



IDENTIFICATION OF FUNGI ISOLATED FROM BATHROOMS IN FEMALE STUDENTS' HOSTEL, UNIVERSITY OF BENIN, BENIN CITY

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ABSTRACT

Fungi are specifically dangerous as they exhibit a significant tolerance to environmental changes and can proliferate under low relative humidity, unlike bacteria. They produce spores that are easily dispersed by air hence they are ubiquitous. The study aimed at identifying the fungal isolates present in the bathrooms located on the three floors of the hostel, University of Benin, Benin City. Samples were collected from the walls of the bathrooms using sterile swab sticks and were identified using standard microbiological techniques. The isolated fungi were *Aspergillus nidulans*, *A. niger*, *A. tamarii*, *A. flavus*, *Candida albicans*, *Penicillium cyclopium*, *P. oxalicum*, *Mucor mucedo*, *Trichophyton rubrum* and *Rhodotorula* species. From the ground floor bathrooms, *Candida albicans* (23.40%) were most frequently isolated, *Aspergillus nidulans* (55.56%) were mostly isolated from the first floor and *Mucor mucedo* (56.00%) were the most isolated from the second floor. After washing the bathrooms, *Mucor mucedo* was scarcely isolated from the walls of the bathrooms. The findings were processed statistically using the two-tailed test to detect the significant difference between the groups of means for the fungal counts from each floor. A significant difference ($p < 0.05$) in the fungi isolated before and after washing was found. Isolated fungi from this study are known to be of public health importance hence, strict hygiene practices should be observed by those using the bathrooms.

Keywords: Bathroom, Fungi, Hostel, Identification, Isolates

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INTRODUCTION

Fungi are eukaryotic heterotrophs, that extensively include species of moulds, yeasts and mushrooms. They could be single celled (yeasts) or multicellular (moulds) and may also be classified as biotrophs, saprotrophs or necrotrophs which reproduce and disperse by spore formation (Carris *et al.*, 2012; Bueno and Silva, 2014). Fungi proliferate in humid environments composed of organic substrate. Moulds are very adaptable and can grow even on damp inorganic materials such as glass, metal, concrete or painted surfaces if a microscopic layer of organic nutrients is available. Such nutrients can be found on household dust and soil particles (Robins and Morell, 2007). Fungi are capable of extracting their food from the organic materials they grow on and the ability to reproduce by way of minute spores makes them ubiquitous (DeHoog *et al.*, 2007). Fungal colonization of buildings is especially common in low-income communities in developing countries, where buildings are situated indiscriminately without consideration for environmental sanitation, urban planning and building regulations (Ahiamba *et al.*, 2008; Ayanbimpe *et al.*, 2010).

Fungi are particularly dangerous because they are able to withstand different conditions of the environment. Moreover, they can survive in environment with low relative humidity (RH) unlike bacteria for their proliferation and formation of spores which are dispersed by wind. The primary reason of contamination in the environment is due to the dispersion of spores and the indoor environment is colonized by moulds through the dispersal of spores within the ventilating system (Jabber *et al.*, 2016). Some pathogenic fungi in soil and vegetation which can induce infections could be transferred by animals or foot wears of humans into their homes (Ayanbimpe *et al.*, 2012).

Occupants of buildings have been reported to develop certain allergies and affected by toxins as a result of the fungal contaminations in indoor environment (Caillaud *et al.*, 2018). Inhalation of fungal spores, fragments (parts), or metabolites (e.g., mycotoxins and volatile organic compounds) from a wide variety of fungi can trigger symptoms of asthma, rhinitis or bronchitis (sick building syndrome) or other building related illnesses (Crook and Burton, 2010). All moulds have the potential to cause health effects such as headaches, breathing difficulties, skin irritation, allergic reaction and aggravation of asthma symptoms (Jabber *et al.*, 2016). The emergence of diseases in the immune-compromised could possibly be triggered by the inhalation of spores of air-borne fungi. Evidence on the association between mould exposure and most human respiratory diseases has been reported (Caillaud *et al.*, 2018). Most people can recognize and react to the mouldy smell negatively with symptoms like headaches, vomiting, nausea, blocked noses, and asthma. Volatile products of indoor moulds have also been suggested to cause toxic illnesses or mycotoxicoses (Sean and Abbott, 2002; Straus and Wilson, 2006). Mycotoxins can be inhaled by people through the air, ingested through food, and can also come in contact with skin, resulting in many illnesses such as gastrointestinal, respiratory, and reproductive disorders (O'connor *et al.*, 2004; Mirabelli *et al.*, 2006). Also reported are eye irritations, poor taste sensations, sore throat and tiredness (U.S. Environmental Protection Agency, 2003). Even severe neurologic conditions such as depression have been linked to indoor mould colonization (Shenassa *et al.*, 2007). The species of *Aspergillus*, *Fusarium* and *Penicillium* are potential toxigenic moulds, hence the awareness of the colonization of such spores on bathrooms' walls and their termination by washing with disinfectants and disinfectants are of public health importance.

MATERIALS AND METHODS

STUDY AREA

Samples were collected from bathrooms located at the female students' hostel, University of Benin, Benin City, and transported to the Department of Microbiology. Analysis was carried out at University of Benin Microbiology Department Laboratory.

SAMPLE COLLECTION

Samples were collected from the walls of the hostel's bathrooms using swabs sticks, before and after cleaning of the bathrooms. A total of seventy-two (72) samples were collected from the bathrooms of the three floors at three consecutive times. A solution of 1% glucose peptone diluents was labeled and used to moisten the swab and about 5 cm of the bathroom's wall was swabbed systematically in two directions at 90° to one another while slowly rotating the swab around its long axis. The cotton end was then placed into the diluents bottle and snapped off so that it falls into the 10 ml bijou bottle containing 1% glucose peptone and transferred to the laboratory and incubated for 24 hours after which a ten-fold serial dilution was carried out on each sample before inoculation (FAO, 2006; Satyada and Sandle, 2016).

PREPARATION AND STERILIZATION OF MEDIA AND MATERIALS

Potato dextrose agar was prepared according to the manufacturer's instruction. The media and glass wares (e.g test tubes and conical flasks) were sterilized in an autoclave at a temperature of 121°C for 15 minutes. The agar media were supplemented with 0.25 mg/ml chloramphenicol (Odds, 1992).

MICROBIOLOGICAL ANALYSIS

INOCULATION

One millilitre of each diluted sample (10^{-4}) was dispensed into empty labeled sterile plates in duplicates. Amended sterile medium (potato dextrose agar supplemented with antibiotics) were poured aseptically into the petri dishes, whirled slowly and left to cool. The agar solidifies and the incubation of the agar plates was done at $28 \pm 2^\circ\text{C}$ (room temperature) for 4 – 7 days (Cheesebrough, 2006).

ISOLATION OF PURE CULTURE

After incubation, appearance of discrete, well separated colonies in culture plates were examined for suspected fungal species base on their colonial morphology. Each distinct colony of fungi were sub-cultured into separate potato dextrose agar plate by picking minute number of hyphae or spores onto a non-inoculated solidified agar plate, much rather at the centre of the agar plate so as to support best colonial growth and spore formation of the fungi. The incubation of the plates was done at room temperature ($28 \pm 2^\circ\text{C}$). Fungal growth (pure culture) were usually seen from three (3) days after incubation (Al-mohanna, 2016).

DIRECT MICROSCOPY

The sub-cultured fungi were identified based on their cultural and morphological characteristics such as mycelia, spore type and other fruiting bodies by viewing a lactophenol cotton blue wet mount at x40 objective lens of a compound microscope and comparing the structures to a fungal atlas. *Candida albicans* were identified by carrying out germ tube test (Arora *et al.*, 2003; Catherine *et al.*, 2015; Kidd *et al.*, 2016).

ENUMERATION

Calculation of fungal counts (cfu/cm²)

$$\text{Count per swab} = \frac{C}{v(n_1 + 0.1n_2)d} \times n_3$$

C = sum of colonies on all plates counted

v = volume applied to each plate

n₁ = number of plates counted at the first dilution

n₂ = number of plates counted at the second dilution

n₃ = original volume of neat suspension i.e. 10

d = dilution from which the first count was obtained e.g. 10⁻⁴

$$\text{CFU/cm}^2 = \frac{\text{Count}}{\text{Area swabbed}}$$

(PHE, 2017)

Fungal contamination was determined by calculating the occurrence percentage for the isolates (Jabber *et al.*, 2016).

$$\text{Occurrence Percentage} = \frac{\text{No. of Isolates for genus or type}}{\text{Total isolate number for all fungi}} \times 100$$

DATA ANALYSIS

Data obtained were presented as mean ± standard error (SE) of duplicate data. Significance difference between different groups was tested with double tailed t-test using SPSS version 20 (Ogbeibu, 2014). The graphs were plotted using Microsoft Excel.

RESULTS

FUNGAL COUNTS FROM THE BATHROOMS OF THE THREE FLOORS OF THE HOSTEL

Table 1 shows a significant reduction in the fungal load of the bathrooms' walls after washing. The ground floor had the highest fungal count, while the first floor had the least fungal count. Before washing of the bathrooms, the fungal count ($\times 10^4$ cfu/cm²) ranged between 6.40 ± 0.04 and 22.80 ± 0.30 , while after washing the fungal count ($\times 10^4$ cfu/cm²) ranged from 0.00 ± 0.00 to 0.80 ± 0.02 . A significant difference between before and after washing was observed on the first week ($p = 0.049$), second week ($p = 0.030$) and third week ($p = 0.048$) in the hostel.

Table 1: Fungal counts from the bathrooms

Bathrooms' Location	Period	Week 1 10 ⁴ (cfu/cm ²)	Week 2 10 ⁴ (cfu/cm ²)	Week 3 10 ⁴ (cfu/cm ²)
Ground Floor	Before washing	18.80 \pm 1.00	14.80 \pm 0.08	22.80 \pm 0.30
	After washing	0.80 \pm 0.02	0.20 \pm 0.02	4.40 \pm 0.02
First Floor	Before washing	7.20 \pm 0.08	8.00 \pm 0.10	6.40 \pm 0.04
	After washing	0.40 \pm 0.00	0.20 \pm 0.02	–
Second Floor	Before washing	10.00 \pm 0.12	8.00 \pm 0.10	12.00 \pm 0.14
	After washing	0.20 \pm 0.02	0.40 \pm 0.00	–
p-value	(before-after washing)	0.049	0.030	0.048

Values are mean \pm S.E. of 2 replicates

OCCURRENCE PERCENTAGE OF FUNGAL ISOLATES BEFORE WASHING OF THE BATHROOMS

Table 2 shows the frequency of occurrence of the fungal isolates from the walls of the bathrooms. *Candida albicans* was predominantly isolated (23.40%) from the ground floor, *Aspergillus nidulans* was the most frequently isolated fungi (55.56%) from the first floor and *Mucor mucedo* was the most frequently occurring fungi from the second floor. The ground floor had more isolated fungal species than the other floors of the hostel.

Table 2: Occurrence percentage of fungal isolates before washing of the bathrooms

Fungal isolates	Ground floor (%)	First floor (%)	Second floor (%)
<i>Mucor mucedo</i>	12.77	nil	56.00
<i>Candida albicans</i>	23.40	5.56	Nil
<i>Penicillium cyclopium</i>	21.40	22.22	8.00
<i>Penicillium oxalicum</i>	14.89	nil	12.00
<i>Aspergillus nidulans</i>	8.51	55.56	20.00
<i>Aspergillus niger</i>	6.38	5.56	Nil
<i>Aspergillus flavus</i>	Nil	11.11	4.00
<i>Aspergillus tamari</i>	4.26	nil	Nil
<i>Trichophyton rubrum</i>	4.26	nil	Nil
<i>Rhodotorulaspp.</i>	4.26	nil	Nil

OCCURRENCE PERCENTAGE OF FUNGAL ISOLATES FROM FIRST WEEK

Figure 1 shows the occurrence percentages of the fungi isolated at the first sample collection. Ten fungal isolates were identified of which *Mucor mucedo* occurred most (22.22%). Other isolates include; *Aspergillus nidulans* (21.11%), *Penicillium cyclopium* (17.78%), *Candida albicans* (13.33%), *P. oxalicum* (11.11%), *A. niger*(4.44%), *A. flavus* (3.33%), *A. tamari* (2.22%), *Trichophyton rubrum* (2.22%) and *Rhodotorula* species (2.22%).

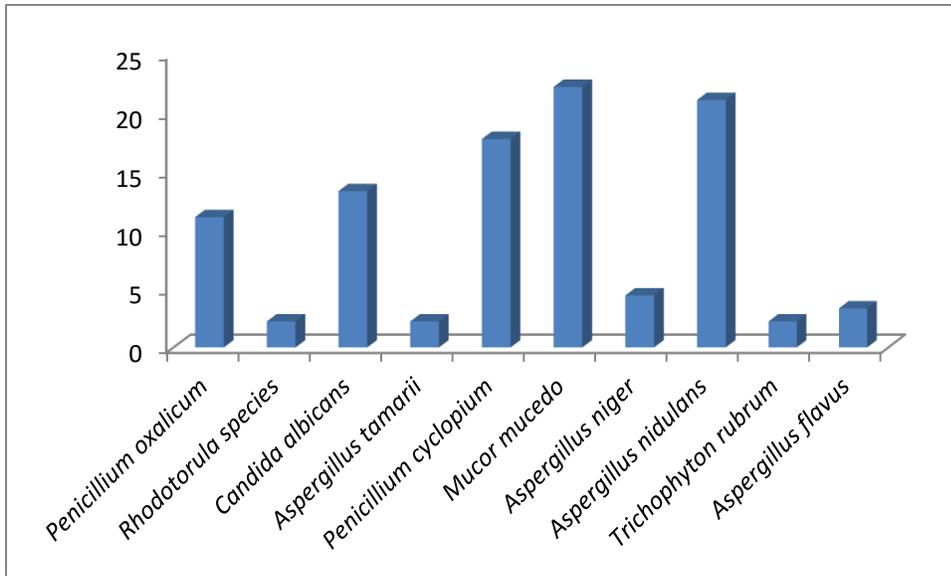


Figure 1: Occurrence percentage of fungal isolates from first week

OCCURRENCE PERCENTAGE OF FUNGAL ISOLATES FROM SECOND WEEK

Figure 2 shows the occurrence percentages of the fungi isolated at the second sample collection. Seven fungal isolates were identified of which *Candida albicans* occurred most (25.97%). *Penicillium cyclopium* (19.48%), *Mucor mucedo* (18.18%), *Aspergillus nidulans* (18.18%), *P. oxalicum* (9.09%), *A. niger* (5.19%) and *A. flavus* (3.90%) were also isolated.

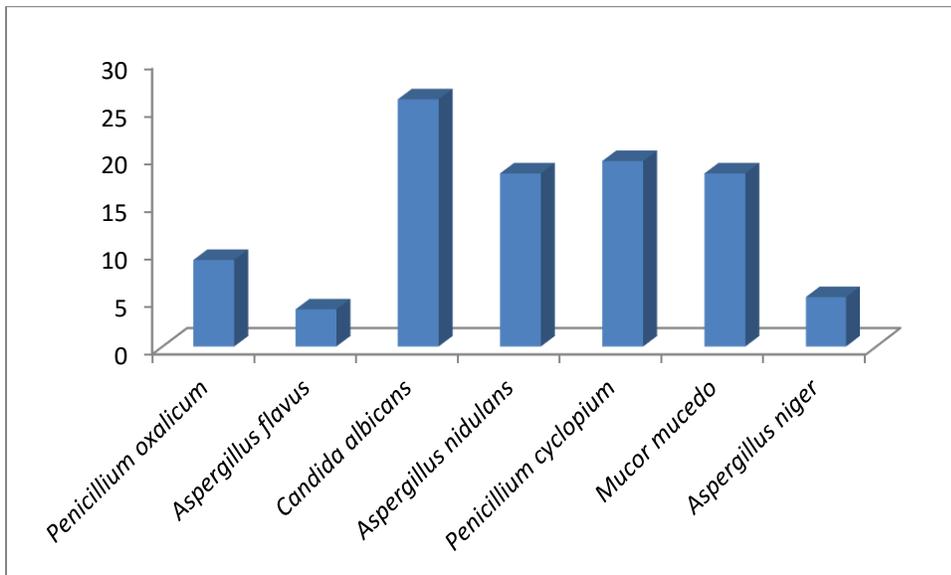


Figure 2: Occurrence percentage of fungal isolates from second week

OCCURRENCE PERCENTAGE OF FUNGAL ISOLATES FROM THIRD WEEK

Figure 3 shows the occurrence percentages of the fungi isolated at the third sample collection. Ten fungal isolates were identified of which *Mucor mucedo* occurred most (25.24%). Other fungi isolated include: *Aspergillus nidulans* (23.30%), *Penicillium cyclopium* (16.51%), *P. oxalicum* (12.62%), *Candida albicans* (3.88%), *Rhodotorula* species (3.88%), *A. niger* (3.88%) *A. tamaritii* (3.88%), *Trichophyton rubrum* (3.88%), and *A. flavus* (2.91%) were also isolated.

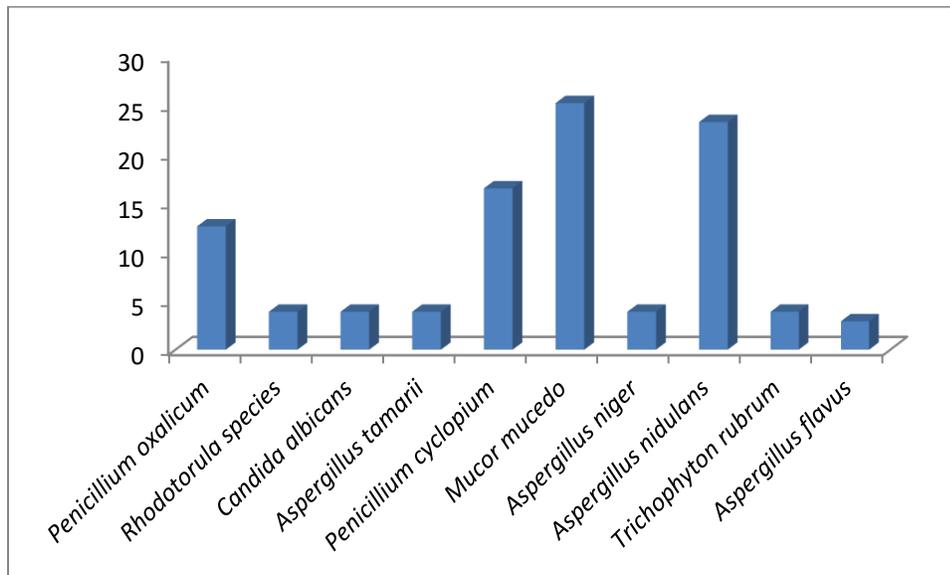


Figure 3: Occurrence percentage of fungal isolates from third week

DISCUSSION

Mould and yeast contamination are higher in the upper part of bathrooms (Hamada, 2004) and the frequency of cleaning bathrooms' floors with detergents and disinfectants is much higher than that of the upper parts of the bathrooms, hence the prevalence of fungi on the walls of such bathrooms. This has led to the study of bathrooms' walls in highly populated (school) environment, for the presence of fungal growth.

Results from Table 1 shows that the highest prevalence of fungi was from the ground floor bathroom. Consistent observations by the ground floor occupants revealed that there was regular usage of the bathroom by passers-by who tend to ease themselves at the ground floor hence increasing the microbial loads by the introduction of foot wears and urine (Ayanbimpe *et al.*, 2012) into the bathroom. The ground floor had the highest fungal count, while the first floor had the least fungal count. Before washing of the bathrooms, the fungal count ($\times 10^4$ cfu/cm²) ranged between 6.40 ± 0.04 and 22.80 ± 0.30 , while after washing the fungal count ($\times 10^4$ cfu/cm²) ranged from 0.00 ± 0.00 to 0.80 ± 0.02 . Fungal isolates of the species *Mucor mucedo* was only isolated and identified after washing of the bathrooms. There was a significant reduction of the fungal loads on the first week ($p = 0.049$), second week ($p = 0.030$) and third week ($p = 0.048$) in the hostel, after cleaning of the bathrooms.

Results from Table 2 shows that *Candida albicans* had the highest prevalence (23.40%) in the ground floor's bathroom, the first floor was mostly contaminated with *Aspergillus nidulans* (55.56%), while the second floor had *Mucor mucedo* (56.00%) as its most occurring fungal isolate. The high prevalence of *Candida albicans* on the ground floor could be as a result of the regular abuse of the bathroom by passers-by, who tend to urinate there. *Candida albicans* is the most commonly reported species in urine culture (Gajdaes *et al.*, 2019). According to the study carried out by Jabber *et al.*, 2016, *Candida albicans* had the highest occurrence percentage in the female bathrooms in Thi-Qar Government. The high prevalence of *Mucor mucedo* on the second floor could be as a result of the dispersal of the bio-particles such as its spores and plant parts by wind (Singh, 2017) from the outdoor to the indoor environment through the windows and door.

Results from Figure 1 and Figure 3 shows that *Mucor mucedo* had the highest occurrence percentage of 22.22% and 25.24%, at the first and third week of the study respectively. Figure 2 shows that at the second week of fungal enumeration of the bathrooms' walls, *Candida albicans* had the highest occurrence percentage of 25.97%. The fungal counts from the hostel bathrooms may had reduced due to the lysing effect of detergents and disinfectants. The bathrooms seldom harbor *Mucor mucedo* when not properly washed, which still pose a threat to human health. Quan and Spellberg (2010) stated that mucormycosis pneumonia has a higher mortality rate than that of most other pathogenic fungi. Thorough cleaning of bathrooms with biocides such as detergents and disinfectants destroy the cell walls of microorganisms and interfere with their growth and metabolism. However, disinfectants can be sporostatic but not necessarily sporicidal (McDonnell and Russell, 2001).

CONCLUSION

Fungal occurrence in the bathrooms reduced after washing with detergents and disinfectants. The opportunistic fungi could be inhaled by students and exacerbate asthmatic attacks, pneumonia and other illnesses. The isolated fungi which are of public health significance, are known to cause aspergillosis, vaginal candidiasis, keratitis and otomycosis. Hence, strict hygiene practices should be observed by those using the bathrooms. Cleaning of bathrooms should be regularly and thoroughly done with detergent and disinfectant solutions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research work.

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