

Keratinophilic Fungi Isolated from Second Hand Wears in Anambra State, Nigeria.

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Abstract: Samples were collected from one hundred and twenty second hand wears and inoculated into Sabouraud Dextrose Agar Petri dishes supplemented with 0.5mg/ml cyclohexamide and 0.05mg/ml chloramphenicol. The fungal isolates were tested for their ability to degrade keratin using modified hair-baiting technique and eight species of fungi were recovered. The organisms isolated belong to dermatophyte and non dermatophyte fungi. The non dermatophyte fungi accounted for 78% of the isolates and include *Aspergillus niger* (12.2%), *Aspergillus terreus* (4.9%), *Aspergillus fumigatus* (12.2%), *Trichosporon asteroides* (7.3%), *Trichosporon cutaneum* (9.7%), *Geotrichum candidum* (14.6%) and *Penicillium chrysogenum* (17.1%). *Trichophyton tonsurans* (22%) was the only dermatophyte isolated. Photomicrographs of the hair strands revealed perforations as a result of the ability of the fungi to degrade keratin. Most of the fungi have been implicated in human and animal mycoses and so pose public health problem. There is therefore, need for users of these second hand wears to disinfect them before use.

Key words: Second hand wears; keratinophilic fungi; hair-baiting technique; dermatophytes; non-dermatophytes.

Introduction

Keratinophilic fungi are groups of fungi that degrade keratinous substances to different components of low molecular weight (Yaser 2013; Gughani *et al.*, 2014). They are made up of hyphomycetes (dermatophytes and non dermatophytic keratinophilic fungi) and fungi from other taxonomic groups (Gughani 2000). These keratinophilic fungi possess the enzyme keratinase and therefore, have strong affinity for keratinaceous materials. Keratin could be found in hair, wool, stratum comeum, horns, nails, claws and hooves of mammals, in scales, claws of reptiles and shells (Khanam and Jain 2002; Shukla 2014). Some of these fungi produce large number of spores which help in their distribution and survival.

Second hand wear locally called "okirika" or "agwuagwu" are popular in Nigeria. They are imported into the country by business men/women and charitable organizations. In the past, second hand wears was synonymous with poverty but today, all classes of people source them because they are cheaper and are believed to be more durable than the new ones. Nigeria is a country made up of about 182 million people with more than half the population under 30 years of age (NPC 2015). This group live with tight budget (mostly youths, unemployed, self employed, young parents) but still want to look good and so shop for these wears (Adegaju 2016). Some of these wears could be contaminated from different sources with spores from air, domestic animals and previous users.

Ringworm infection, piedra, onychomycosis and aspergillosis are some of the diseases that can be caused by these keratinophilic fungi and have attracted the attention of dermatologists and mycologists (Papini

et al., 1998; Awad and Kraume 2011; Grumbt *et al.*, 2011; Ekwealor *et al.*, 2012). Dermatophytes are spread by direct contact with infected people, animal and soil and indirectly from formites (Hainer 2003). These second hand wears could serve as formites for indirect transmission of spores or fragments of these fungi to users of second hand wears when not properly treated, hence the need to sample these wears. Recently, there have been reports of newly emerging non dermatophyte fungi causing infections of moderate or serious symptoms (Sarma and Borthakur 2007; Ekwealor and Oyeka 2013; Malejezyk *et al.*, 2013).

Many researchers have investigated the keratinophilic activities of fungi from soil (Papina *et al.*, 1998; Deshmukh and Verakar 2006; Shrivastav *et al.*, 2013; Ugoh and Ijigbade 2013; Gughani *et al.*, 2014), poultry farm (Anbu *et al.*, 2004; Mini *et al.*, 2012) and student house (Yaser 2013), but there have been no report of isolation of these fungi from second hand wears. This is very important especially in Nigeria, where a good percentage of the population depend on these wears. The aim of this work, therefore, is to isolate keratinophilic fungi from second hand wears.

Materials and Methods

Sample collection: One hundred and twenty samples were collected from second hand wears (40 shoes, 40 handbags and 40 cloths) sold in two major markets (Awka and Onitsha main markets) in Anambra State, Nigeria using moistened sterile swab sticks. These were transported to Microbiology laboratory, Nnamdi Azikiwe University, Awka and processed within 24h.

Fungal isolation: samples were inoculated into duplicate plates of Sabouraud Dextrose Agar (SDA) medium supplemented with 0.5mg/ml cyclohexamide and 0.05mg/ml chloramphenicol using spread plate method. The plates were incubated at 25°C for 21 days. Positive plates with fungal growth were sub-cultured into fresh SDA slants without antibiotics and stored at 4°C.

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Identification of isolates: Identification of isolates was based on detailed study of their macroscopic and microscopic morphologies in lactophenol cotton blue mount and compared with the standard fungal descriptions given by Campbell and Stewart (1980); Onion et al., (1981); Guy St-Germain and Richard (1996); De Hooget al., (2000).

Determination of keratinophilic abilities of the isolates: Modified hair baiting technique of Vanbreuseghem (1952) was employed. Freshly cut human hairs were collected from young school children. These were washed with detergents, dried and sterilized at 121°C for 15min. The sterile hairs spread on Petri dish were moistened with sterile water and inoculated with fungal isolates. Petri dish containing sterile moistened hairs not inoculated with any fungi served as control. Photomicrographs of the control and inoculated hair strands were taken.

Result

Sample collection: A total of eighty two (82) fungi were isolated from the second hand wears.

Identification of isolates: Eighty two isolates made up of 8 species of the fungi capable of utilizing keratin were recovered. The fungal organisms are species of *Aspergillus*, *Trichosporon*, *Geotrichum*, *Penicillium* and *Trichophyton* (Table 1). All the fungal isolates are non dermatophyte fungi except *Trichophyton tonsurans*. Non dermatophyte fungi accounted for 78% of the isolates, with *Aspergillus* species (29.3%) as the most prevalent. *Trichophyton tonsurans* was the only dermatophyte isolated, with prevalence rate of 22%. Keratinophilic fungi were isolated more in shoes than from other wears. *Aspergillus* species were not isolated from cloths while *Trichosporon asteroides* was isolated only from clothes.

Determination of keratinophilic abilities of the isolates: The ability of the fungal isolates to degrade keratin as observed from the photomicrographs revealed perforations of the hair strands (Plates 1a and 1b). The perforations are as a result of the degradation of the keratin in the hair strand by the fungi. Non perforations of the control hair strand indicate that keratin was not degraded (Plate 1c).

Discussion

A total of 82 fungal organisms with the ability to utilize keratin were isolated from fairly used wears. The isolated fungi belong to the dermatophyte and non dermatophyte keratinophilic fungi. The non dermatophyte fungi accounted for 78% of the isolates. These include *Aspergillus niger*(12.2%), *Aspergillus terreus* (4.9%), *Aspergillus fumigatus* (12.2%), *Trichosporon asteroides* (7.3%), *Trichosporon cutaneum* (9.7%), *Geotrichum candidum* (14.6%) and *Penicillium chrysogenum* (17.1%) (Table 1). *Trichophyton tonsurans*, the only dermatophyte isolated accounted for 22% of the total fungal isolates.

Aspergillus sp. was the most prevalent (29.3%) while *G. candidum* was the least prevalent (14.6%)(Table 1). Keratinophilic fungi were isolated more from shoes than from other wears.

The fungal organisms isolated in this work had also been reported by other researchers to possess keratinophilic activities. Anbu (2004); Zarim and Haghighi (2011); Mini et al., (2012); Shrivastav et al., (2013); Ugoh and Jigbade (2013); Gugnani et al., 2014; Shukla (2014) and Sharma and Chaudhary (2015) had reported the isolation of many keratinophilic fungi including *Aspergillus* sp, *Geotrichum candidum*, *Penicillium chrysogenum*, *Trichophyton tonsurans* obtained in this work from soil while Awad and Kraume (2011), and Yaser (2013) isolated these keratinophilic fungi from activated sludge and students house in Egypt respectively.

The isolation of *Aspergillus* sp as the most predominantly keratinophilic fungi, is in agreement with the reports of Awad and Kraume (2011), Ugoh and Jigbade (2013) and Yaser (2013), who recorded *Aspergillus* sp as the most frequently isolated keratinophilic fungi. Contrary to the observation of *T. tonsurans* as the only dermatophyte in this study, with a prevalence rate of 22%, Sharma and Choudhary (2015) in their work isolated other dermatophytes and reported *T. tonsurans* as the most prevalent.

Photomicrographs revealed perforations of the hair strands (Plates 1a and 1b) as a result of the ability of the fungi to degrade keratin in the hair. There was no perforation in control hair strand indicating that keratin was not degraded (Plate c).

Although the disease conditions of these fungal isolates were not studied, all the organisms have been implicated in various types of infection which had been attributed to their keratinophilic abilities (Aly et al., 2000; Ozer et al., 2009; Colombo et al., 2011; Ekwealor et al., 2012; Mochizuki et al., 2012; Ekwealor and Oyeka 2013; Hachem et al., 2014; Issachraichkul et al., 2014). The presence of these fungi in second hand wears, especially shoes, show that these wears could serve as sources of transmission of these organisms to users of these wears, thereby posing public health problems.

Conclusion

Dermatophyte and non dermatophyte keratinophilic fungi were isolated from second hand wears in Anambra State, Nigeria. Most of the isolated fungi have been implicated in human and animal mycoses and can be transferred through fomites. Users of these wears need to know the risk involved in using these wears without proper disinfection.

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Table 1: Prevalence of different keratinophilic fungi in second hand wears

Fungi isolated		shoes	bags	Wears clothes	Total	% prevalence
<i>Aspergillus niger</i>	6	4	-	10	12.2	
<i>Aspergillus terreus</i>	2	2	-	4	4.9	
<i>Aspergillus fumigatus</i>		4	6	-	10	12.2
<i>Trichosporon asteroides</i>	-	-	6	6	7.3	
<i>Trichosporon cutaneum</i>	2	2	4	8	9.7	
<i>Geotrichum candidum</i>		6	2	4	12	14.6
<i>Penicillium chrysogenum</i>	8	2	4	14	17.1	
<i>Trichophyton tonsurans</i>	12	4	2	18	22	
Total		40	22	20	82	100

Plate 1(a): Keratin degradation by *Aspergillus niger*Plate 1(b): Keratin degradation by *Trichophyton tonsurans*

Plate 1(c): Control hair strand

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