MYCOLOGICAL ASSESSMENT OF DERMATOLOGICAL AGENTS FROM ABATTOIR WORKERS IN AWKA METROPOLIS.

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ABSTRACT

Fungal dermatological conditions are caused by a group of fungi called dermatophytes. They cause infections in almost all parts of the body. The most common cause of skin infections are dermatophytes and opportunistic fungi. The main objective of this study is to isolate and identify fungal dermatological agents from samples from slaughterhouse workers in Amansea and Awka, Anambra State. The study also examined the possible predisposing factors to fungal infections. The specimens were cultured on duplicate plates of Sabouraud’s Dextrose Agar supplemented with 0.05mg/ml chloramphenicol and on another set of duplicate SDA plates containing 0.05mg/ml chloramphenicol and 0.5mg/ml cycloheximide. Fungal isolates were studied for their microscopic and macroscopic features. Out of the 140 samples collected from 35 individuals, a total of 114 isolations were made, consisting of 18 different fungi distributed thus; Aspergillus nidulans (6.14%), Trichosporon asahii (8.77%), Aspergillus terreus (5.26%), Penicillium verrucosum (4.39%), Acrophialophora fusicora (3.51%), Aspergillus flavus (7.89%), Trichophyton concentricum (5.26%), Rhizopus oryzae (6.14%), Scedosporium apiospermum (4.39%), Aspergillus niger (4.39%), Trichophyton tonsurans (4.39%), Trichophyton erinacei (3.51%), Microsporum ferrugineum (4.39%), Trichosporon ovoides (5.26%), Rhodotorula mucilaginosa (9.65%), Candida krusei (2.63%), Candida kefyr (5.26%), and Candida guillermondii (8.77%). The higher isolation was from the finger webs with 57.02%, followed by that of the toe webs with 42.98% of the isolates, this indicates the occurrence of fungal infections more as a result of what their hands touch than through their feet. This study recommends routine mycological investigations in workers with suspected mycoses for better management of dermatological conditions in the abattoir.

Keywords: Abattoir, Meat sellers, Mycological investigations, Fungi, dermatophytes.

INTRODUCTION

Infectious diseases, particularly those involving the skin and mucosal surfaces, are a serious problem all over the world, mainly people of the “Third World”, due to deficient sanitation and education. An important group of these skin pathogens are fungi[1,2]. Fungal infections may be classified as "Superficial": affecting only the skin, hair, nails and mucous membrane, or "Systemic": affecting the body as a whole. Fungal infections may also be described as "Local" when they are restricted to one body area, as "invasive" when there is spread into the tissue, or as "disseminated" when the infection has spread from primary site to other organs throughout the body [3,4]. Dermatophytoses have been reported to be encouraged by hot and humid conditions and poor hygiene and occur throughout tropical and temperate regions of the world [5,6].

Abattoir operation could be very beneficial to man; in that it provides meat for human consumption and other by-products, still it can be very hazardous to public health in respect to the waste it generates [7,8]. It produces
highly characteristic organic waste with relatively high level of suspended solids, liquids and fats. These include condemned meat, undigested ingesta, bones, hairs, aborted fetuses, gut contents, blood, urine and water [9].

Fungal disorders are emerging significant infections in the world [10]. In recent years they have become an important clinical condition that deserves public health attention [11]. Mycology is a somewhat ignored field in medical research limiting the availability documented data on the overall prevalence of fungal infections in the world. However, recent literature suggests a prevalence of dermatological conditions as high as 30% depending on the type of fungal agent and the country [12,13]. The burden is more in developing countries and also ranges from one country to another, for example the prevalence of dermatophytoses in Tunisia is 30.3%, in Brazil 38.4% and in Iran 21.1 % [13,14].

Superficial fungal infections of the skin are among the most common diseases seen in our daily practice. These infections affect the outer layers of the skin, the nails and hair. In contrary to many of the other infections affecting the other organ systems in humans, the fungi may cause dermatological conditions that do not involve tissue invasion. On the other hand, the skin surface is the habitat of some of these fungi and is liable to environmental contamination. In Nigeria the prevalence and distribution of fungal disorders and the causative agents are not well documented hence the situation is not known. One of the major risk factors for microbial infections is poor hygiene and sanitary condition, such as can be found in slaughter houses all over the country.

This study was designed to identify the common superficial fungal organisms associated with abattoir workers.

**METHODOLOGY**

Sample collection was done by using moist or dry swab stick on the skin. Samples were collected from the finger webs (inter-digital area) and toe webs of participants. Sample size involved four specimens from each individual (both hands and both feet) and taken to the laboratory for immediate processing.

**Study Area**

The observational and informative study was carried out between June and August 2014. The research on prevalence of fungal organisms among slaughter house workers was carried out in prominent slaughter houses (Amansea and Awka) in Anambra state, Nigeria. The abattoirs are not fully operational 24 hours of the day; the busiest period is from 6:30am to 12 noon. The major activities were carried out during this period, starting from the sequential and simultaneous killing of the animals, butchering and selling of the meat.

**Study Population and Size**

The study population was mostly adult males of different age bracket, who complied with the research and permitted sample collection. Structured questionnaire was used to obtain information on their age, years of service, family size, type of accommodation, educational level and presence or absence of fungal lesion. The only criterion for eligibility was being an abattoir worker. The sample was taken from thirty five (35) abattoir workers, which included toe and finger webs of both hands and both feet using one sterile swab stick for each, summing the number of samples to 140.

Mathematically,
Total number of individuals μ= 35;
Number of sampling sites per person () = 4
Therefore, total number of samples x = μ= 35 samples.

**Sample Collection**

Samples were collected by random from thirty-five (35) abattoir workers, who were without symptoms of fungal infection, by swabbing of finger and toe webs of the workers with sterile swab stick. For the purpose of the study, a variety of samples were taken from; workers who have not touched any meat products for the day (have not being involved in either killing, butchering or selling), those who were already taking part of the animal slaughter, those who were butchering the animal parts, and those involved in selling the meat products.
and processed within 2h.

**Isolation of Fungal Organisms**

Samples were inoculated on duplicate plates of Saboraud’s Dextrose agar (SDA) supplemented with 0.05mg/ml chloramphenicol and on another set of duplicate SDA plates containing 0.05mg/ml chloramphenicol and 0.5mg/ml cycloheximide. The samples were incubated at room temperature (25°C) for minimum of 48 hours and maximum of 4 weeks for the isolation of some fungi and confirmation of dermatophytes respectively.

**Purification of isolates**

Different colonies isolated were purified by sub-culturing unto sterile SDA plates without any drug supplements.

**Identification of Fungal Isolates**

Identification of fungal isolates from positive cultures was based on colonial characteristics in pure culture and microscopic morphology of fungi using lactophenol blue. These were compared with the standard description [15].

**Wet Mount examination**

Microscopic examination of culture was done by slide preparation for immediate use containing the organism and lacto phenol cotton blue as stain, then viewed under the microscope.

**Slide culture technique**

This involved preparing a thick layer of SDA which was infused with 3-4 cover slips in a slanting position separated from each other and inoculation of the specimen underneath these slants. This enables us to observe the mold colonies at different stages of growth within 2 to 7 days.

**Biochemical Tests**

**Urease**

The organism was streaked in a test tube containing a mixture of urea base agar and sterile urea. The test tube was incubated at 25°C for 24 to 48 hours. Pink colour on the agar surface was observed for positive results. Organisms that showed negative after 48 hours were incubated for another 5 days to confirm negativity.

**Sugar fermentation test**

Peptone broth was prepared in a test tube and an indicator (bromothymol blue) was added. Sugar (glucose and lactose) was added in the tube at a concentration of 1g in 100ml of broth. A Durham tube was placed inside the test tube in an inverted position and the solution is then heated in a water bath at 110°C for 10 minutes. The test organism is inoculated and incubated in the broth at 25°C for 24 hours. Gas production was observed as a clear space inside the Durham tube while a change in colour of the broth indicated acid production.

**Germ tube test**

*Candida* cells were diluted with serum or, and incubated at 37°C or 39°C for 1–2 hrs. The germ tube was observed with a light microscope. In order to investigate the germ tube structure, the elongated daughter cells from the round mother cell without constriction at their origin were referred to as germ tubes.

**RESULTS**

Of the 140 samples collected, a total of 114 isolates were obtained, consisting of 18 different fungi species distributed thus: *Aspergillus nidulans* (6.14%), *Trichosporon asahii* (8.77), *Aspergillus terreus* (5.26%), *Penicillium verrucosum* (4.39%), *Acrophialophora fusiispora* (3.51%), *Aspergillus flavus* (7.89%), *Trichophyton concentricum* (5.26%), *Rhizopus oryzae* (6.14%), *Scedosporium apiospermum* (4.39%), *Aspergillus niger*
(4.39), \textit{Trichophyton tonsurans} (4.39%), \textit{Trichophyton erinacei} (3.51), \textit{Microsporum ferrugineum} (4.39%), \textit{Trichosporon ovoides} (5.26%), \textit{Rhodotorula mucilaginosa} (9.65%), \textit{Candida krusei} (2.63%), \textit{Candida kefyr} (5.26%), and \textit{Candida guillermondii} (8.77%). This is represented in figures 1a and 1b.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1a.png}
\caption{Percentage occurrence of dermatophytes and non-dermatophyte molds}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1b.png}
\caption{Percentage occurrence of yeast}
\end{figure}
Table 1: Biochemical tests of isolated yeasts

<table>
<thead>
<tr>
<th>S/N</th>
<th>MICROORGANISMS</th>
<th>GLUCOSE</th>
<th>LACTOSE</th>
<th>SUCROSE</th>
<th>GERM TUBE</th>
<th>UREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Rhodotorula mucilaginosa</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><em>Trichosporon asahii</em></td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><em>Candida krusei</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td><em>Candida kefyr</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td><em>Candida guilliermondii</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td><em>Trichosporon ovoides</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Keys:**
- Positive
- Negative
- Variable

Further tests were also run on *Trichosporon asahii,* and *Trichosporon ovoides* by confirming their growth on an SDA medium at 37°C.

From the results obtained, more organisms (66) were isolated from the finger webs with 57.89%, having 10 dermatophytes (15.15%), 26 non-dermatophyte molds (39.39%) and 30 yeast (45.45%). While 48 organisms were gotten from the toe webs, having 10 dermatophytes (20.83%), 22 non-dermatophyte molds (45.83%) and 16 yeast (33.33%). This is represented in figures 2a, 2b, 3a and 3b.

**Figure 2a:** The occurrence of dermatophytes and non-dermatophyte molds on finger webs
Figure 2b: The occurrence of yeasts on finger webs

Figure 3a: The occurrence of dermatophytes and non-dermatophyte molds on toe webs
Figure 3b: The occurrence of yeasts on toe webs

The distribution of fungal isolates based on the number of years spent is shown on figure 4. Organisms were isolated more from workers that have spent for more than five years in the abattoir with 46.49% of isolates, followed by who have worked between two to three years (2-3 yrs) with 28.95%, those who have worked for four to five (4-5) years with 15.79%, and then, those have served for less than or equal to one year (0-1 yr) with 8.77%.

Figure 4: The occurrence of fungal agent according to years of service

According to the level of education, those who stopped at or after secondary school have the highest number of occurrence with 68.42%, followed by those who stopped at or after primary with 26.32%, while, those who attended tertiary or did not attend any school rank the lowest with 2.69% each.
Information obtained from the workers showed that the age of the workers ranges from 19 years to 65 years. Out of the 114 isolated organisms, 56 (49.12%) came from those ranging from 21 to 30 years, 34 (29.82%) fungi were gotten from those 31 to 40 years old, 14 (12.28%) of the organisms from those 20 years old and below while 10 (8.77%) was gotten from those above 40 years.

Figure 5: The occurrence of fungal agents by level of education

Figure 6: The occurrence of fungal agents according to age

while 10 (8.77%) was gotten from those above 40 years.

Figure 6: The occurrence of fungal agents according to age
From the result obtained, varying numbers of fungal agents were found in different ranges of family size, with those who fall within the range of one to four (1-4) having the highest number of occurrence with 63 fungal agents (55.26%), where the total number of dermatophytes are 13 (20.63%) and the total number of non dermatophytes are 50 (79.37%). Those who fall within the range of 5-10 family size are 16 in number with 49 fungal agents (42.98%) with a total number of 10 dermatophytes (20.41%) and 39 (79.58%) non dermatophytes. Finally, one person falls within the range of 11-15 family size with two fungal agents 2/114 (1.75%).

![Figure 7: Percentage occurrence of fungal agents by family size](image)

Of the total of 135 samples, the 114 isolated fungal agents were distributed according to the accommodation of the workers. Those who live in one room are 6 in number with a total of 20 fungi organisms and percentage occurrence of 17.54%. The dermatophytes are 3 out of 20 (15%), while the non dermatophytes are 17 in number with 85%. Those who live in two rooms are 14 in number, with total of 48 organisms, i.e. 42.11% of total isolates. The occupants of 3 rooms are 13 in number with a total of 43 organisms, giving 37.72%. The inhabitants of 4 rooms and above are two with a total of 5 organisms, giving 4.39%.

![Figure 8: The distribution of fungal agents by accommodation](image)
DISCUSSION

From the 140 samples collected from the abattoir, 114 (81.43%) fungal agents were recovered. Out of these, *Trichophyton sp.* occurred 15 times (13.16%) with *T. concentricum* as the highest occurring species, *Microsporon* 5 (4.39%), yeasts 46 (40.35%) and other non dermatophyte molds 48 (42.11%). These results showed that *Trichophyton sp.* was a leading fungal agent of dermatophytosis followed by *Microsporon sp.*. These results are similar to those obtained by Ellabib and Khalifa, (2001) [16], Chepchirchir et al., (2009) [17], and Ekwealor and Oyeka (2013) [18] who also recorded *Trichophyton sp.* as the most frequently isolated dermatophyte, even though their studies were not among abattoir workers and varies in species at different regions. This could be due to the fact that *Trichophyton* is a keratinophilic filamentous fungus with a high ability to invade keratinized tissue due to possession of several enzymes such as acid proteinases, elatinases, keratinases and other proteinases while *Epidermophyton* species lack the ability to perforate the hair hence they are low in occurrence [19].

Non dermatophyte fungi that were isolated include *Aspergillus niger, Aspergillus nidulans, Trichosporon asahii, Aspergillus terreus, Penicillium verrucosum, Acrophiophora fastispora, Aspergillus flavus, Rhizopus oryzae, Scedosporium apiospermum, Trichosporon ovoides, Rhodotorula mucilaginosa, Candida krusei, Candida kefyr, and Candida guillermondii*. This is similar to findings of Maruthi et al., (2008) [20] where *Aspergillus flavus, Fusarium oxysporum and Penicillium spp* were isolated. Presence of other non-dermatophytes particularly *Aspergillus* and *Penicillium* species may be due to the ubiquitous nature of their spores in our environment carried transiently on healthy skin [20]. Among the non-dermatophytic fungi, *Aspergillus sp.* was the most common and isolated in 27 cases, this is related to the findings from the work of Ekwealor and Oyeka (2013) [18] where *A. candidus* was the most frequently isolated non-dermatophyte. *Candida* species were isolated in 19 cases in this study; this isolation rate is comparable to that of other studies [21].

Results show that workers who have served for more than five years in the abattoir had the highest occurrence of fungal agents; this could be as a result of prolonged and constant exposure to the organisms. It was also observed from the study that level of education attained by the workers had no obvious effect on the occurrence of fungal agents. The most active age bracket in the abattoir are those between 21 to 30 years, which is probably the reason why most of the isolates were recovered from them, those 20 years old and below had minor tasks to do while those above 40 did very little, this is why these two groups recorded less number of isolates. Comparing family size and nature of accommodation, higher occurrence of fungal agents were among workers who live in crowded areas. Fungi thrive on warm and moist skin and also survive directly on the hair shaft or in their interiors [11,22]. This may be why the finger webs and toe webs of the abattoir workers were a major site of fungal occurrence.

CONCLUSION

Dermatophyte infections are very common in our country where hot and humid climate in association with poor hygienic conditions play an important role in the growth of these fungi. The findings of this study suggest that the finger and toe webs of these abattoir workers harbour dermatophytes, non-dermatophytes and yeast which could be transferred to other members of their family and the general public. There was some observable association in isolation between different ages, residence and years of service. We conclude that along with dermatophytes, non dermatophytic fungi are also emerging as important causes of superficial mycosis. Therefore, these fungi should also be considered for tropical antifungal treatment.

REFERENCES


