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**MYCOLOGICAL ASSESEMENT OF MALE SECOND HAND WEARS SOLD IN  
ABAKALIKI AND NKWO AREAS OF EBONYI STATE, NIGERIA**

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**ABSTRACT**

This study was designed to assess and compare the mycological profile of male second hand wears sold at Abakaliki and Ikwo areas of Ebonyi State. This study involved a laboratory-based survey design. The laboratory-based survey was incorporated to assess the mycological profile of the second hand wears (singlets, boxers, socks, shoes, trousers and shirts). The study was conducted on a total of sixty male second hand wears procured from distinct locations at Ebonyi north and central senatorial districts. The categories of male wears procured were singlets, boxers, socks, shoes, trousers and shirts. Each wear was procured in isolation using a separate sterile polythene bag to avoid cross contamination. Also, new male wears of same category were procured from the aforementioned locations and were used as control. A total of sixty second hand wears (singlets, boxers, socks, shoes, trousers and shirts) were assessed for fungi growth using standard procedures. Filamentous fungi isolated were identified by macroscopic examination. A total of ten different species of fungi were isolated; nine were moulds and one was a yeast. The mould isolates were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Cladosporium* spp, *Mucor* spp, *Penicillium* spp, *Rhizopus* spp, and *Trichoderma* spp. *Candida albicans* was the only yeast isolated. The different categories of male second hand wears showed significant variation in

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the species of associated fungi. *Aspergillus spp* are recognized for their role in toxicoses, opportunistic infections and allergic states.

**Keywords: Mycological profile, Male, Second hand Wear (Okirika) and Ebonyi north and Central district**

## INTRODUCTION

Second hand wears with its multiple sobriquets in Nigeria namely “okrika, bend down select or wash and wear”, are categorized as different wears that have been previously used prior to procurement by another individual. They range from used pants, socks, boxers, brassieres, shirts, trousers, knickers, skirts, blouses, shoes, curtains, towels, singlet, and handkerchiefs.

Sellers of second hand wears are patronized by all categories of people including undergraduates and graduates; unemployed and working class; privileged and less privileged individuals. Indeed this brand of business is currently a growing, thriving and booming enterprise in Nigeria. Although prohibited in Nigeria and seen as a threat to locally manufactured textiles, second hand wears are imported illegally from neighbouring countries [1]. Countries frequently patronized by “okrika” sellers in Nigeria include United Kingdom, United States, Dubai, China, Korea, Cotonou in the Republic of Benin and Togo [2]. Adow and Arsenault (2012) through the popular news network, Aljazeera, reported that although the Nigeria Government imposed a ban on the acquisition and sale of second hand wears, about 80% of Nigerians adorn themselves in these illicit garments. Owing to high market demands and profits, it is highly improbable that the trade of second hand wears in Nigeria will stop anytime soon.

Mycology is the study of fungi. Fungi encompass lichens, mushrooms, molds, slime molds and yeasts. While fungi are ubiquitous, only a few are considered pathogenic. The pathogenic species give rise to infections in both man and animals. Fungal infections are referred to as mycoses. Fungal infections pose a serious health challenge in Nigeria and all over the world. The most prevalent fungal infections are those of the skin and nails (dermatomycoses) primarily caused by dermatophytes of the genera *Trichophyton*, *Microsporum*, *Epidermophyton* [2].

Clothing and related materials can act as vehicles for possible transmission of infectious diseases. Infectious agents that can potentially be spread via clothing includes Bacteria such as *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli*, *Clostridium difficile*, *Staphylococcus aureus*; Viral strains such as *Norovirus*, *Rotavirus*, *Adenovirus*, *Astrovirus*, *Herpes*; Yeast such as *Candida albicans*; Dermatophytic fungal strains such as *Trichophyton*

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rubrum and Trichophyton mentagrophytes. In a study conducted within a UK hospital, Staphylococcus aureus and Clostridium difficile were isolated from the clothing and bed linens of patients inflicted with infections caused by the aforementioned organisms. Similarly, organisms isolated from a random sampling of clothing and hospital linen were mostly normal skin flora viz. Staphylococci, Corynebacteria, Micrococci [3].

Second hand wears has become the choice clothing line for majority of Nigerians irrespective of ethnicity, social and economic status. The strong affinity towards second hand wears has been attributed to consumer aspirations and desire for foreign fashion, affordability, popularity amongst low income earners, presumed quality and longevity. Conversations with top-notch and elite Nigerians at home and in diaspora has led to a mindboggling revelation that Nigeria serve as both a dumping ground for undesirable textile and also a means through which developed countries manage their environmental waste problems [4].

Second hand wears have a high propensity for transmission of infectious agents. Worthy of particular mention are second hand undergarments [5]. Body fluids in form of sweat, penial and vaginal discharges can be absorbed by undergarments and consequently when procured as second hand wears can act as vehicles for transmission of infections such as genital candidiasis, ringworm. Research has revealed that for every 100-500g of faeces excreted per day by an individual, an estimated 0.1g remains on the undergarment [5]. Furthermore, most microorganisms can survive on fabrics as spores and germinate once the environmental conditions become favourable. The idea that laundering translates to an infection free fabric is not necessarily true as bacteria, fungi and viruses can still survive on improperly washed fabric. Second hand wears are packaged, stockpiled and stored in bales giving rise to cross contamination [6]. This study was to assess and compare the mycological profile of male second hand wears sold at Ebonyi North and Central Senatorial districts, Ebonyi state.

## **MATERIALS AND METHODS**

### **Description of the Study area**

The study was conducted within Ebonyi North and Central Senatorial districts, Ebonyi State, Nigeria. With Abakaliki as its capital, Ebonyi State is located in the South Eastern region of Nigeria and derives its name from the River Aboine. It is bounded to the North by Benue State, to the West by Enugu State, to the East by Cross River State and to the South by Abia State. Ebonyi state has a land area of about 5,935 sq.km with coordinates, 6° 15'N 8° 05'E / 6.250°N 8.083°E. There are thirteen Local Government Areas (LGAs) in Ebonyi State.

Ebonyi North senatorial district comprises of Abakaliki, Ebonyi, Ishielu, Ohaukwu and Izzi

LGAs while Ebonyi Central senatorial district comprises of Ikwo, Ezza North and Ezza South LGAs.

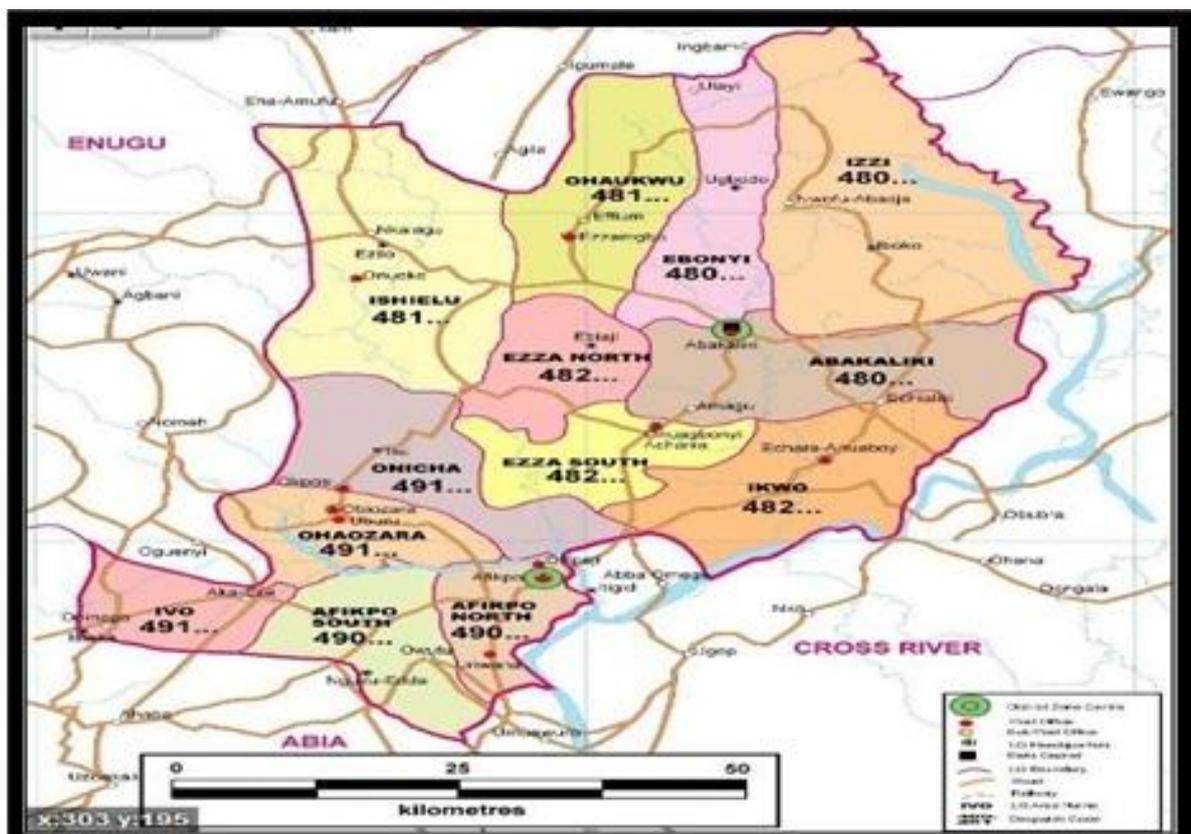


Figure 1: Map of Ebonyi State showing the thirteen LGAs

### Study design

This study involved a laboratory-based survey design. The laboratory-based survey was incorporated to assess the mycological profile of the second hand wears (singlets, boxers, socks, shoes, trousers and shirts). Informal oral interview with few residents of the study area was also conducted in order to ascertain whether cases of fungal infections linked to usage of second hand wears existed.

### Sample collection

The study was conducted on a total of sixty male second hand wears procured from distinct locations at Ebonyi North and Central senatorial districts. The categories of male wears procured were singlets, boxers, socks, shoes, trousers and shirts. Five of each category of wear was procured from separate outlets at Ebonyi North senatorial district (thirty male second hand wears in all). Same was done at Ebonyi central senatorial district (thirty male wears in all). Each wear was procured in isolation using a separate sterile polythene bag to avoid cross contamination. Also, new male wears of same category were procured from the aforementioned locations and were used as control.

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## Laboratory investigations

### Media preparation

The medium utilized for culture was Sabouraud Dextrose Agar (SDA). The medium was prepared according to the manufacturer's specification and sterilized by autoclaving at 15psi (121°C) for 15 minutes prior to culture. After cooling to 45-50°C, the medium was aseptically dispensed into sterile Petri dishes and allowed to gel. Peptone water was also employed in the course of the study and was sterilized by autoclaving.

### Isolation of fungi from male second hand wears

Samples (inoculum) from shoes were collected using a sterile cotton swab stick dipped in sterilized peptone water. The cotton swab sticks were then streaked on surface of the gelled medium (SDA) while maintaining an aseptic working condition. Afterwards, the culture plates were incubated at 25-28°C for one week. Collection of samples (inoculum) from other wears apart from shoes was as follows:

A portion of each wear was cut and soaked separately for 30 minutes in small beakers containing sterile peptone water. The beakers were swirled at regular intervals to dislodge the cells into suspension.

Afterwards, 1ml of peptone water from each beaker was transferred to separate petri dishes after which SDA was poured into each petri dish.

The medium was swirled and allowed to gel.

### Identification of fungi

A total of sixty second hand wears (singlets, boxers, socks, shoes, trousers and shirts) were assessed for fungi growth using standard procedures. Yeast and mould isolates on SDA were identified using procedures described by [7]. An identification chart (atlas) was also employed for identification of fungi isolates [8].

### Identification of filamentous fungi (moulds)

Filamentous fungi isolated on SDA were identified by;

- a) Macroscopic examination: This was based on the growth rate and colonial morphology which includes colour, texture, colonial topography and diffusible pigments (pigment production).
- b) Microscopic examination: This was carried out to elucidate the morphology of spores and hyphae. Microscopic examination was conducted by wet mount using lactophenol cotton blue. The procedure is as follows:

- A drop of 95% ethanol was placed on a clean grease free microscope slide.

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- A sterilized straight inoculating needle was used to remove a small portion of the fungal growth on SDA from midway between the colony centre and the edge.
  - Afterwards, the fungus was gently teased and spread out thinly in the mounting medium.
  - After most of the ethanol had evaporated, a drop of lactophenol cotton blue was added.
  - The microscope slide was then covered with a cover slip and examined under the microscope using 10x and 40x objective lens.

### Identification of Yeasts

Colonies of yeasts on SDA were identified based on;

a) Cultural Characteristics: Colonial morphology was used to differentiate yeasts from filamentous moulds.

b) Microscopic morphology: The morphology of yeast isolates were investigated microscopically by temporary direct mount (wet preparation) and Germ tube test. Temporary direct mount was used to observe the morphology (size and shape) of the yeast cells and also to observe the presence and absence of structures such as pseudohyphae or chlamydo spores. The procedure used to carry out temporary direct mount is as follows:

- A small drop of sterile distilled water was placed on a clean grease free microscope slide.
- Afterwards, a small portion of the yeast colony was mixed with the distilled water.
- The microscope slide was covered using a clean cover slip and then subsequently observed under the microscope using 10x and 40x objective lens.

Germ tube test was used for tentative identification of *Candida albicans*. The procedure was as follows:

- 0.5ml of human serum was first placed in a sterile test tube.
- The suspected colony of *Candida albicans* was inoculated into the serum using a sterile inoculating wire loop.
- The tube was then incubated at 37°C for 3 hours.
- Afterwards, a drop of the serum yeast culture was transferred to a clean microscope slide after which it was covered with a clean cover slip.
- The microscope slide was then observed under the microscope using 10x and 40x objective lens.
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## RESULTS

### Fungal Isolates

A total of ten different species of fungi were isolated; nine were moulds and one was a yeast. The mould isolates were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Cladosporium* spp, *Mucor* spp, *Penicillium* spp, *Rhizopus* spp, and *Trichoderma* spp. *Candida albicans* was the only yeast isolated. Table 1 shows the macroscopic and microscopic characteristics of isolated fungal species. Microscopically, the genus of *Aspergillus* had a notable characteristic conidial head that looked like a brush used for sprinkling holy water. When viewed using the microscope, colonies of *Candida albicans* inoculated in human serum and left for three hours showed a characteristic tube-like extensions. Microscopically, conidia of *Cladosporium* spp were observed to have a characteristic dark spots like the hilum of a bean. The absence of rhizoids was used to differentiate *Mucor* spp from *Rhizopus* spp. *Penicillium* spp were identified based on their fork-shaped conidia heads. *Trichoderma* spp were identified macroscopically based on their grey coloured obverse and microscopically based on observation of septate hyphae with spherical clusters of conidia on the sides of the hyphae.

### Distribution of Fungal Isolates with Respect to Districts

Two districts were studied viz., Ebonyi North and Ebonyi Central Senatorial districts. Figure 3 shows the number of fungal species isolated from each category of male second hand wear procured at Ebonyi North and Central Senatorial districts. The number of fungal species isolated from boxers, singlets, shirts, shoes, socks and trousers procured at Ebonyi North Senatorial district was 8, 5, 4, 5, 6, and 6 respectively. The number of fungi species isolated from boxers, singlets, shirts, shoes, socks, and trousers procured at Ebonyi Central Senatorial district was 6, 3, 5, 4, 6, and 6 respectively.

The different categories of male second hand wears showed significant variation in the species of associated fungi. Table 2 provided information on fungal species isolated from six categories of male second hand wears (boxers, singlets, shirts, shoes, socks and trousers) procured from Ebonyi North Senatorial district. Ten different species were isolated in total (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans*, *Cladosporium* spp, *Mucor* spp, *Penicillium* spp, *Rhizopus* spp, and *Trichoderma* spp).

Table 2 provided information on fungal species isolated from six categories of male second hand wears (boxers, singlets, shirts, shoes, socks and trousers) procured from Ebonyi Central

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Senatorial district. Eight different species were isolated in total (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans*, *Mucor spp*, *Penicillium spp*, *Rhizopus spp*).

Table 3 and Table 4 shows the prevalence of fungal isolates with respect to second hand wears procured at Ebonyi North Senatorial district and Ebonyi Central Senatorial district. On comparison of the two districts, the percentage prevalence of fungal isolates in second hand wears procured at Ebonyi North Senatorial districts was in the following order; *Aspergillus niger* (43.33%); *Mucor spp* (43.33%); *Rhizopus spp* (30%); *Aspergillus fumigatus*; *Candida albicans* (23.33%); *Aspergillus flavus* (20%); *Aspergillus terreus* (20%); *Penicillium spp* (16.67%); *Cladosporium spp* (6.67%); and *Trichoderma spp* (3.33%). Percentage prevalence of fungal isolates in second hand wears procured at Ebonyi Central Senatorial district was in the following order; *Mucor spp* (46.67%); *Aspergillus niger*(40%); *Rhizopus spp* (36.67%); *Candida albicans* (30%); *Aspergillus terreus* (26.67%); *Aspergillus flavus* (20%); *Penicillium spp* (20%); and *Aspergillus fumigatus* (16.67%).

## DISCUSSION

Findings from this study provide an insight on the different fungal species associated with male second hand wears. Fungal species isolated from six categories of male second hand wears (boxers, singlets, shirts, shoes, socks, trousers) procured at Ebonyi North Senatorial district includes *Aspergillus niger* (43.33%); *Mucor spp* (43.33%); *Rhizopus spp* (30%); *Aspergillus fumigates* (26.67%); *Candida albicans* (23.33%); *Aspergillus flavus* (20%); *Aspergillus terreus*(20%); *Penicillium spp* (16.67%); *Cladosporium spp* (6.67%); and *Trichoderma spp* (3.33%). Fungal species isolated from six categories of male second hand wears (boxers, singlets, shirts, shoes, socks, trousers) procured at Ebonyi Central Senatorial district includes *Mucor spp*(46.67%); *Aspergillus niger* (40%); *Rhizopus spp* (36.67%); *Candida albicans* (30%); *Aspergillus terreus* (26.67%); *Aspergillus flavus* (20%); *Penicillium spp* (20%); and *Aspergillus fumigatus*(16.67%). Male second hand wears procured from the two districts had eight fungal isolates in common, with an additional two fungal species present only in wears procured at Ebonyi North Senatorial district. At Ebonyi North Senatorial district, boxers had the highest number of fungi isolates with a total of 8; socks and trousers had 6 each; singlets and shoes had 5 each; shirts had 4. At Ebonyi Central Senatorial districts, boxers, socks and trousers each had a total of 6; shirts, shoes and singlets each had 5, 4 and 3 respectively.

This work compares favourably with a closely related work conducted by Agbulu *et al.* (2015) who isolated *Candida albicans*(29.9%),*Candida tropicalis* (65.5%), *Penicillium spp* (1.8%),

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Rhodotorula rubra(1.8%),and Trichophyton rubrum (1.8%), from second hand female undergarments and children's wear sold in Makurdi Metropolis, Nigeria. This study also affirms that fungi species are capable of surviving in clothing materials and this conforms to the findings of [8].

Most of the fungal isolates are cosmopolitan and are capable of causing human infections. Skin and body fluids are among the primary sources of contamination of clothing materials. Since most of these wears are packed in bundles or bales, cross contamination is highly probable. There is also the possibility of cross contamination during laundry. Due to the notion that laundry translates to a clean and sterile fabric, people often ignore to iron wrinkle resistant second hand fabrics after washing. As asserted by Bloomfield et al. (2011), the availability of detergents active at ambient water temperature is of great concern.

The genus *Aspergillus* comprises of filamentous and ubiquitous fungal species. *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* are among the common species in this genus. *Aspergillus* spp are recognized for their role in toxicoses, opportunistic infections and allergic states. Infection caused by this specie of fungi is referred to as Aspergillosis. Forms of Aspergillosis include invasive aspergillosis, central nervous aspergillosis, osteomyelitis, endophthalmitis, endocarditis and disseminated aspergillosis. They are the most common cause of invasive infections and second only to *Candida* among the fungi implicated in opportunistic mycoses. *Aspergillus fumigatus* and *Aspergillus terreus* are notable species capable of causing opportunistic infections in individual with compromised immune system. *Aspergillus* spp has also been implicated in the contamination of food with the production of mycotoxin such as aflatoxin which when ingested can cause aflatoxicosis [8].

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**Table 1: Fungal Species Isolated from Different Categories of Male Second Hand Wears procured at Ebonyi North Senatorial District**

Categories of male second hand wears						
S/No.	Fungal isolates	B <sub>1</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>
1.	<i>Aspergillus flavus</i>	+	+	-	+	+
2.	<i>Aspergillus fumigatus</i>	+	-	+	-	+
3.	<i>Aspergillus niger</i>	+	+	+	+	+
4.	<i>Aspergillus terreus</i>	-	-	-	-	+
5.	<i>Candida albicans</i>	+	+	+	-	-
6.	<i>Cladosporium spp</i>	+	-	-	-	-
7.	<i>Mucor spp</i>	+	+	+	+	+
8.	<i>Penicillium spp</i>	-	-	-	+	+
9.	<i>Rhizopus spp</i>	+	+	-	+	+
10.	<i>Trichoderma spp</i>	+	-	-	-	-

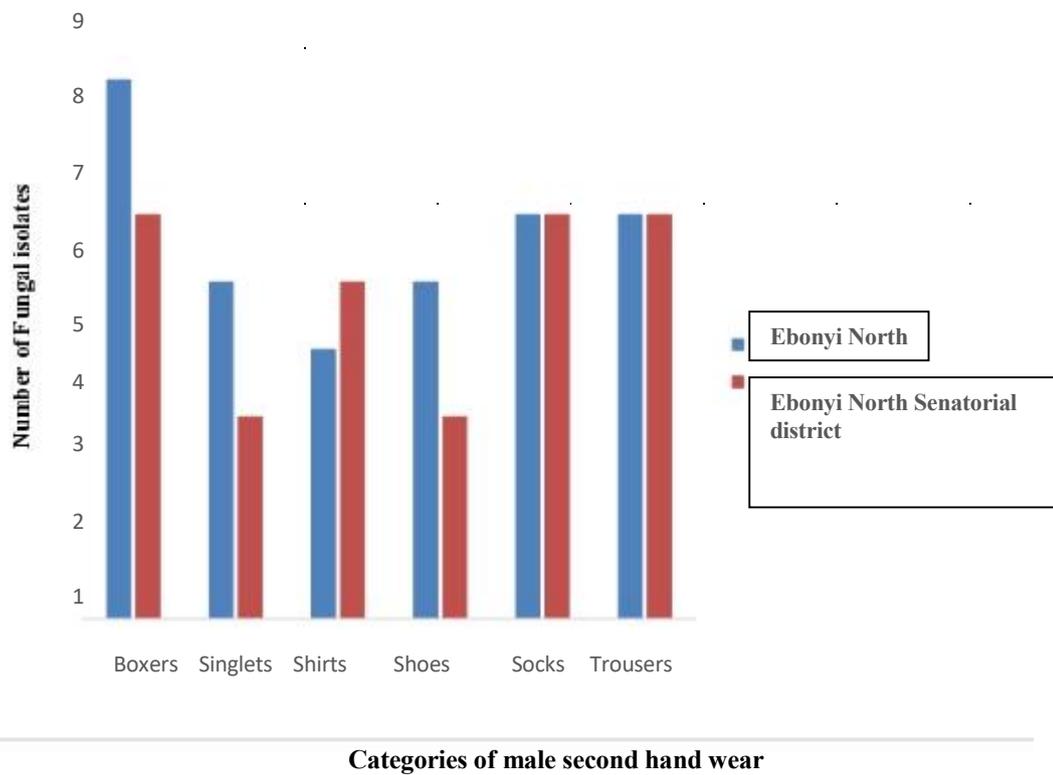


Figure 2: Number of fungal species isolated from each category of male second hand wear procured at Ebonyi North and Central Senatorial districts

Categories of male second hand wears						
S/No.	Fungal isolates	B <sub>1</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>
1.	<i>Aspergillus flavus</i>	+	-	+	-	+
2.	<i>Aspergillus fumigatus</i>	-	+	-	-	+
3.	<i>Aspergillus niger</i>	+	-	+	+	+
4.	<i>Aspergillus terreus</i>	+	-	+	-	+
5.	<i>Candida albicans</i>	+	+	+	-	-
6.	<i>Mucor spp</i>	-	+	+	-	+
7.	<i>Penicillium spp</i>	+	-	-	+	-
8.	<i>Rhizopus spp</i>	+	-	-	+	+

Keys: B<sub>1</sub>: Boxers; S<sub>1</sub>: Singlets; S<sub>2</sub>: Shirts; S<sub>3</sub>: Shoes; S<sub>4</sub>: Socks; T<sub>1</sub>: Trousers +ve: Present; -ve: Absent

**Table 3 Prevalence of fungal isolates with respect to male second hand wears procured at Ebonyi North Senatorial district Prevalence of fungal isolates n(%P)**

S/No	Categories of second hand wears	Total no of samples procured in each category	<i>Aspergillus Flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus Niger</i>	<i>Aspergillus terreus</i>	<i>Candida albicans</i>	<i>Cladosporium spp</i>	<i>Mucor spp</i>	<i>Penicillium spp</i>	<i>Rhizopus Spp</i>	<i>Trichoderma spp</i>
1.	B <sub>1</sub>	5	1 (20)	3 (60)	2 (40)	-	1 (20)	2 (40)	2 (40)	-	3 (60)	1 (20)
2.	S <sub>1</sub>	5	1 (20)	-	3 (60)	-	3 (60)	-	3 (60)	-	1 (20)	-
3.	S <sub>2</sub>	5	-	2 (40)	2 (40)	-	3 (60)	-	1 (20)	-	-	-
4.	S <sub>3</sub>	5	2 (40)	-	3 (60)	-	-	-	2 (40)	2 (40)	1 (20)	-
5.	S <sub>4</sub>	5	-	2 (40)	1 (20)	2 (40)	-	-	3 (60)	3 (60)	2 (40)	-
6.	T <sub>4</sub>	5	2 (40)	1 (20)	2 (40)	4 (80)	-	-	2 (40)	-	2 (40)	-
		30	6 (20)	8 (26.67)	13 (43.33)	6 (20)	7(23.33)	2 (6.67)	13(43.33)	5 (16.67)	9 (30)	1 (3.33)

Keys: B<sub>1</sub>: Boxers; S<sub>1</sub>: Singlets; S<sub>2</sub>: Shirts; S<sub>3</sub>: Shoes; S<sub>4</sub>: Socks; T<sub>1</sub>: Trousers; -ve: Absent, \*n: No. of samples in each category from which a given fungus was isolated. %P:Prevalence in percent

**Table 4: Prevalence of fungal isolates with respect to male second hand wears procured at Ebonyi Central Senatorial districtPrevalence of fungal isolates**

S/No	Categories of second hand wears	Total no of samples procured in each category	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Aspergillus terreus</i>	<i>Candida albicans</i>	<i>Mucor Spp.</i>	<i>Penicilliu m Spp.</i>	<i>Rhizopus Spp.</i>
1.	B <sub>1</sub>	5	2 (40)	-	3 (60)	1 (20)	3 (60)	-	3 (60)	3 (60)
2.	S <sub>1</sub>	5	-	2 (40)	-	-	2 (40)	3 (60)	-	-
3.	S <sub>2</sub>	5	2 (40)	-	2 (40)	2 (40)	1 (20)	3 (60)	-	-
4.	S <sub>3</sub>	5	-	-	2 (40)	-	-	-	3 (60)	3 (60)
5.	S <sub>4</sub>	5	2 (40)	2 (40)	2 (40)	3 (60)	-	4 (80)	-	2 (40)
6.	T <sub>1</sub>	5	-	1 (40)	3 (60)	2 (40)	3 (60)	4 (80)	-	3 (60)
Total		30	6 (20)	5 (16.67)	12 (40)	8 (26.67)	9 (30)	14(46.67)	6 (20)	11 (36.67)

Keys: B<sub>1</sub>: Boxers; S<sub>1</sub>: Singlets; S<sub>2</sub>: Shirts; S<sub>3</sub>: Shoes; S<sub>4</sub>: Socks; T<sub>1</sub>: Trousers, -ve: Absent, \*n: No. of samples in each category from which a given fungus was isolated %P: Prevalence in

