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

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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, 2010;2(1):1-5]. (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^{o} 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^{o}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 cultre 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.sloofiae* (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	(%)
Malassezia restricta	119	(71.7)	27	(55.1)
Malassezia globosa	38	(22.9)	18	(36.7)
Malassezia obtusa	6	(3.6)	4	(8.2)
Malassezia slooffiae	3	(1.8)	0	(0.0)
Total	**166**	**(100.0)**	**49**	**(100.0)**

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

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

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)
Total	**166**	**(100.0)**	**67**	**(100.0)**

$X^2 = 4.56$, $P < 0.05$

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

Malassezia fungemia	**Symptomatic PV**		**Asymptomatic PV**	
	n	**(%)**	**n**	**(%)**
Positive	81	(48.8)	11	(16.4)
Negative	85	(51.2)	56	(83.6)
Total	**166**	**(100.0)**	**67**	**(100.0)**

$X^2 = 20.60$, $P < 0.05$

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

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

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; ≥ 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 epidemis, Pseudomonas specie*s 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.

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