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Dermatophytes and Bacterial Super infections in antimicrobial resistant Tinea pedis patients in Dour city, Iraq

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Abstract

Background: Tinea pedis is a dermatophyte infection of the feet, especially the toes and soles of the feet.

Aim of this study: This study aims to evaluate prevalence, etiology, and risk factors of tinea pedis and bacterial super infections in Dour city population.

Patients and methods: This is a cross sectional study, was carried out in one year duration from March 2019 to March 2020 on 150 patients, both genders, ages ranging from 20-70 years old. Methods include sampling by scraping, direct microscopical exam. with KOH., culture on different media that are used to isolate dermatophytes, as well as culture on different media and different biochemical tests to isolate bacteria, and finally calculating MIC.s. of Lawsonia inermis in killing dermatophytes.

Results: T. pedis is the most prevalent in the age 40-49(31.3%). I t is most prevalent during Spring(28%). There are nonsignifcant associations in the relation between diabetes, vascular disease and psoriasis with gender(p value>0.05), while the relations of smoking, obesity, and family history of foot mycoses with gender are statistically significant(p value<0.05). Trichophyton rubrum is the most prevalent causative dermatophyte(36.7%), while Trichophyton verrucosum is the least prevalent(8.7%). Staphylococcus aureus is the most prevalent bacterial species accompanying dermatophytes(80%). MIC. of petroleum ether extract of Lawsonia inermis for T. tonsurans and T. verrucosum is 128 µg/ml., while MIC. of petroleum ether extract of L. inermis is 256 µg/ml. for T. rubrum, T. interdigitale, and E. floccosum. MIC.s of ethanol extract of L. inermis is 16 µg/ml. for T. verrucosum, 32 µg/ml. for T. tonsurans, 64 µg./ml. for T. interdigitale and E. floccosum, and 128 µg./ml. for T.rubrum.

Conclusions: T. rubrum was the commonest dermatophyte isolated from cases of Tinea pedis and Staphylococcus aureus was the most common bacterial species. The disease is contagious between the family members. Tinea pedis is usually diagnosed via clinical observation, but there are variety of other methods used to diagnose it.

Key words: Tinea pedis, dermatophytes, Lawsonia inermis.

Introduction

Tinea pedis is estimated to affect 10%of the world's population. The cause of T. pedis is dermatophyte. The most common dermatophytes in T. pedis lesions are *Trichophyton rubrum*, *Trichophyton mentagrophytes var interdigitale* and *Epidermophyton floccosum*[(1)]. These fungi can collectively be known as keratinolytic fungi which affect individuals who are healthy as well as people with compromized immune system[(2)]. Symptoms include erythema, scaling, fissures, maceration, and pruritus between the toes extending to the soles, borders, and sometimes the dorsum of the foot. The most commonly affected space is between the fourth and fifth toes because for anatomical reason this web space tends to be the most occluded[(3)]. Tinea pedis is the most common form of dermatophytosis in the post pubescent period; this condition is a public health problem because of its contagious and recurrent nature[(4)].

Rippon revealed that 70% of the population is infected with athlet's foot at some point in their life[(5)]. Studies demonstrated that men are infected 2-4 times more often than women[(6)]. Historically it is believed to have been a rare condition, that became more frequent in the 1900.s. due to the greater use of shoes, health clubs, war, and travel[(7)].

As the disease progresses, the skin may crack, leading to bacterial skin infection and inflammation of lymphatic vessels. If allowed to grow for too long, athlet's foot fungus may spread to infect the toe nails feeding on the keratin in them, a condition called onychomycosis[(8)]. Some individuals may experience an allergic response to the fungus called an id reaction in which blisters or vesicles can appear in areas such as the hands, chest, and arms. Treatment of the underlying infection typically results in the disappearance of the id reaction[(9)].

The prevalence of T. pedis increases with age and it is more frequent in adults aged 31-60 years old followed by adults aged more than 60 years old, it is rare in children. The risk is more in developed countries. Certain occupational groups are exposed to a higher risk of infection such as miners, soldiers, marathon runners, mosque attendees. The exposure of these special populations to sweating, trauma, occlusive foot wear and communal areas predisposes these groups to an increased incidence of T. pedis as well as the use of common showers, psoriasis, DM. which are associated with interdigital maceration[(4)].

Lawsonia inermis(Henna)grows naturally in hot, semi-arid climates. It has been used for both cosmetic purposes as well as practical applications. Studies have shown the effectiveness of henna and/or solutions derived from henna and its compounds to be effective in antifungal, antimicrobial and anti-inflammatory uses. This plant molecule strengthens keratin, lessens pain sensation, speeds wound healing and decreases inflammation[(10)]. Water extracts of this plant leaves exhibit an absolute toxicity against *Microsporum gypseum, Trichophyton mentagrophytes* and aspergillus[(11)].

Gram +ve and gram –ve bacteria could be isolated from cases of T. pedis. The best results of treatment were seen with azole compounds that have mixed antibacterial and antifungal properties[12].

The aim of this study is to estimate the prevalence of Tinea pedis in Dour city population.

Objectives:

1- to estimate the relation between infection and some demographic parameters like age, sex, diabetes, obesity, seasonal variation, presence of pets, and others.

2- to evaluate secondary bacterial infection in some cases of tinea pedis.

3- to estimate the therapeutic effect of Henna in tinea pedis patients through MIC. study.

Patients and Methods

Samples were taken from 150 patients throughout a period of one year from March 2019 to March 2020, ages ranging from 20-70 years old, 86 females and 64 males. The questionnaire allowed documentation of potential predisposing factors, Diabetes mellitus, vascular diseases, immunosuppressive drugs treatment, psoriasis, associated fingernail onychomycosis, family history of foot mycosis, ritual religion washing, used shoes, occlusive shoes, swimming pools, smoking, walking barefoot, thermal station, and pedicure.

Samples were taken by skin scraping from the center of the lesion to the edge crossing the lesion margin using a sterile scalpel blade and collected in sterile petri dishes for direct exam. and culture. Suppurating lesions may be sampled with a swab when it is impractical to obtain scraping[13],[14]. All specimens were submitted to a microscopic exam. by KOH.10% and inoculated into SDA. with chloramphenicol with cycloheximide all in duplicate. The culture was incubated at 27^0C and examined after 48-72 hr.s. for yeast detection and every 4 days for at least 4 weeks for fungal detection. The identification of filamentous fungi was based on macroscopic appearance and microscopic in lactophenol cotton blue[13],[15].

Dermatophyte test medium DTM: It is used for selective isolation of dermatophytes from cutaneous specimens. They are presumptively identified by gross colonial morphology and the production of alkaline metabolites which cause a colour change in the medium from yellow to red (phenol red is the pH indicator).

Hair Perforation Test:-

*blond hair autoclaved and cut into short pieces (1cm),10-20hairs. *sterile D.W.5ml in a vial.

*place hairs in water in vial.

*inoculate with small fragments of the test fungus.

*incubate at room temperature.

*individual hairs are removed at intervals up to 4weeks and examined microscopically in LCB. Isolates of *T. mentagrophytes* produce marked localized areas of pitting and marked erosion where as those of *T.rubrum* do not.

Urease test:- recommended to differentiate between *T. mentagrophytes* +ve while *T.rubrum –ve.*

BCP agar:- For the differentiation of Trichophyton spp.
Solution A: *DW. 1000ml *Skim milk powder 80gm *Bromocresol purple (1.6% solution in alcohol) 2ml. Dissolved in 2liters flask and autoclaved 10psi/15min.s.
Solution B: *Glucose 40gm *DW. 200ml Dissolved and autoclaved at 10psi/8min.s.
Solution C: *Bacto agar{BD 214010} 30gm *DW. 800 ml Soak for 15 min.s in 3liters flask, autoclave at 15psi/15min.s. To make media: add solution A and B to solution C, aseptically dispense in slopes (7ml).
Trichophyton test medium:- Trichophyton agars 1-7 are solid media recommended for differentiation and identification of Trichophyton spp. based on nutritional requirements (vitamins and amino acids).
*agar 1 is the Casein agar base (vitamin free) composed per liter purified water:-
_acid hydrolysate of casein 2.5gm
_dextrose 40 gm
_monopotassium phosphate 1.8gm
_magnesium sulfate 0.1gm
_agar 15gm
*agar 2 : agar 1+inositol 50mg
*agar 3: agar 2+thiamine HCL 200µg
*agar 4: agar 1+thiamine HCL 200µg
*agar 5: agar 1+nicotinic acid 2mg
*agar 6: agar 1+ammonium nitrate 1.5gm
*agar 7: agar 6+histidine HCL 30mg. Inoculate, incubate cultures at 25-30^0 C for 7days up to 14 days and record growth. Some Trichophyton spp. can grow at 37^0 C like *T. verrucosum.*
Rice grain medium:- This is to induce sporulation and for differentiation of *M. audoinii* which does not grow on this medium from *M. canis* which grows rapidly on it.
_place 0.5 teaspoon rice grains into wide neck 20ml vials.
_add 8ml DW. to each vial.
_ensure even distribution of rice grains, autoclave.
Dermatophyte strains were maintained on Saboraud dextrose agar (SDA) and potato dextrose agar (PDA) at 27-30^{0}C. They were subcultured at 3 months intervals, but observed on a weekly bases. Just before use, the samples were transferred to SDA. and maintained for 7 days at 27^{0}C-30^{0}C[(16),(17),(18),(19)].
The identification of bacterial species was based on culture on different media and different biochemical tests.
MIC. Calculation of Henna:
- Preparation of crude extracts:
150 gm. of powder of *Lawsonia inermis* was extracted with petroleum ether at 60-80^0 C. and 150 gm. of the powder was extracted with 80% ethanol[(20)]. The extraction can be performed also by taking 25 gm. of powder and adding 100ml. of petroleum ether and keep it on shaker overnight, next day centrifugation of the contents and taking the supernatant into a fresh container and allowing it to dry[(21)]. One gram of each extract

was weighed and dissolved in 5 ml. of the solvent used for extraction to give 200gm./ml. Serial dilutions of the extracts were prepared to determine MIC[20].
The microorganisms were standardized according to the 0.5 McFarland's scale, 100µl. of the stock solution was thoroughly mixed with 20 ml. molten sterile SDA. and maintained at 25^0 C. The agar plates were left to set[20].
BMI.(body mass index)is calculated as weight/$(height)^2$.
A BMI. of 25 kg/m^2 or more is overweight, while the healthy range s 18.5-24.9 kg/m^2[22].
Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 25. The quantitative data were presented as mean, and standard deviations while qualitative data were presented as number and percentages. The comparison between two independent groups with qualitative data was done by using Chi-square test. The comparison between two independent groups with quantitative data and parametric distribution was done by using
Independent t-test. Pearson correlation was calculated to obtain the relation among quantitate variables. The p value was considered significant as the following: $P > 0.05$: Non-significant. $P < 0.05$: Significant.

Results

This study was conducted on 150 patients(64 males and 86 females), ages ranging from 20-70 years old throughout a period of one year, 65 patients were diabetic, 50 were complaining from vascular diseases, 15 were having psoriasis, 55 were smokers, 70 were obese, and 100 patients were having family history of foot mycoses.
The main dermatophytes that could be isolated were:
T.rubrum in 55 cases, *E. floccosum* in 32 cases, *T. interdigitale* in 27 cases, *T. tonsurans* in 23 cases, and *T. verrucosum* in 13 cases. The main bacterial species that could be isolated were: *S. aureus* in 120 cases, *Pseudomonas* spp. in 17 cases, and *Strept.* Spp. in 7 cases.

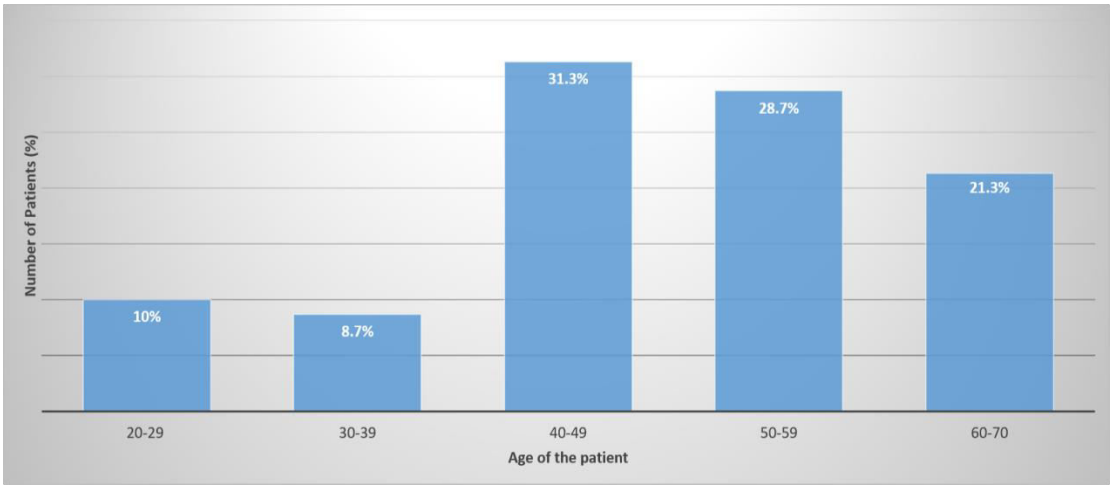

Figure 1. The distribution of patients according to age

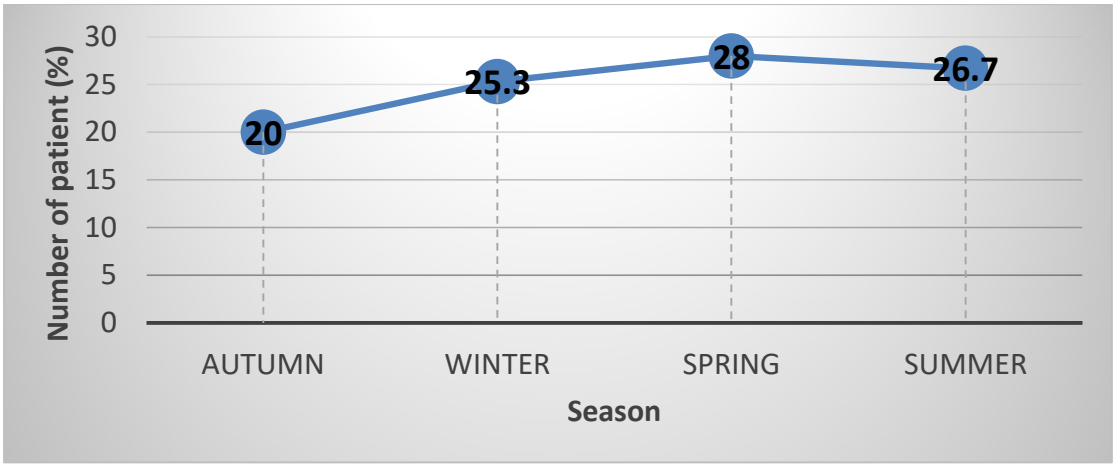

Figure 2. The distribution of patients according to season

Table 1. The patient distribution according to risk factor

Risk factors	Female		Male		P value
	Freq.	%	Freq.	%	
DM					>0.05 NS
Present	35	40.7	30	46.9	
Absent	51	59.3	34	53.1	
Vascular disease					>0.05 NS
Present	30	34.9	20	31.3	
Absent	56	65.1	44	68.8	
Psoriasis					>0.05 NS
Present	10	11.6	5	7.8	
Absent	76	88.4	59	92.2	
Smoking					<0.05 S
Present	5	5.8	50	78.1	
Absent	81	94.2	14	21.9	
Obesity					<0.05 S
Present	44	74.4	24	37.5	
Absent	42	25.6	40	62.5	
Family history					
Positive	37	43.0	63	98.4	<0.05 S
Negative	49	57.0	1	1.6	
Total	86	57.3	64	42.7	

Ns = not significant, S= significant

Table 2. The causative agents among the patients

Causative agents	Freq.	%
T. rubrum	55	36.7
T. tonsurans	23	15.3
T. verrucosum	13	8.7

T. interdigitale	27	18.0
E. floccosum	32	21.3
Staph. aureus	120	80.0
Pseudomonas	17	11.3
Strept.	7	4.7

Table 3. The MIC of Petroleum ether extract of *L. inermis* against dermatophytes.

Extract	**conc. µg/ml**	***T. rubrum***	***E. floccosum***	***T. verrucosum***	***T. tonsurans***	***T. interdigitale***
of Petroleum ether	256	-	-	-	-	-
	128	+	+	-	-	+
	64	+	+	+	+	+

(+) indicates growth of fungi, (-) indicate inhibition of growth

Table 4. The MIC of Ethanol extract of *L. inermis* against dermatophytes.

Extract	**conc. µg/ml**	***T. rubrum***	***E. floccosum***	***T. verrucosum***	***T. tonsurans***	***T. interdigitale***
of Ethanol	128	-	-	-	-	-
	64	+	-	-	-	-
	32	+	+	-	-	+
	16	+	+	-	+	+
	8	+	+	+	+	+

(+) indicates growth of fungi, (-) indicate inhibition of growth

Discussion

Tinea pedis - or athlete's foot - is a superficial inflammatory infection of the skin of the feet caused by dermatophyte fungi. Among the fungi responsible for athlete's foot are *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum.* There are three clinical subtypes of athlete's foot: interdigital, moccasin, and vesiculobullous, with interdigital being the most common. The name 'athlete's foot' was derived from the increased occurrence of the condition among athletes, because of their use of occlusive footwear, which create the ideal conditions for dermatophyte reproduction. Athlete's foot is characterized by whitish macerated skin and itchy or asymptomatic erythema between the toes, usually in the fourth and fifth spaces. Blisters and cracks in the skin between the toes may cause pain and inflammation of the exposed raw tissue. A concomitant bacterial infection may also be present and require antibiotic treatment. Athlete's foot is typically diagnosed by visual inspection of the lesions, microscopy, and culture and may be differentially diagnosed as bacterial or candidal intertrigo, dermatitis, eczema, idiopathic

keratoderma, or psoriasis. Athlete's foot has been said to occur in roughly 1 in 5 adults, with a prevalence rate of approximately 10% in developed countries. This rate is presumably higher in most developing countries whose warm and humid climates are conducive to fungal infection[23]. The study by Vena etal. showed that the infection being most common among men between the ages of 25-44 years while the current study found the highest incidence of infection between the ages of 40-59[24]. The study by Ilkit M. etal. revealed that prevalence of T. pedis is highest among people aged 31-60 years and it is more common in males than in females[4]while the current study revealed higher prevalence in females(57.3%)than in males(42.7%). The difference in gender distribution of tinea pedis between various studies is due to their physical outdoor labors, nature of work and frequent interactions with different people of society. The study by Balamuruganvelu S. etal. revealed that *T. rubrum* is the most commonly isolated dermatophyte which agrees with the current study[25]. The same study observed that maximum prevalence of tinea pedis was recorded during Summer months(40%)while the current study shows the highest prevalence during Spring(28%)[25]. A higher incidence of cutaneous infections has been reported in obese compared with non obese patients. Intertrigo, candidiasis, furunculosis, erythrasma, tinea cruris, and folliculitis are frequent skin infections among obese patients. Tinea pedis and onychomycosis are more common in obese than non obese patients and in the long run may predispose the affected patients to acute bacterial cellulitis of the lower extremities. Adipose tissue participates actively in inflammation producing and releasing a variety of proinflammatory and anti-inflammatory factors including adipokines leptin and adiponectin, as well as cytokines and chemokines. Adiponectin is potently immunosuppressive, while leptin activates polymorphonuclear neutrophils, exerts proliferative and anti-apoptotic activities on T lymphocytes, affects cytokine production, and regulates the activation of monocytes/macrophages. Leptin induction seems to be a protective component of the immune response and genetic leptin deficiency in human beings has been associated with increased mortality due to infections. The genetic defect of leptin-deficient mice, which causes a severe obese phenotype, is associated with increased sensitivity to proinfl ammatory monocyte/ macrophage-activating stimuli and impairment of phagocytic functions, as well as reduced T-cell function. These mutant mice are highly susceptible to bacterial infections with, for example, Listeria monocytogenes, Klebsiella pneumoniae, etc.[22]. It was shown in a prospective observational study of 330 patients with venous stasis leg ulcers that a high BMI was linearly associated with poor healing of the ulcers[26]. The study by Alazab RM. etal. observed that the prevalence of tinea pedis in obese patients was 41.2% while the current study reveals 58.7% prevalence in obese patients[27]. The loss of protective sensation associated with diabetic neuropathy may result in patients being unaware of the fungal infection. Equally, if a patient has peripheral arterial disease and vascular insufficiency, this can reduce the tissue viability and protection from damage. Many studies reviewed the relationship between fungal infection of the feet (tinea pedis and onychomycosis) and cellulitis of the low extremities in people with diabetes. The results showed that fungal infection of the feet was a significant risk factor for cellulitis, a risk that

manifested in interdigital mycoses, onychomycosis, and sole infection[28]. The responsible agents are aerobic fungi that grow within the layers of stratum corneum in the form of branching hyphae, and that lack the ability to penetrate through the keratinized layer into the viable epidermis and internal structures. The feet pose a favorable environment for dermatophyte proliferation owing to their lack of sebaceous glands that produce fungistatic lipids. Masri-Fridling GD. found that *T. rubrum* is the most commonly isolated dermatophyte found in 76% of cases, *T. mentagrophytes* in 17% and *E. floccosum* in 1% of cases and candida in 6% of cases in addition to *T. tonsurans*. The current study demonstrates that *T. rubrum* is the commonest isolated dermatophyte 36.7%, *T. mentagrophytes var interdigitale* 18%, and *E. floccosum* in 21.3%. The difference between various studies is due mainly to sample size. Organisms that can be cultured from normal toe webs include such microflora as *Micrococcae*(staph.), aerobic coryneforms, and few G-ve bacteria. The interspaces can also be colonized by dermatophytes and yeasts such as Candida. Once the stratum corneum barrier is disrupted by dermatophytes, producing maceration and inflammation, bacteria are able to proliferate. Masri-Fridling GD. found staph. in 11% and G-ve in 35% of cases[29]while the current study finds *Staph. aureus* in 80%, Pseudomonas in 11.3%, and Strept. species in 4.7% of cases. Shiri M. etal. found that the most species isolated were *Microsporum audoinii,* candida, and *E. floccosum*[30]. Henna is believed as a medicinal plant, because of its antibacterial effects especially on gram positive bacteria, antifungal activity against dermatophytes, wound healing, antitumor effects, hypotensive, astringent and sedative effects.

Several studies are being carried towards its activities like cytotoxic, hypoglycemic, antimicrobial, antibacterial, antioxidant, trypsin inhibitory, wound Healing, analgesic, anti-corrosion, anti-inflammatory, antiparasitic, tuberculostatic , and hepatoprotective. Lawsone (2-hydroxynapthoquinone) is the most important constituent of the plant. Henna also contains flavonoids, sterols, tannins, saponins, tannic acid, gallic acid and etc. Antimicrobial activity may be due to these numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. Inhibitory action of henna was shown a wide range of microorganisms such as gram negative, gram positive bacteria and dermatophytes. Most of the studies showed that the leaves of *L. inermis* were found to exhibit strong fungitoxicity. The growth in culture media of the different clinical fungal isolates was suppressed when the water leaf extract of henna was used in different concentrations[30],[31],[32]. The study by Soares MM. etal. demonstrated MICs. Of some antifungal drugs against dermatophytes isolated from cases of T. pedis. It showed that MICs. of terbinafen, cetoconazole and ciclopirox olamine were 0.015, 32, and 32 µg/ml. against *T. rubrum, T. mentagrophytes,* and *E. floccosum* respectively. The study demonstrated also MICs. of some antiseptic solutions such as PVPI. about 128 µg/ml., boric acid 512 µg/ml., and propolis 2000 µg/ml.[33]. The study by Mesquita I. etal. showed that MICs. of various antifungal drugs such as Ketoconazole, Amphotericin B., Itraconazole and Fluconazole were varying between 32- 128 µg/ml. against some candida isolates[34]. These MIC values were nearly close to MIC values of Henna of the current study.

Conclusions

1- Tinea pedis is mostly caused by *T. rubrum* in Dour city population.

2- Most common secondary bacterial infection is caused by *Staph. aureus.*

3- Almost all ages were found susceptible to dermatophytosis with maximum number of cases was in the range of 40-49 years old.

4- Cases were isolated along the whole year with maximum number isolated in Spring season.

5- Higher prevalence of tinea pedis was in females than males.

6-DTM. can be used as a rapid screening medium for the isolation and identification of dermatophytes compared to SDA.

7- Ethanol and petroleum ether extracts of *L. inermis* (Henna) revealed antimycotic activity against tested fungi.

Recommendations

1- Accurate clinical data, including the appropriate diagnosis of the clinical forms of tinea pedis and the reliable identification of the causative fungus, will improve the education and knowledge of medical practitioners in the field. Molecular tools, such as PCR, improve the consistency and quality of diagnoses and patient care and are required to obtain clues regarding the source of infection.

2- Healthcare tips for managing athlete's foot include:

•Wear clean socks daily.

• Use suitable foot powder, or a medicated foot spray, both on the feet and inside the shoes.

• Air the feet as frequently as possible and dry shoes in the sun when not on the feet.

• Practice good foot hygiene, washing feet daily with soap and water, washing between the toes, and then also drying feet properly before putting on socks and shoes.

• Try to alternate between different pairs of shoes on a daily basis.

• Use topical and/or systemic treatment, as recommended or prescribed by a healthcare professional.

3- Consider oral antifungal agents in:

- Patients with a more treatment-resistant subtype of tinea pedis, e.g. moccasin, vesicular or ulcerative.

- Patients with interdigital tinea pedis that is severe and involves multiple interdigital spaces or has spread to the plantar aspect of the foot.

- Patients with a co-existing fungal nail infection If topical treatment has been unsuccessful.

4- Avoid sharing nail tools, such as clippers and scissors.

5- Avoid sharing sports gear, shoes, and towels with others.

6- When washing clothing, use hot water and bleach to increase the chance of killing fungi. Washing clothes in soapy, warm water may not kill the fungi that cause athlete's foot.

7- Some home remedies used to treat athlete's foot include: - *Hydrogen peroxide can effectively kill the fungus on the surface level of the foot, as well as any surface bacteria that could cause an infection. Pour hydrogen peroxide directly onto the

affected area. Note that it may sting, and it should bubble, especially if you have open wounds. Do this twice daily until the infection subsides.

* Tea tree oil: has antifungal and antibacterial properties. To treat athlete's foot, mix a carrier oil like warm coconut oil with tea tree oil for a concentration of 25 to 50% tea tree oil. Apply it to the affected area two times a day.

*Rubbing alcohol: can help kill off the fungus that is on the surface level of the skin. You can apply it directly to the affected area or soak your feet in a foot bath of 70% rubbing alcohol and 30% water for 30 minutes.

*Garlic: it may have a strong scent, but it can be an effective topical treatment for athlete's foot. One older study even found that a derivative of garlic, alone, resulted in a complete cure in 7 days. To use garlic to treat athlete's foot, crush 4-5 cloves of garlic. Once smashed, rub them over the affected area. Do this twice daily.

* Sea salt baths: sea salt has strong antibacterial and antifungal properties. It is used by mixing sea salt with other natural treatments like vinegar, to make a sort of paste.

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