Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Research Article

Sensitivity Profile of Fungal Pathogens Responsible for Lower Respiratory Tract Infections in Yaounde

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Received: February 08, 2022; **Accepted:** April 21, 2022; **Published:** April 28, 2022

Abstract

Background: Infectious diseases of the respiratory tract are known as respiratory tract infections (RTIs). An infection of this type usually is further classified as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). LRIs are the leading cause of death among all infectious diseases. The objective of our study was to bring out the sensitivity profile of fungal pathogens responsible for lower respiratory tract infections in Yaounde

Methods: We carried out a transverse and descriptive study during a 6 month period (February to June 2021), at the Jamot hospital in Yaounde. Included in this study were patients suffering from a lower respiratory tract infection from whom the medical practitioner had requested a sputum or broncho alveolar liquid analysis A macroscopic, microscopic, fungal culture of the sample was carried out and a germ tube test, fungal sensitivity test as well as specie identification using the ID 32 C gallery was carried out on the positive cultures Statistical analysis was carried out using the R version 3.6.1 software. The mean was calculated with the aid of the Kruskal Wallis rank sum test.

Results: 300 patients participated in this study. They had mean age ± standard deviation of 41.59 ± 17.5 years and extremities of 1 and 91years. The male /female ratio was 2:1 Fungal infection was positive in 127 patients (42.33 %), 75 (59%) *Candida albicans*, 25 (19.68%) *Cryptococcus humicola*, 10 (7.87%) *Candida tropicalis*, 6 (4.72%) *Candida krusei*, 4 (3.14%) *Candida famata*, 4 (3.14%) *Candida sake* and 3 (2.36%) *Cryptococcus curvatus.* As far as antifungigram is concerned, the total drug susceptibility was Nystatine (98.47%), Amphotericine B (86.91%), Miconazole (55.42%), Econazole (52.61%), Ketoconazole (52.57%) and Fluconazole (14.42%).The prevalence of fungal pathogens was 42.33%. Of the 300 patients, 71 had tuberculosis, 24 were HIV positive and 6 were diabetic we had 5 patients with HIV, tuberculosis and fungal co-infection, 16 with HIV and fungal co-infection and 6 with HIV and tuberculosis co-infection.

Conclusion: This study shows a relative high prevalence (42.33%) of the colonization of the respiratory tract by fungal pathogens. The fungal pathogens responsible for lower respiratory tract infections are *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida famata*, *Candida sake*, *Cryptococcus humicola* and *Cryptococcus curvatus*. The drug of choice is Nystatine and Fluconazole presents a very limited activity. Additional studies should be carried out in other towns in order to better document this issue in Cameroon.

Keywords: Respiratory tract infection; Candida species; Fluconazole; Nystatine; Fungal pathpgens

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; AJRCCM: American Journal of Respiratory and Critical Care Medicine; BAL: Broncho Alveolar Liquid; CDC: Centers for Disease Control and Prevention; CHROMagar™: Chromogenic agar; CLSI: Clinical Laboratory Standard Institute; °C: Degree Celsius; GT: Germ Tube; HIV: Human Immunodeficiency Virus; ICU: Intensive Care Unit; KOH: Potassium Hydroxide; LRTI: Lower Respiratory Tract Infection; LRI: Lower Respiratory Infection; mg/L: Milligram per Liter; RSV: Respiratory Syncytial Virus; SAB: Sabouraud; Spp: Species; TB: Tuberculosis; URI: Upper Respiratory Infection; URTI: Upper Respiratory Tract Infection

Introduction

Infectious diseases of the respiratory tract are known as respiratory tract infections (RTIs). An infection of this type usually is further classified as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). LRIs are the leading cause of death among all infectious diseases. The objective of our study was to bring out the sensitivity profile of fungal pathogens responsible for lower respiratory tract infections in Yaounde. The specific objectives were to:

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Citation: Claris K, Laure N, Leopold N, Ekobo S and Hortense G. Sensitivity Profile of Fungal Pathogens Responsible for Lower Respiratory Tract Infections in Yaounde. J Bacteriol Mycol. 2022; 9(1): 1196.

Identify fungal pathogens responsible for lower respiratory tract infections, bring out the sensitivity profile of the different fungal pathogens causing lower respiratory tract infections and determine the prevalence of lower respiratory tract infections caused by fungal pathogens. Candida spp. is the most common cause of intensive care unit (ICU) invasive fungal infections worldwide. The isolation of Candida spp. from respiratory tract secretions of non-immunocompromised, mechanically ventilated patients varies between 20% and 55%, but it might represent colonization rather than infection [1].

The few studies performed in the 90's used traditional culture methods [2]. Although culture is still considered to be the gold standard, the method has important disadvantages, such as a longer time to result, the stringent specimen collection and transport condition and the risk of inhibited growth of the pathogens due to previous antibiotic treatment [3,4].

As a consequence, many patients in African healthcare centers remain undiagnosed despite clinical evidence of LRTIs. In critically ill patients Candida spp. are frequently isolated from respiratory tract secretions such as endotracheal aspirates and bronchoalveolar lavages (BAL) and are most often considered as colonizers of the respiratory tract. In contrast, pneumonia due to infection with Candida spp. is rare and is diagnosed by histological demonstration of the yeast in lung tissue with associated inflammation. In spite of this, preemptive antifungal therapy based on isolation of Candida spp. from the respiratory tract is often initiated in critically ill patients. The disadvantages of this approach include increased selective pressure for the development of antimicrobial resistance, potential risks of adverse drug reactions and high treatment costs. On the other hand, immediate administration of appropriate antifungal therapy has been shown to be an important predictor of favorable outcome for patients with invasive fungal infections. Therefore, the development of reliable diagnostic measures for the detection of invasive pulmonary candidiasis is crucial [5]. Candida spp. colonization promotes bacterial pneumonia in animal models.

- Candida spp. colonization could clinically increase the risk for *Pseudomonas aeruginosa* ventilator-associated pneumonia, prolong mechanical ventilation and stay and worsen outcomes, but to date contrasting data are available.
- Available evidence is not sufficient to support routine antifungal therapy in non-immunocompromised patients.

Candida spp. is part of the normal skin, oropharyngeal, mucosal membranes and upper respiratory tract flora. Candida spp. can reach the lungs through either haematogenous dissemination or aspiration of colonized oropharyngeal or gastric contents [1]. The isolation of Candida spp. from respiratory tract secretions is frequent in non immunocompromised, mechanically ventilated patients. Several studies have reported the presence of Candida spp. in the sputum of 20-55% of patients receiving antibiotics [6,7]. Candida spp. is the most common cause of invasive fungal infections, with an incidence estimated at 72.8 cases per million inhabitants per year [8]. The five main species of Candida spp (*C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis* and *C. krusei*) are responsible for more than 90% of invasive fungal infections, in both intensive care unit (ICU) and non-ICU patients [9]. *Candida pneumonia* is a rare lung infection with a high morbidity and mortality, commonly observed as part of a disseminated Candida infection and associated with predisposing clinical circumstances (i.e. long-term antibiotic use, haematologic malignancy or severe immunosuppressive states). The majority of *Candida pneumonia* cases are secondary to haematological dissemination of Candida spp. [10]. There is no specific clinical or radiological presentation of Candida pneumonia. This aspect of the disease makes the diagnosis difficult to perform. A definitive diagnosis of candida pneumonia is now based on histopathological identification of yeast parenchymal invasion with associated inflammation [11-14].

This study was necessary because there wasn't any existing data as to the prevalence of fungal pathogens responsible for lower respiratory tract infections in Yaounde [15-20].

Materials and Methods

The aims of our research work were to identify fungal pathogens responsible for lower respiratory tract infections, bring out the sensitivity profile of the different fungal pathogens causing lower respiratory tract infections and determine the prevalence of lower respiratory tract infections caused by fungal pathogens (Table 1 and 2). We carried out a transverse and descriptive study during a 6 month period (February to June 2021), at the Jamot hospital in Yaounde. The study was conducted in Yaounde, the political capital of Cameroon at the Jamot hospital. It is located at Mballa II neighbourhood in Yaounde and specialized in the management and follow up of patients with mental illness. However, the hospital also hosts some other departments apart from the psychiatric department. It has a psychiatric center as well as a pneumology department". Those with pulmonary diseases (tuberculosis notably) are treated at the pneumology department. General medicine is equally practiced at the hospital center. Samples were collected at the Jamot hospital and transported to the Bacteriology laboratory of the Yaounde teaching hospital for analysis.

The target population was composed of patients presenting signs and symptoms of a lower respiratory tract infection and from whom a Mycobacterium tuberculosis investigation of their sputum or broncho alveolar liquid had been asked for by the physician and who gave an oral or written consent or assent

The samples were labelled with the patient information and date and transported in a cooler from the Jamot hospital to the Yaounde University teaching hospital laboratory where:

- The macroscopic examination of biologic samples was done and consisted in recording the aspect (cloudy, bloody, purulent, mucopurulent, salivary) and colour of the different collected samples. The sample was directly streaked on Sabauraud + Chloramphenicol agar (culture media that enables the growth of fungi) Liofilchem® using a 10µl calibrated wire loop. The culture plates were incubated at 37 degrees centigrade and examined everyday for a one week period. No growth indicated a negative result and was recorded as such. Every isolated colony except contaminants was considered a potential pathogen and further investigation to identify the species was done including a germ tube test, regrowth on chrom agar, identification using ID 32C fungus gallery and an antifungigram was carried out.
- The microscopy was done in which a drop of the sample

was placed on a slide and a coverslip was applied and observed under the microscope using the 10X objective and then moved to the 40X objective, yeast appeared as small, oval, budding, thin-walled yeast cells, 2-4 micros in size. Then followed Gram Stain procedure in which the smear was heat fixed onto the slide by passing the slide through a bunsen flame (just above the inner blue cone of the flame) three (3) times. The slide was then placed on a wire rack over a laboratory sink. And the smear was flooded with Crystal Violet for 30 seconds and gently rinsed with tap water, Lugol's Iodine was then applied for 30 seconds and gently rinsed with tap water, the smear was then decolorized by lifting one end of the slide and running ethanol down the slide and across the smear for about 5 seconds and gently rinsed with tap water and then counterstained with Carbol Fuchsin or Safranin for 30 seconds followed by gentle rinsing with tap water and allowed to dry. It was then examined under the microscope, using a 100x objective and oil immersion.

- On the Positive growths from Sabauraud + Chloramphenicol agar, the aspect of the colonies were taken note of and registered, then followed the preparation of a 0.5 McFarland suspension and re streaking on CHROMagar™ Candida Plus and then incubation at 37 degrees centigrade for 24 hours after which the colours of the colonies were taken note of. A germ tube test was equally done. This consisted in putting 0.5ml of human serum into a small tube and using a Pasteur pipette, a colony of yeast was gently emulsified in the serum. The tube was then incubated at 37°C for 2 to 4 hours and a drop transferred to a slide for examination. A coverslip was then applied and examined microscopically under low and high power objectives. A positive test was observed as a short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. Germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus. Examples: *Candida albicans* and *Candida dubliniensis*. A negative test was observed as no hyphal (filamentous) extension arising from a yeast cell or a short hyphal extension constricted at the point of origin. Examples: *C. tropicalis*, *C. glabrata* and other.

Then followed Biochemical testing using ID 32 C which is a standardized system for the identification of yeasts, which uses 32 miniaturized assimilation tests and a database. Reading and interpretation were carried out automatically or manually. The ID 32 C strip consists of 32 cupules, each containing a dehydrated carbohydrate substrate. A semi-solid, minimal medium is inoculated with a suspension of the yeast organism to be tested. After 24-48 hours of incubation, growth in each cupule is read either using the ATB TM Expression™ or mini API® instruments, or visually. Identification is obtained using the identification software. The identification was done following the manufacturer's instructions. After 24-48 hours incubation, wells showing turbidity were registered as positive and those with no turbidity were registered as negative. A code was then generated and a decoder used to identify the species.

Then followed the susceptibility testing in which the Rapid Labs tablet assay was performed according to the manufacturer's instructions (Rapid Labs user's guide; Rapid Labs Ltd, Essex, UK) and CLSI guidelines. Briefly, the isolated fungal species were cultured on Sabouraud + Chloramphenicol agar at 35°C for 24h. Then, the yeasts were suspended in 5mL of sterile physiological serum and thoroughly vortexed to achieve a smooth suspension. The optical density (OD) of the suspensions was adjusted to 0.08 to 0.1 at a wavelength of 625nm to yield turbidity equal to 0.5 McFarland standards. A sterile cotton swab moistened with the inoculums suspension was used and applied to a 90mm diameter plate, containing Sabouraud + Chloramphenicol agar. The plates were allowed to dry for 3-10 minutes. To determine the antifungal susceptibility patterns of the isolates, a Rapid labs disk of each antifungal drugs, including FLU (10μg/disk), amphotericin B (10μg/disk), MIC (10μg/disk), ECN (10μg/disk), NYS (100μg/disk), and ketoconazole (10μg/disk) was dispensed onto the inoculated plates. Zones of inhibition around the disk were measured following incubation of the plates for 18-24 hours at 35-37°C. When insufficient growth was encountered at the 24-hour reading, the plates were re-evaluated after a further 24 hours. The susceptibility of all species was evaluated based on the zone interpretive criteria of the manufacturer (Rapid labs). Quality control was censured by testing the Rapid Labs user's guide and CLSI recommended control strains C. parapsilosis ATCC 22019 (AMP:24-28mm, KET: 30-33mm, FLU: 27-30mm) and *C. krusei* ATCC 6258 (AMP:19-22mm, KET: 22-24mm, FLU: 9-12mm). All control strains were included in each series of tests.

Culture strains were then conserved in Brain heart media and conserved at -20 degrees centigrade in the dip freezer. Enzymatic digest of animal tissues and brain-heart infusion provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Disodium phosphate is the buffering agent. The infusion was prepared by suspending 37g of the powder in 1-liter of distilled or deionized water, well mixed and then heated to boil while shaking frequently until completely dissolved. 10% glycerol was then added and sterilized in the autoclave at 121°C for 15 minutes after which distributed in cryotubes and freezed. At the point of usage, they were removed and allowed to thaw and the colonies were then introduced and freezed.

- Data was collected using a data collection sheet constructed for the study and the data was registered in Excel then transported to R version 3.6.1 software. Results were summarized as percentages and frequencies and presented in graphs and tables. The R version 3.6.1 enabled us to analyze the data. For quantitative data and for descriptive analyses we used the mean, median and standard deviation to describe socio demographic data. As far as qualitative data is concerned we used confidence intervals for uni varied analysis: Chi square to compare two variables in order to define the link of dependence. Graphics were plotted using Microsoft Office Excel 2010.

Results

We observed that of the 316 patients contacted, 300 ended up filling the questionnaires making a participation of 94.9% (300/316) and non participation of 5.0% (16/316) (Figure 1).

Of the 300 patients who participated in this study, 101 were females and 199 were males giving a sex ratio (female/male) of 1:2 and a 33.66% (101/300) participation of females as compared to 66.33% (199/300) participation of males. The P-value of 0.2 showed that sex distribution was statistically non significant. Of the 300 patients who participated in our study, 11% (33/300) came from the rural area whereas 87.7% (236/300) came from the urban area giving a p value

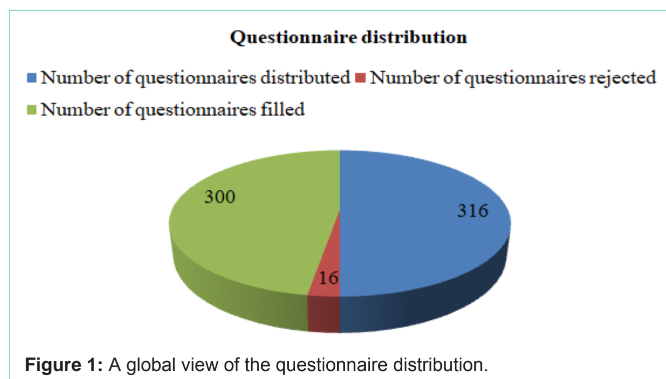

Figure 1: A global view of the questionnaire distribution.

Table 1: List of equipment used during the study.

Devices	Function
Incubator 30°C	Incubation of culture media at 30°C.
Deep freezer -20°C	Conservation of colonies in brain heart media.
Vortex	Homogeinise fungal inoculum.
Optical microscope	Examination of wet preparations and coloured slides.
Refridgerator	Conservation of samples.
Water bath	Incubation of serum-colony mixture for germ tube test.
Autoclave	Preparation of culture media.

Table 2: List of Material used.

Material	
Biologic	Respiratory tract secretions (BAL and Sputum)
Sample	Sputum, BAL
Transport	Cooler
Analysis	Sputum container, culture media, scale, round bottom flask, bunsen burner, funnel, petri dishes, scale, wire loop, cryotubes, cotton swab, antifungal discs, ruler, micropipette, micropipette tips, tubes, sterile water, physiologic water, watman paper, sucker, slide rack, optical microscope,0.5 McFarland standard, slides and cover slides, trash can.
• Protection	Gloves, Lab jacket, fume hood, decontaminating solution (bleach diluted 1 in 10), mask, cover shoes, guggles.
Reagents	**Fungal Diagnosis:** Culture media, antifungal discs, KOH, crystal violet, lugol, carbol fuschin, alcohol acetone, immersion oil.
Data analysis	Complete laptop or desktop, Epi info 3.5 software, Excel.
Waste management and decontamination	Liquid soap, bleach diluted 1 in 10, tap water, sharps container, toilet tissue, gloves, incinerator.

Table 3: Representation of sex distribution.

Variable	Levels	Sex: Men (199) N (%)	Sex: Women (101) N (%)	Total (300) N (%)	P-value
Age	Mean (SD)	40.8 (17.0)	43.1 (18.7)	41.6 (17.6)	0.286*
Sample	BAL	5 (2.5)	1 (1.0)	6 (2.0)	0.402**
	Bronchial aspiration	2 (1.0)	0 (0.0)	2 (0.7)	
	Sputum	192 (96.5)	100 (99.0)	292 (97.3)	
Neighbourhood	Rural	18 (9.1)	15 (15.0)	33 (11.0)	0.161**
	Urban	180 (90.9)	83 (83.0)	263(87.7)	

*P-value of Kruskal-Wallis rank sum test.
**P-value of Pearson's Chi-squared test.

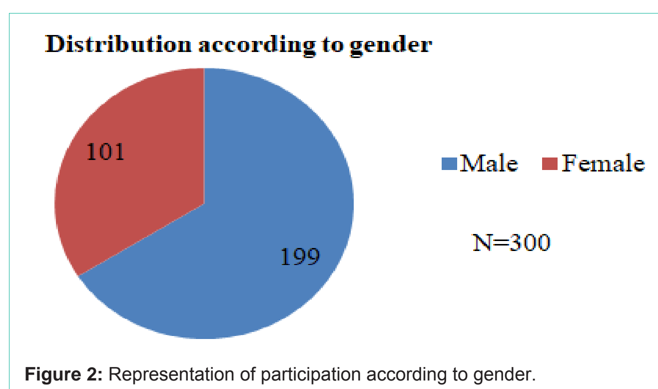

Figure 2: Representation of participation according to gender.

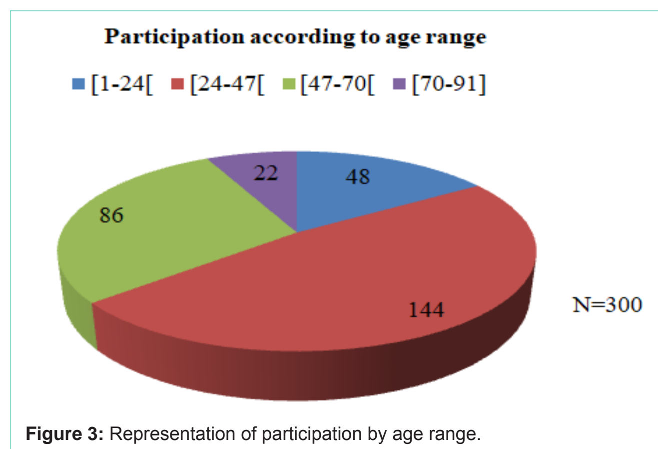

Figure 3: Representation of participation by age range.

of 0.16 this was statistically non significant cf Table 3 and Figure 2.

We noticed that the highest participation 47.6% (143/300) was observed among the 24-47 age range and the lowest 7.33% (22/300) among the 70-91 age range. The patients had a mean age of 41.59 ± 17.5 years with the youngest patient being 1-year old and the oldest 91 years and a p value of < 0.001 showing that age range was statistically significant. The greatest number of sample collected was sputum 97.0% (292/300) and the least was bronchial aspirate 4.2% (02/300). As far as habitat is concerned the greatest number of patients came from the urban area 88% (264/300) and the least 11% (33/300) from the rural area Cf Table 4 and Figure 3.

The most isolated species was *Candida albicans* 59.0% (75/ 127) followed by *Cryptococcus humicola* 19.7% (25/127), *Candida tropicalis* 7.9% (10/127), *Candida krusei* 4.7%(6/127), *Candida famata* 3.1% (4/127), *Candida sake* 3.1% (4/127) and *Cryptococcus curvatus* 2.4% (3/127) Cf Table 5 and Figure 4. The prevalence of fungal infection in the population being 42.33% (127/300).

Candida albicans and *Cryptococcus curvatus* were isolated most in the (24-47) age range and least in the (70-91) age range, *Candida famata* was isolated most in the [47-70] age range. *Candida sake* was mostly isolated in the (1-24) and (24-47) age ranges meanwhile *Candida tropicalis* was mostly isolated in the (47-70) age range. Our overall prevalence showed that the isolated species were statistically significant Cf Table 6 and Figure 5.

The highest number of the different isolated pathogens except

Table 4: Representation of participation according to age range.

Variable	Levels	Age groups (years) [1-24] (48) N (%)	[24-47] (143) N (%)	[47-70] (86) N (%)	[70-91] (22) N (%)	Total (300) N (%)	P-value
Age	Mean (SD)	18.2 (4.7)	34.9 (6.1)	56.7 (6.7)	77.4 (5.8)	41.6 (17.5)	<0.001*
Sample	BAL	0 (0.0)	1 (0.7)	4 (4.7)	1 (4.5)	6 (2.0)	0.012**
	Bronchial aspiration	2 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)	
	Sputum	46(95.8)	143(99.3)	82(95.3)	21(95.5)	292(97.0)	
Neighbourhood	Rural	8 (16.7)	13 (9.1)	10(11.6)	2 (9.1)	33 (11.0)	0.536**
	Urban	40(83.3)	129(90.2)	75(87.2)	20(90.9)	264(88.0)	

*P-value of Kruskal-Wallis rank sum test.

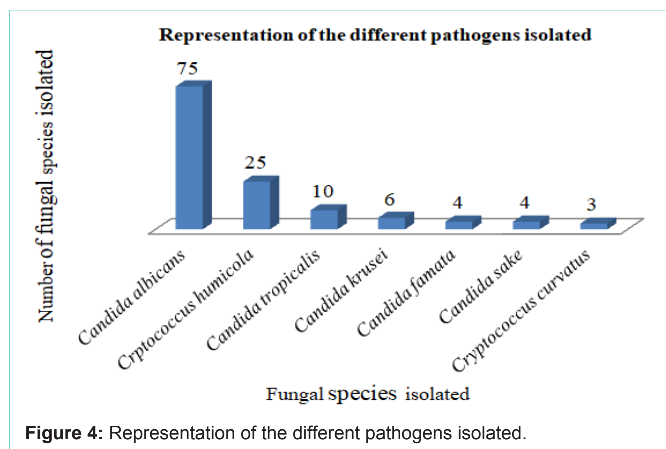

Figure 4: Representation of the different pathogens isolated.

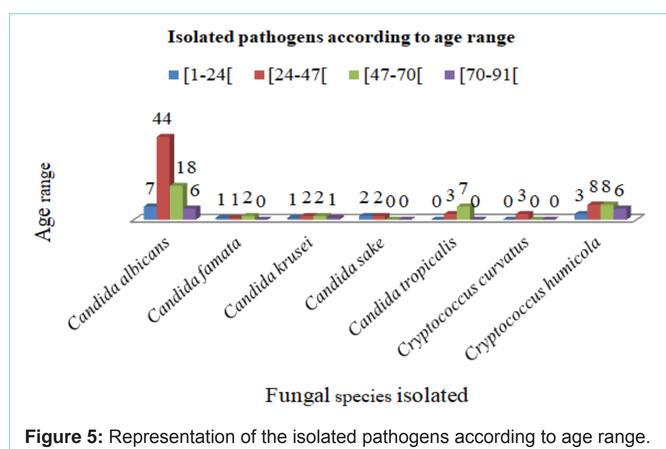

Figure 5: Representation of the isolated pathogens according to age range.

Table 5: Representation of the different pathogens isolated.

Ranking	Organism	No. of isolates	Total in %
	Fungi		
1	*Candida albicans*	75	59
2	*Cryptococcushumicola*	25	19.7
3	*Candida tropicalis*	10	7.9
4	*Candida krusei*	6	4.7
5	*Candida famata*	4	3.1
6	*Candida sake*	4	3.1
7	*Cryptococcus curvatus*	3	2.4
	Total	127	100

Table 6: Representation of the different pathogens isolated according to age range.

Isolated organism	Age groups (years) [1-24] (48) N (%)	[24-47] (143) N (%)	[47-70] (86) N (%)	[70-91] (22) N (%)	Total (300) N (%)	P-value
Candida albicans	7 (14.6)	44 (30.8)	18 (20.9)	6 (27.3)	75 (25.0)	0.0829**
Candida famata	1 (2.1)	1 (0.7)	2 (2.3)	0 (0.0)	4 (1.3)	0.5456*
Candida krusei	1 (2.1)	2 (1.4)	2 (2.3)	1 (4.5)	6 (2.0)	0.5133*
Candida sake	2 (4.2)	2 (1.4)	0 (0.0)	0 (0.0)	4 (1.3)	0.1135*
Candida tropicalis	0 (0.0)	3 (2.1)	7 (8.1)	0 (0.0)	10 (3.3)	0.05829*
Cryptococcus curvatus	0 (0.0)	3 (2.1)	0 (0.0)	0 (0.0)	3 (1.0)	0.6582*
Cryptococcus humicola	3 (6.3)	8 (4.9)	8 (9.3)	6 (27.3)	25 (8.0)	0.03182*
Overall prevalence	14 (29.2)	63 (43.4)	37 (43.0)	13 (59.1)	127(42.0)	0.026**

*P-value of Fisher's exact test.
**P-value of Pearson's Chi-squared test.

Candida tropicalis 6.0% (6/101) was from the male gender. The same number of *Candida sake* 2 was isolated from both genders Cf Table 7 and Figure 6.

As for the sensitivity profile, the greatest resistance to Miconazole (16.7%) and Amphotericine B (33.3%) was observed in *Candida krusei*, to Econazole (24.0%), Nystatin (8.0%), Ketoconazole (100.0%) in *Cryptococcus humicola* and *Cryptococcus curvatus* respectively and to Fluconazole (100.0%) observed in *Candida Krusei*, *Candida sake* and *Cryptococcus curvatus*. As for sensitivity we observed the greatest to Miconazole (75.0%) and Ketoconazole (75.0%) in *Candida famata* and *Candida sake*, greatest to Econazole (83.3%) in *Candida krusei*, Nystatin (100.0%) in *Candida famata*, *Candida krusei*, *Candida sake*, *Candida tropicalis* and *Cryptococcus curvatus*, Amphotericine B (100.0%) in *Candida sake* and *Cryptococcus curvatus* and lastly Fluconazole (30.7%) in *Candida albicans* (Figure 7-13).

Of the 300 patients who participated in this study, 8.3% (25/300) were HIV positive and 91.7% (275/300) were HIV negative giving us a p value of < 0.0001*according to the Pearson's Chi-squared test showing that HIV status was statistically significant (Figure 14).

As of the tuberculosis status of the patients, 23.7% (71/300) were TB positive and 76.3% (229/300) were TB negative giving us a p value of < 0.0001*according to the Pearson's Chi-squared test showing that TB status was statistically significant (Figure 15).

According to the HIV and positive culture co infection, 56%

Table 7: Representation of the different pathogens isolated according to sex.

Isolated organism	Sex		Total (300) N (%)	OR (95% CI)	P-value
	Men (199) N (%)	Women (101) N (%)			
Candida albicans	51 (25.3)	24 (23.0)	75 (24.3)	1.15 (0.50-2.61)	0.8469*
Candida famata	4 (2.0)	0 (0.0)	4 (1.3)	-	0.3019*
Candida krusei	5 (2.5)	1 (1.0)	6 (2.0)	2.52 (0.26-122.61)	0.6627*
Candida sake	2 (1.0)	2 (2.0)	4 (1.3)	0.48 (0.03-6.83)	0.5969*
Candida tropicalis	4 (2.0)	6 (6.0)	10 (3.3)	0.29 (0.06-1.33)	0.0786*
Cryptococcus curvatus	3 (1.5)	0 (0.0)	3 (1.0)	-	0.5502*
Cryptococcus humicola	16 (8.1)	9 (9.0)	25 (8.3)	0.83 (0.31-2.40)	0.8123*
Overall prevalence	85 (42.4)	42 (41.0)	127(41.7)	-	0.225**

*P-value of Fisher's exact test.
**P-value of Pearson's Chi-squared test.

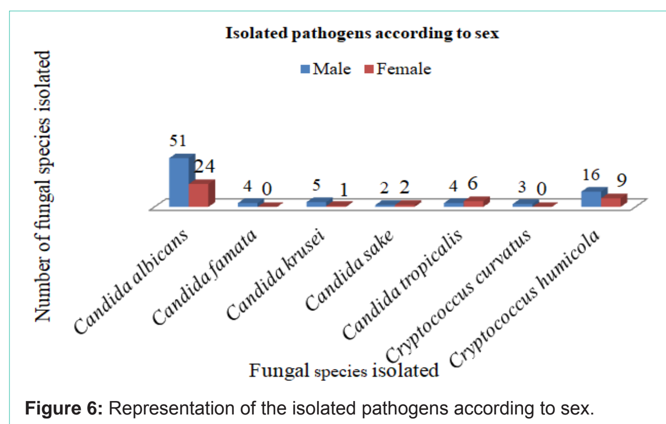

Figure 6: Representation of the isolated pathogens according to sex.

(14/25) of HIV positive patients had a *Candida albicans* infection giving us a p-value of 0.5976*according to the Fisher's exact test showing that this was statistically non significant (Figure 16).

According to the TB and positive culture co infection, 29.6% (21/71) of TB positive patients had a *Candida albicans* infection giving us a p value of 0.032*according to the Fisher's exact test showing that this was statistically significant. We equally observed that 5 patients were positive to HIV, TB and *Candida albicans*.

Discussion

Drawbacks or challenges of the study

Our study had as goal to provide the sensitivity profile of Candida species responsible for lower respiratory tract infections [21-26]. In order to achieve this we described the sociodermographic characteristics of our study population, calculated the prevalence of respiratory mycosis, identified the different fungi and produced the sensitivity pattern of the identified species to different antifungal drugs [27-30]. Our challenge was the slow pace of data and sample collection due to skeptism because of the present covid 19 pandemic [31-34].

Fungal species identified

Of the species identified, we had 59% (75/127) prevalence for *Candida albicans*. This result is close to that of a similar study carried out in Brazil by Ana et al. in 2017 [35] in which the prevalence of

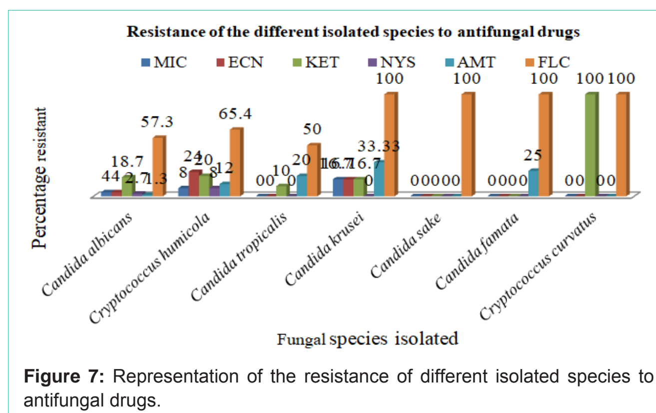

Figure 7: Representation of the resistance of different isolated species to antifungal drugs.

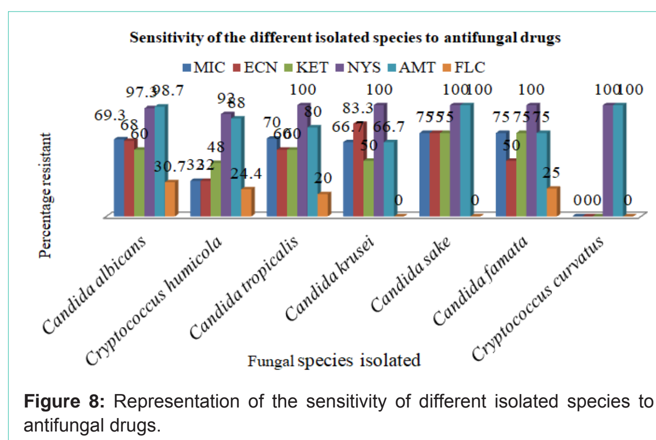

Figure 8: Representation of the sensitivity of different isolated species to antifungal drugs.

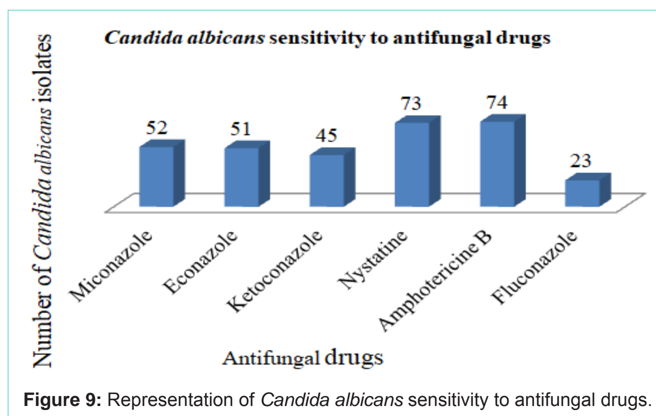

Figure 9: Representation of *Candida albicans* sensitivity to antifungal drugs.

Candida albicans was 56% and to that carried out by Sundar et al. [36] in Nepal in the same year with the same prevalence. These results can be explained by the fact that *Candida albicans* is a common fungal pathogen of humans that colonizes the skin and mucosal surfaces of most healthy individuals [37]. Our least isolated species was *Cryptococcus curvatus* 2.36% (3/127) which is contrary to the least isolated species *Candida tropicalis* (1.7%) in a study by P Badiee et al. [43] in Iran in 2010.

Sensitivity profile of species identified

As far as the sensitivity profile is concerned we discovered that all our identified species were resistant to Fluconazole. This finding is contrary to that by Kamiar et al. [38] in 2011 in Iran in which the candida isolates were sensitive to fluconazole at a percentage of 96.6.

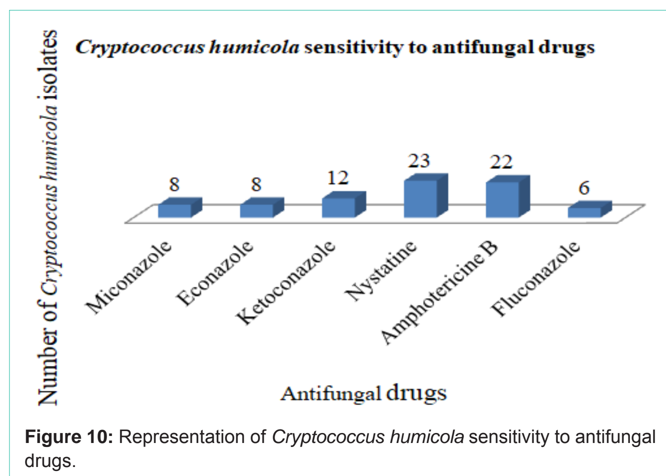

Figure 10: Representation of *Cryptococcus humicola* sensitivity to antifungal drugs.

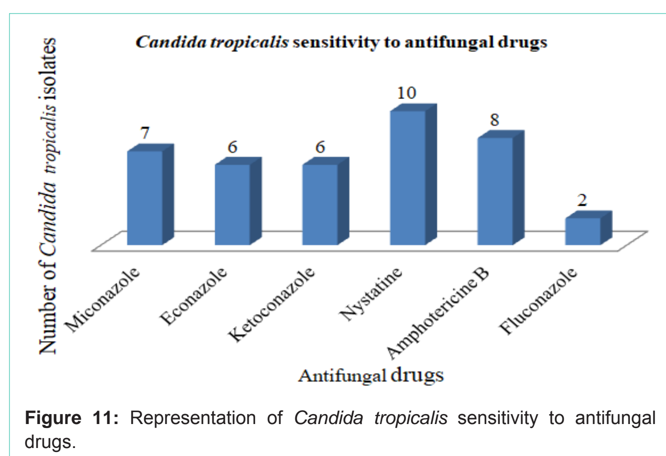

Figure 11: Representation of *Candida tropicalis* sensitivity to antifungal drugs.

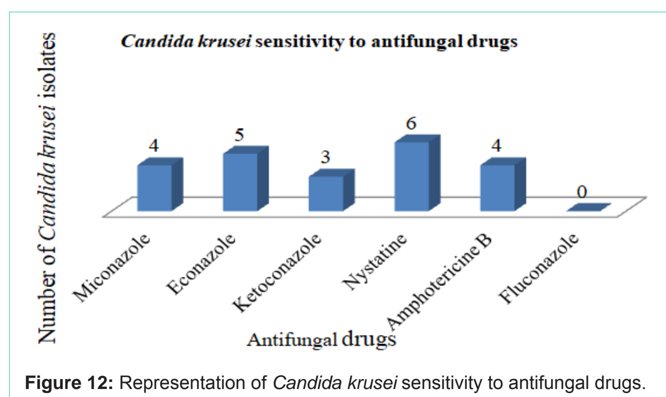

Figure 12: Representation of *Candida krusei* sensitivity to antifungal drugs.

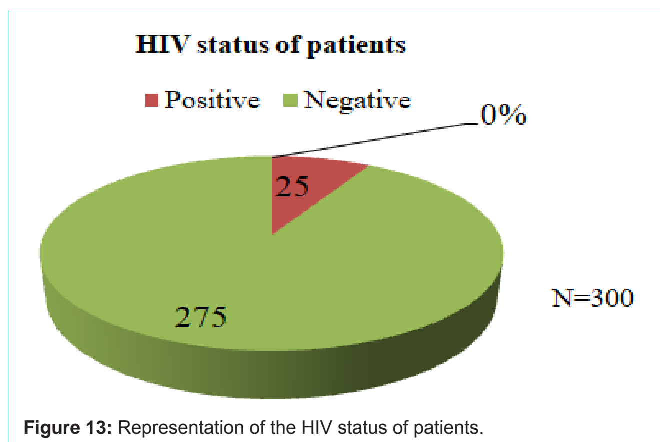

Figure 13: Representation of the HIV status of patients.

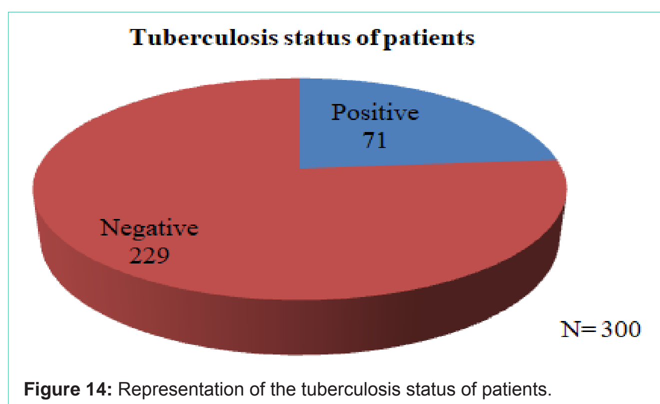

Figure 14: Representation of the tuberculosis status of patients.

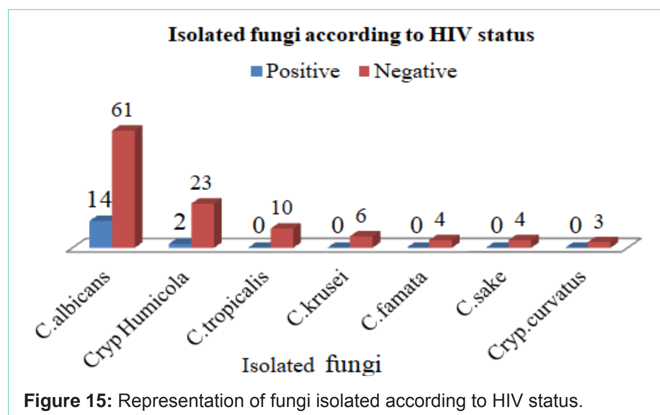

Figure 15: Representation of fungi isolated according to HIV status.

This loss of sensitivity in our study as compared to that in Iran in 2011 can be explained by the fact that there has been a misuse of fluconazole antifungals over the years i.e. consumption without medical prescription that has led to resistance. Our results ties with that by Gonsu Kamga et al. [39] in a study carried out in Cameroon in 2014 on the antifungal susceptibility patterns among the clinical isolates of Candida Spp in digestive candidiasis in HIV-positive subjects in which fluconazole activity was limited [40-42]. This makes us understand that resistance to fluconazole is not only limited to isolates from the respiratory tract but from the digestive tract as well. The drug of choice from our study is Nystatine [44]. This is contrary to the result obtained from a study carried out in 2018 in Iran by Simin et al. [45] in which their drug of choice was Miconazole. This difference can be explained by the fact that nystatine was not among the list of antifungal discs used in their study. In our study Candida sake was susceptible to Amphotericine B (100%) [46-49]. This result is contrary to that obtained in a study carried out by Patricio et al in 2000 in Brazil [50] in which *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* were susceptible to both amphotericine B and fluconazole. This difference is due to the misuse of fluconazole along the years.

Prevalence

In this study the prevalence of respiratory mycosis was 42.33% with a sample size of 300 patients and positive culture of 127. This

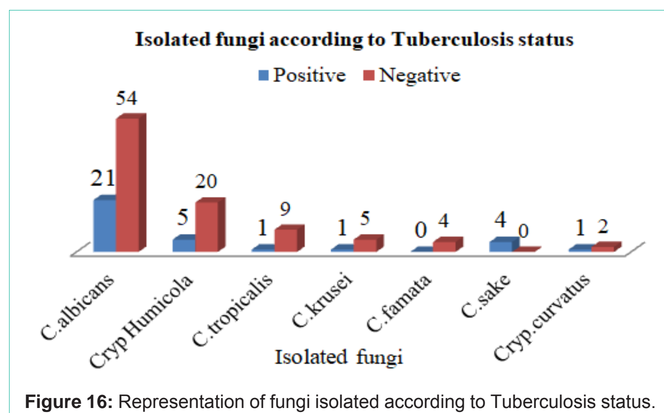

Figure 16: Representation of fungi isolated according to Tuberculosis status.

prevalence is half of that described in a similar study by Ana et al. in Brazil [35] in the year 2017 with a sample size of 52 patients, positive culture of 43 giving a prevalence of 83% This difference is explained by the fact that all recruited patients for that study were AIDS patients and we know that fungal infections are opportunistic in AIDS patients therefore resulting in the high prevalence whereas our study included both HIV positive and HIV negative patients. We had a 4.72% prevalence of *Candida krusei* similar to 5.3% *Candida krusei* in a study carried out by Hossein et al in Iran in 2018 [44].

Age range

The age range of our study participants was 1-91 years. This is close to that of a study carried out by Singh et al. in 2013 [42] in India with age range 9 months-77 years. This similarity can be explained by the fact that no restrictions were put as of the age participation in both studies.

Most resistance species to all antifungals

In this study the specie with the highest resistance 200 to antifungals was *Cryptococcus curvatus*. This is contrary to *Candida krusei* which was found to be the most resistant specie in a study carried out by Parissa et al. in 2017 in Iran [46].

Participation according to sex

As far as sex is concerned the 33.6% (101/300) participation of females and 66.33% (199/300) participation of males in our study giving a ratio of 1:2 is contrary to the 53.3% (158/296) participation of females and 46.62% (138/296) males (ratio 1:1) in a study carried out by Maiz et al. in 2015 in Spain [47]. This difference can be explained by the fact that there was no sex restriction in both studies.

Conclusion

At the end of our study on 'The sensitivity profile of fungal pathogens responsible for lower respiratory tract infections in Yaounde' which had as objectives to identify fungal pathogens responsible for lower respiratory tract infections, bring out the sensitivity profile of the different fungal pathogens causing lower respiratory tract infections and determine the prevalence of lower respiratory tract infections caused by fungal species, we conclude that:

- These objectives were met according to the following results obtained and we confirm that the different fungal pathogens responsible for lower respiratory tract infections in Yaounde are *Candida albicans*, *Cryptococcus humicola*, *Candida tropicalis*, *Candida krusei*, *Candida famata*, *Candida sake* and *Cryptococcus curvatus*.

- Most fungal pathogens are resistant to fluconazole but sensitive to nystatine.

- The prevalence of respiratory tract infections caused by fungal pathogens is 42.33%.

Acknowledgement

- My Supervisor Pr. Same Ekobo for the strict follow up of this study.

- My Co-supervisor Pr. Gonsu Hortense for advice, correction and supervision of this study.

- The Dean of the Faculty of Medicine and Biomedical Sciences Pr. Ze Minkande Jacqueline for her supervision.

- The members of jury Pr. Ama Moor Vicky and Pr. Perfura for accepting to bring out critics on this study.

- The President of jury Pr. Luma Henry for accepting to bring out critics on this study.

- Pr. Ama Moor Vicky for permitting me to collect serum from her laboratory in order to carry out the germ tube test.

- Dr. Ngando Laure and Dr. Mbamyah Lyonga for bringing out the necessary corrections.

- Dr. Olinga Medjo Ubald of the Jamot hospital Yaounde for collecting the Broncho alveolar fluid and permitting me to participate in this delicate collection process.

The laboratory staff of the Jamot hospital and that of the Yaounde University teaching hospital for their collaboration.

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