IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 11 Ver. V (Nov. 2015), PP 17-19
www.iosrjournals.org

Identification of Dermatophyte species causing tinea capitis using conventional methods in comparison with MALDI-TOF Mass spectrometry

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Abstract : *identification of dermatophyte to the species level is very important for the correct line of treatment. Sometimes identification of dermatophyte is difficult specially it takes more than one week to grow .So this study shows that Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)could be a useful tool for identification of dermatophyte species specially Trichophyton spp., with vars. 111 dermatophyte isolates of 9 different species were analyzed using (MALDI-TOF MS)in comparison to conventional methods including hair perforation test and urease test. The results of conventional method matched (MALDI-TOF MS) for 95% of the isolates analyzed .With conventional method incorrect identification were mostly between closely related species of Trichophyton isolates. MALDI-TOF MS showed accurate results with T.mentagrophytes vars and M.canis but three isolates of T.violaceum failed to be identified by MALDI-TOF MS . In conclusion , MALDI-TOF MS considered a fast and accurate method for species identification of dermatophytes specially closely related species of T.mentagrophytes complex and M.canis .*
Keywords: *–Conventional method ,Dermatophytes ,MALDI-TOF MS, Identification*

I. Introduction

Dermatophytes considered one of the most important group of skin infection which can invade the keratinized layer of the skin causing cutaneous infection . *Trichophyton,Epidermophyton* and *Microsporum* are the three genera of Dermatophyte species [1]. *Trichophyton and Microsporum* are the two genera that can invade the hair shaft which clinically appears as single or multiple area of hair loss with inflammation, itching and sometimes pustules [2]. Some of these two genera are easy to identify but others comes to be a diagnostic challenge , the conventional methods of identification depend on macroscopic and microscopic characters of the micro organism also biochemical tests needed for confirmation of the results which is time consuming [3] . The common dermatophyte species isolated from the tinea of the head are *M.canis(M.distortum),T.verrucosum, T.Mentagrophytes var equinum* and *var erinacei* as a zoophilic source of infection and *T.violaceum ,T.schoenleinii ,T.soudunense, T.rubrum, M.audounii and M.ferruginem* as the anthropophilic source of infection [4,5] . Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can detects directly the weight of phenotypical proteins from the growth of micro-organism on the media without purification [6]. The present study was done to compare between conventional methods of identification of dermatophytes causes tinea capitis in children(in Hail region, KSA) and MALDI-TOF Mass as new technology for identification of filamentous fungi . A total of 111 strain belonging to 10 different species were investigated .

II. Materials And Methods

1.1. Patients and Strains:

Samples were collected from children both male and female in primary schools in Hail region-KSA age (4 to 10 years old) Saudi and non Saudi. Skin scraping and hair roots were collected from children heads infected with tinea capitis . Isolates were maintained for 3 to 4 weeks at 25°C on Sabouraud's Dextrose agar(SDA) with and without cycloheximide and on Bromocresol purple milk solids dextrose agar . Altogether 111 dermatophytes isolates belonging to 10 different species .

1.2. Dermatophyte species identification by conventional methods:

The isolates were identified by standard macroscopic characters e.g surface color, reverse color ,constancy of the colony , and microscopic features e.g the development of macroconidia and microcodia ,chlamydosopres . In addition to biochemical and physiological tests e.g urease activity and hair perforation test.

1.3. MALDI-TOF mass spectrometry :

SDA with cycloheximid and Bromocresol purple milk solids dextrose agar were used at first to isolate dermatophyte species. Most of the isolates took 3 weeks but *T.violaceum* and *T.verrucosum* took 2 more weeks for sufficient colony growth then subcultures of the isolates on SDA without cycloheximid for three more weeks to be used in MALDI-TOF MS analysis .

50µg were removed from the growth of single colony ,without agar residue by using wire loope and transferred to stainless steel templates and then extracted with 0.3 ml matrix solution (10mg of α-acyano-4-hydroxycinnamic acid CHCA in Epependorf® and then add 1 ml of solution[50:50 water/acetonitril with 0.1 % trifoloroacetic acid ATF) final concentration . Positive ion mass were recorded for each strain using MALDI-TOF MS (AXIMA confidence Schimadzu Europe GmbH) adjusted on reflectron mode(curved-field reflectron (CFR) to provide high resolution and mass accuracy . Laser pulse (nitrogen laser firing at 337 nm strikes the samples ,the matrix absorbs the energy and the heat vaporize and ionize the peptide samples . As the ion running down the flight tube, the speed is a function of its m/z ratio. Hence, a particle's m/z ratio is measured based up on its time of flight .The resulting peaks were compared with BioMèrieux group (AnagnosTEC Gesellschaft für Analytische Biochemie and Diagnostik mbH) SARAMIS™ database .

III. Results

111 as a total strains were investigated belonging to 10 different species of dermatophyte genera(Table 1). Macro morphology and micro morphology characteristics for the isolates were detected specially for *T.violaceum , T.soudanens and T.schoenleinii* e.g *T.violaceum* showed deep violet folded colony as macro morphology and no macro conidia found under microscope only pyriform micro conidia and numerous number of chlamydocondia on the other hand *T.schoenleinii* showed favic chandleries under microscope and deeply folded honey comb like colony. About 95% of all isolates were identified successfully with urease test and hair perforation test . Each isolates were subjected to MALDI-TOF MS for analysis and the majority of results were in the range of 2.000 and 15,000 Da the difference in readings is due to different morphological character for each type of dermatophytes and 97% of the isolates were identified .

Species	No.	Conventional method	MALDI-TOF MS
T.violaceum	18	18	15
T.verrucosum	12	12	12
T.mentagrophytes var equinum	4	3	4
T.mentagrophytes var erinacei	3	2	3
T.soudunense	39	39	39
T.rubrum	4	4	4
T.schoenleinii	11	11	11
M.canis	12	10	12
M. audounii	5	4	5
M.ferruginum	3	2	3
total	111	105	108
%	100%	95%	97%

Table 1 The results of species differentiation for all strain ,the species names are based on the conventional methods . 111 isolates were tested for both conventional methods including hair perforation test , urease test and MALDI-TOF MS.

IV. Discussion

MALDI-TOF MS is a new technology since 1987 when Koichi Tanaka of Shimadzu Corporation and his co-workers used ultra fine metal plus liquid matrix method, MALDI-TOF MS conceder a sensitive technique for determining the mass of proteins, peptides, or polymers [7] and become popular diagnostic tool for identification of micro organisms like bacteria and conceder fast and reliable. Dermatophyte species identification can be done by conventional methods which depend on morphological and biochemical analysis [8,9] . Genus *Microsporum* can be easily identified and differentially diagnosed from genus *Trichophyton* with macroconidia but in case of *Trichophyton spp.,* many additional tests should be used such as hair perforation test ,*Trichophyton* agars ,urease test and dermatophytes test medium. The technology of MALDI-TOF MS is producing a characteristic mass spectra which based on protein ions[10]. All 111 strains were analyzed by conventional method with hair perforation test , urease test and also with MALDI-TOF MS which showed a reliable results [11] .

The 111 dermatophyte strains were investigated in the present study belonging to 10 species of two genera *Tricophyton* and *Microsporum* which are the main cause of tinea capitis . *Trichophyton spp.,* was the most prevalent causative agent of infection specially *T.soudunense* and then followed by *Microsporum spp.,* [12]. Species identification in dermatophytes are important because the most of species have a nature habitat

and therefore are related to the source of infection which shows mostly in *T.verrucosum, M.canis* as a zoonotic infection. Human traveling and emigration are influencing the distribution of endemic fungi [13] .

V. Conclusion

In conclusion , MALDI-TOF MS can provide a rapid reliable result for identification of dermatophyte species but in case of *T.violaceum* MALDI-TOF MS could not identify all isolates and conventional method did .on the other hand conventional method incorrect identification were for some closely related isolates as *T.mentagrophyte* complex . .MALDI-TOF MS can be useful with species with more than one var like *T.mentagrophytes* and also with *M.canis* . but expensive, however further studies should be done by using PCR which consider the most accurate method for identification of dermatophyte cases which conventional methods fail to identify with comparison to MALDI-TOF MS.

Acknowledgements

I would like to thank my sister Marwa for helping and supporting me , and the research unit in Hail University KSA, Microbiology unit Medicine collage in Zagazig University Egypt and Dr. Yvonne Gräser Konsiliarlabor für Dermatophyten, Institut für Mikrobiologie und Hygiene (Charité), Berlin, Germany my mentor .

Declaration of interest: the author report no conflicts of interest. the author alone is responsible for the content and writing of the paper.

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