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Isolation and identification of microbial and fungal flora from female hair samples in Riyadh Saudi Arabia

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***Abstract*—** *The human hair harbors several species of fungi and also bacteria. The present study was performed to determine the prevalence of keratinophilic fungi and bacteria from hair samples of femalesfrom November 2016 to April 2017. A total of 50 human hair samples were examinedusing hair-baiting techniques for isolation. After the incubation period, the number of colony forming unit was counted. The microorganisms were identified based on the colony morphology from culture and microscopic features. After purification, each representative colony was gram-stained and examined for cell morphology and gram reaction under a light microscope. Fungal isolates included were Aspergillus niger, Aspergillus flavus, Penicilliumspp, Alternaria alternata, Chrysosporium keratinophilum. Cladosporium cladosporioides and Trichosporon mucoides. Isolated bacterial species included gram positive bacteria such as Leuconostoc mesenteroidess spcremoris, Kocuriarosea, Staphylococcus haemolyticus, and the gram negative bacteria Kocurikristinae, Stenotrophomonas maltophilia, and Micrococcus luteu/ lylae. Human hair samples from females studied were found have several fungal and bacterial isolates, some of which can cause some serious disease in humans. Health authorities need to heighten up their health information campaigns that will include not only prevention and treatment of serious illnesses but also body hygiene.*

Key words*— *keratinophilic fungi, microbial and fungal flora,female hair.

I. INTRODUCTION

The human hair is one part of our body that is always exposed to environmental pollutants, and also to fungal and bacterial contamination. In Saudi Arabia, women wear the "hijab" to cover their hair. Fungal disorders are emerging significant infections in the world (WHO, 2005). In recent years, they have become an important clinical condition that deserves public health attention because of the fact that some of them are potentially harmful to human health (Anbu, 2004; Ganaie 2010; Deshmukh, 2010; Lee *et al*., 2011). Keratinophilic fungi are usually isolated from the soil and from keratinous tissues such as the skin, hair and nails. This includes the dermatophyte*Microsporumgypseum* (Shukia*et al*, 2003), and some species of *Aspergillus*, *Fusariumsolani*, and *Bipolarisspicifera*. (Shadzi, 2002; Gherbawy, 2006; Anbu, 2004;Ganaie, 2010, Ali, 2008; Zarrin, 2011; Chepchirchir, 2009; Kannan, 2006; Ali-Shtayeh, 2001)

Bacteria, on the other hand were known to reside in the hair follicles, in which 85% of the bacterial population if found in the superficial layers of the skin and hair follicles (Lange-Asschenfeldt et al., 2011) Bacteria such as *Micrococcaceae* represents the most common isolated specie. (Lange-Asschenfeldt et al., 2011) The human hair is also a reservoir of bacterial including *Staphycoccusintermedius* and coagulase-negative *Staphylococci* (Mase et al, 2000), and *Staphylococcus aureus* (Jappe, 2003).

There were very limited reports on keratinophilic fungi and bacterial colonization on the hair. This study aimed to determine the prevalence of keratinophilic fungi and bacteria in the hair of females in Riyadh, Saudi Arabia.

II. METHODS

Collection of human hair samples

Participants were recruited from various areas in Riyadh, Saudi Arabia fromNovember 2016 until April 2017. Participants were informed about the aim and objectives of the study and consent forms were obtained. The study protocol was reviewed and approved by the Princess NourahbintAbdulrahman University Research Ethics Committee. Hair samples were collected from consenting participants aged 14 to 50 years old.

Isolation of fungi from hair samples

Hair samples were placed separately in clean plastic bags and then transferred directly to the laboratory, and kept in a cool place (3-5°C) until fungal assay was performed. Two different techniques were used: hair baiting as recommended by Vanbreuseghem and described by Sharma in 2003. (Sharma, 2003) Fragments of hair samples (10 cm in length) were sprinkled on the surface of double sterilized soil. The soil was moistened with sterilized distilled water and remoistened whenever necessary and incubated at 28 ° C

for three months.The plates were examined every week. The moulds that appeared on the hair were transferred onto a Sabouraud`s Dextrose Agar which contained (g/l): glucose, 20; peptone, 10; agar, 20 and chloramphenicol 40. (Ellis et al., 2007)The other technique used was the direct plating of the hair onto Sabouraud`s Dextrose agar which contained chloramphenicol. (Gherbawy et al., 2006) Blood agar plate for bacteria Plates were incubated at 28°C for 2-10 days and the cultures were examined periodically for fungal and bacteria growth.

Bacterial isolation and identification

After the incubation period, the number of colony forming units was counted using the CFU/mL. The microorganisms were identified based on the different types of colonies.Colony morphologies were recorded and purified to obtain pure colonies for the identification purposes. Each representative colony was gram-stained and examined for cell morphology and gram reaction under a light microscope. Fungi samples were all identified on the basis of their morphological characteristics, whereas the bacterial isolates were identified by the use of Vitekanalyzer (bioMerieux, UK).

Preparation of plant extract

One gram of henna powder, Ziziphusspina-christipowder, roselle powder (*Hibiscus sabdariffa*) and Trigonellafoenum-graecum) were mixed in 10 ml. of distilled water. The content of the flask was then filtered through antibacterial filter to obtain clear infusion of 1 ml. fresh Garlic, Daber oil were used directly. The fungal inoculum was prepared by incubating samples in old culture grown on Potato dextrose agar medium for 5 to 10 days. The petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. A final concentration of approximately 1 ml of each fungus was then spread onto the surface of SDA plate.

Plant extracts which suppressed the fungal growth were tested for their efficiency against the fungi isolated from hair by tested the disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in millimeters.

III. RESULTS

Fifty females participated in the study. The mean age was 27.5 years old. A total of 27colonies of different keratinophilic fungi were isolated from 50 hair samples. The isolated keratinophilic fungi included *Aspergillus niger, Aspergillus flavus, Penicilliumspp, Alternaria alternate, Chrysosporiumkeratinophilum. Cladosporiumcladosporioides, Trichosporon-mucoides.*(Tables 1 and 2)

The isolated bacterial species included gram positive bacteria such as *Leuconostocmesenteroidessspcremoris, Kocuriarosea, Staphylococcus haemolyticus*, and gram negative bacteria including *Kocurikristinae* , *Micrococcus luteu/ lylae,*and *Stenotrophomonas maltophilia*. Dual infection with both gram positive and gram-negative bacteria was also seen. (Table 3)

Table 4 shows the bacterial count in different clinical subsets of females. It was observed that high bacterial count, was found in females who were having dandruff, who were (and were not) using antibiotics and those who were using corticosteroids. Henna users and those using antibiotics had lower bacterial counts. Table 5 represents the antifungal activity of plant extracts by disc diffusion. Henna extract and Dabur oil gave most promising results and were protective against fungal infection.

IV. DISCUSSION

The presence of keratinophilic fungi in different soil has been reported from all over the world. (Anbu, 2004; Ganaie, 2010; Deshmukh, 2010, Lee, 2011, Mahmoudabadi, 2008) Keratinophilic fungi are small, well defined and important group of fungi that colonize various keratinous substrate and degrade them to components of low molecular weight. These fungi are present in the environment with variable distribution patterns.Keratinolytic fungi are associated with human and animal mycoses 26-30 (FilipelloMarchisio, 1996; Shadzi, 2002; Zarrin, 2011; Chepchirchir, 2009; Nakagawa, 1999) Very few studies are reported regarding isolation of *keratinophilic* fungi from human hair samples. (Kannan, 2006; Ali-Shtayeh, 2001)

This study shows the most prevalent isolate both in terms of its percent occurrence and frequency of occurrence *Aspergillus niger*, which some of the isolates are found to be pathogenic to humans. It can cause fatal invasive aspergillosis and pulmonary disease in immunocompromised patients and they are associated with the production of oxalate crystals in clinical specimens. (Atchade et al., 2017; Oda et al., 2013) *Aspergillus flavus* was also isolated in this study. *A. flavus* was reported to have keratinase activity and a strong producer of extracellular keratinase. (Kim, 2007)

On the other hand, bacterial isolates that included *Leuconostoc mesenteroides ssp cremoris, Kocuriarosea, Staphylococcus haemolyticus, Kocurikristinae, Micrococcus luteu/ lylae,* and *Stenotrophomonas maltophilia*. *Leuconostoc mesenteroides* were known to cause nosocomial outbreaks and brain abscess. (De Boni*et.al.*,2011 ,Albanese et al., 2006)

*Kocuriarosea*has been found to cause a significantly wide spectrum of human infections including peritonitis. (Purty et al., 2013) *Staphylococcus haemolyticus* is an opportunistic bacteria that is highly resistant to antibiotics and can cause meningitis, skin and soft tissue infections, endocarditis and bacteremia. (Falcone et al., 2007)*Kocuriakristinae* on the other hand are found to cause urinary tract infection among catheterized children (Chen et al., 2015) *Stenotrophomonas maltophilia* cause respiratory infections (Dignani et al., 2003)

The present research gave us a recent insight about the existence of keratinophilic fungi in the hairs. In many clinical and epidemiological studies, fungal infections of the skin and scalp represent a relatively common problem especially in the tropical and subtropical regions of the world where warm and humid climate provides a favorable environment for fungi. They have become a significant health problem affecting children, adolescents and adults They (these diseases) are transmitted from person to person directly infected (infecting) skin scales or hairs(hair follicles). They can also be acquired by humans from infected animals and by direct exposure to infected soils.

The fungal and bacterial contaminations in the surrounding atmosphere affects the health of human beings and needs knowledge, awareness and maintenance of hygiene to avoid the development of disease. Keratinolytic activity of fungi is important ecologically. The impact of keratinophilic fungi on human health seems unexplored. Knowledge of the frequency and extension of etiological agents of humans and animal mycosis and other potentially pathogenic fungi on the healthy hairs is of prime importance for understanding of epidemiological cycle of these fungi, apart from ecology point of view. Therefore hygiene protocol should be taken to prevent the spread of pathogenic fungi in these environments as there is a risk of fungal infections of human.

V. CONCLUSION

A variety of keratinolytic fungi and pathogenic bacteria exists in the hair. The hair could serve as a vector for disease transmission of pathogenic microorganisms and fungal elements.There is a need for a hygiene protocol to prevent the spread of pathogenic fungi, and also invasion of the deeper structures of the head including the meninges and the brain parenchyma. These findings should be taken into consideration and necessary treatment methods should be taken up periodically.

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Table.1: Frequency of fungal isolates from 50 human hair samples on Sabouraud`s Dextrose Agar:

Sample no.	AGE	Fungal species (SDA)	Number	Percentage
9	20	*Aspergillus niger*	1	3.7
19, 28	29	*Aspergillus niger*	2	7.4
33	31	*Aspergillus flavus*	1	3.7
16	23	*Penicillium spp.*	1	3.7
20	30	*Cladosporium cladosporioides*	1	3.7
35	26	*Trichosporon mucoides*	1	3.7
39	19	*Alternaria alternata*	1	3.7

Table.2: Frequency of fungal isolates from human hair samples of 50 Females grown on sterile soil

Age	Fungal species	n	incubation period	%
29	*Penicilliumspp*	1	50	3.7
26	*Chrysosporium keratinophilum*	1	60	3.7
31	*Chrysosporium keratinophilum*	1	81	3.7
38	*Chrysosporium keratinophilum*	1	50	3.7

Table.3: Bacterial isolates from hair samples

Age	Bacterial Type	Gram stain
29	*Leuconostoc mesenteroides ssp cremoris*	(+ve)
28	*Kocuri kristinae*	(-ve)
16	*Stenotrophomonas maltophilia*	(+ve)
22	*Kocuriarosea*	(+ve)
38	*Micrococcus luteu/ lylae*	(-ve)
23	*Staphylococcus haemolyticus*	(+ve)

Table 4. Frequency of different baseline characteristics within the sample and corresponding mean microbial counts:

Variable	Henna Users	Non-henna Users	with dandruff	No dandruff	receiving antibiotic	Not receiving antibiotic	Using corticosteroids	Not using cortico steroids	Suffering from asthma	No asthma
Number subjects within sample (%)	26%	74%	42%	58%	24%	76%	6%	94%	6%	94%
Mean of total microbial count (units)	11.9	21.5	21.1	17.4	15	20.2	36	17.9	13.3	19.3

Table.5: Antifungal activity of plant extracts (1/10 ml) , and plantpowder by disc diffusion

Zone of inhibition (mm)	Henna powder	water extracts of henna	Cidir Ziziphus spina-christi powder	water extracts of Cidir (Ziziphus spina-christi)	Roselle (Hibiscus sabdariffa) powder	water extracts of Roselle (Hibiscus sabdariffa)	Garlic fresh	Fenugreek Seeds Powder	Daber oil
Aspergillus niger	(-)		(-)		(-)		(-)	(-)	(-)
Aspergillus flavus	2.5mm	(-)	(-)		(-)		(-)	(-)	(-)
Penicillium spp.	2mm	(-)	(-)		(-)		(-)	(-)	2.2mm
Alternaria alternata	(-)		(-)		(-)		(-)	(-)	(-)