

## Incidence of Malassezia Fungemia and Bacteremia in School Children with Pityriasis Versicolor in Ogun State, Nigeria.

Afolabi Ogunledun<sup>1\*</sup>, Hyacinth. Izuka Effedua<sup>1</sup>, Adebayo Adetola Amballi<sup>2</sup>, Francis Ademola Oluwole<sup>3</sup>, Albert Adekunle Salako<sup>3</sup>, Kolawole Sunday Oritogun<sup>1</sup>  
[afolabiogunledun@yahoo.com](mailto:afolabiogunledun@yahoo.com)

1. Department of Medical Microbiology and Parasitology, College of Health Sciences, Olabisi Onabanjo University, P.M.B 2022, Sagamu, Ogun State, Nigeria.
2. Department of Chemical Pathology and Immunology, College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.
3. Department of Community Medicine & Primary Care, College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

**ABSTRACT: Background:** *Malassezia* species are dimorphic mycoflora of human skin with colonization as early as the neonatal period. However, they are often associated with superficial skin infection known as pityriasis/tinea versicolor with little reports on their involvement in systemic diseases in developing nations. Recent rise in cases of morbidity and mortality due to fungal sepsis among children in developing countries warrants the present study to determine the occurrence of *Malassezia* fungemia with cases of pityriasis versicolor (PV) and bacteremia among pupils attending public primary schools in Ogun State, Nigeria. **Materials and Methods:** Venous blood samples of 232 pupils with symptoms of PV and those of 67 asymptomatic pupils were cultured in pairs of glucose broth with and without olive oil before subculturing on Sabouraud Dextrose agar, Blood agar and MacConkey agar plates. Skin scrapings of the symptomatic pupils were separately cultured on the three agar plates. The microbial isolates were speciated using cultural, morphological and biochemical methods. **Results:** Of the 232 skin scrapings of symptomatic pupils, 166 (71.6%) were found to be cultural positive for *Malassezia* species in, which *M. restricta* gave the highest isolation frequency of 71.7% followed by *M. globosa* (22.9%), *M. obtusa* (3.6%) and *M. slooffiae* (1.8%) while the blood cultures of 49 (21.1%) showed occurrence of these respective fungi to be 55.1%, 36.7%, 8.2% and 0.0%. The frequencies of bacteria in the blood cultures of symptomatic pupils were: *Pseudomonas* spp. 27.5%, *Staphylococcus aureus* 20.9%, *Proteus* spp. 18.7%, *Klebsiella* spp. 14.3%, *Enterococcus faecalis* 9.9% and *Staphylococcus epidermidis* 8.8%. Fungemia and bacteremia were found to be significantly associated with symptomatic PV ( $P < 0.05$ ) when compared with asymptomatic cases. Ten patterns of fungi and bacteria blood co-infections were obtained in the symptomatic PV. **Discussion:** The result of this study has shown that symptomatic PV is both a superficial and systemic mycosis and could occur as a co-infection with bacteremia in pupils. These findings should be considered in the management of this common mycotic infection of the skin. [Academia Arena, 2009;1(6):37-41]. (ISSN 1553-992X).

**Keywords:** - Pityriasis versicolor, *Malassezia* spp, Fungemia, Bacteremia

### 1. Introduction

Pityriasis versicolor is a superficial mycotic infection caused by yeasts of the genus *Malassezia*, which may also be found on normal human skin (Arzumanian, 2001; Ashbee *et al*, 2002; Salah *et al*, 2005; Gaitanis *et al*, 2006). Some species of *Malassezia* have complex lipid requirements for growth, which also explains their occurrence on the skin (Thoma *et al*, 2004). In recent years, rare cases of systemic infections and fungemias caused by *Malassezia* have been reported (Schmidt, 1997; Ashbee *et al*, 2002; Thoma *et al*, 2004). The distribution and ecology of *Malassezia* species and cutaneous bacteria on human skin has also been reported (Leeming *et al*, 1989). However, there is paucity of report on systemic

co-infections of *Malassezia* and bacteria especially in developing nations where tropical and subtropical climates, poor hygiene, malnutrition and immunosuppression are very common. All these have been reported as possible predisposing factors to the infections caused by *Malassezia* species and bacteria (Leeming *et al*, 1989; Dutta *et al*, 2002, Gulec *et al*, 2003). Regarding these probable and possible risk factors, we thought that pupils attending public primary schools form a unique population affected by these infectious agents. Therefore, we decided to evaluate some aspects of systemic co-infections between *Malassezia* and bacteria species in them.

### 2. Materials and Methods

## 2.1 Sample Collection

This cross sectional study was conducted in the year 2008 in six public primary schools in Sagamu, a town in Ogun State in Nigeria with a tropical climate. Two hundred and thirty two male and female pupils with depigmented skin lesions resembling pityriasis versicolor (PV) were randomly selected among 1048 pupils in the schools. Also, 67 asymptomatic pupils were randomly selected as controls among the pupils in the schools. The study protocol was approved by the research committee of Olabisi Onabanjo University Teaching Hospital (OOUTH) and the parents of the selected pupils gave informed consent before enrollment in the study. Skin scrapings were obtained from only the pupils with depigmented skin lesions by means of sellotape as described by Tarazooie *et al*, 2004, while venous blood samples were aseptically taken from both subjects and controls. Pupils with skin lesions were treated with To-To ointment and soap products in line with a clinical trial conducted by Alebiosu *et al*, (2003).

## 2.2 Isolation and Identification

A portion of the skin scrapings were observed in wet mount prepared with 10% KOH and methylene blue for direct microscopy and the remaining portion was cultured on pairs of Sabouraud Dextrose Agar (SDA) plates with and without olive oil disks. Infection was assessed by observing morphological features of Malassezia including budding cells and/or hyphae under x40 objective lens. Also the rapid and luxuriant growth of Malassezia in 5 days at 30 – 37° C on SDA plates in the presence of olive oil disks coupled with the results obtained from the physiological and biochemical tests including catalase, urease splitting of esculin and Tween assimilation were further used in speciating Malassezia isolates according to methods described by Salah *et al* (2005). The venous blood samples were first cultured in pairs of glucose broth enriched with and without olive oil at 37°C for 48h to isolate Malassezia species and for 7-14 days to isolate bacteria before subculturing on blood agar, chocolate agar, MacConkey agar and SDA. The bacteria were then identified by means of cultural, morphological

and biochemical methods as described by Cheesebrough (1985).

## 2.3 Statistical Analysis

Chi-square ( $X^2$ ) was used as a test of significant association between PV, malassezia fungemia and bacteremia at 95% confidence interval. P values equal or less than 0.05 were considered significant.

## 3. Results

One hundred and sixty six skin scrapings (71.6%) out of the two hundred and thirty two (232) pupils with skin lesions resembling PV were found to be culture positive for Malassezia species while 49 (21.2%) were blood culture positive for these yeasts. The frequency distribution of the yeasts from the skin lesions showed that *M. restricta* (71.7%) was the most prevalent followed by *M. globosa* (22.9%) while low frequencies were recorded for *M. obtusa* (3.6%) and *M. slooffiae* (1.8%). The blood cultures of 49 (21.2%) depicted occurrence of these yeasts to be 55.1%, 36.7%, 8.2% and 0.0% respectively (Table 1). Ninety one (39.2%) of the 166 pupils with skin lesions were positive for bacteria blood culture with *Pseudomonas* species having the highest frequency of 27.5%, followed by *Staphylococcus aureus* 20.9%, *Proteus* species (18.7%), *Klebsiella* spp (14.3%), *Enterococcus faecalis* (9.9%) and *Staphylococcus epidermidis* (8.8%) (Table 2). When the frequencies of malassezia were compared between symptomatic and asymptomatic PV, fungemia was found to be significantly associated with symptomatic PV ( $X^2 = 4.56$ ,  $P < 0.05$ ) (Table 3). Similar comparison of the results of bacteria blood cultures between the two groups showed that bacteremia was significantly associated with symptomatic PV ( $X^2 = 20.60$ ,  $P < 0.05$ ) (Table 4). Seventeen (10.2%) of the 166 pupils with culture positive skin lesions exhibited ten patterns of Malassezia and bacteria species co-infections in their blood with highest frequency of 17.6% each for *M. restricta* and *S. aureus*, *M. restricta* and *Proteus* spp, and *M. restricta* and *Pseudomonas* spp (Table 5).

**Table 1.** Frequency Distribution of *Malassezia* species Isolated from Skin Lesions and Blood of Pupils with Pityriasis Versicolor.

Malassezia isolates	Skin lesions		Blood	
	n	(%)	n	(%)
<i>Malassezia restricta</i>	119	(71.7)	27	(55.1)
<i>Malassezia globosa</i>	38	(22.9)	18	(36.7)
<i>Malassezia obtusa</i>	6	(3.6)	4	(8.2)
<i>Malassezia slooffiae</i>	3	(1.8)	0	(0.0)
<b>Total</b>	<b>166</b>	<b>(100.0)</b>	<b>49</b>	<b>(100.0)</b>

**Table 2.** Frequency Distribution of Bacteria species Isolated from Blood Culture of Pupils with Pityriasis Versicolor.

Bacteria Isolates	n	(%)
<i>Pseudomonas</i> species	25	(27.4)
<i>Staphylococcus aureus</i>	19	(20.9)
<i>Proteus</i> species	17	(18.7)
<i>Klebsiella</i> species	13	(14.3)
<i>Enterococcus faecalis</i>	9	(9.9)
<i>Staphylococcus epidermidis</i>	8	(8.8)
<b>Total</b>	<b>91</b>	<b>(100.0)</b>

**Table 3:** Association of Pityriasis Versicolor (PV) with Malassezia Fungemia in Pupils.

Malassezia fungemia	Symptomatic PV		Asymptomatic PV	
	n	(%)	n	(%)
Yes	49	(29.5)	8	(12.0)
No	117	(70.5)	59	(88.0)
<b>Total</b>	<b>166</b>	<b>(100.0)</b>	<b>67</b>	<b>(100.0)</b>

$X^2 = 4.56, P < 0.05$

**Table 4:** Association of Pityriasis Versicolor (PV) with Bacteremia in Pupils.

Bacteria Blood Culture	Symptomatic PV		Asymptomatic PV	
	n	(%)	n	(%)
Positive	81	(48.8)	11	(16.4)
Negative	85	(51.2)	56	(83.6)
<b>Total</b>	<b>166</b>	<b>(100.0)</b>	<b>67</b>	<b>(100.0)</b>

$X^2 = 20.60, P < 0.05$

**Table 5.** Frequency Distribution of Malassezia and Bacteria species Co-infection in Blood of Pupils with Pityriasis Versicolor.

Malassezia and Bacteria spp.	n	(%)
<i>M. restricta</i> & <i>S. aureus</i>	3	(17.6)
<i>M. restricta</i> & <i>S. epidermidis</i>	1	(5.9)
<i>M. restricta</i> & <i>E. faecalis</i>	1	(5.9)
<i>M. restricta</i> & <i>Proteus</i> spp.	3	(17.6)
<i>M. restricta</i> & <i>Klebsiella</i> spp.	1	(5.9)
<i>M. restricta</i> & <i>Pseudomonas</i> spp.	3	(17.6)
<i>M. globosa</i> & <i>S. Aureus</i>	1	(5.9)
<i>M. globosa</i> & <i>Klebsiella</i> spp.	2	(11.8)
<i>M. globosa</i> & <i>Pseudomonas</i> spp.	1	(5.9)
<i>M. obtusa</i> & <i>Proteus</i> spp.	1	(5.9)
<b>Total</b>	<b>17</b>	<b>(100.0)</b>

#### 4. Discussion

Out of 232 pupils with lesions suggestive of pityriasis versicolor, 166 (71.6 percent) of them were confirmed to be having the disease by positive culture results. Among the primary school pupils with skin lesions recruited in this study, a significant association was found between PV and malassezia fungemia indicating malassezia invasive infection ( $X^2=4.56, P < 0.05$ ). This result corroborated an earlier report credited to Devlin (2006), which stated that invasive malassezia infection is usually preceded by skin colonization, and if the situation is not arrested it might lead to disease conditions such as meningitis, vasculitis, dacryocystitis, mastitis, peritonitis and thrombo-embolic disorders with death consequences.

Contrary to most findings in other places of the world, where *Malassezia globosa* was the leading

etiological agent of pityriasis versicolor in Iran (Tarazooie *et al*, 2004), and Tunisia (Salah *et al*, 2005), it was uncovered in this study that *Malassezia restricta* was the most predominant species of Malassezia with occurrences of 71.7% and 55.1% in skin lesions and in the blood of the infected pupils respectively. *Malassezia globosa* was the second most frequently isolated species among the agents. However, no *Malassezia furfur*, *Malassezia pachydermatis* nor *Malassezia sympodialis* was isolated. Only one of the subjects showed co-infection of *Malassezia globosa* with *Malassezia obtusa*. This was in accordance with report by Tarazooie *et al* (2004), which stated that more than one species of Malassezia can be recovered from one sample.

*Malassezia* species are part of the human-associated skin flora and are also associated with disease under conditions where their ecology is disturbed and/ or under impaired host immunity (Cassadevall, 2006). Since opportunistic fungal infections generally occur after a breach of some aspects of the host defense systems, it is not surprising that mixed infections by multiple pathogens are a common phenomenon. Bacterial flora including *Enterobacter* species, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* accompanying *Candida* yeast in clinical specimens has been reported (Hermann *et al*, 1999). The majority of research on bacterial-fungal interactions has focused on the fungus *Candida albicans*. Much less is known about the interactions between bacteria and *Malassezia*.

Bacteremia as observed in this study, has been strongly associated with PV ( $P < 0.05$ ). Response from the questionnaire indicated that some of the affected pupils have taken antibacterials in the past, and since the clinical history of the pupils prior to the study was unknown, it was difficult to predict which of these two clinical conditions (i.e PV and bacteremia) precedes each other; and so one cannot state categorically which of the conditions predisposes to the other as both cases are likely.

Documented evidence exists that;  $\geq 3$  episodes of sepsis may increase skin colonization by *Malassezia* species (Feja *et al*, 2005; Devlin, 2006). On the other hand downregulation of the immune system by *Malassezia* due to inhibition of pro-inflammatory cytokines by its lamellar (lipid layer of cell-wall), impairment of phagocytic killing by inhibition of hydrogen peroxide production as a result of azelaic acid production coupled with induction of interleukin - 10 (IL-10) which is inhibitory to macrophages as stated by Ashbee and Evans (2002); may encourage dissemination of bacterial skin flora, with resultant cases of systemic infections such as bacteremia and septicemia.

Though, detailed mechanism behind Malassezia-bacterial co-isolation in the blood of the studied

subjects with PV in this study is unknown, significant association between PV (skin colonization) and Malassezia fungemia ( $P < 0.05$ ), coupled with co-isolation of Malassezia with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas species* and enteric (*Enterococcus faecalis*, *Proteus species*, *Klebsiella species*) contaminating bacteria in the blood of the subjects suggested that these bacterial isolates might have gained their entry exogenously via skin surfaces or endogenously through the intestine. Since the microbial skin flora can attach themselves to the sticky lamellar of Malassezia (Ashbee and Evans, 2002), they might gain entry into the blood during malassezial invasion. On the other hand, capillary invasion by enteric bacteria may also assist in the dissemination of Malassezia into the blood streams of the hosts.

There is, therefore, the need to learn about different strategies that bacteria use to interact with fungi and vice-versa in the body of human beings and other animals. From the perspective of the bacterium, Hogan and Kolter (2006) postulated that a fungus could represent a synergistic partner for the degradation of complex substrates, a competitor for scarce nutrients or the producer of lethal antibiotics. Survival of the bacterium can depend on its being able to control these interactions. Thus, it is likely that bacteria have evolved numerous ways to manipulate fungal behavior.

The data obtained in this study emphasize the point that the persistence of *Malassezia* species with bacteria in the blood of the pupils is determined by both their ability to interact with the hosts and their success in competing or acting synergistically with bacteria. While the effects of mixed fungal-bacterial infections on the host have not been well characterized, one can speculate many ways by which these microbial interactions could impact virulence factor production, host immune responses and / or susceptibility to antibiotic therapy.

The result of this study has revealed *Malassezia restricta* and *Malassezia globosa* as the predominant etiological agents of pityriasis versicolor among primary school pupils in Ogun State, Nigeria. It has further established that symptomatic PV is both a superficial and systemic mycosis, which could occur as a co-infection with bacteremia in primary school pupils.

It is recommended that the findings of this study be considered in the management of PV, which is a common mycotic infection of the skin. Future research incorporating molecular study is hereby advocated in this area.

#### Acknowledgements

We are grateful to the management of Smooford International Ltd, Iperu-Remo, Ogun State, Nigeria for

donating To-To ointment and soap used in treating the pupils with lesions resembling PV and also to the management of the primary schools where clinical specimens were collected from the pupils.

#### Correspondence to:

Afolabi Ogunledun  
Department of Medical Microbiology and Parasitology,  
College of Health Sciences,  
Olabisi Onabanjo University, P.M.B 2022, Sagamu,  
Ogun State, Nigeria.  
Tel. +234-8033871945  
Email: [afolabiogunledun@yahoo.com](mailto:afolabiogunledun@yahoo.com)

#### References

1. Alebiosu CO, Ogunledun A and Ogunleye DS. A report of clinical trial conducted on To-To ointment and soap products. Nat. Med. Assoc. 2003; 95:95-105.
2. Ashbee HR, Leck AK, Puntis JWL, Parsons WJ and Evans EGV. Colonisation of the skin of newborns and infants by Malassezia – Implications for the acquisition of intravascular catheter related fungaemia. Infect. Control Hosp. Epidemiol. 2002; 23: 212-216.
3. Ashbee HR and Evans EGV. Immunology of diseases associated with Malassezia species. Clin. Microbiol. Rev. 2002; 15: 21-57.
4. Arzumaniyan VG. The yeast Malassezia on the skin of healthy individuals and patients with atopic dermatitis. Vestn. Ross. Akad. Nauk. 2001: 29-31.
5. Casadevall, A. Amoeba and slime mold: Hosts of virulence evolution. In: Eds. Hietman J, Filler SG, Edwards JE and Mitchell AP. Molecular Principles of fungal pathogenesis. ASM Press, Washington DC 2006: 227-234.
6. Cheesebrough LM. Identification of bacteria. Medical Laboratory manual for tropical countries. Vol II. Butterworth & Co. Publishers. London, UK 1985: 63-69.
7. Devlin RK. Invasive fungal infections caused by Candida and Malassezia species in the Neonatal Intensive Care Unit. Adv. Neonatal Care 2006; 6 (2): 68-77.
8. Dutta S, Bajay AK, Basu S, Dikshit A. Pityriasis versicolor: Socio-economic and clinico-mycologic study in India. Int J Dermatology 2002; 41:823-824.
9. Feja KN, Wu F, Roberts K. Risk factors of candidaemia in critically ill infants: a matched case-control study. J. Pediatr. 2005; 147(2): 156-61.
10. Gaitanis G, Velegraki A, Alexopoulos EC, Chasapi V, Tsigonia A and Katsambas A.

- Distribution of *Malassezia* species in pityriasis versicolor and Seborrheic dermatitis in Greece. Typing of the major pityriasis versicolor isolate; *M.globosa*. British J. of Dermatol 2006; 154: 854-859.
11. Gulec AT, Demirbilek M, Seckin D, Can F, Saray Y, Sarifakioglu E *et al*. Superficial fungal infections in 102 renal transplant recipients: A case control study. J. Am. Acad. Dermatol. 2003; 49: 187-192.
  12. Hermann, C, Hermann J, Munzel U, and Ruchel R Bacterial flora accompanying *Candida* yeasts in clinical specimens. Mycoses 1999; 42: 619-627.
  13. Hogan DA and Kolter R. Fungal-bacterial interactions. In Eds. Hietman J, Filler SG, Edwards JE and Mitchell AP. Molecular Principles of fungal pathogenesis ASM Press, Washington DC 2006: 261-269.
  14. Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. J Appl Bacteriol 1989; 67: 47-52.
  15. Salah SB, Makni F, Marrakchi S, Sellami H, Cheikhrouhou F, Bouassida S, Zahaf A and Ayadi A. Identification of *Malassezia* species from Tunisian patients with pityriasis versicolor and normal subjects. Mycoses 2005; 48 (4): 242-245.
  16. Schmidt A. *Malassezia furfur*: A fungus belonging to the physiological skin flora and its relevance in skin disorders. Cutis 1997; 59: 21-24.
  17. Tarazooie B, Kordbacheh P, Zaini F, Zomorodian K, Saadat F, Zeraati H, Hallaji Z and Rezaie S. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. BMC Dermatology 2004; 4(5) : 1 – 6.
  18. Thoma W, Kramer HJ, Mayser P. Pityriasis versicolor alba. Journal of the European Academy of Dermatology and Venereology 2004; 19: 147-152.

5/10/2009