

Isolated and identification of mycosis infection associated with psoriasis

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ABSTRACT

At the AL-Ramadi Teaching Hospital in Al-Ramadi, seventy samples of hair, nails, and skin were collected from patients with probable dermatophytosis of various ages and genders. between the 8th of December and the 2nd of February (2020-2021). There were 32 males and 38 females among the 70 patients tested, with ages ranging from 8 to 76. The largest rate of dermatophyte infection was found in the age group 21-40, with 33 cases, followed by the age group 0-20, with 20 cases, and the age group 41-60, with 8 cases, while the lowest rate was found in the age group 61-80, with 3 cases (4.3 percent). All of the specimens To identify the dermatophyte species, they were examined directly (KOH 10%) and cultivated on Sabourauds dextrose agar media. In both males and females, direct microscopic inspection indicated positive results, and cases were verified by culture (containing dermatophytes and *Candida* spp.). *Tinea pedis*, *Tinea capitis*, *Tinea corporis*, and onychomycosis were the most prevalent clinical types. *Tinea cruris* and *Tinea faciei* are two species of *Tinea* that grow together. had the lowest infection rates because they were both. Males were found to have more *T. pedis*, *T. corporis*, *T. cruris*, and *T. faciei*, whereas females had more *T. pedis*, *T. corporis*, *T. cruris*, and *T. faciei*. were found in greater numbers in males. Six species from two genera were discovered among 26 isolated dermatophytes species. *Trichophyton rubrum*, followed by *Trichophyton verrucosum*, was the most commonly isolated etiologic agent in the total. Other dermatophytes included *Microsporum canis*, *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, and *Microsporum gypseum*, in that order. The CTAB technique was used to extract genomic DNA from all isolated samples (dermatophytes and *Candida* spp.) with a purity of 1.7-1.8. To identify dermatophytes and *Candida* species, the ITS regions of all extracted DNA were amplified using the ITS1/ITS4 primer combination. The goods were excellent. Electrophoresed 2 percent agarose gels in TBE buffer were electrophoresed, and the results were visualized using ethidium bromide staining under UV irradiation.

Keywords: mycosis infection, psoriasis

Introduction

Psoriasis is a genetically established, systemic immune-mediated chronic inflammatory disease that mostly affects the skin and joints, as is widely recognized. Psoriasis is a skin condition in which skin cells develop up to ten times faster than they should. The skin becomes rough red spots covered with white scales as a result of this. They can grow on any part of the body, although the majority of them appear on the scalp, elbows, knees, and lower back.

Psoriasis is a chronic inflammatory skin disease whose pathogenesis involves a deregulated interplay among immune cells, keratinocytes, and environmental triggers, including microbiota. Bacterial and fungal dysbiosis has recently been linked to a number of chronic immune-mediated diseases, including psoriasis M. Surcel, A Munteanu, G Isvoranu, A Ibram, and others (2021). Psoriasis can be mentally and physically disabling. Patients do not only have to deal with their highly visible skin disease. Studies have shown that psoriasis causes as much disability as dermatophytosis and cannot be easily diagnosed based on clinical manifestations as many other conditions mimic the clinical presentation. R. Bubnov, M. Spivak, M. Spivak, M. Spivak, M. Spivak, M Dermatophytosis has a wide range of differential diagnoses, including seborrhoeic dermatitis, atopic dermatitis, contact dermatitis, psoriasis, candidalintertrigo, erythrasma, and eczema. Furthermore, diagnosing dermatophytosis in immunocompromised patients is more difficult because of the unique clinical presentation. A. Hovnanian, E. Petrova (2020). In order to implement suitable treatment and prevention measures, good

laboratory methods must be available for rapid and exact identification of the dermatophytes implicated (Garg et al., 2009 and F Abdallah, L Mijouin, C Pichon-Mediators of inflammation, 2017).

In the past, dermatophyte infections in humans were dismissed as minor and non-life threatening. L Abel, JL Casanova, L Abel, L Casanova, JL Casanova, L Casanova, JL Casanova, JL Casanova Furthermore, many of the most important etiologic agents were limited to specific geographic locations and did not cause widespread epidemics. However, over the last two decades, demographic shifts have resulted in widespread dermatophyte diseases. Dermatophytes fungi-caused cutaneous illnesses now affect people of all ages around the globe. TA Bitencourt, AL Fachin, MF Petrucelli, etc. (2021)

The millions of people infected with these viruses contribute to annual healthcare expenses in the tens of millions of euros, particularly for treatment when the diagnosis is not made soon (C de Andrade Monteiro et al., 2019; Roque et al., 2006).

Since the early nineteenth century, a traditional method has been used to investigate macroscopic and microscopic aspects of fungal cultures. However, due to the polymorphic nature of these features, these methods appear to be difficult to amplify, even when amplified by a factor of ten.

Differences in medium components, temperature swings, and other culture variables are all factors to consider. Furthermore, in rare situations, dermatophytes do not form reproductive structure in culture (sterile mycelia), making final identification problematic. Aside from that, due to anomalous microscopic or macroscopic morphology, the traditional approach is frequently challenging. Molecular investigations have become increasingly important and vital for identifying pathogenic fungus (KD Hyde, AMS Al-Hatmi, B Andersen, T Boekhout, 2018)

The internal transcribed spacer (ITS) ,sections of fungal ribosomal DNA (rDNA) have been employed as one of the approaches for species identification since they are quicker, more accurate, and less susceptible to external influences , such as temperature fluctuations and chemotherapy (Aala et al., 2012).Alignments of consensus sequences by MEGA software showed that almost all dermatophyte species have an expectedly similar sequence in the 5.8S subunit but are different in the ITS1 and ITS2 non-coding regions of the rDNA complex (Rezaei-Matehkolaei et al., 2012). This entry was posted on July 8, 2010.

This work used universal primers for amplification of the ITS gene to identify dermatophytes species in clinical isolates using both traditional and molecular approaches. Furthermore, using the PCR technique, pathogenic fungi were isolated from patients with various cutaneous illnesses in Ramadi city and identified at the species level. -..., JR Köhler, B Hube, R Puccia, A Casadevall.. (2017)

Collection of Specimens

Seventy-five samples were collected from the AL-Ramadi Teaching Hospital in Ramadi City. Hair, nails, and skin samples were taken from suspected patients. Dermatophytosis occurs in people of all ages and both sexes between the ages of 8 and 8. Using sterilized forceps, fine scissors, and a scalpel, samples were taken. Scraping skin is a good example of suitable material. Knives, nail scissors, and 70% ethanol Depending on the affected site, hair or nail clippings were used, and the affected area was disinfected with 70% ethanol before sample collection. A portion of the samples was used for direct KOH 10% screening, and the rest was used for inoculationSDA culture media for isolation of pathogenic dermatophytes.

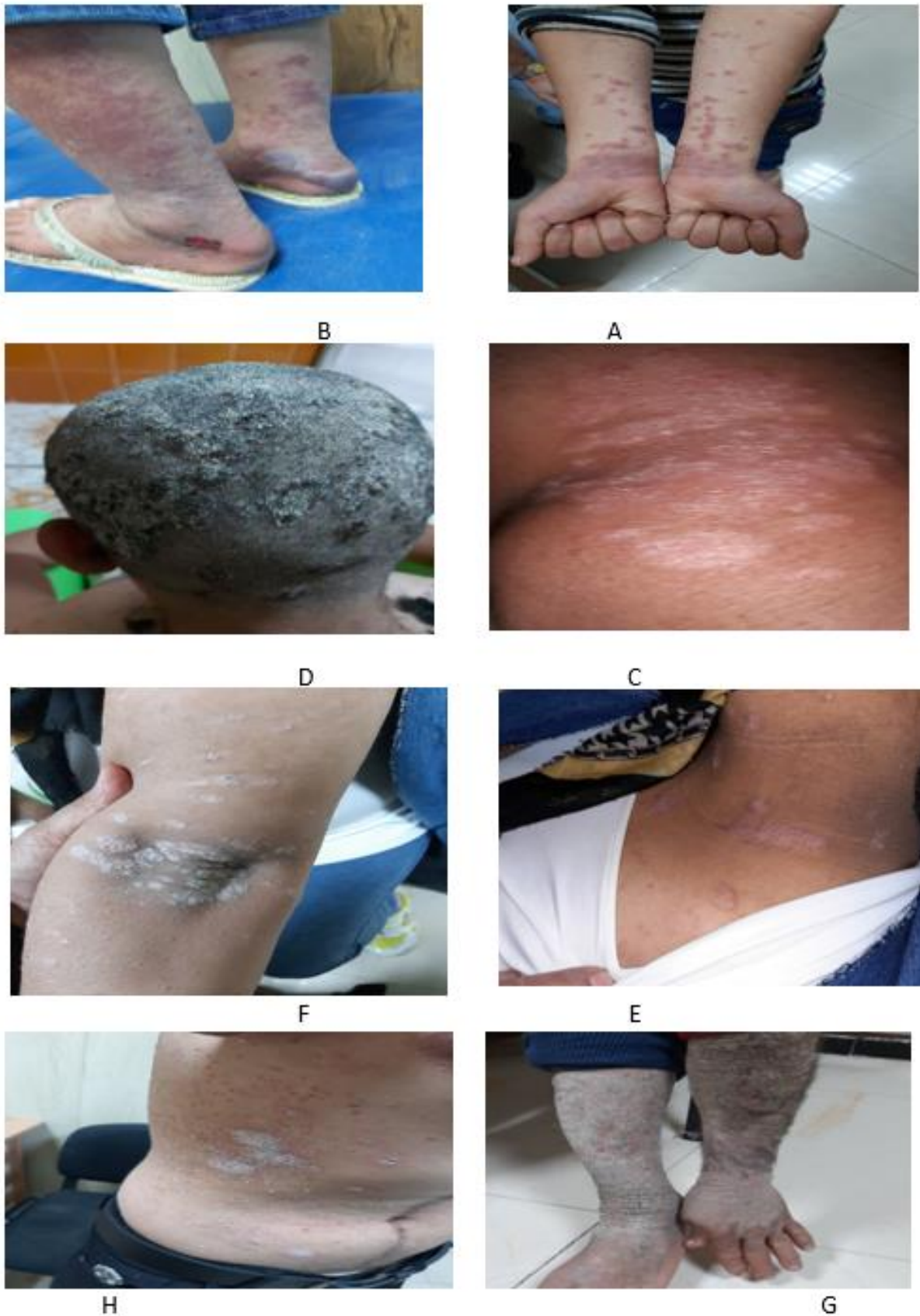


Figure 4.3. Dermatophytoses in various clinical manifestations from various individuals

A,D,G ,H ; T. manuum

C ; T. pedis

E;T. capitis H; T. faciei

F , B , I , J ;T. corporis

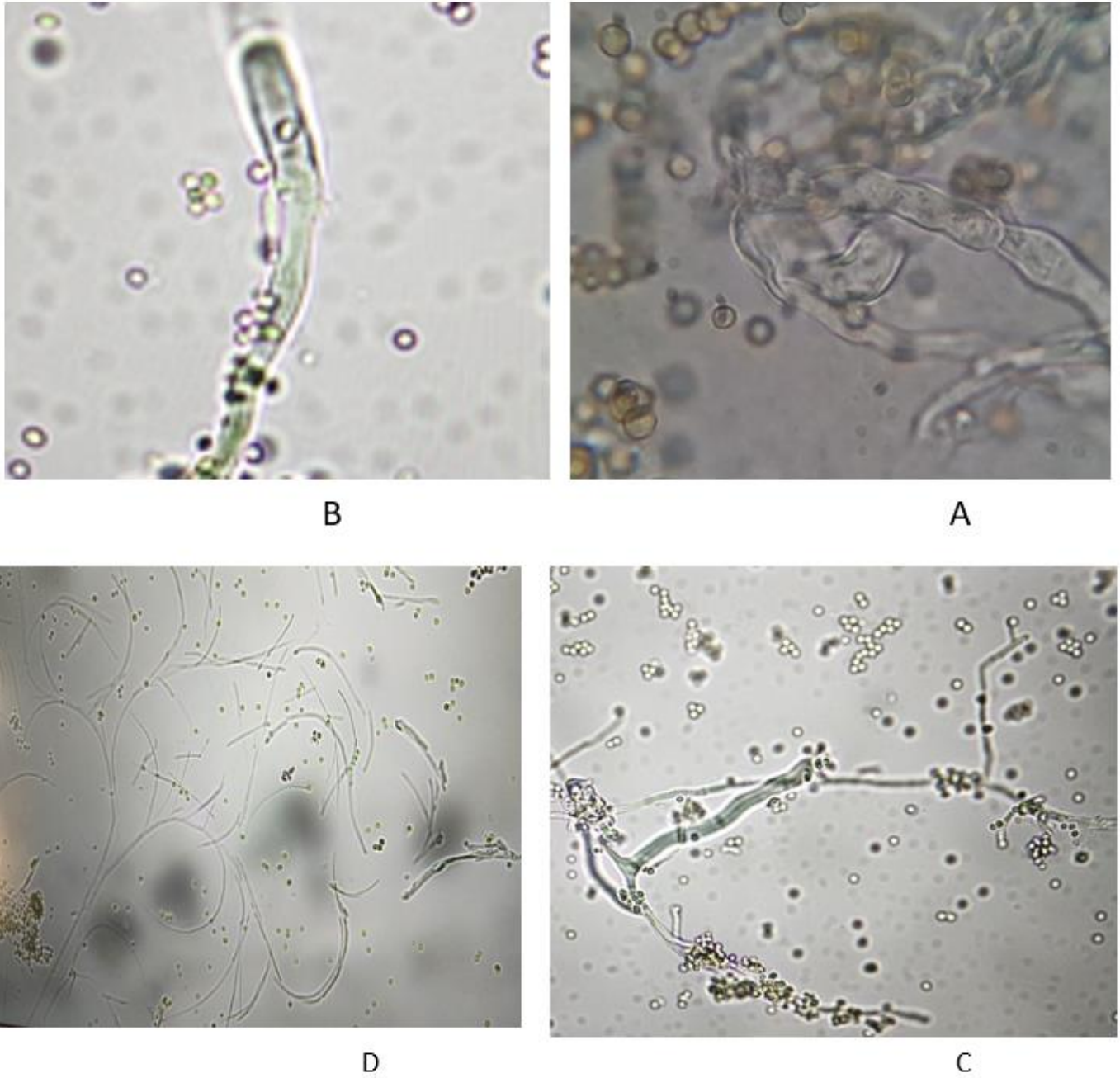


Figure 4.7. Microscopic morphology of different dermatophyte species isolated from patients with dermatophytosis

macro and micro conidia(A; *M. canis* , B; *T. rubrum* (microconidia) , C;*T. interdigitale* (microconidia) , D;*T. verrucosum* (chlamydospore)

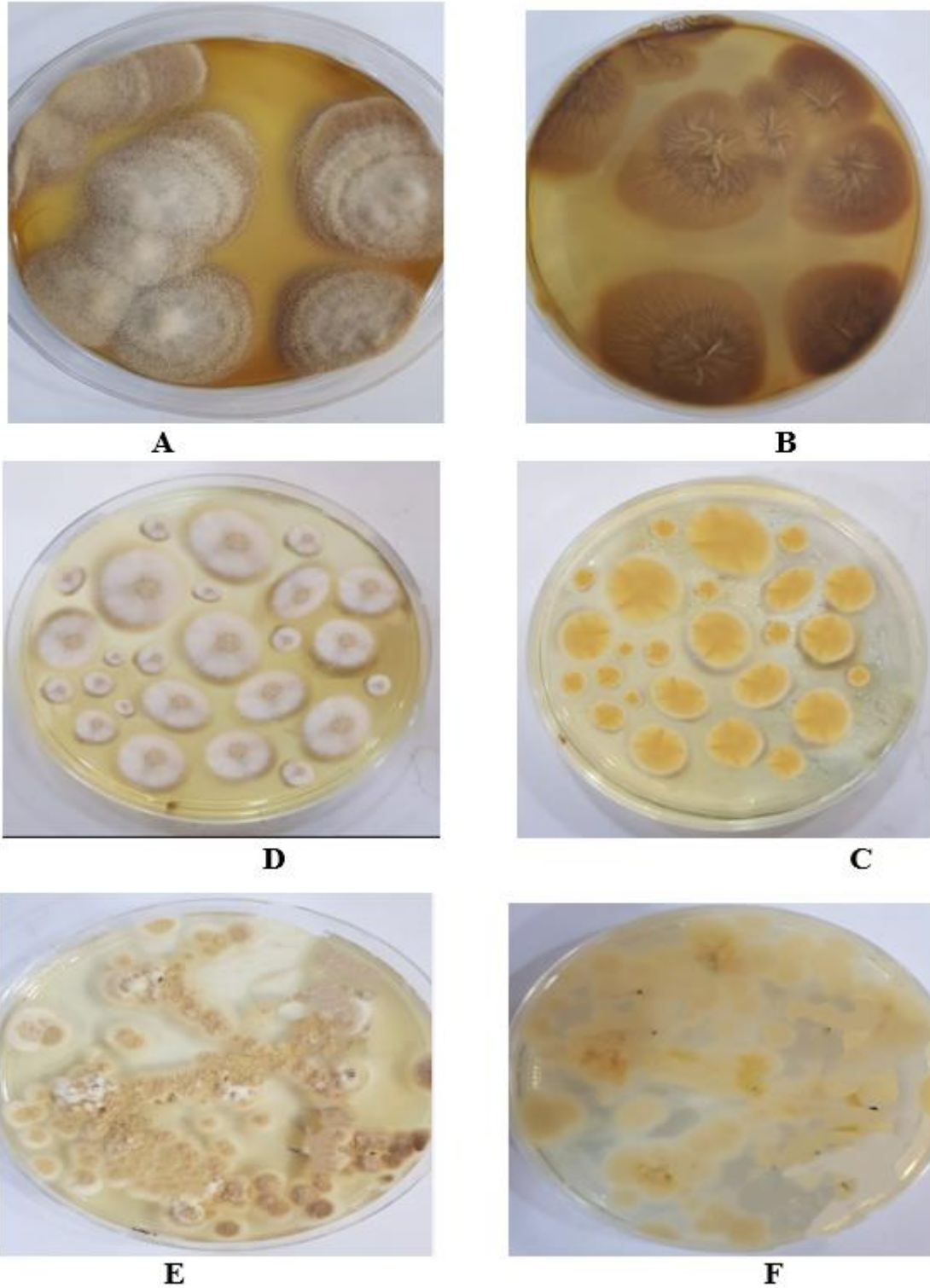


Figure 4.8: Morphological appearance of colonies of different dermatophyte species
A: : culture of *T. rubrum* (front) **B: culture of *T. rubrum* (reverse)**
C: culture of *M. canis* (front) **D: B: culture of *M. canis* (reverse)**
E: culture of *M. gypseum* (front) **F: culture of *M. gypseum* (reverse)**

Diagnostic kits and their Companies and Origins

Kits	Company	Origin
latex kit	Oxoid	England
LT master mix	Bioneer	Korea
Primers	Bioneer	Korea
DNA Marker (100-1000) bp	Bioneer	Korea
DNA extraction kit	Promega	USA
TBE buffer 1x	Promega	USA

Molecular Diagnosis

Extraction of Genomic DNA from fungi culture and purification:

Total fungi genomic DNA was isolated according to the Promega kit (USA) as mentioned in the following procedure:

Procedure

Fungi growth was taken by loop and placed in brain heart infusion broth to do suspension and incubated for 24 h. when suspension become turbid that was meaning bacteria growth , after this process DNA could be isolated by using Promega kit.

1. One ml of an overnight culture was added to a 1.5ml micro centrifuge tube.
2. The sample was Centrifuged at 13,000–16,000× g for 2 minutes to pellet the cells. The supernatant was removed.
3. Six hundred µlof NucleiLysis Solution was added. Gently pipet until the cells were resuspended.
4. The tube was incubated at 80°C for 5 minutes to lyse the cells; then cool to room temperature.
5. Amount of 3µl of RNase Solution was added to the cell lysate. The tube was inverted 2–5 times to mix.
6. The tube was incubated at 37°C for 15–60 minutes. The sample was cool to room temperature.
7. Two hundred µl of Protein Precipitation Solution was added to the RNase-treated cell lysate . The tube was vortexed vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.
8. The sample was incubated on ice for 5 minutes.
9. The tube was centrifuge at 13,000–16,000 × g for 3 minutes.
10. The supernatant containing the DNA was transfered to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.
11. The tube was gently mixed by inverted until the thread-like strands of DNA form a visible mass.
12. The tube was centrifuge at 13,000–16,000 × g for 2 minutes.
13. The supernatant was carefully poured off and the tube was drained on clean absorbent paper. 600µl of room temperature 70% ethanol was added and the tube was gently inverted several times to wash the DNA pellet.
14. Then the pellet was centrifuge at 13000-16000 g for 2 minutes. The ethanol was carefully aspirated.
15. The tube was drained on clean absorbent paper and the pellet was allowed to air-dry for 10–15 minutes.
16. One hundred µl of DNA Rehydration Solution was added to the tube and rehydrated the DNA by incubating at 65°C for 1 hour. Periodically the solution was mixed by gently tapping the tube.

Alternatively, DNA rehydrate by incubating the solution overnight at room temperature or at 4°C.
17. The DNA was Stored at 2–8°C.

DNA quantitation

A spectrophotometer 2600 uv/vis Unico was used to measure the DNA samples isolated from fungus growth (USA). At 260 and 280 nanometers, measurements were taken (Sambrook et al., 1989). All samples were stored at -20°C until they were utilized.

1- For 30 minutes, the DNA for testing was removed from cold storage, and the bottoms of tubes were wiped clean. were flicked to verify that the complete DNA was properly resuspended.

2-After that, the samples were centrifuged for 1 minute at 12000 x g.

3-A 1000 l distil water blank was utilized to the first Q quartz cuvette of the spectrophotometer

4- 10 liters of DNA were mixed with 990 liters of D.W. in the second quartz cuvette (sample cuvette). The 2600 uv/vis Unico was put on the first cuvette after the spectrophotometer was zeroed to get an absorbance reading.

5- The spectrophotometer was rezeroid between each wavelength reading, with readings obtained at 260 nm (OD260) for sample DNA and 280 nm (OD 280) for sample protein concentration.

6-The ratio of the readings (OD260/OD280) was used to estimate sample purity, which should be between 1.3-2.

Protein contamination of the DNA is indicated by a value less than 1.3. (Sambrook et al., 1989).

7-The following equation was used to calculate the concentration of DNA (g/l):

Concentration (g/l) = OD 260 reading x total/sample volume x constant 50.

The DNA concentration ratio was found to be in the range (3-12).

Agarose Gel Electrophoresis:

Agarose gel electrophoresis is a procedure that include running DNA in to agarose gel and then apply an electric current to the gel.

Component of Gel Electrophoresis

- Agarose
- TBE buffer (1x)
- Loading dye (6x)
- Ethidium bromide (10 mg/ ml)

A-Agarose Gel Preparation for Pre PCR:

After DNA Quantitation the next step including pre PCR for see DNA present or no. that use agarose 0.70 g dissolved in (10 ml TBE buffer +90ml D.W.) and heating on flame for 5-10 min. Until completely melted and leaves to cool at room temperature and placed in the tank for15- 30 min. The gel chamber end with sticky plastic tapes. When the agarose gel had cooled down and become solid, the comb was removed carefully by gently pulling it straight up with the tray surrounding tapes (Sambrook et al., 1989).

Followed by added 4 µl methylin blue Loading Dye +10 µl DNA sample mixed both and take 14 µl by micropipette in well. The gel with tray was laid into the chamber with 1x TBE , and assured that the gel was completely covered With TBE, until top surface of the gel submerged with approximately 2 min, and that the wells were at the negative electrode . The safety cover was placed onto the chamber carefully ensuring that both plugs were secured and connected with power supply (Sambrook et al., 1989). Electrophoresis condition was set up at 125 volts for 1 hours if use large tanke while if use small tanke Electrophoresis condition was set up at 75 volts for 1hour. After that the power supply was turned off, and disconnected the leads. The DNA fragments were observed by examining the gel under UV light of

transilluminator with protective glasses (Sambrook et al., 1989).

B- Agarose Gel Preparation for Post PCR :

As a result , the small DNA fragments move faster than the larger fragments through the gel toward the positive electrode. All PCR product of the samples were detected and analyzed after amplification by

Agarose gel electrophoresis followed by detection of the specific bands in ultra violet light at (302 nm).

Agarose gel was prepared in 2% for hly A that same to procedure of Paton and Paton, (1998), and 1.5% for fliCH7, rfbO157 that same to procedure of Reza and Sakineh, (2013), by dissolving 1.5 g and 2 g agarose in 100 ml of 1x TBE buffer by boiling on flame for 5-10 min. until completely melted.

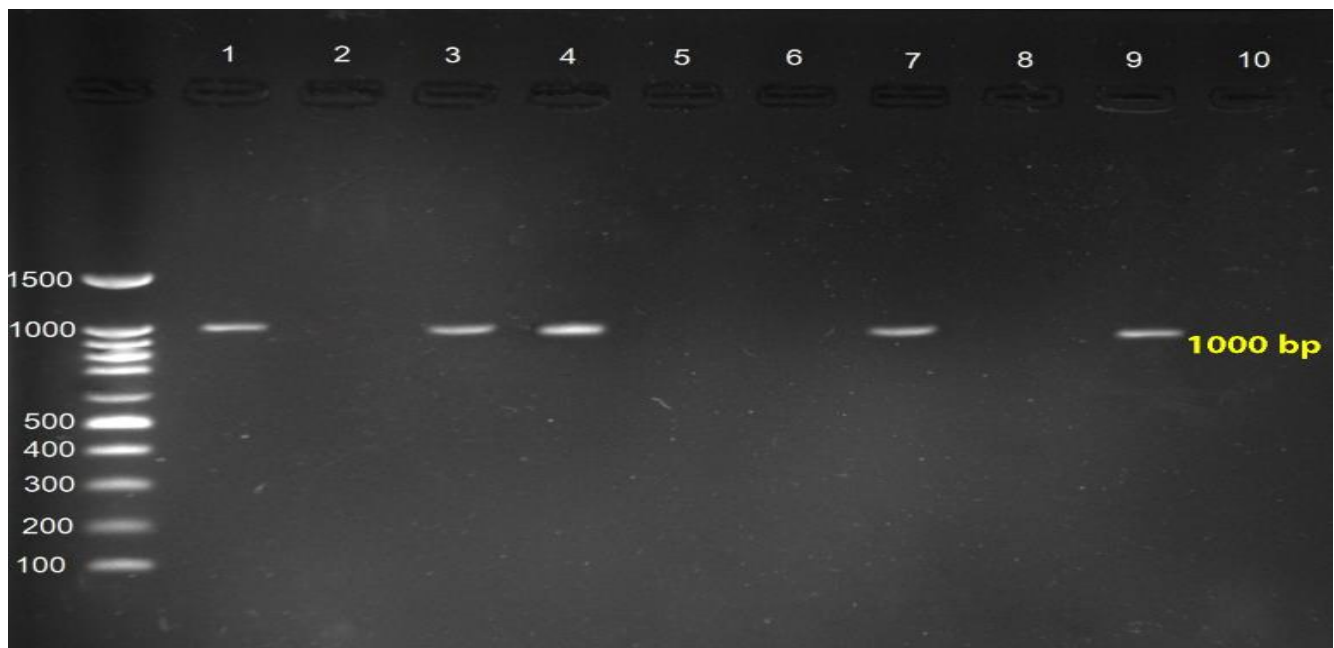


Figure1: Agarose gel electrophoresis 2 % of PCR product of the ITS1 , 5.8S, and ITS2 regions of different organisms , 5.8S Lanes L: DNA ladder of 50 bp and 1kb respectively, lanes 1, 3, 4: *M. canis* , and lanes 7: *T. rubrum*, and lane 9: *T. mentagrophytes* and lane 2,5,6 9 : negative control

Conclusions

Dermatomycosis is a frequent skin illness in Ramadi, where dermatophytes are the most common cause of skin infection, with *Candida* spp. accounting for a small percentage of cases. *Tinea manuum* and *tinea pedis* were the culprits. Ringworm is the most common kind of fungus. While the ringworm was artificial and the fungus was visible, the illness was less common. In this study, it was discovered that females were more afflicted by dermatophytosis than males, and that they had the greatest infection rate. It was detected in people aged 22 to 45. *Trichophyton rubrum*, and *Trichophyton wart* Isolated dermatophyte infections were the most prevalent causal agent. PCR-based genetic differentiation provides a quick and easy way to identify dermatophytosis isolates by type and strain.

RECOMMENDATIONS

- 1-Determining the most appropriate and precise treatment for dermatomycosis, which necessitates research into antifungals. Allergy to dermatophytes isolated
- 2-Using the PCR approach, dermatophytes can be detected quickly. Dermatophytosis sufferers' nail, skin, and hair samples PCR-
- 3- identification Dermatophytes and the use of RFLP are tightly linked. The ITS region of rDNA was analyzed and sequenced using cutting-edge technology.
- 4-Evaluation of Dermatophytes' immunological markers, including

References

- JW Guo, YP Cheng, CY Liu - Experimental and , 2021 - spandidos-publications.com
1. G Raut, S Wairkar - Complementary therapies in clinical practice, 2018 - Elsevier
 2. M Surcel, A Munteanu, G Isvoranu, A Ibram - Journal of Personalized , 2021 - mdpi.com
 3. RV Bubnov, LP Babenko, LM Lazarenko, VV Mokrozub - EPMA Journal, 2018 - Springer
 4. F Abdallah, L Mijouin, C Pichon - Mediators of inflammation, 2017 - hindawi.com
 5. AL Fachin, MF Petrucelli, TA Bitencourt - Dermatophytes and , 2021 - Springer, A

- Souza Monteiro, C Andrade Monteiro - Pathogens, 2019 - mdpi.com
6. KD Hyde, AMS Al-Hatmi, B Andersen, T Boekhout - Fungal Diversity, 2018 - Springer
 7. F Aala, UK Yusuf, F Jamal, S Rezaie - Brazilian Journal of , 2012 - SciELO Brasil
 8. F Aala, R Nulit, UK Yusuf - African Journal of , 2012 - academicjournals.org
 9. A Rezaei-Matehkolaei, K Makimura - Iranian journal of , 2012 - ncbi.nlm.nih.gov
 10. A Rezaei-Matehkolaei, K Makimura - Journal of medical , 2012 - microbiologyresearch.org
 11. A Rezaei-Matehkolaei, K Makimura- Medical , 2012 - academic.oup.com
 12. JR Köhler, B Hube, R Puccia, A Casadevall - Microbiology , 2017 - Am Soc Microbiol, C Viscoli, B De Pauw, W Dismukes - Clinical infectious , 2003 - academic.oup.com
 13. KS Coyner - Clinical Atlas of Canine and Feline Dermatology, 2019 - Wiley Online Library
 14. KE Harr - Veterinary Clinics: Exotic Animal Practice, 2018 - vetexotic.theclinics.com
 15. IC Senanayake, AR Rathnayaka, DS Marasinghe - , 2020 - mycosphere.org
 16. GT Bailes - 2017 - search.proquest.com
 17. JE Kirby, W Branch-Elliman, MT LaSalvia, Journal of clinical , 2017 - Am Soc Microbiol
 18. E Kang, M Zheng, Y Zhang, M Yuan - Plant , 2017 - academic.oup.com
 19. WE Dismukes, PG Pappas, JD Sobel - 2003 - books.google.com
 20. MR McGinnis - Annual Reviews in Microbiology, 1980 - annualreviews.org
 21. J Sambrook, EF Fritsch, T Maniatis - 1989 - cabdirect.org