Identification of malassezia species from tunisian patients with pityriasis versicolor

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Identification des espèces de Malassezia chez des patients atteint de pityriasis versicolor dans un échantillon de la population de Tunis

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RÉSUMÉ

Introduction : Le pityriasis versicolor est une mycose cosmopolite parmi les plus fréquentes. L'agent responsable appartient au genre Malassezia. Actuellement onze espèces du genre Malassezia sont connues et sont identifier par leurs caractères morphologiques et biochimiques et par la biologie moléculaire.
le But de cette étude est l'identification des espèces de Malassezia chez des patients atteint de pityriasis versicolor dans un échantillon de la population de Tunis.
Méthodes : 58 patients ont été inclus dans cette étude. Pour chaque patient, un prélèvement cutané par grattage a permis de recueillir des squames qui sont ensemencés sur deux milieux Sabouraud-Chloramphénicol, dont l'un est additionné d'huile d'olive. L'identification s'est basée sur les caractères morphologiques et surtout physiologiques par la recherche de l'uréase, de la catalase et l'assimilation des Tween 20, 40 et 80.
Résultats : nous avons isolés 5 espèces du genre Malassezia : M globosa chez 76.2% des patients,
Malassezia furfur (9.55%), Malassezia sympodialis (4.75%), Malassezia slooffiae (4.75%) et Malassezia pachydermaties (4.75%).
Conclusion : dans notre étude Malassezia globosa représente la principale espèce en cause impliquée dans la pathogénicité de pityriasis versicolor, suivie de loin par Malassezia furfur.

Mots-clés
Pityriasis versicolor - Identification - Espèce de malassezia

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SUMMARY

Background: Pityriasis versicolor is caused by Malassezia sp. It is a common worldwide mycosis. Recently, eleven species are known of the Malassezia genus, and are identified in vitro by their morphological characteristics, biochemical tests and by molecular biology.
The aim of this study is the identification of Malassezia species from Tunisian patients with pityriasis versicolor.
Methods: Specimens were taken from 58 patients with pityriasis versicolor. All samples were both inoculated in Sabouraud dextrose agar and Sabouraud agar overlaid with olive oil. Malassezia species were identified by morphological and physiological methods: macroscopy, microscopy, catalase, urease and lipid assimilation tests.
Results: we have isolated five Malassezia species: Malassezia globosa being isolated in 76.2% of patients, followed by Malassezia furfur (9.55%), Malassezia sympodialis (4.75%), Malassezia slooffiae (4.75%) and Malassezia pachydermaties (4.75%).
Conclusion: in our study Malassezia globosa presents the main species implicated in the pathogenicity of pityriasis versicolor and Malassezia furfur as the second agent of importance.

Key-words
Pityriasis versicolor - Identification - Malassezia species.

Pityriasis versicolor (PV) is a common worldwide mycosis. It is a superficial infection of the stratum corneum which caused by Malassezia (M) spp. PV is a mild, chronic condition, usually affecting the upper trunk; it is characterized by scaly hypo- or hyperpigmented lesions with minimal pruritus. The condition occurs mainly between adolescence and middle age, when the sebaceous glands are more active [1, 2, 3].

The genus Malassezia includes a group of lipophilic yeasts whose natural habitat is the skin of humans and other warmblooded animals[1.4] As other micro-organisms of the normal human cutaneous commensal flora, Malassezia produces an enzyme with lipase activity, as demonstrated by its ability to release fatty acids from the triglycerides of the sebum[5]. Malassezia species are dimorphic, existing in both yeast and mycelial phases. PV is caused by conversion from the yeast to the mycelial form, which is then able to invade the stratum corneum, penetrating both between and through the corneocytes [5].

The genus of Malassezia has undergone several taxonomic revisions. In the reclassification by Gueho and al, in 1996 seven distinct species were recognized within this genus namely M. furfur, M.pachydermatis, M. sympodialis, M. globosa, M. obtusa, M. restricta and M. slooffiae [6]. Furthermore, in recent years, ribosomal DNA sequencing techniques have added another six species: Malassezia dermatis, Malassezia japonica, Malassezia yamatoensis, Malassezia equina, Malassezia caprae and Malassezia nana [7,8] .

The aim of this study is the identification of Malassezia species from Tunisian patients with pityriasis versicolor using morphological, biochemical and physiological criteria.

MATERIALS AND METHODS

This is a transversal study of 58 patients with pityriasis versicolor during the period of 3 months at the department of Dermatology in Charles Nicolle University Hospital.

PV is diagnosed by its clinical appearance and the observation of many yeast cells and hyphae (so-called spaghetti and meatballs) in scotch tape in microscopic examination. The range of pathological change is observed by fluorescence in Wood's lamp examination.

Specimens were taken by scraping the lesions with a scalpel. All samples were both inoculated in Sabouraud dextrose agar and Sabouraud agar overlaid with olive oil. The plates were incubated at 32°C for two weeks and examined at frequent intervals for developing colonies.

Identification

Malassezia species were identified according to their morphological features and physiological properties. Isolated colonies were used for identification. Among Malassezia species, only M. pachydermatis is able to grow on the lipid-free culture medium [6, 9].

However, further tests are essential for identification of other Malassezia species such as Tween assimilation test, catalase reaction and urease reaction.

Tween assimilation test

According to the method reported by Gueho and al [6], ability to utilize different Tween compounds as a unique lipid supplement by Malassezia species was evaluated. Briefly, yeast suspension (at least 107 cfu/ml) was made in 2 ml sterilized distilled water and poured into plate containing Sabouraud dextrose agar at 45°C. The inoculums were then spread evenly. After solidification of each plate, four wells were made and filled with 30 Ìl of a Tween compound, in Tween 20, 40 and 80, respectively. These plates were incubated for a week at 32°C and the growth was assessed around the individual wells after 2, 4 and 7 days.

Catalase reaction

Presence of catalase was determined by using a drop of hydrogen peroxide (3% solution) and production of gas bubbles was considered as a positive reaction. Lack of catalase activity is a characteristic feature of M. restricta [6].

Urease reaction

Some colonies are introduced into a medium containing urea and an indicator such as phenol red. The urease hydrolyzes urea to ammonia, which raises the pH of the medium, and changes the color of the specimen. This reaction is positive only in M. furfur and M. pachydermatis.

RESULTS

Culture of scales was positive in 21 PV lesions, in which beige mucous colonies were seen.

We have isolated five Malassezia species.

One case of M. pachydermatis was identified. It was able to grow on lipid-free culture medium and to have a positive urease reaction.

For the other specimens the catalase reaction was positive.

The assimilation of Tween 40 was absent for 16 of positive cultures ; the microscopic observation of these yeasts found spherical yeasts giving birth to buds which can lengthen to form very short cylindrical strands. This allows the identification of M globosa.

The assimilation of Tween 40 was present for the 4 others. The assimilation of Tween 20 and 80 differentiated then in: one isolate of M sloofiae which utilised only Tween 20, one isolate of M sympodialis which assimilated only Tween 80 and 2 isolates of M furfur which utilised Tween 20 and Tween 80 and have also a positive urease reaction.

DISCUSSION

Diagnosis of PV is generally simple and lies on the clinical manifestations and microscopic examinations of the lesions [10, 11, 12]. Direct examination of samples by unskillful technicians, may fail to reveal the infection. Culture is necessary to distinguish the Malassezia species by morphological and physiological methods [12].

We have isolated five Malassezia species: M globosa being

isolated in 76.2% of patients, followed by M furfur (9.55%), M sympodialis (4.75%), M slooffiae (4.75%) and M. pachydermatis (4.75%).

We noticed a high prevalence of M. globosa in lesional skin of PV, which was consistent with most of the studies that reported a frequency of more than 55% [13, 14, 15]. M. sympodialis was isolated in lower frequency than in other studies in which it was reported as a secondary species [16]. In our study, M furfur was the secondary species. However, some studies conducted mainly in areas with tropical or subtropical climates show a clear predominance of M furfur in PV lesions [17].

Malassezia restricta was not isolated in our study. M. restricta was infrequently detected with culture-based system identification of Malassezia species from lesions of PV, because of its poor growth [3, 18].In order to resolve this identification problem, non-culture-based methods using of nested PCR with specific primers are done [19].

On the other hand, the results presented here indicated that the method reported by Gueho and al is a useful and inexpensive tool for the routine identification of clinically important Malassezia species.

CONCLUSIONS

M globosa seems to be the predominant species, if not the only one, in the etiology of PV, at least in temperate climates. However, M furfur is predominantly isolated in some studies conducted in tropical areas. These results suggest that subtle changes in the environment, such as higher temperatures or humidity, are one of the factors that transform the yeast into its mycelia form.

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