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Prevalence of dermatophytosis among Suse federal prison inmates using conventional identification methods and PCR-RFLP typing

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Abstract

Dermatophytosis is a common skin infection especially in closed area with high temperature and humidity. Prisons are considered as closed areas especially for long times existed inmates in addition to some risk factors that may increase dermatophytosis. To investigate the prevalence and the risk factors of dermatophytosis in Suse federal prison for men at Sulaimani, northern Iraq, a total of 1620 inmates were clinically screened. Clinically suspected dermatophytosis by specialists were 139 (8.5%), they distributed on 102 (73%) Tinea pedis, 18 (12%) Tinea cruris, 14 (10%) Tinea corporis, 4 (3%) Onychomycosis, and 1 (0.7%) tinea mannum. By microscopical, cultural and PCR-RFLP typing procedures 50 cases were confirmed that distributed on 25 (50%) tinea pedis, 15 (30%) tinea cruris, 9 (18%) tinea corporis and 1 (2%) onychomycosis. The causative agents were *Trichophyton rubrum* 16 (32%), *Trichophyton mentagrophytes and Trichophyton interdigitale* 14 (28%) for each, *Epidermophyton floccosum* 4 (8%) and *Trichophyton schoenleinii* 2 (4%). *Trichophyton interdigitale* was the most frequently isolated from tinea pedis (40%), *Trichophyton rubrum* and *Trichophyton mentagrophytes* from tinea corporis (33% for each) and *Trichophyton rubrum* from onychomycosis and tinea cruris (%100 and 40% respectively). Antifungal administration and frequency of bathing were with significant effect on identification of the causative agent by direct KOH test (P= 0.000 and P= 0.026 respectively) and on its recovery by culture method (P= 0.000 and P= 0.016 respectively). The non-significant risk factors were age (P= 0.07), education level (P= 0.82), smoking (P= 0.61), and duration of imprison (P= 0.557). KOH exam was fast and cheap but it is less specific and sensitive. Culture method of identification is essential to identify species of dermatophytes, but it was time consuming and with problems related with confusion in dissemination between species morphologically that could be avoided by PCR-RFLP method which we consider it as a golden method for identification in spite of its high cost but it is with less time, correct identification, and highest sensitivity.

Introduction

Dermatophytes are filamentous fungi responsible for many superficial mycoses in human and animals because they can use and dissolve the keratin layer of the skin. Their pathogenicity depends on the ability to secret keratinases which regarded to their virulence factors, hence keratin is a main part of skin, hair, nails, hooves, horn and claws, so that they can infect broad range of hosts [1, 2]. Dermatophytes are opportunistic pathogens can accidentally infect human particularly during alterations in immune system. The pathogens cannot penetrate deeper tissue because they cannot well grow at body temperature (37° C) and/or the presence of immune system [3–5]. Dermatophytes are zoophilic and

geophilic, moreover they transmit through direct contact with infected humans (anthropophilic). Infections with dermatophytes mistakenly called tinea or ringworm, because of their raised and circular lesions. Based on the shape of macroconidia dermatophytes can be divided into three genera *Trichophyton* infect hair (tinea capitis), nails (tinea unguium or onychomycosis) and skin which may be tinea corporis (body), tinea cruris (groin), tinea mannum (usually hands) and tinea pedis (feet). *Microsporum* infects hair and skin while *Epidermophyton* infect nails and skin [6]. Dermatophytes infections usually affect immune suppressed, children, prisoners, soldiers and overcrowded area in parallel with environmental condition and socioeconomic situation [7]. Prisons are punishment places that are houses for those who committed the offences. Dermal infections are the common health problem in prisons with high prevalence [8]. It was reported that prisons are suitable condition for spreading various diseases especially skin diseases because of lack of proper treatment, high security concern and low medical supplies, therefore their health problems usually neglected, these are in addition to psychological conditions, low sanitary quality, overcrowding, sharing of footwear, bed and fomites increase skin contact helps to aggravate skin problems like dermatophytosis [9, 10].

Epidemiology is an effective way to disease control and global health problems concerned with various kinds of dermatophytosis [5]. Dermatophytosis epidemiology altered by effect of some factors such as change in life style, economic situation, population density, transportation and migration; *Trichophyton rubrum* has become the most prevalent species across the world, while in Mediterranean countries *Microsporum canis* is the main cause of tinea corporis and capitis. Tinea pedis has the higher frequency rate among others around the world especially among certain groups that has particular higher risk to infection such as miners, armies and runners of marathons due to sweating, trauma, footwear for a long time [11], also nearly 30% of mosque attendees [12]. The most prevalent infection in Europe is tinea pedis, while in USA and Mexico, onychomycoses, *T. rubrum* is the most etiologic agent for both types of infection [13–15]. In southern cities of Iraq *Microsporum canis* is the most common etiologic agent of dermatophytosis [16, 17]. In Baghdad *T. rubrum* was reported to be the most prevalent to cause tinea capitis which is the most common infection [18]. In Kurdistan region of Iraq, it was reported that *T. mentagrophytes* is the most common species causes dermatophytosis especially at Kalar and Erbil districts [19, 20]. In fact, there are some studies reported dermatophytes in Iraqi community, but there is no any report on dermatophytes in jails. This study aimed to survey the prevalence of dermatophytosis among prison inmates in Suse federal prison in Slemani province, to investigate the risk factors that increase the infection rates by using routine mycological methods and molecular identification of the causative agents including PCR amplification of the internal transcribed spacers of rDNA and typing by Restriction Fragment Length Polymorphism (RFLP).

Materials and methods

Study setting

The prison: fort Suse Federal Prison located at Suse village 38 Km Northwest Sulaimani city (35.7631, 45.1379), Kurdistan region / Iraq. It is inhabited by a total of 1620 heavy sentenced prisoners. All inmates were male with ages over 18 years old.

Specimen collection

Total of 1620 prisoners were observed by dermatologist of the Jail from Nov 2018 to May 2019. Nails were sampled by using sterile nail clipper whereas skin by scrapping with sterile surgical blade after whipped out with 70% ethanol [6]. All specimens were transferred immediately to laboratory for direct microscopy and culture.

Direct test and culture

Direct microscopy was performed by using 20% KOH and examining under magnification X10 and X40 [21]. Culture methods were performed by using Sabouraud Dextrose Agar (SDA) (Oxoid, UK) and SDA supplemented with 0.04 g/L Chloramphenicol (sigma-Aldrich) and 0.5 g/L cycloheximide (sigma-Aldrich) to exclude bacterial and other fungal species contamination. Plates were incubated at 30ºC for up to 3 weeks [6], [22].

Macro- and microscopical identification

Depending on phenotypic characteristics of fungi, macroscopically included colony growth, colony morphology, colony color and reverse colony color whereas microscopically by simple staining using safranin and lactophenol cotton blue followed by microscopical examination to observe the presence or absence of macro-microconidia and their shapes and arrangements. Differentiation between the species was according to colony morphology on Potato dextrose agar PDA (Accumedia, UK), urease test (Biolife, Italy), Cornmeal Dextrose Agar (Oxoid, UK). and Trichophyton Agar No.4 (Quelab, UK) [6].

PCR-RFLP

DNA extraction: liquid nitrogen was used to break out the cell wall of seven young colonies. Extraction was performed by using Easy pure genomic DNA kit (Trans gene, Korea). Yielded DNA was checked by Nanodrop spectrophotometer C2000 (Thermoscientific).

Polymerase Chain Reaction: Universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to polymerize the internal transcribed spacers of rDNA including ITS1 and ITS2 of the dermatophytes (Macrogene, Korea).[23]. The thermal cycler (Biorad T100, USA) was optimized; initial denaturation temperature 94ºC for 5 min, denaturation at 94ºC for 30 sec, annealing temperature 55ºC for 30 sec., extension 72ºC for 30 sec., and final extension at 72ºC for 5 min. The PCR products were checked on 1% agarose gel and photographed by using Gel Doc system (Biorad, USA). RFLP was performed for PCR products by using two endonucleases *Dde1* and *Mva1* separately according to the manufacturer instructions (Thermo Scientific, Lithuania). After incubation the digested DNA was run on 2% agarose gel [22]. The RFLP patterns were compared to [23]–[25], where for *Mva1 T. mentagrophytes* gives bands of 410/140/90 bp, *T. schoenleinii* 420/ 140/125, *T. interdigitale* 310/160/140/100 bp, *E. floccosum* 365/230/170 bp, and *T. rubrum* 380/180/110 bp, and size of fragments for *Dde1* are for *T. mentagrophytes* 427/ 155/101 bp, *T. schoenleinii* 343/156/100 bp, *T. interdigitale* 427/256 bp, *E. floccosum* 484/258 bp and *T. rubrum* 317/263/111 bp. The microscopical and molecular identifications were performed at department of Biology, college of science research laboratories.

Statistical analysis

Statistical analyses were performed using SPSS (V.26). Frequencies, percentages and data compared by using Chi-square test. P-values below 0.05 were considered significant. Specificity and sensitivity of direct test was calculated in which sensitivity is the proportion of true positives that are correctly identified by the test, specificity is the proportion of true negatives that are correctly identified by the test according to [26].

Results

Direct examination test

Out of 1620 prison inmates, 139 were clinically suspected to have dermatophytosis by the prison's dermatologist; 102 (73%) *T. pedis*, 18 (12%) *T. cruris* and 14 (10%) *T. corporis*, 4 (3%) Onychomycosis, and 1 (0.7%) tinea mannum. All suspected cases were subjected to direct KOH examination that showed 57 out of 139 with fungal infection through appearance of long tubular septated branched hyphae, but no spores or sporangia, while the others (82) were with no signs of fungal elements (figure, 1). The prevalence of dermatophytosis among inmates was 8.5%.

All the 139 clinical specimens of suspected cases, were cultured on SDA, 50 (36%) were positive for dermatophytes growth while the other 89 (64%) were failed to grow. Out of the culture positive specimens, 6 (13%) have showed negative KOH test. The culture negative specimens were excluded from further identification process even the thirteen KOH test positive specimens. Negative for both culture and KOH were 76 while 44 were positive for both and so the rate of false negative KOH test became 12%. The Sensitivity and the specificity of the direct examination test for dermatophytosis were 88% and 85% respectively.

Culture

Cultural results revealed *Trichophyton rubrum* the most prevalent species 16 (32%) followed by *T. mentagrophytes and T. interdigitale* 14 (28% for each), *E. floccosum* 4 (8%) and *T. schoenleinii* 2 (4%) (figure 2). The main causative agent of tinea pedis was *T. interdigitale* (40%), tinea corporis was *T. rubrum* and *T. mentagrophytes* (33% for each), onychomycosis and tinea cruris was *T. rubrum* (100%) (40%) respectively (table 1). The three species *T. rubrum, Epidermophyton floccosum* and *T. schoenleinii* were easy to identify by using PDA, cornmeal agar, and urease test while the identification of *T. mentagrophytes* and *T. interdigitale* remained with some doubt because of lack in clarity of their morphology which led us to use PCR-RFLP typing technique along with the three confirmed isolates to completely confirm them.

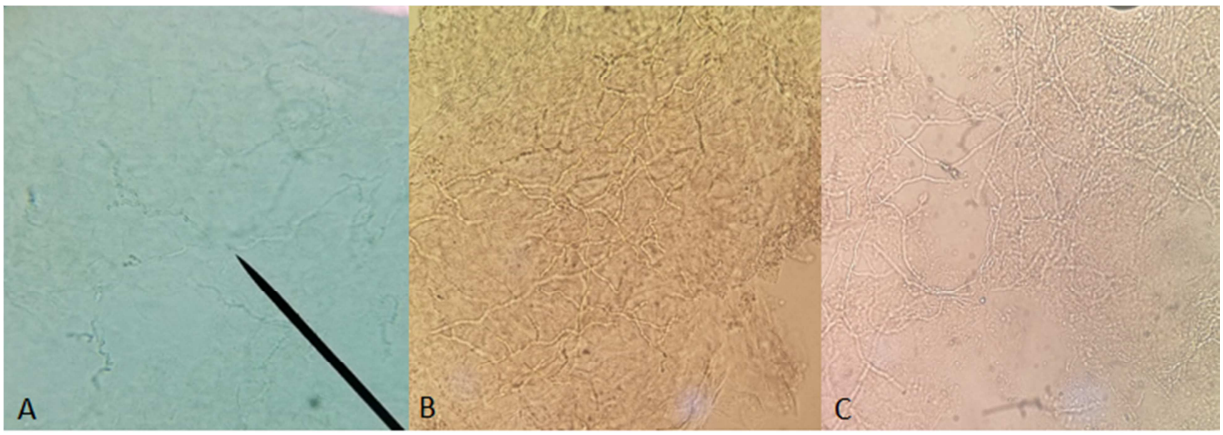

Figure 1. Potassium hydroxide preparations of skin specimens shows hyaline septated hyphae. A: staining with lactophenol cotton blue to enhance contrast. B and C were unstained. Compound light microscope (400X),

Table 1. Distribution and percentage of the Dermatophytes and infections according to each other.

		Dermatophyte Species					Total
		E. floccosum	T. interdigitale	T. mentagrophytes	T. rubrum	T. schoenleinii	
Onychomycosis	No. **	0 **(0 %)	0 **(0 %)	0 **(0 %)	1**(100%)	0 **(0 %)	1
	* %	0.0%	0.0%	0.0%	6.3%	0.0%	2.0%
Tinea Corporis	No. **	0 **(0 %)	2 **(22.2%)	3 **(33.33%)	3 **(33.33%)	1 **(11.11%)	9
	%*	0.0%	14.3%	21.4%	18.8%	50.0%	18.0%
Tinea Cruris	No. **	1 **(6.66%)	2 **(13.33%)	5 **(33.33%)	6 **(40%)	1** (6.66%)	15
	%	25.0%	14.3%	35.7%	37.5%	50.0%	30.0%
Tinea Pedis	No. **	3 **(12%)	10 **(40)	6 **(24%)	6 **(24%)	0 **(0%)	25
	*%	75.0%	71.4%	42.9%	37.5%	0.0%	50.0%
Total	No. **	4 **(8%)	14 **(28%)	14 **(28%)	16 **(32%)	2 **(4%)	50
	*%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

*% = percentage of species within each infection.

**%= percentage of the infection caused by each dermatophyte

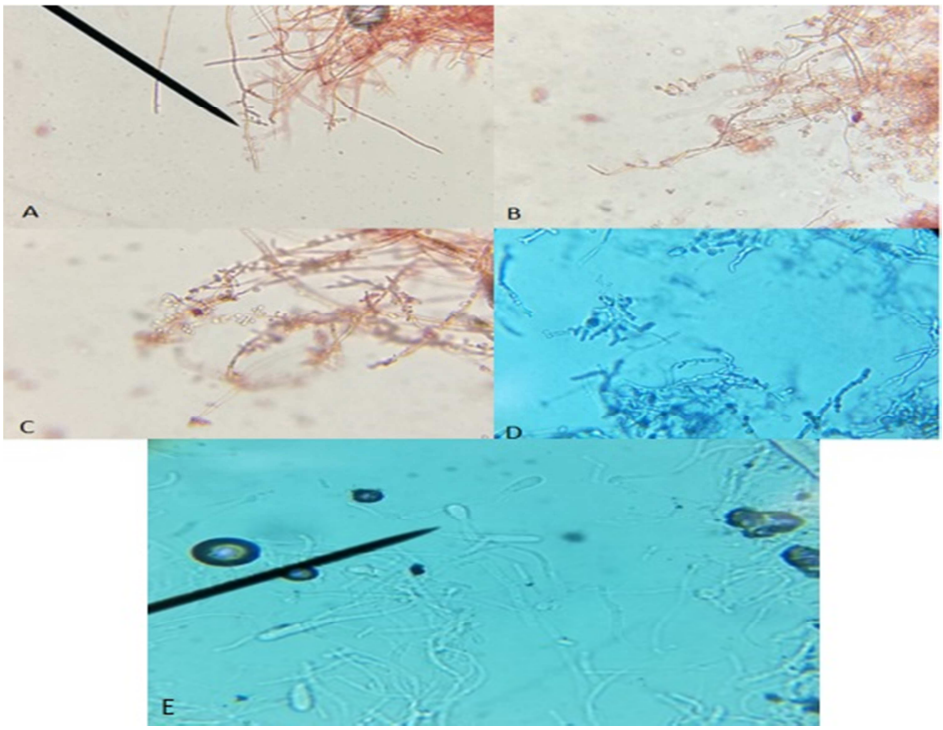

Figure 2. Slide preparations from culture using safranin (red color) and lactophenol cotton blue (blue color). (A) club-shaped microconidia of *T. rubrum*, (B) round shaped microconidia with spiral hyphae of *T. interdigitale*, (C) grape shaped microconidia of *T. mentagrophytes*, (D) antler shaped hyphae with no conidiospore of *T. schoenleinii*, (E) large smooth walled clavate shaped macroconidia of *E. floccosum* without microconidia. Compound light microscope (400X).

The amplification of the ITS regions of the five isolates gave the expected bands of the fungal rDNA on 1 % agarose gel between 640 and 790 bp. *T. mentagrophytes* gave a band of 683 bp, *T. schoenleinii* 690 bp, *T. interdigitale* 685, *E. floccosum* 790 bp and *T. rubrum* 690bp (figure, 3). Digestion by *Mva1* for the amplicons gave digestion patterns on 2% agarose gel, so that confirmed the mycological identification of the five species; *T. mentagrophytes* gives three bands 410/140/90 bp, *T. schoenleinii* 420/ 140/125, *T. interdigitale* 310/160/140/100 bp, *E. floccosum* 425/100/115 bp, and *T. rubrum* 380/180/110 (figure, 4). Also, for *Dde1* the DNA fragments were *T. mentagrophytes* 427/ 155/101 bp. *T. schoenleinii* 343/156/100 bp. *T. interdigitale* 427/256 bp. *E. floccosum* 484/258bp. and *T. rubrum* 317/263/111 bp. (figure 5).

The impact of antifungal administration was significant on recovery of fungi on plates (P= 0.000), and on positive direct KOH test (P= 0.000). The impact of bathing frequency/week on recovery of fungi on plates was significant (P= 0.016) and on the direct KOH test (P= 0.028). The other risk factors were non-significant which included age (P= 0.07), education level (P= 0.82), smoking (P= 0.61), and duration of imprison (P= 0.557).

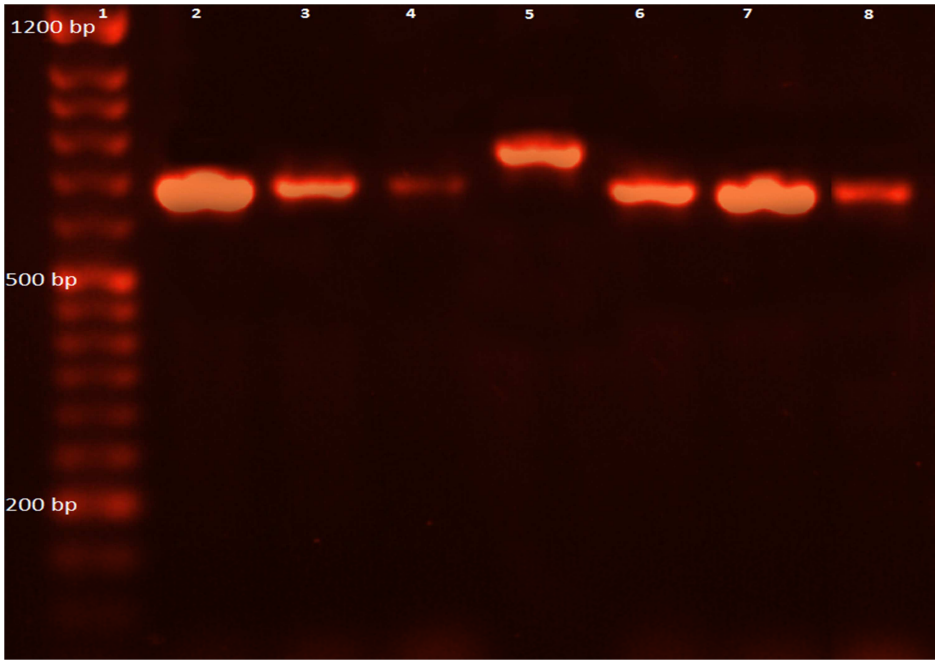

Figure 3. Agarose gel electrophoresis for PCR products of ITS regions. Lane 1: DNA ladder, Lanes 2,6: *T. mentagrophytes*, lane 3: *T. schoenleinii*, lanes 4,7: *T. interdigitale*, lane 5: *E. floccosum*, lane 8: *T. rubrum*.

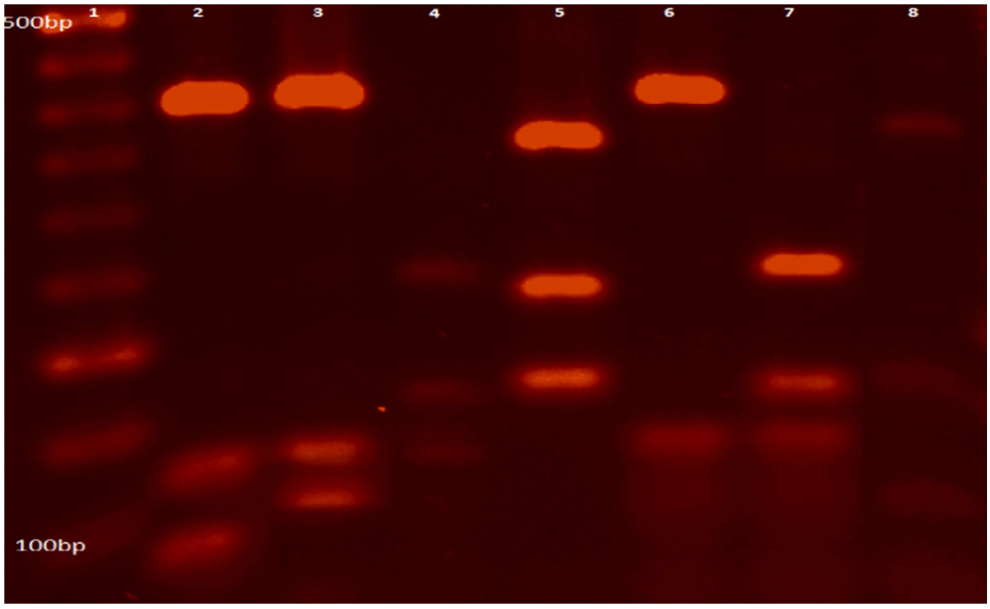

Figure 4. RFLP patterns by using *Mva1* endonuclease; Lane 1: DNA ladder,. Lanes 2,6: *T. mentagrophytes*, lane 3: *T. schoenleinii*, lanes 4,7: *T. interdigitale*, lane 5: *E. floccosum*, lane 8: *T. rubrum*.

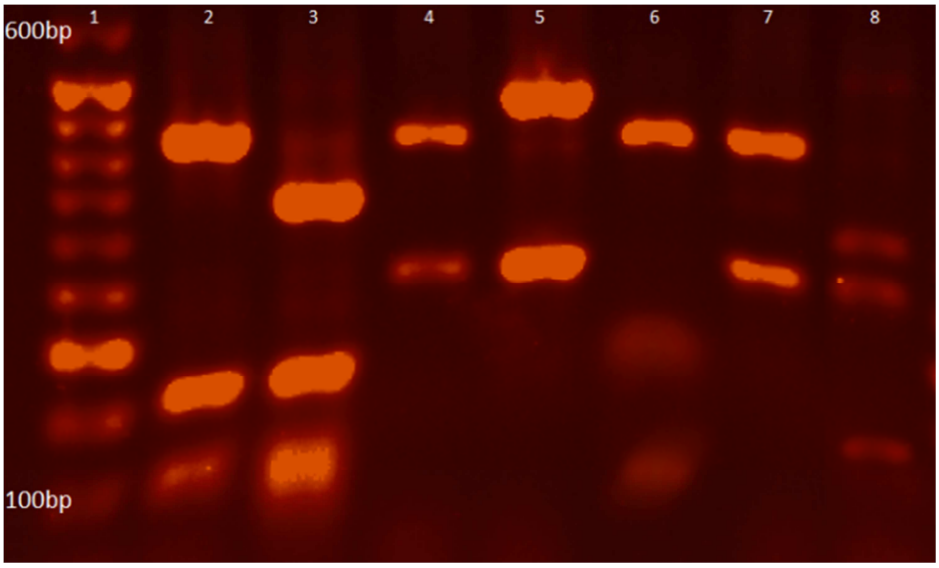

Figure 5. RFLP patterns of *Dde1* endonuclease; Lane 1: DNA ladder, Lanes 2,6*: T. mentagrophytes,* lane 3*: T. schoenleinii*, lanes 4,7: *T. interdigitale,* lane 5: *E. floccosum*, lane 8: *T. rubrum.*

Discussion

The most common dermatosis are fungal infections, especially dermatophytosis. These problems can affect prisons and inhabitants of crowded area with regarding environmental and socioeconomic conditions [7]. Therefore, a proper identification is needed to control the infection. The sensitivity of the direct test for primary identification was 88% and the false negative was 12%, it is normal that false negative results are up to 15% [5]. It was mentioned that false negative results are due to lack of enough experiences to detect fungal elements and the amount of the samples are not enough to be diagnosed easily [27]. Here, the treatment by antifungals has effect on identification of the causative agents. In fact, the direct KOH examination declared that it is very important in diagnosis of dermatophytosis because of its fast, low requirement, inexpensive, and it can give the clinician good information. Although the direct KOH test has low specificity and sensitivity, it is effective to the diagnosis of dermatophytosis. Close results were obtained in Nigeria from prisons which indicate the impact of bathing on dermatophytosis [10].

Inmates who bath fewer times per week showed more dermatophytosis frequency, this may be due to excess sweating and repeat expose to the inoculum without bathing. Antifungal therapies have significant impact on culture results (p-value = 0.000), this means that the recovery of dermatophytes from those who used antifungals were impeded. Results revealed that age is not significant, may due to the small available space for physical activities and low climate temperatures during sampling. Whereas in previous study age groups play important role in rate and types of dermatophyte infections, in which the prevalence of dermatophytes is higher at lower ages due to higher activity rate and excessive sweating [10]. With respect to duration, the physician specialist of the prison has mentioned that patients have got infection at first months of imprison, this may due to psychological conditions [28], overcrowding which increase skin contact and rapid changing environment. smoking was non-significant to the infection as it was reported also [29] whereas other reported it significant but without a proper interpretation [30], [31]. Indeed, smoking causes alterations in host immune responses and increases risks for common infections for both active and passive smokers [32]. Education levels has no significant effect on dermatophytosis among inmates as it has been also reported by[29] which may due to the absence of cleaning supplies and antimicrobial treatments to prevent spread and develop of infection or may despite of their levels of education they do not have awareness for fungal infection. It was reported that tinea pedis is more prevalent than others especially after pubescence period, due to its transmissible rout and reinfection nature [33].

In Erbil 146 Km in northwest Sulaimani, it was reported that tinea pedis is the most prevalent [20]. Among prison inmates, tinea pedis was mentioned to be the more prevalent [34]. Our results revealed the high prevalence tinea pedis as reported in other literatures; [35] reported that 70% of all population had tinea pedis at a time of their life. The common occurrence of tinea pedis may be due to sharing space, clothes and personal requirements; dermatophytes have been isolated from shoes and socks of infected persons [36]. Because of the prisoners shared bathroom, slippers, towels and foot wears and even barefoot we hypothesize that these may be

additive risk factors lead to spread fungal infections among them. The justification of this hypothesis is potentiated in a previous finding also; dermatophytes were isolated from floor of bath, swimming pools and changing room, fungal elements can survive for months in shed skin scaled infected with fungi, desquamation due to alkaline soap as well as hot and humid environment can help to disperse infection [37]. In the heavy sentenced prisons, the prisoners are staying for long times, therefore they are continually exposed to favor conditions for invasion by fungi without suitable treatment [10]. Another reason of wide spreading of infection among inmates may be due to psychological conditions of prisoners since prison inmates suffer severely from stresses than normal population and so prisoners are more susceptible for infections [28]. Tinea pedis can serve as reservoir for other parts of the patient body to get infection by autoinoculation [38]. Some of inmates had history of infection before they have been put in the prison may served as reseviors for infection of other parts of their bodies and even other prisoners which may increase the prevalence rate**.** Here, the most common etiologic agents of Tinea pedis was *T. interdigitale* followed by *T. rubrum* and *T. mentagrophytes* then *E. floccosum.* It was reported that *T. interdigitale* is the common causative of tinea pedis [14], [35], [39], whilst a study revealed that *T. rubrum* and *T. mentagrophytes* are the common species [40]. So that it can be predicted that *T. interdigitale T. rubrum* and *T. mentagrophytes* are the common causatives of tinea pedis either in prisons or out of them. This conclusion is adequate also for all types of tinea that these species are common in spite of that *T. rubrum* is the most common among all types of tinea [41], [42]. Borman *et al*. (2007) [41]suggested that there is no definitive explanation to how those dermatophytes that cause Tinea pedis are more prevalent, but Seebacher *et al*. (2008) [14] thought that increase in prevalence of Tinea pedis leads to increase the prevalence of its etiologic agents. However, Tinea cruris and tinea corporis were the most prevalent after tinea pedis. Overcrowd, environmental condition like humidity, temperature, and personal activity help to spread infection more, skin sweating and macerations provide adequate condition to get infection, autoinoculation is always occurs especially by tinea pedis [44]. The frequent causative agents for tinea cruris and tinea corporis were *T. rubrum, T. mentagrophytes,* while *T. interdigitale, T. schoenleinii* and *E. floccosum* were less common respectively. These results are in agreement with Smith and McGinnis (2011) [45], while Muhsin *et al.* (1999) [44] who worked in Iraq reported different findings, they said *E. floccosum* is the most common one. The climate of southern Iraq and Saudi Arabia are nearly similar [46], so Khaled *et al.* (2015) [47] in Saudi Arabia and Najem (2015) [16] in southern Iraq reported identical results but different from that in the current study; they reported that tinea capitis is the common and *M. Canis* is the common etiologic agent.

In fact, tinea capitis mainly occurs at childhood and rarely in adults since the secretion of fatty acids after puberty protects the hair against infection [48]. The current results have also revealed the same findings even if it was conducted at prison. Onychomycosis was less prevalent (3%). Indeed, Onychomycosis has been reported as less prevalent [39] and the most common causative is *T. rubrum* [13], [49], the same results are also obtained here.

Jackson *et al.* (1999) [50]used universal primers for identification of major common species of dermatophytes through amplification of ITS regions. ITS1-5.8S-ITS2 regions of all dermatophyte species are ranges from 614 bp for *M. gallinae* to 780 bp for *E. floccosum*, all of them have a same sequence at 5.8S region but differ in internal transcribed spacers (ITS1 and ITS2). Thus, ITS regions have enough property to identify dermatophyte species [25]. PCR product length have the ability to discriminate dermatophytes at the species level for example *E. floccosum* which shows ideal band size of 780 bp [51]. The PCR identification was, as had been expected, in agreement with those amplifications conducted by Rezaei-Matehkolaei *et al*. (2012) and Shin *et al*. (2003) [23], [25]. Although the amplifications were with the predicted results but the variation at the ITS spacers may make some interruption in the identification at the species level or even between varieties, so a complementary procedure should be done to discriminate dermatophyte species. For this purpose the RFLP was suggested which is considered as the most common, reproducible and easy [50]. So that, this test is needed to confirm the identification by the mycological ways and the amplification of rDNA fragments. The results were expected for the five species that have been identified mycologically as they were agreed with the results of [23, 25]. Two isolates that were identified as *Trichophyton* have not been differentiated easily at the species level because that the hyphae were not typical to either the zoophilic *T. mentagrophytes* or the anthropophilic *T. interdigitale* while the PCR-RFLP revealed discrimination between them into *T. mentagrophytes* and *T. interdigitale* easily and so this test appeared to be more essential in identification of dermatophyte species. In fact, nowadays all anthropophilic *T. mentagrophytes* are taxonomically under *T. interdigitale* [13].

In Africa, among inmates in Nigeria and Burkina Faso, reports have mentioned that prevalence of dermatophytosis is higher than that of the current study [8, 52, 53]. Excluding the only one report in USA because it is old, which reported lower prevalence [54], in Europe, Belgium and France, the prevalence has been reported to be lower but close to the current study [30, 55]. The climate of prison may be affected by the geographical location which may discuss the interruption in prevalence rate between tropical and temperate zone where Suse prison of northern Iraq is located in a zone of Mediterranean climate close to that of Europe and so it is logically that the infection becomes lower than at Africa. Other factors are the level of health awareness of the prisoners and the healthcare services of the prisons. From the parameters regarded here it was appeared that the level of awareness and healthcare services at Suse prison is somewhat good that mimics the low prevalence of infection.

Conclusion

Prevalence of dermatophytosis was 8.5% among Suse federal prison. The high prevalence of dermatophytosis among inmates was tinea pedis which frequently caused by *T. interdigital* whereas *T. rubrum* was the most frequent species among all species. The significant factors decreased dermatophytosis were bathing frequency and administration of antifungal therapy. KOH exam was fast and cheap but it is less specific and sensitive. Culture method of identification is essential to identify dermatophytes species but it was time consuming and has problems related with confusion in dissemination between species morphologically that could be

avoided by PCR-RFLP method which we consider it as a golden method for identification with highest sensitivity and less time in spite of its high cost .

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