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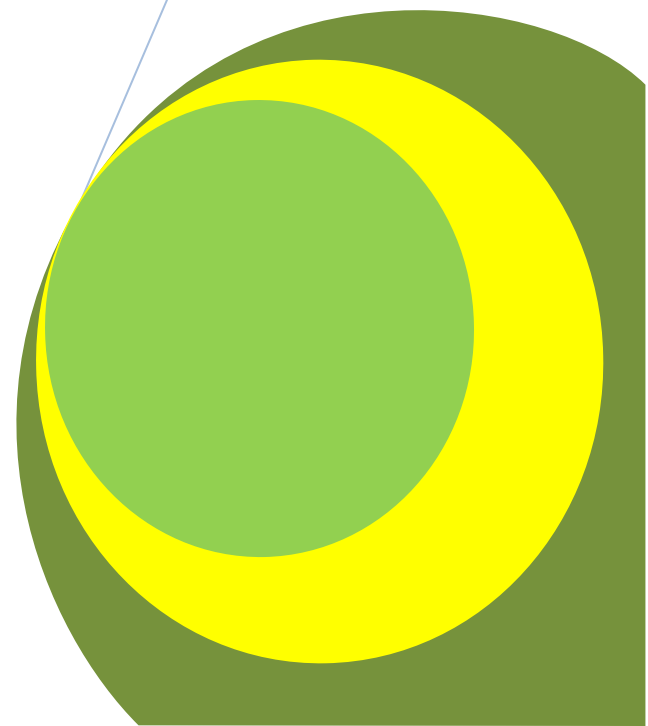
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Research Article



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ABSTRACT

In the last ten (10) years, different studies have shown interesting geographical variations in the prevalence of different *Malassezia* species in Pityriasis versicolor. In 200 patients with dandruff, *Malassezia* species were identified by culture on Potato Dextrose Agar (PDA) containing Chloramphenicol to get rid of bacterial contaminants. The isolates were identified by morphological and physiological characteristics. Biochemical tests were carried out, yielding negative esculin hydrolysis test and positive catalase reaction; typical of *Malassezia globosa*. Microscopically using 10% KOH+ parker blue ink, smear of the samples from 50 patients showed negative while in 150 patients the smear showed characteristic spaghetti and meatball (globose) appearance of hyphal growth and microconidia respectively; the only characteristic microscopic appearance that differentiates *M. globosa* from other species of *Malassezia*. In this regard, growth was obtained on PDA and 75% of the isolates obtained were *M. globosa*.

Keywords: *Malassezia globosa*, Dandruff, Isolation, Identification.

INTRODUCTION

Malassezia (formerly known as Pityriasis) is a genus of related fungi. These yeasts are naturally found on skin surfaces of many animals including man. In occasional opportunistic infections, some species can cause hypopigmentation of the trunk and other locations in humans.

Malassezia belongs to the division of fungi Basidiomycota in the class Exobasidiomycetes (those found outside the host body) and in the order Malasseziales. They were originally identified by a French scientist Louis Charles Malassez in the 19th century. Later Raymond Saboraud identified the dandruff-causing organism in 1904 and named it *Pityrosporum malassez*, honoring Malassez but at the species level. When it was found out that the organisms were the same, the term "Malassezia" was judged to possess priority (Inamadar and Palit, 2003; De Angelis *et al.*, 2007).

Dandruff is a condition characterized by flaking of skin (most commonly scalp skin) resulting from rapid turnover and release of skin cells. Dandruff is reliant on three (3) factors that favor its survival and reproduction of the yeasts, they are:

- * Sebum production
- * Microbial metabolism
- * Susceptibility of individual.

Recently, identification of *Malassezia* on skin has been aided by the application of molecular or DNA based techniques. These investigations show that the *Malassezia* species causing most skin diseases in humans the most common cause of dandruff and *Seborrhoeic dermatitis* is *M. globosa*. The skin rash of *Tinea versicolor* is also due to this fungus (De Angelis *et al.*, 2007).

As the fungus requires fat to grow, it is most commonly found in areas with many sebaceous glands: on the scalp, face and upper part of the body. But when the fungus grows too rapidly, the natural renewal of cells is

disturbed and dandruff appears with itching (a similar process may also occur with other fungi or bacteria) (BBC News, 2007). The yeast produces substances that irritate and inflame the skin. Patients with *Seborrhoeic dermatitis* appear to have a reduced resistance to the yeast. However, the colonization rate at affected skin may be lower than that of unaffected skin.

Only saturated fatty acids have been shown to support *Malassezia* growth. It has also been shown that while number density of *M. globosa* do not directly correlate to dandruff presence or severity, removal correlates directly with amelioration of flaking. Furthermore, in dandruff susceptible individuals, pure oleic acid, and unsaturated fatty acid and *Malassezia* metabolites induce flaking in the absence of *Malassezia* by direct effects on the host's skin barrier.

Genetic, environmental, hormonal and immune system factors have been shown to be involved in the manifestation of *Seborrhoeic dermatitis*. *Seborrhoeic dermatitis* may be aggravated by illness, physiological stress, fatigue or sleep deprivation.

Dandruff is the shedding of dead skin cells from scalp (Rapini *et al.*, 2007). Dandruff is a common scalp disorder affecting almost half of the population at the post-pubertal age and of any gender and ethnicity. It often causes itching. It has been well established that keratinocytes play a key role in the expression and generation of immunological reactions during dandruff formation. The severity of dandruff may fluctuate with season as it often worsens in winter (Ranganathan and Mukhopadhyay, 2010).

Dandruff is characterized by patches of loosely adherent flakes, usually accompanied by itching. Dandruff has the clinical feature of small white or gray flakes that accumulate diffusely on the scalp in localized patches. It does not exhibit apparent inflammation and is confined to the scalp.

For most individuals, as the epidermal layer continually replaces itself, cells are pushed outward where they eventually die and flake off. These flakes of skin are too small to be visible. However, certain conditions cause cell turnover to be unusually rapid, especially in the scalp. For people with dandruff, skin cells may mature and be shed in 2–7 days, as opposed to around a month in people without dandruff. The result is that dead skin cells are shed in large, oily clumps, which appear as white or grayish patches on the scalp, skin and clothes (De Angelis *et al.*, 2005). Dandruff has been shown to be the result of three required factors:

1. Skin oil commonly referred to as sebum or sebaceous secretions (Ro and Dawson, 2005).
2. The metabolic by-products of skin micro-organisms (most specifically *Malassezia* yeasts) (Ashbee and Evans, 2002; Batra *et al.*, 2005; Dawson 2006; Gemmer *et al.*, 2002; Gupta *et al.*, 2004).
3. Individual susceptibility

Sebaceous gland activity shows strong association with most disorders that cause scalp flaking. Early sebum production in the neonate may manifest as cradle cap. Once maternal hormone control subsides, sebum production does not begin again until puberty, at which time sex hormone control affects sebum production. As sebum production increases, *Malassezia* proliferates in response to new food sources. Proliferation of *Malassezia* increases lipid metabolism, which causes scalp itching and flaking.

Malassezia species are lipid-dependent microorganisms that adapt to the narrow niche provided by sebum-rich skin. *Malassezia globosa* and a second species *M. restricta* predominate on dandruff scalp. *M. globosa* likely initiates dandruff formation due to its lipase activity. *M. globosa* lacks the ability to synthesize fatty acids: it is highly adaptive but niche dependent and is commonly found on the scalp, back, face and chest where the highest levels of sebum are produced. *M. globosa* excretes more than 50 different enzymes to help metabolize hair and scalp. *Malassezia* metabolism results in increased oleic acid levels that lead to the symptoms of dandruff and other forms of dermatitis in some individuals (De Angelis *et al.*, 2007; Dawson, 2007; Ro and Dawson, 2005).

It had been previously cited that the fungus *Malassezia furfur* (previously known as *Pityrosporum ovale*) as the cause of dandruff. While this species does occur naturally on the skin surface of both healthy people and those with dandruff, it was discovered in 2007 that the responsible agent is a scalp specific fungus, *Malassezia globosa* (BBC News, 2007), that metabolizes triglycerides present in sebum by the expression of lipase, resulting in a lipid byproduct, the oleic acid (OA). During dandruff, the levels of *Malassezia* increase by 1.5 to 2 times its normal level (Ranganathan Mukhopadhyay, 2010). Penetrating the top layer of the epidermis and the stratum corneum, by Oleic acid (OA), results in an inflammatory response in susceptible persons which disturbs homeostasis and results in erratic cleavage of stratum corneum cells (Dawson, 2006).

Gueho *et al.*, (1996) studied a protocol of the biochemical, morphological and molecular characteristics of *M. globosa* and its consent forms have been approved by the research and ethic committee of Imandade Santa Case de Misericordia de Sau Paulo. He classified the fungal body into Light (23%); Moderate (56.86%); Decimated (19.60%).

The objective of this project was to isolate and identify the fungus *M. globosa*, the dandruff-causing using the appropriate methods employed, with a view to finding the appropriate treatment to the incessant scalp and hair flaking and loss as complained by the female students of the university.

MATERIALS AND METHODS

Sample collection

A sharp and sterile epilation forceps was used to detach the hair and scalp samples from the infected persons (Cheesbrough, 2000). The infected region was first washed with 70% ethanol followed by scraping with the sterile epilation forceps which was held at an angle of 90° with the head. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. Each sample was labeled with the patient's name. The samples were then taken to the laboratory for analysis.

Sample Analysis

Direct microscopy

A drop of 10% potassium hydroxide, KOH with blue parker ink was introduced onto a slide containing the sample and covered with cover slip (Kindo *et al.*, 2004;). The purpose of applying the alkali was to digest the keratin surrounding the fungi so that the hyphae and conidia (spores) could be seen (Cheesbrough, 2000). The sample was then heated over a Bunsen flame to remove bubbles. The slides were viewed using x10 and x40 objectives with closed iris diaphragm of the lens of the condenser (Cheesbrough, 2000). Direct microscopy that showed the typical mixture of globose blastoconidia and pseudomycelia coupled with mycelia in the form of "spaghetti" indicated the most identifiable difference of *M. globosa* from the other species of *Malassezia* (Crespo *et al.*, 2000; Kindo *et al.*, 2004).

Culture

The samples collected were cultured on SDA (Saboraud Dextrose Agar) incorporated with chloramphenicol to rid the media of bacterial contaminants. Small amounts of the samples collected were introduced into Petri dishes containing the media using sterile forceps. The Petri dishes were then labelled and incubated at room temperature (25°C) for three days and then, up to a week (Kindo *et al.*, 2004; Cheesbrough, 2000).

Biochemical tests

Catalase test

Catalase test was carried out to ascertain the presence of *M. globosa* as it is, unlike *M. restricta* that is catalase negative, catalase positive. 3mls of 3% hydrogen peroxide (H₂O₂) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using a sterile glass rod. Active bubbling observed, signifying the release of oxygen from the H₂O₂ indicated positive test for catalase production (Cheesbrough, 2000; Khosravi *et al.*, 2009).

Esculin hydrolysis test

The medium used was bile esculin agar slant; a nutrient agar-based medium containing 0.1% esculin and 10% bile salts, and allowed to solidify at a slant. The bile salts inhibit some bacteria, and so the ability to grow in the presence of bile salts represents a second test use for the medium. An inoculum from a pure culture was aseptically transferred to a sterile tube of bile esculin agar and streaked along the slant. The medium was not stabbed anyway. The inoculated tube was incubated at 35-37°C for 24 hours and the results were determined. Abundant growth on the slant indicated a positive test for growth in the presence of bile. Esculin hydrolysis was observed when the medium changed to chocolate brown colour. *M. globosa* is negative for this test (Khosravi *et al.*, 2009).

Gram's staining

A smear of the pure culture obtained after two weeks of incubation at 32°C and gram stained as demonstrated by Cheesbrough 2000. This is with a view to studying the morphology of the yeast cells. A typical positive test is indicative of *M. globosa* (Kindo *et al.*, 2004).

RESULTS

Out of the 200 samples, 150 showed hyphae and conidiospores exhibiting the characteristic “spaghetti and meatball” in KOH appearance preparation. All the 200 samples exhibited growth on SDA.

Table 1: Identification of *M. globosa* from the dandruff patients showing characteristic “spaghetti and meatball” appearance by direct microscopy using KOH with blue parker ink.

Fungal pathogen	Samples positive (%)	Samples negative (%)	Total
<i>M. globosa</i>	150 (75)	0.00	150
Other Malassezia spp.	0.00	50 (25)	50
Total	150	50	200

Table 2: Shows the enzyme **Catalase** production test. In this, some fungal pathogens isolated were found positive for catalase test because it is not *M. globosa* alone that produces catalase.

Samples positive	Percentage positive	Samples negative	Percentage negative
170	85	30	15

Table 3: Shows the Esculin hydrolysis test, a test in which *M. globosa* is negative for. Other organisms than *M. globosa* were also negative for this test.

Total samples collected	Samples positive	Percentage positive	Samples negative	Percentage negative
200	33	16.5	167	83.5

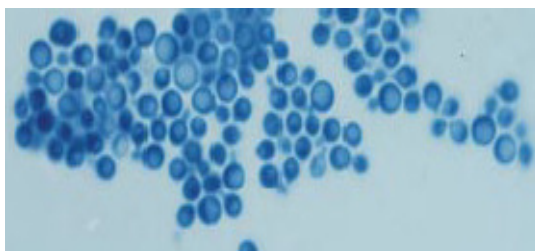


Fig. 1: (a) direct microscopy of scales of hair and scalp from dandruff patients showing globose blastoconidia in clusters, typical characteristics of *M. globosa*

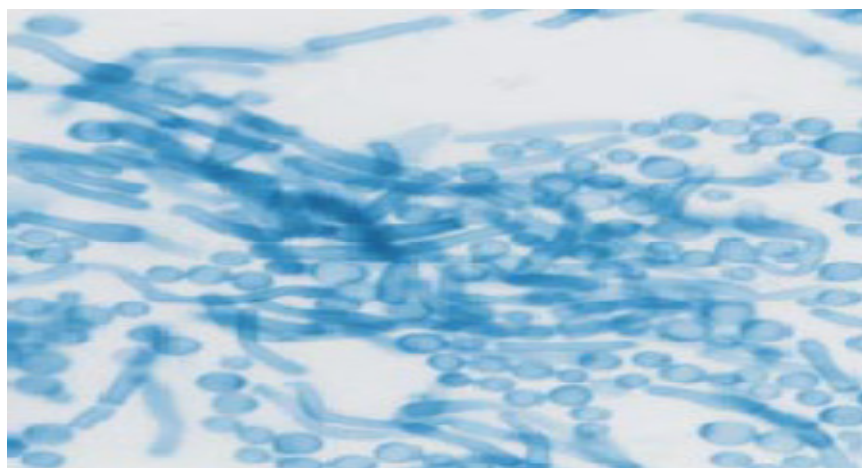


Fig. 2: (b) direct microscopy of hair and scalp from dandruff patients showing typical globose cells (meatball appearance) and mycelia (spaghetti shape) of *M. globosa* using KOH and blue parker ink.

DISCUSSION

The result of the identification of *Malassezia* species from the scrapings of scalp of the female students of Gombe state university by direct microscopy (Table 1) conducted using 10%KOH with blue parker ink. Based on the microscopy (Crespo *et al.*, 2000; Kindo *et al.*, 2004, Trabelsi *et al.*, 2010), *M. globosa* was the predominant species which shows sphagetti and meatball appearance of mycelia and microconidia respectively, an only ideal method for the differentiation of *M. globosa* from other *Malassezia*. Other samples (50%) proved negative. This corresponds with the work of Crespo *et al.*, 2000, Kindo *et al.*, 2004 and Khosravi *et al.*, 2009 who worked independently and variously on Pityriasis versicolor caused by different *Malassezia* species.

Table 2 however, displays the result of the catalase production test in which out of 200 samples obtained, 170 (80%) were found to be positive. This shows that in addition to *M. globosa* which is catalase positive, some other *Malassezia* species are also positive, including *M. sympodialis*, *M. obtusa* and so on. The only negative one is *M. restricta*. This work is in line with that of Kindo *et al.*, 2004 who worked on the isolation of *Malassezia* species.

It can be seen in Table 3 that, since *M. globosa*, *M. restricta* and few other forms of *Malassezia* are negative for esculin hydrolysis test (Khosravi *et al.*, 2009), only 33 samples out of the 200 were found to be positive.

CONCLUSION

Based on the catalase reaction, direct microscopic morphology of the scalp and hair scrapings as well as the microscopic morphology in Gram stained smears, the 150 specimens were identified as *M. globosa* (Kindo *et al.*, 2004).

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