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Research Article

FUNGAL ASSESSMENT OF ARTIFICIAL NAILS OF FEMALE STUDENTS OF NNAMDI AZIKIWE UNIVERSITY, AWKA

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Abstract

This study was conducted to isolate and identify fungi from artificial female nails of female students. A total of ten nail samples were collected. The samples were collected from random female students of Nnamdi Azikiwe University, Awka. The experiment was carried out at the Department of Applied Microbiology and Brewing, Faculty of Bioscience, Nnamdi Azikiwe University, Awka. The isolated pathogens from finger nails include *Cladosporium* spp., *Candida albicans,* and *Trichophyton* spp. Highest contamination of *Cladosporium* spp. was isolated followed by *Candida albicans* and then *Trichophyton* spp. with low frequency. After the colonies were being isolated, they were further characterized on the basis of morphological and microscopic features including Lactophenol cotton blue stain and Germ tube test to identify fungi type. The frequency of occurrence of various fungal isolates in female artificial fingernails were attained showing a high level of *Cladosporium* spp. with 64%, followed by *Candida albicans* with 31% and *Trichophyton* spp. with the least occurrence of 5%. Fingernails remain one of the channels of food contamination and it serves as probable reservoir for pathogenic organisms in this study especially the females.

Keywords: Fungi, Artificial nails, *Cladosporium, Trichophyton*.

INTRODUCTION

Microorganisms are widely distributed all over the world be it air, water, soil and even human body. Water born disease are those disease which have water as their vehicle of transmission (Agu *et al.,* 2014; Agu *et al.,* 2017; Agu *et al.,* 2023; Victor-aduloju *et al.,* 2023).Human body is said to be the shelter of millions of bacteria, viruses, fungi, and other many other invisible organisms. These organisms are collectively called as microbes. The microbes belong to different communities and together called as micro biome. The human micro biome is a source of various genetic diversity and no two human micro biomes can be absolutely same. Different microbes reside on different places of human body and they are adapted to the conditions in which they live. These microorganisms play an important role in maintaining the human health. (Kumar *et al.,* 2017). The hands of the human body are in most contact with the outer world. The human hands are located at the end of each arm. Normally, a human has five fingers on each hand which includes: a thumb, index finger, middle finger and little finger (Baran *et al.,* 2002). People use their hands for multiple purposes every day. Therefore, it is very easy to come in contact with different microbes and to transfer them to objects and even to people. Surprisingly, fingernails are the home for most of the bacteria found on human hands (Wachukwue *et al.,* 2017). Finger tips are the areas of the human skin that contains the highest concentration of receptors. These areas of the nerve endings make the fingers very sensitive to heat, cold, moisture, vibration, pressure and various other stimuli (Wootton *et al.,* 2007). Fingernails are attached to the distal end of each finger.

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A nail is tough envelope like covering the terminal phalanges of fingers and toes in human (Wang *et al.,* 2016). The area under the fingernail is difficult to clean and therefore there resides the most pathogenic organisms. Activities which can increase the risk of fungal nail infection include: having constantly wet hands, severe nail biting, and eczema around the fingernails. It is seen that the fingernails are more sensitive to accumulate different kinds of bacterial pathogens due to constant change in the environment of the host and its surrounding (Larson *et al.,* 2003). The aim of this work is to isolate, identify and characterize fungi from artificial fingernails of female students of Nnamdi Azikiwe University, Awka.

Materials and Methods

Study Area

The study areas for this research were some selected areas in Nnamdi Azikiwe University, Awka metropolis, Anambra state, Nigeria known for their high population.

Sample Population

The target population was female students of Nnamdi Azikiwe University, Awka, within the selected study area. The inclusion criteria were female students who use artificial fingernails to carry out several personal activities, while the exclusion criteria were female students without artificial fingernails.

Sample Collection

Fingernails of different female students around Nnamdi Azikiwe University, Awka metropolis were sampled. The

samples obtained were asceptically wrapped to prevent contamination and then transported to microbiology section in the Departmental laboratory for microbial analysis.

Preparation of Culture Media and Normal Saline

Sabouraud Dextrose Agar was used in this research to isolate and identify the fungi; and the media was prepared according to manufacturer's instruction 11g of Sabouraud Dextrose Agar and 2g of Antibiotics was weighed and dissolved into 160ml of distilled water in a conical flask. Cotton wool was then placed to cover it and wrapped with aluminum foil. After which the solution was then autoclaved at 121oc for 15 minutes, it was then allowed to cool and dispensed into Petri-dishes.8.5g of Sodium chloride (NaCl) was weighed and tipped into 100ml of distilled water after which it was sterilized in the autoclave. 1ml of the sample was dropped into 9ml of normal saline and shake vigorously to form a uniform solution of 10^{-1} concentration.

Sample Preparation

The finger nails were cut and inoculated in a sabouraud dextrose broth and incubated overnight at 25-28^{o}C.

Inoculation and Incubation

1ml pipette was employed to drop 0.01ml of the inoculums into the Petri-dishes and evenly spread all over the surface of the agar plate using stirring rod. All plates were incubated immediately after inoculation and placed upside down to prevent drops of condensations from collecting on the inoculated surface. Sabouraud Dextrose Agar plates were incubated for 28oC for 72hrs, after which pure culture was prepared from the distinct fungal isolate observed (Harrigan, 1998; Ogbo *et al.,* 2015).

Subculturing and Storage of the Fungal Isolates

A loopful of the inoculated broth was sub cultured on Sabouraud Dextrose Agar (SDA) and further incubated for 24 hours. The isolated organisms were purified through repeated subculture method. Streak plate methods were used for this purpose. Sabouraud Dextrose Agar (SDA) was used as media. When a plate yielded only one type of colony, the organisms were considered to be pure.

Isolation and Characterizaton of the Fungi: This was done based on the gross morphological appearance of fungal colonies on the SDA culture medium and the slide culture as described by Agu and Chidozie (2021) and lactophenol cotton for microscopic evaluation under X10 and X40 magnification of the microscope; with reference to the Manual of Fungal Atlase (Ellis *et al.,* 2007).

Germ Tube Test: A suitable yeast colony and emulsify it in a tube containing 0.5ml human serum (HIV &HBSAg negative serum). Incubate at 35°C for 1½ - 3 hours. After incubation, place a drop of the suspension on a glass slide, cover with cover slip and examine under low power magnification for the presence of germ tubes.

RESULTS

Table 1 shows the colony morphologies and microscopic features of fungal isolates Note: S-Sample

Isolates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Morphological features	White to cream colored, smooth, and yeast-like appearance	Green to black from the front and texture is velvety to powdery in appearance	Green to black from the front and texture is velvety to powdery in appearance	Green to black from the front and texture is velvety to powdery in appearance	Green to black from the front and texture is velvety to powdery in appearance	White to cream colored, smooth, and yeast-like appearance	White to cream colored, smooth, and yeast-like appearance	Flat, white to cream in color with a powdery surface	Green to black from the front and texture is velvety to powdery in appearance	Green to black from the front and texture is velvety to powdery in appearance
Microscopic features	Short and inflated conidiophores, hyphae are septate, hyaline.	Long and branched conidiophores, hyphae is septate and brown	Short and inflated conidiophores, hyphae are septate, hyaline	Short and inflated conidiophores, hyphae are septate, hyaline	Short and inflated conidiophores, hyphae are septate, hyaline	Short and inflated conidiophores, hyphae are septate, hyaline	Short and inflated conidiophores, hyphae are septate, hyaline	Spiral and branched hyphae	Long and branched conidiophores, hyphae is septate and brown	Long and branched conidiophores, hyphae is septate and brown
Probable organisms	*Candida albicans*	*Cladosporium* spp.	*Cladosporium* spp.	*Cladosporium* spp.	*Cladosporium* spp.	*Candida albicans*	*Candida albicans*	*Trichophyton* spp.	*Cladosporium* spp.	*Cladosporium* spp.

Table 2. The Lactophenol cotton blue stain

Isolates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Fungal type	Yeast	Mold	Mold	Yeast	Mold	Yeast	Yeast	Mold	Mold	Mold

Note: S-Sample

Table 3. The Germ Tube Test

Isolates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Germ tube test	positive	-	-	negative	-	positive	positive	-	-	-

Note: S-Sample

Table 4. The Frequency of occurrence of various fungal isolates in female artificial fingernails

S/N	Organisms Identified	Frequency (%)
1	*Cladosporium* spp.	64%
2	*Candida albicans*	31%
3	*Trichophyton* spp.	5%

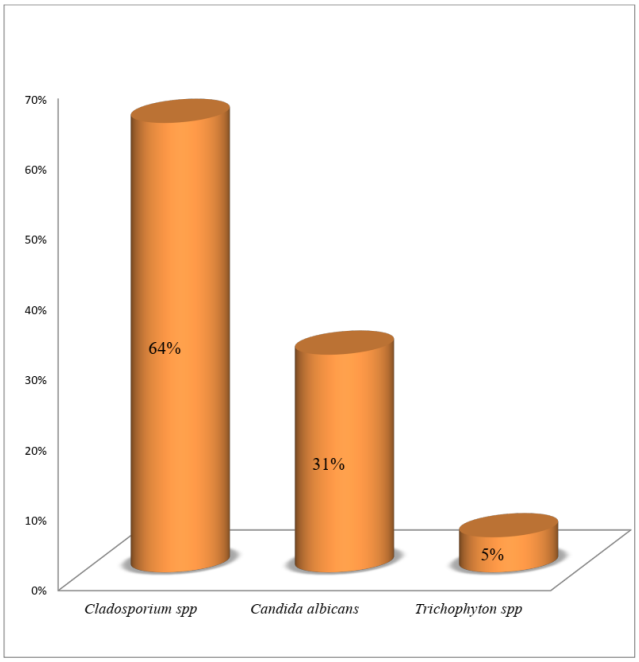

Fig.1. The percentage of fungal isolates from artificial fingernail samples

DISCUSSION

Microbial contamination of the female artificial fingernails has become a global health problem. This study shows that *Candida albicans, Cladosporium* spp., and *Trichophyton* spp. were found in the female artificial nails, *Cladosporium* spp. and *Candida albicans* have the highest frequency of 64% and 31% respectively, followed by *Trichophyton* spp. with 5% which is the least frequency of occurrence. Some of these pathogens have been reportedly isolated from cooked foods in Nigeria (Baiyewu *et al.,* 2017; Chukwuka *et al.,* 2013). Out of the fungi isolated, *Cladosporium* spp. has the highest frequency of occurrence (64%) followed by *Candida albicans* (31%) and *Trichophyton* spp. with 5% frequency of occurrence. This is however in agreement with Ifeanyi, (2015) and Bello (2013) whom both isolated about seven different fungal genera from different artificial fingernails from vendors and when these isolates were aseptically inoculated into healthy foods, the characteristic symptoms originally observed were also noticed. Figure 1 showed the percentage of fungal isolates from the artificial fingernails sample after calculating the total percentage of each isolate *Cladosporium* spp. 64%, *Candida albicans* 31% and *Trichophyton* spp. 5% as the number and types of fungi associated with the hands are of greater concern for health. Opportunistic pathogens such as Fungi can survive on inanimate surfaces for long periods of time and items such as watches, pens, and mobile phones are permanent surfaces for transmission of these types of infections (Akinyemi *et al.,* 2019). Ryan *et al.,* (2014) Explain *Cladosporium* spp. are Rugged and opportunistic without highest prevalence of 64%. The identification of isolated organisms was determined using Lactophenol cotton blue staining technique in Table 2.

Rayan and Flournoy (2017) had reported heavy fungal growth under fingernails that were more than 1mm in length and showed that food vendors with short finger nails (properly cut) had 64% fungal contamination (fungal count) and food vendors with long finger nails showed more (67%) contamination of fungal count on their hands. Lin *et al.,* (2013) reported that long fingernail tends to harbors more microorganisms than short nails. Visibly clean nails were observed merely by appearance of finger nails of students, showed presence of 62% bacterial contamination ontheir hands. Ray *et al.,* (2019) observed a decrease in colony count following hand washing with soap in 60% of the samples.

Ray *et al.,* (2014) found that hand swab samples of 61% children harbors potential pathogens before taking food, also reported presence of pathogenic microbes on the hands of the students which included *Cladosporium* spp., *Aspergillusniger, Candida albicans, Trichophyton spp.* Tambekar and Shirsat, (2012) reported the presence of *Cladosporium* spp., *Aspergillusniger, Candida albicans, Trichophyton* spp., Rhizopus spp., *Fusarium spp.*, from the hand swabs of students. Chinakwe *et al.,* (2012) also isolated *E. coli, Aspergillusniger, Candida albicans, Trichophyton* spp., *Cladosporium* spp., from the hand-wash water samples. Oniya *et al.,* (2006) isolated microorganisms transmissible through hand-shake and also reported prevalence of microorganisms was higher in primary and secondary school students than in the under graduate students. The reduction in the number of pathogens after hand washing was also reported by Tambekar *et al.,* (2009). Generally, most fungi are considered toxigenic or pathogenic (Al-Hindi *et al.,* 2014). Some molds may produce mycotoxins (Tournas and Stack, 2014). The fungi isolated in this study have been reported to produce secondary metabolites in foods. These secondary metabolites are potentially harmful to humans and animals (Eaton and Groopman, 2014; Baiyewu *et al.,* 2017). A good example is Aflatoxin which has been implicated in cancer of the liver (heplatoma), aflatoxicosis and also acute hepatitis in humans, especially in the developing world (Baiyewu *et al.,* 2017). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2014).

Conclusion

Fingernails remain one of the channels of food contamination and it serves as probable reservoir for pathogenic organisms in this study especially the females who prepare meals for both domestic and commercial purpose.

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