Growth on the media was checked on a weekly basis for a maximum of 8 weeks. The identification of the *Mycobacterium tuberculosis* was based on the following: presence of brown granular colonies on the media, susceptibility to P-nitrobenzoic acid (PNB), niacin positive and catalase negative at 68°C.

2.5.2 Parasitological analysis

Paragonimiasis was diagnosed based on clinical presentation of chest pain and cough with the production of rusty-brown or blood-tinged sputum, and confirmed by the microscopic demonstration of ova in the sputum samples collected, firstly by direct microscopic examination and secondly, by concentrating the sputum samples using 4% w/v NaOH for the detection of low intensity infection. *Paragonimus africanus* was identified by their characteristic brownish and elongated ova^[15]. Ova of *P. africanus* are larger than ova of *P. uterobilateralis* measuring 72-124 X 42-59µm and slightly thinner compared to *P. westermani*^[16].

2.5.3 Mycological analysis

2.5.3.1 Direct microscopy: All sputum samples were subjected to 10% potassium hydroxide (KOH) preparation for the detection of fungal elements.

2.5.4 Culture: All sputum samples were inoculated onto Sabouraud dextrose agar (SDA) and incubated aerobically at 30°C for 48hrs and observed for growth. The culture plates were observed for growth after 48hrs. Where there was no growth after 48hrs, the culture plates were left and observed on a weekly basis for 3 weeks before the culture was reported as negative.

2.5.4.1 Cultural Identification: After appropriate incubation, cultural characteristics: growth form, rate of growth, surface and reversed coloration on Sabouraud dextrose agar plates were noted. Pure isolates were obtained by sub-culturing on new plates and colonies growing out of the inoculation area were regarded as contaminants. Identification was based on the culture standard methods^[17].

2.5.5 Germ tube test: 50ul of serum was pipette into test tubes. With the aid of a wire loop, SDA cultured sample pre-suspended in distilled water were mixed with serum and incubated at 37°C for three hours. The presence of short lateral filament (germ tube) is strongly indicative of *Candida albicans*.

2.5.6 Microscopy: Needle mount staining technique was performed for identification. The *Aspergillus* species were identified base on the various morphological features associated with the characteristic sporing head according to Collier et al.^[18] while yeasts were identified by their smell, pseudomycellia and budding cells. Further investigations included: germ tube test, urease test, chlamidospore formation, Fermentation reaction and Sugar assimilation.

2.5.7 Gram stain

The sputum samples were all Gram stained to detect the presence of bacteria, yeast or fungal elements in the samples.

2.6 Statistical analysis

Data were entered into Microsoft Excel spreadsheet and analysed using Stata® version 12.1 (StataCorp LP). Tests performed included the Chi-square test for group comparison. Statistical significance was set at $p < 0.05$.

3. RESULTS

Two hundred and sixty (260) participants were successfully enrolled into the study including 129 (49.6%) males and 131 (50.4%) females. The ages of the participants ranged between 3 and 95 years (mean $\pm$ SD = 36.5 ± 17.16).

Mycobacteria was detected in 44 of the 260 sputum samples giving a prevalence of 16.92% (95% CI: 12.33 – 21.51). There was no significant difference in the prevalence between males 23 (17.83%) and females 21 (16.03%) ($\chi^2 = 3.8$, $P = .70$). The prevalence was highest in the age group 21-40 years 26 (20.80). However, no significant difference was observed between the prevalence and age ($\chi^2 = 3.875$, $P = .42$) (Table 1).

Aspergillus spp. was detected in 42 of the 260 sputum samples giving a prevalence of 16.15% (95% CI: 11.65 – 20.66). The prevalence was higher in males 30 (23.26%) than females 12 (9.16%). However there was no significant association with gender ($\chi^2 = 0.279$, $P = .60$) (Table 1). The prevalence was highest in the age group 41-50 years 29 (44.62). There was a significant association between prevalence and age ($\chi^2 = 55.42$, $P = .000$) (Table 1).

Table 1: Prevalence (%) *Mycobacterium tuberculosis* and *Aspergillus spp.* in the study population stratified by Age and Gender

Age group	N	<i>Mycobacterium tuberculosis</i>			<i>Aspergillus spp.</i>		
		Male	Female	Total (%)	Male	Female	Total (%)
< 20	40	4	0	4 (10.0)	0	0	0 (0.0)
21 – 40	125	10	16	26 (20.80)	10	3	13 (10.4)
41 – 60	65	6	50	11 (16.92)	20	9	29 (44.62)
61 – 80	28	3	0	3 (10.71)	0	0	0 (0.0)
>81	2	0	0	0 (0.0)	0	0	0 (0.0)
Total (%)	260	23 (17.83)	21 (16.03)	44 (16.92)	30 (23.26)	12 (9.16)	42 (16.15)

Paragonimus africanus was detected in 7 of the 260 sputum samples, giving a prevalence of 2.69% (95% CI: 0.7 – 4.67). The highest prevalence was observed in females 6 (4.58%) than in males 1 (0.78%). However, no significant difference was observed between prevalence and gender ($\chi^2 = 3.59$, $P = 0.06$).

Among the 42 sputum samples that were positive for *Aspergillus*, *Aspergillus fumigatus* was the most prevalent 20 (47.62%) (Figure 1).

Among the 42 patients that were infected with Aspergillosis, the majority were builders 19 (45%) (Figure 2). There was a significant association between prevalence of aspergillosis and profession ($P = 0.013$).

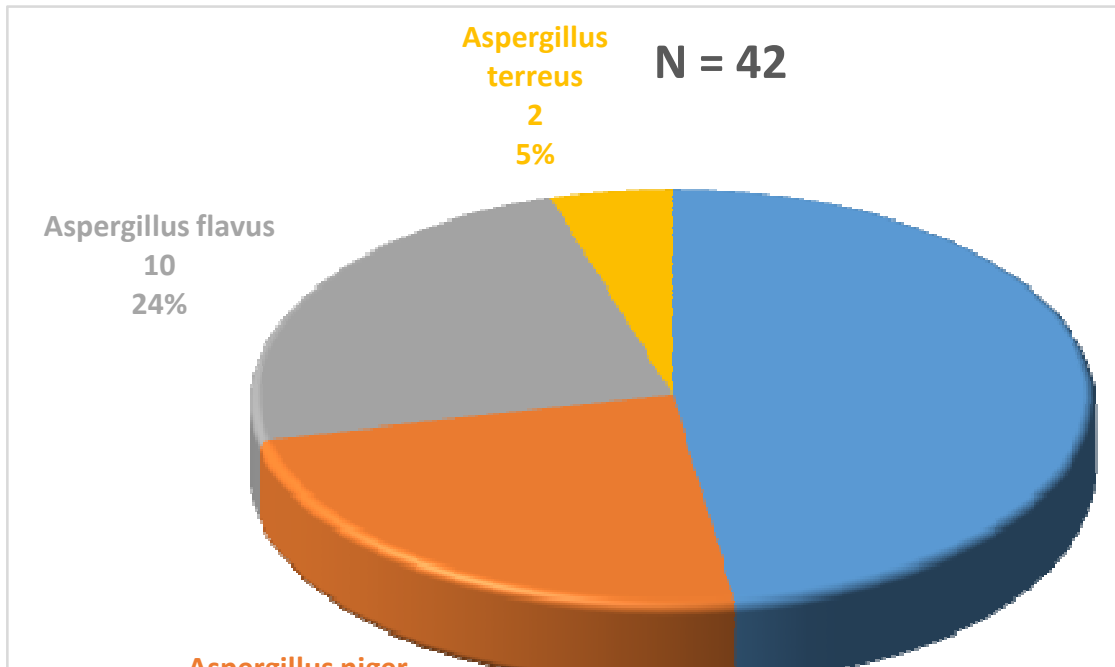


Figure 1: Prevalence of the different *Aspergillus* spp. isolated

The figure shows species, number of isolates, and proportion (%) with respect to all isolates

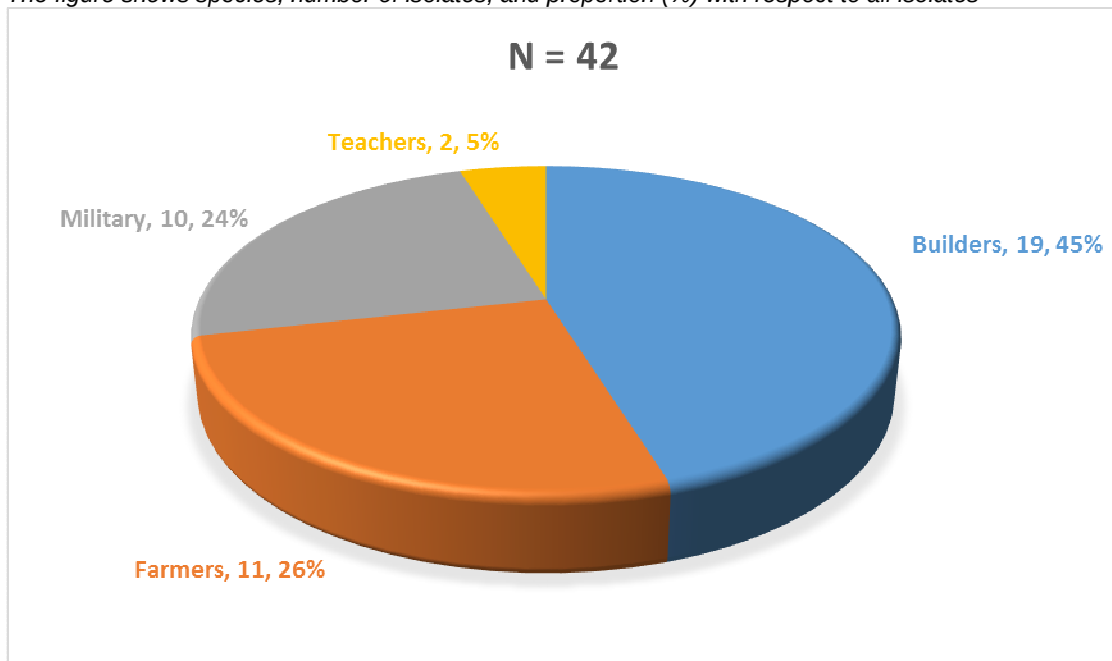


Figure 2: Prevalence of *Aspergillus* spp. with respect to the occupation of the participants.

Figure shows profession, number infected with *Aspergillus* spp. and proportion (%) with respect all infection

The other fungal pathogens isolated were *Candida albicans* 80 (30.8%) and *Histoplasma capsulatum* var *duboisii* 21 (8.1%). Coinfection between *Mycobacterium tuberculosis* and *Aspergillus* spp. was observed in 9 (3.46%) of the 260 sputum samples. Among the 9 cases, the most prevalent coinfection was observed with *Aspergillus fumigatus* 5 (55.56%), followed by *Aspergillus flavus* 3 (33.33%) and *Aspergillus Niger* 1 (11.11%). Twenty (7.69%) of the 260 sputum samples examined were found to be coinfecting with *Mycobacterium tuberculosis* and *Candida albicans*. No coinfection was observed between *Mycobacterium tuberculosis* and *Paragonimus africanus*. Gram staining revealed that out of the 260 sputum samples examined, 20 (7.7%) were positive for fungal elements, 80 (30.8%) for yeast, 5 (1.9%) positive for both yeast and gram positive rods, 40 (15.4%) positive for gram negative rods and 30 (11.5%) positive for gram positive cocci.

3. DISCUSSION

The prevalence of pulmonary aspergillosis in patients suspected of tuberculosis in Yaounde (Centre region of Cameroon) was 16.15% (95% CI: 11.65 – 20.66), which is not very different from the 15% reported in a similar study performed in the South West region of the country^[19], but low compared to the study performed India by Kurhade et al.^[20]. There was a significant association in the prevalence of aspergillosis with age, with the infection being more common in the age group 41-60years ($P = .000$). This is contrary to the study performed in the South West region where infection was observed to be more common in individuals above 60years^[19]. No significant association was observed in the prevalence of aspergillosis and sex in this study ($P = .60$), which is in accordance with the study by Njunda et al.^[19]. Furthermore, an association was observed between the prevalence of aspergillosis and occupation where the prevalence was observed to be higher among builders and lowest among teachers ($P = .013$). This could be as a result of the inhalation of dust containing *Aspergillus* spores in construction sites. The most predominant *Aspergillus* species isolated was *Aspergillus fumigatus* (47%) which is in accordance with earlier studies^[19,20]. Other species isolated in decreasing order of prevalence were *A. flavus* 24%, *A. niger* 24%, *A. terreus* 5%. The prevalence of *A. flavus* observed in this study was higher compared to the study performed in the South West region^[19], but similar to the prevalence reported by Shahid and Malik^[17]. This difference may be attributed to the geographical differences in the study population^[21].

The prevalence of *Paragonimus africanus* observed in this study was 2.69% (95% CI: 0.7 – 4.67) which was low compared to the prevalence observed by Strobel et al.^[22]. *Paragonimus africanus* has been known to be endemic in 5 foci in the South West Region of Cameroon^[7,8,23]. A 6th foci has recently been identified in the Manjo health district in the Littoral Region^[24]. To the best of our knowledge, this is the first study to report *Paragonimus africanus* in the Centre Region of Cameroon. Further studies will be needed to establish Yaounde as the 7th foci. Crayfish and crabs which are the intermediate hosts for *Paragonimus* are a delicacy in the Cameroonian cuisine which are sometimes eaten raw or pickled. This together with the constant eating of pork, snail and undercooked fish along major streets in Yaounde may be the major factor accounting for the transmission in the area. In this study, females were found to be more affected than their male counterparts, 4.58% vs 0.78% which is in accordance with the findings of Kagawa et al^[25]. This observation could be attributed to the fact that women are the ones who are most in contact with the intermediate hosts in the kitchen.

The prevalence of tuberculosis in this study was 16.92% (95% CI: 12.33 – 21.51), which is not significantly different from the 13.5% reported in the South West region of Cameroon^[19]. The prevalence of coinfection between *M. tuberculosis* and *Aspergillus* observed in this

study was 3.46%, which is very similar to that reported in the South West region.^[19] Tuberculosis has been observed to leave behind scarred pulmonary parenchyma which can be colonized by fungi, thereby serving as a predisposing factor for colonizing aspergillosis^[26]. No coinfection was observed in this study between *Paragonimus africanus* and *M. tuberculosis*.

Other fungal pathogens observed in this study included *Candida albicans* (30.8%) and *Histoplasma capsulatum var duboisii* (8.1%). *Histoplasma capsulatum var duboisii* was identified by the production of colonies on SDA which are initially smooth, but become filamentous, cottony, and brownish with age, and confirmed microscopically by the observation of macroconidia which is larger in size (8 - 15µm) and thicker walls compared to *Histoplasma capsulatum var capsulatum*. The prevalence of *Candida albicans* observed here was very high compared to that reported in similar study by Mwaura et al.^[27], and also high compared to a similar study performed in the South West Region of the country^[19]. An immediate explanation for this was not evitable. Coinfection between tuberculosis and candidiasis was observed in 7.69% of the participants which is similar to that reported by Mwaura et al.^[27], but higher than the prevalence of coinfection between *M. tuberculosis* and *Aspergillus spp.* observed in this study. It has been shown that a syntropic relationship exists between *Candida* and *Mycobacterium tuberculosis* whereby *Candida albicans* was observed to enhance the growth of *Mycobacterium tuberculosis*^[28]. The prevalence of *Histoplasma capsulatum var duboisii* (8.1%) reported in this study is also worth noting. *Histoplasma capsulatum var duboisii* is responsible for the so called "African histoplasmosis" and usually coexists with *Histoplasma capsulatum var capsulatum*. The earliest report on *Histoplasma capsulatum var duboisii* in Cameroon dates back to 1984^[29]. Histoplasmosis caused by *var duboisii* is generally regarded by skin, bone and lymph node involvement and only few case reports have described pulmonary involvement^[30,31].

In this study, Gram staining was performed on all the sputum samples. Gram reaction is a useful indicator of possible pathogens whenever culture is not feasible, and has been used for the preliminary diagnosis in clinical practice. It was observed that 7.7% of the sputum samples were positive for fungal hyphae, 30.8% for yeasts, 15.4% for gram-negative rods, and 11.5% for gram-positive cocci. These findings are higher compared to that reported in Nigeria by Mwaura et al^[27]. This could be attributed to the geographical differences in the study populations.

In this study, we could not established if there was actually any pulmonary involvement with *Aspergillus spp.*, *Candida spp.*, and the other isolates or just a mere colonisation due to the lack of sufficient clinical data and radiologic images. This serves as a major limitation to this study. The findings of this study should therefore be interpreted in an epidemiological rather than in the clinical context.

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256 4. CONCLUSION

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In this study, the prevalence of Aspergillosis and *Paragonimus africanus* observed were 16.15% and 2.69% respectively. *Aspergillus fumigatus* was the most predominant *Aspergillus spp.* isolated. Coinfection between *Mycobacterium tuberculosis* and *Aspergillus spp.* or *candida albicans* were also common, with the most frequent being coinfection between *Mycobacterium tuberculosis* and *Candida albicans*. No coinfection was observed between *M. tuberculosis* and *Paragonimus africanus*. Other pathogens observed in this study included *Histoplasma capsulatum var duboisii* (8.1%). These findings further underscores the importance of including the diagnosis of Aspergillosis and Paragonimiasis in patients suspected of tuberculosis in the study area.

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