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Genotyping of dermatophyte fungal species by AP-PCR technique: Pre-publication draft

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***Abstract*---**Dermatophytes fungi are causative agents of dermatophytosis. Surface skin fungi are called dermatophytes after the anatomical localization of the lesions. A total of 164 specimens were collected from patients with dermatophytosis (ring worm) distributed into 89/164(54.26%) specimens from male and 75/164(45.73%) specimens from female. The DTM culture medium was prepared by adding 0.2g of phenol red dye to 1 litter of PDA culture medium, utilized the short oligonucleotide (GACA)4 as a primer for identification of the tested dermatophyte isolates by AP-PCR. AP-PCR products for T. verrucosum isolates consisted of three bands at 150 and 300 bp and faint band in 600 bp. For the T. mentagrophytes isolates consists of four bright bands approximately at 200, 400,500 and 1500 bp. All T. rubrum isolates produced nearly similar band pattern, which consisted of five bright bands (approximately 100, 250, 320,500 bp) and one faint band at 2500 bp. M. canis strains revealed the most complex profiles, with up to 7 bands, ranging from 150 bp to 800 bp in size. These results indicated of the current that the AP-PCR technique is simple, reliable, accurate and easy to performed method for the identification dermatophytes fungi to the species level.

***Keywords*---**dermatophytes, dermatophytosis, (GACA)4, tinea, AP-PCR.

Introduction

Dermatophytes fungi are causative agents of dermatophytosis. Surface skin fungi are called dermatophytes after the anatomical localization of the lesions.

Dermatomycosis (tinea or ringworm) is a generic name for acute to mild and chronic lesions of the outer layers of the keratinized tissue caused by the skin fungus. Includes, Tinea capitis, Tinea corporis, Tinea pedis, Tinea barbae Tinea cruris, Tinea unguium, and Tinea manuum (Mahmoudabadi, 2005). Dermatophytes includes a wide range of filamentous pathogenic fungi consists of three important genera infection the human: Epidermophyton, Microsporum, and Trichophyton which may cause superficial and cutaneous infections. However, Malassezia furfur, Saccharomyces cerevisiae, and Candida spp. as opportunistic pathogenic fungi are capable of causing superficial mycotic infections in human beings. On the basis of environmental habitats, cutaneous fungi are divided into three groups of anthropophilic microorganisms (from person to person), zoophilic microorganisms (from animal to animal or human), and geophilic microorganisms (transmitted from soil to animals or humans) (Moriarty et al., 2012).

Methods

Isolation and Collection of Specimens

A total of 164 specimens were collected from patients with dermatophytosis (ring worm) distributed into 89/164(54.26%) specimens from male and 75/164(45.73%) specimens were obtained from female who clinically diagnosed by Dermatologist, were included in the current study after attending to the consultant of Dermatology clinic in Al-Hussein Teaching Hospital in Samawah Province and from Samawah Central Prison, From some animal breeders in veterinary clinics and private medical clinics in Al-Samawah Province , in the period from November 2021 to January 2022 , the patients age were ranging between few months to fifty years old.

Dermatophytes Test Medium (DTM)

Dermatophytes Test Medium was formulated by (Taplin et al., 1969) for use in locations where specialized training and microscopic examination is not available. a pH indicator and two antimicrobial agents are incorporated into the agar to provides a differential and selective medium for isolation of dermatophytes belonging to the genera Microsporum, Trichophyton or Epidermophyton The DTM culture medium was prepared by adding 0.2g of phenol red dye to 1 litter of PDA culture medium (Hi-media, India) because this medium contains the same nutrients listed in the preparation protocol of DTM which instructed by Taplin et al., (1969)

Identification of Dermatophytes by AP-PCR

Utilized the short oligonucleotide (GACA)4 as a primer for identification of the tested dermatophyte isolates. All of the studied isolates were amplified with this simple, repetitive primer, and the numbers of the resulting PCR bands ranged from 4 to 11 (size range, 100 bp to 1500 bp) AP-PCR was conducted for all isolates. Short oligonucleotide (GACA)4 was used as a primer for identification of the tested dermatophyte isolates. Amplification reactions were performed with volumes of 20 µL containing reaction buffer, 4 µL template

DNA,10 µL master mix,2 µL Nuclease-Free Water, 4 pmol of random primer (5'-GACAGACAGACAGACA-'3). The PCR products were electrophoresed in 2% agarose gel in 1× TAE buffer and stained with DS Red Nucleic Acid Stain.

Results and Discussion

Isolation and Collection of Specimens

Collection of 164 specimens which included 89(54.26%) from males and 75(45.73%) from females, all these specimens were belonging to various clinical cases of dermatophytosis. The results revealed that 48/164 (29.26%) specimens were positive on culture, 116/164 (70.73%) specimens have been shown negative result in culture for dermatophytes.As for the negative culture results the most common cause was belonged to inappropriate topical use of corticosteroid drugs, which had been taken randomly by the patients without consulting a specialist doctor to get rid of the painful symptoms associated with dermatophytosis (Collee, 1996 ; Hayette and Sacheli, 2015), Or the reason for this may be that the amount of the sample collected was insufficient to show a positive result (Milne, 1996).

Infections caused by dermatophytes are widespread, are increasing in prevalence on a global scale, and have been considered a major public health concern in some areas of the world, which accounts for as many as 69.5% in humans (Chen and Friedlander, 2001). Furthermore, dermatophytes are parasitic fungi that infect skin, hair and nails of both humans and animals. They are the primary causative agents of dermatophytosis, a major public health concern in some geographic regions (Chermette et al., 2008).

Dermatophytes Test Medium (DTM)

All the 48 isolates that gave positive results in microscopy tests and phenotypic diagnosis of dermatophytes, were cultured on Dermatophyte Test Medium (DTM) which is a differential media for dermatophytes, the results revealed that 41 (85.41%) were positive (red color change) for dermatophytes, and 7 (14.58%) were negative (no color change) as illustrated in figures (1). The results of the current study indicated that this method was useful in the initial isolation and early detection of dermatophytes from clinical samples to some extent, because not all the isolates gave a positive result on it, the same results were reported by previous studies (Th. N. Singh et al., 2016; Salim Naseif Alzubaidy et al., 2018), which revealed that DTM is a good reliable medium for the isolation of dermatophytes. Also Parmar, (2018) confirm that DTM is advocated for the regular confirmative diagnosis of dermatophytosis.
The results of the current study contradict the findings of Gromadzki et al., (2003) who reported that DTM has limited usefulness for presumptive identification of dermatophytes, a positive result with DTM may lead to misidentification of these organisms as dermatophytes (Kane and Summerbell, 1999).

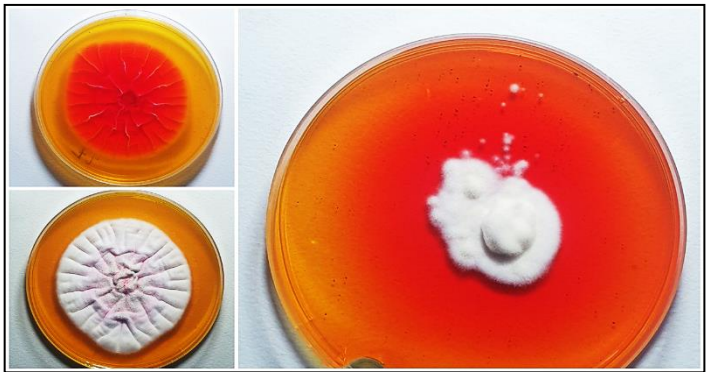

Figure 1: the isolates that gave a positive result in DTM with changed color (red color).

Dermatophyte Identification using AP-PCR

In the present study, a short oligonucleotide (GACA)4 primer was utilized for the dermatophyte identification of 48 clinical isolates: T. rubrum (21), T. mentagrophytes (15), T. verrucosum (3), M. canis (9). The studied strains were amplified with this simple, repetitive primer, and the numbers of the resulting PCR bands ranged from 4 to 7 (size range, 100 bp to 1500 bp). The bands profile of the AP-PCR products for T. verrucosum isolates consisted of three bands at 150 and 300 bp and faint band in 600 bp. For the T. mentagrophytes isolates consists of four bright bands approximately at 200, 400,500 and 1500 bp. All T. rubrum isolates produced nearly similar band pattern, which consisted of five bright bands (approximately 100, 250, 320,500 bp) and one faint band at 2500 bp. M. canis strains revealed the most complex profiles, with up to 7 bands, ranging from 150 bp to 800 bp in size (Figure 2). This study agrees with the results of the Spesso et al., (2013), study which used the (GACA)4-based PCR to determine the different types of dermatophytes.

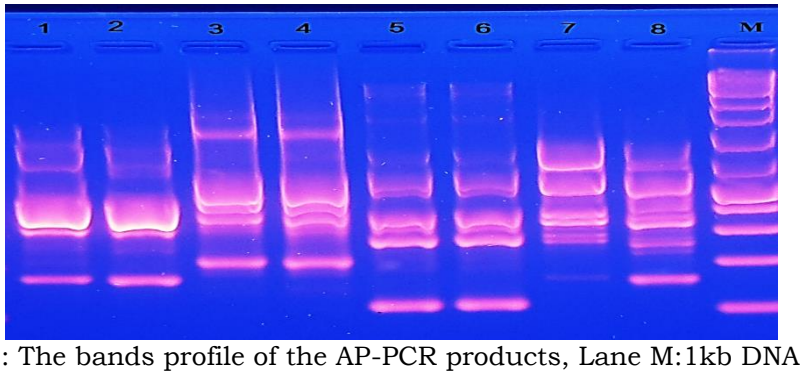

Figure 2: The bands profile of the AP-PCR products, Lane M:1kb DNA ladder; Lane:1-2, *T. verrucosum* isolates; lane 3-4: *T. mentagrophytes* isolates; Lane 5-6: *T. rubrum* isolates; Lane 7-8 *M. canis* isolates.

Conclusions

The results of the current study concluded that the AP-PCR technique is simple, reliable, accurate and easy to performed method for the identification dermatophytes fungi to the species level.

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