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Isolation and Identification of Dermatophytes from Environmental Specimens Comparing with Species Isolated from Clinical Specimens of Scalp Infection among Sudanese Basic School, Kosti, White Nile State, Sudan -2020

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ABSTRACT

Background: Tinea capitis (TC) or scalp ringworm is common in pediatric population with reported rates of infection ranging from 4% to 15% .T.rubrum is rarely reported in countries with a temperate climate . There is always hair loss with varying degrees of scaling and erythema [1-3].

Objectives: To identify the role of soil, plant & sewage in transmission of ringworm of scalp infection. This study was carried out on Kosti basic school students who suffering from ringworm of scalp (fungal infection) in White Nile state at Sudan in 2020.

Methodology, Data Analyses and Results: Due to preservation of this fungal infection among school children in Kosti basic school students, a total of fifty specimens collected from different sources to determine the source of this infection in the area of study, twenty five students who suffering from ring worm of scalp and twenty five from soil, sewage, water and trees in the period between January – December -2020. The samples collected by scraping the scalp by surgical blade and removing the hair with needle and forceps from the peripheral lesion and gathered in filter paper and closed tightly, 10% KOH was used to clarify and keratinized tissues. Trees samples were collected by washing the tree's leaves and with all soil, sewage, water were collected into container. All samples were cultured on sabourad dextrose agar medium for one week at room temperature. After growth, lactophenol cotton blue staining was used to identify depending on the colonial morphology under microscope and to look for color, septate, aseptate hyphae, macro, micro conidia and spores. The results showed that there was 68% of isolates from soil, sewage, water and trees were matched to scalp isolates. The soil is significant one of these when Chi-square (X²) was used (P value = 0.027) that means, the source of infection among students may be due to soil transmission.

Conclusion and Recommendation: Our results highlight the epidemiology of fungal infection of Kosti basic school students. Further studies are necessary to verify these findings considering the relatively high rate of fungal infection to cover all expected area with similar observation.

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Introduction

The superficial fungal infections or superficial mycoses affect the hair, nails and skin [4-6]. On the skin and scalp, the lesions are often roughly circular with raised border, but may coalesce to form confluent areas of dry, scaling skin, inducing itching and scratching which in severe cases may ulcerate [7]. Adult cases of Trichophyton tonsurans infection may be seen in adults with acquired immune deficiency syndrome (AIDS). The infection has a worldwide distribution [1,8]. TC is a fungal infection of the

skin and hair scalp characterized by scaling and patchy alopecia. T. tonsurans is the most common etiology in the USA, while M.canis remains the most common cause of Tinea capitis in Europe [5,7]. T.rubrum is rarely reported in countries with a temperate climate [7]. Except for two species, Epidermophyton floccosum and T.concentricum, all Dermatophytes are able to invade hair. Infection of hair by dermatophytes follows 3 main patterns: ectothrix, endothrix, and favus [9,10].

The prevalence of superficial mycoses (Tinea capitis) has been studied in different parts of the world. The relative occurrence

of the etiologic agents of these infections varies from country to country and from one climatic region to another. In tropical countries, a warm and humid climate, crowded living and poor sanitary conditions all promote the spread of these infections [11-18].

The spectrum of dermatophytes isolated from skin lesions had changed in last 70 years. Before the Second World War in Germany, *Microsporum audouinii* and *Epidermophyton floccosum* ranked the first, whereas *Trichophyton rubrum* is the most common dermatophytes since the fifties of last century, accounting for 80–90% of the strains, followed by *T. mentagrophyte*. This evolution is typical for Central and North Europe and it needs to be connected with the increase in the incidence of *Tinea pedis* [19].

History and Source of Infection

Trichophyton interdigitale – anthropophilic strains in 1914 in Berlin Marie Kaufmann-Wolf distinguished the dermatophyte *Trichophyton interdigitale* (later named after her) from the “red fungus”, i.e., *T. rubrum*, as the cause of *tinea pedis*. The anthropophilic *Trichophyton interdigitale* species, or variety, became known as the “white fungus”, and was later classified taxonomically as *T. mentagrophytes* var. *T. interdigitale*. During the 1950s and 1960s Blaschke-Hellmessen studied fungal flora in the Dresden area and reported that from 1956–1964 there was a change in the frequency of variants of certain dermatophytic species, particularly *T. mentagrophytes* complex. Its widespread variant from 20 years before – “interdigitale” (also known as “Kaufmann-Wolf fungus”) - was frequently isolated between 1956 and 1960 but was rarely identified after that. Around the same time, Schönborn and Friedrich and Kruse [20-24].

Virulence Factor of Dermatophyte

These substances include enzymes such as keratinases, elastases, deoxyribonuclease (DNase). Collagenases and lipases [25].

Methodology

Study Design, Duration and Area

This was an observational, cross-sectional study conducted from January to December 2020. The data and samples were collected from Kosti basic school students (New Village). The practical side was performed in Department of microbiological lab. At University of El Imam El Mahdi, which is a one of major universities in Kosti city, White Nile State, Sudan.

Study Population and Data Collection

Everyone attended to Kosti basic school students (New Village) with the clinical suspicion of infection by ringworm infection was included in this study. Other skin infection and patients with a history of antifungal use before at one week were excluded. A total of 25 cases and 25 non-clinical were enrolled. The study data were collected using interviewing questionnaire, which include personal information, medical information, and outcomes of laboratory investigations.

Sample Collection and Laboratory Analysis

The samples collected by scraping the scalp by surgical blade and removing the hair with needle and forceps and samples taken from the peripheral lesion not from center and gathered in filter paper and closed tightly. Samples of soil, sewage, water and trees collected in filter and closed. then used 10% KOH (appendix 3.2) to help in show samples under microscope by put the samples in clean sterile slide and added a few drops of 10% KOH and covered by cover glass and waited a few minute until keratinize tissue dissolve and put it under microscope and used 10x & 40x

for detection and identification and looked for septate and aseptate hyphae, macro and micro conidia, spore. All samples cultured on sabouraud dextrose agar medium (appendix 3.3) for one week at room temperature. After growth, colonial morphology were observed. By using sterile wire loop take the colonies beside the flame and transferred to sterile slide and then added a few drops of lactophenol cotton blue staining (appendix 3.4), then covered with cover glass and waited for a few second and put it under microscope (10x and 40x) for detection and identification septate and aseptate hyphae, macro and micro conidia and spore to identified organisms which are *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Microsporum canis*, *Microsporum audouinii* and *Microsporum gypseum*.

Data Analysis

The study data were analyzed using statistical package for social sciences (SPSS version 22) software and the results were presented in form of tables, and figures. Person chi-squared and Fisher exact test (For categorical data) besides the two independent samples Willxon test (For numerical data) were used to assess the difference between the groups. A (P value ≤ 0.05) was considered significant.

Sample Collection and Examination

A total of twenty five basic school student who suffering from ring worm of scalp and twenty five from soil, sewage, water and trees were subjected to carry out this study.

Information was collected regarding individual number, history of infection and physical examination for the classic lesion is an erythematous, raised, scaly ring with central clearing and multiple lesion may be present. The severity of infection can range from mild, scaly lesion, to erythematous, exudates lesion characteristic of superimposed bacterial infection.

Sample Processing

Direct Microscopic Examination

Direct microscopic examination of scales and broken-off hairs placed on a microscope slide with one or two drop of 10% potassium hydroxide (KOH) and a cover slip was performed. The sample was warmed for five minutes over a flame as described by Hainer (4). Each treated slide was then carefully examined under low (X10) and high (X40) power objective for the presence of hyphae and/or arthroconidia.

Ethical Consideration

The study was approved by the Ethic Review committee of Department of Microbiology (University of El Imam El Mahdi) also provided the authorization. All samples and information were collected from basic school students after their guardian expectance to be part of the study and signed written informed consent.

Results

Fifty specimens were collected and divided into twenty-five samples from skin scalp and fifty-five samples from soil, plants, sewage and water. The mean age of the cases, groups were 7-13 years old. The isolates in the study belonged to two genera: *Microsporum* 35 isolates —the most prevalent cause of dermatophytosis in our study, accounting for 70% of isolates— and *Trichophyton* (15 isolates; 30 %). The isolates included four species: *M. audouinii* {anthropophilic} 15 (30%), *M. gypseum* {geophilic} 11 (22%), *M. canis* {zoophilic} 9 (18%), *T. tonsurans* 8 (16%) and *T. mentagrophytes* {zoophilic} 2 (4%), as in Table-2, Figure_2. According to SPSS analysis {Chi-square (X² = P < 0.05

) when C.I = 95% and SD ± 2} were used, the study revealed that in Table-1; the frequency and percentage of specimens types, in Table-2; the distribution and percentage of isolated Fungi, in Table-3; which showed the relationship between scalp specimen and isolated organism, Table-4; it revealed relationships between scalp and plants samples, Table-5; illustrated the relationship between scalp infection and sewage samples, Table-6; showed relationship between scalp and water samples, the four last tables all were insignificant (P value > 0.05) as 0.67, 0.35, 0.15, 0.11) respectively. But in Table-7; which revealed the significant value between the soil isolates (mainly *M. audouinii* constitutes 8%) and scalp infection in which (P value = 0.027 which is < 0.05) so that means the soil may be the reason for transmission of ring worm for students as in Table-7, Figure-3.

Table 1: Shows the Frequency and Percentage of Specimen Types

Specimen Types	Frequency	Percent
Scalp	25	50
Soil	8	16
Plants	5	10
Sewage	6	12
Water	6	12
Total	50	100

Table 2: Reveals the Distribution and Percentage of Isolated Fungi

Isolated Fungi	Frequency	Percent (%)
<i>M. audouinii</i>	15	30
<i>M. canis</i>	9	18
<i>M. gypseum</i>	11	22
<i>T. mentagrophyte</i>	2	4
<i>T. tonsourans</i>	8	16
Unknown	5	10
Total	50	100

Table3: Shows the Relationship between Scalp Specimen and Isolated Organism

Specimen	Isolated Organism						P value
	<i>M. audouinii</i>	<i>M. canis</i>	<i>M. gypseum</i>	<i>T. mentagrophyte</i>	<i>T. tonsourans</i>	Total	
Scalp	3	5	7	1	4	20	0.669
Soil	4	2	0	1	1	8	
Plants	2	1	1	0	1	5	
Sewage	3	0	2	0	1	6	
Water	3	1	1	0	1	6	
Total	15	9	11	2	8	45	

Table 4: Reveals Relationships between Scalp and Plants Samples

	Plants				Total	P value
		<i>M. audouinii</i>	<i>M. canis</i>	<i>M. gypseum</i>		
Scalp	25	0	0	0	25	0.345
<i>M. audouinii</i>	3	2	1	1	8	
<i>M. canis</i>	5	0	0	0	5	
<i>M. gypseum</i>	7	0	0	0	7	
<i>T. mentagrophyte</i>	1	0	0	0	1	
<i>T. tonsourans</i>	4	0	0	0	4	
Total	45	2	1	1	50	

Table 5: Shows Relationship Between Scalp and Sewage Samples

	Sewage				Total	P value
		M.audouinii	M.gypseum	T.tonsourans		
Scalp	25	0	0	0	25	0.108
M. audouinii	3	3	1	1	8	
M.canis	4	1	0	0	5	
M.gypseum	7	0	0	0	7	
T.mentagrophyte	1	0	0	0	1	
T.tonsourans	4	0	0	0	4	
Total	44	4	2	1	50	

Table 6: Illustrates Relationship between Scalp Infection and Water Samples

		Water				Total	P value
		M.audouinii	M.canis	M.gypseum	T.tonsourans		
Scalp	25	0	0	0	0	25	0.150
M. audouinii	3	3	1	1	0	8	
M.canis	4	0	0	0	1	5	
M.gypseum	7	0	0	0	0	7	
T.mentagrophyte	1	0	0	0	0	1	
T.tonsourans	4	0	0	0	0	4	
Total	44	3	1	1	1	50	

Table 7: Reveals Relationships between Scalp Infection and Soil Samples

	Soil			Total	P value
	M.audouinii	M.canis	T.mentagrophyte		
Scalp	25	0	0	25	0.027
M. audouinii	3	3	1	8	
M.canis	2	1	1	5	
M.gypseum	7	0	0	7	
T.mentagrophyte	1	0	0	1	
T.tonsourans	4	0	0	4	
Total	42	4	2	50	

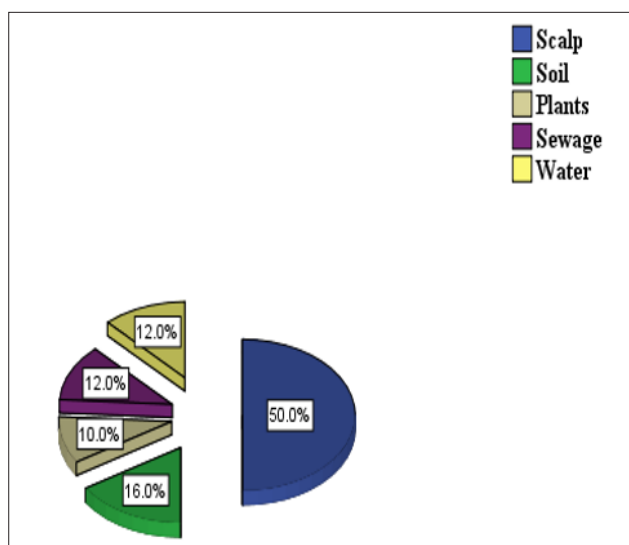


Figure 1: Shows the distribution of Specimen types

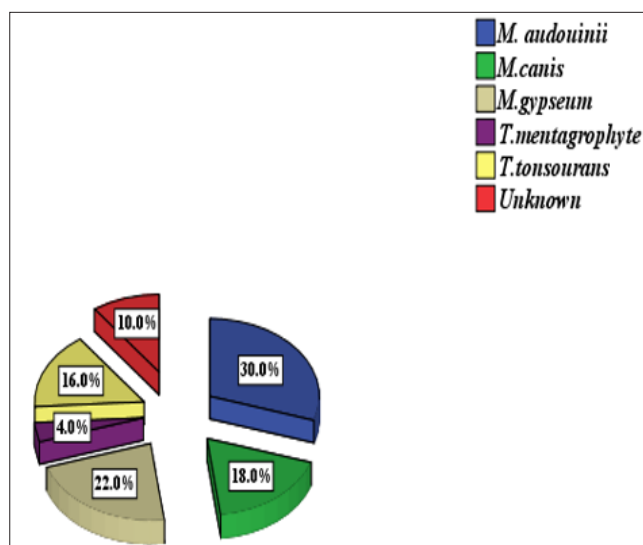


Figure 2: Shows distribution of isolated fungi

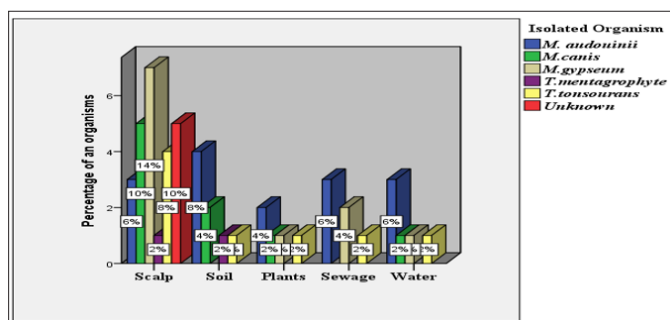


Figure 3: Illustrate the distribution of fungi among the type of specimens

Discussion

The dermatophytosis transfers from animal (zoophilic dermatophytes) and soil (geophilic dermatophytes) to man or through direct infection by personal contact (anthropophilic dermatophytes). For the past 8 to 10 years, the common agent of ringworm of the scalp in the United States has been *Microsporum audouinii* and this organism has been responsible for widespread epidemics in many communities. This present study there were five types of dermatophytes (divided into two genera; 70% of *Microsporum* and 30% *Trichophyton*) isolated from clinical specimens, also *M. audouinii* represent 30% of the total isolates and was the most common isolated fungi in the target area as in globally, followed by *M. gypseum* (22%), *M. canis* (18%), *T. tonsurans* (16%) and *T. mentagrophyte* (4%). Which similar to study conducted by, who said that the isolates were into two genera *Microsporum* and *Trichophyton* but differ to some extent in distribution of isolates percentage which were 83.6% and 16.4% for his study among both genera while in our study were 70% and 30% respectively as in Table-1. In addition, our study represented that *Microsporum* were the most cause of scalp infection (70%) which disagreed with study that performed by, who concluded that *Trichophyton* had predominant cause of scalp infection and counted 69.8%, this may be due to different study area and the activity of population in different target study areas [26-28].

Conclusion

The soil may assist in transmission of scalp infection of Kosti basic school students, while the plants, sewage and water may not help in transmission of scalp infection in the target area.

The hot and humid climate in tropical and subtropical countries like Sudan makes dermatophytosis a very common fungal skin infection. Generally, they are superficial infections, but in immunocompromised patients, they are experienced as a disseminated disease.

Poor of personal hygiene, awareness less and over-crowded of students in classroom may be considered predisposing factor in transmission of scalp infection.

Recommendations

- Further study will be of value to complete the picture about the trends of scalp infection in the entire area in Kosti Basic Schools – Kosti - White Nile State - Sudan.
- More efforts and attention should be planned towards students who revealed a dermatophytosis.
- Hospital laboratories and microbiological laboratory centers should be provided and supplied with essential materials that necessary for the detection of superficial mycoses.

- New strategies and techniques should be implemented for the covering and rapid screening medium for the isolation and identification of dermatophytes compared to SDA.

Declarations

Ethics Approval and Consent to Participate

The study was approved by the Ethic Review committee of Kosti Teaching Hospital. Department of Microbiology (University of El Imam El Mahdi) also provided the authorization. All samples and information were collected from patients after their expectance to be part of the study and signed written informed consent.

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

We declare that they were no competing interests

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None

Authors' Contributions

All authors contributed to study design and protocol and performed the collection the samples and data. Also conducted the laboratory test, statistical analysis, article writing, responsible of results interpretation, manuscript revision and editing. All the authors read and approved the last version of manuscript for submission.

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