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Association of Bacteria with Fungal Infection of Skin and Soft Tissue Lesions in Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AC conceived the study, its design and coordination. Author OON drafted the manuscript and managed the literature searches. All authors participated in sample collection, isolation and enumeration of all fungi and bacteria. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To identify and determine the bacteria associated with skin and soft tissue conditions of fungal infections.
Place and Duration of Study: Sample area was Plateau State Nigeria and sample collection and analysis was done in Dermatophilosis Research Centre, National Veterinary Research Institute Vom, Plateau State, Nigeria, between September 2011 and December 2012.
Methodology: Nine hundred and forty (940) human skin and nail scraping samples from different parts of the body were collected from subjects referred to the Centre from different hospitals with visible skin infections. Sample analysis were carried out using standard microbiological methods which include: Wet mount, tease mount, culture and biochemical tests were used to process and analyze for the isolation and identification of fungi and bacteria.
Results: Out of 940 samples, 892(94.9%) yielded fungal species which include: *Microsporum* 45(4.8%), *Trichophyton* 176(18.7%), *Aspergillus* 216(22.9%), *Epidermophyton* 32(3.4%), *Candida* 72(7.7%), *Mucor* 141(15.0%), *Rhizopus* 52(5.5%), *Fusarium* 12(1.3%), *Bipolaris* 23(2.5%), *Sporothrix* 74(7.9%), *Penicillium* 32(3.4%) and *Curvularia* 17(1.8%). All samples 940 (100%) yielded an array of bacteria which include:

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Staphylococcus aureus 125(13.3%), *Staphylococcus epidermidis* 145(15.8%), *Micrococcus luteus* 233(24.8%), *α-hemolytic Streptococci* 89(9.5%), *Escherichia coli* 59(6.3%), *Proteus mirabilis* 113(12%), *Bacillus subtilis* 78(8.3%) and *Klebsiella pneumonia* 98(10.4%). *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus* were isolated from all sites of infection while *Micrococcus luteus* was isolated from all moist ulcerous and dry scaly skin infections.

Conclusion: This study showed the presence of bacteria in high frequency in and around skin and soft tissue infection sites on the body. *Micrococcus luteus* was the most prevalent bacterial organism associated with skin and soft tissue conditions of fungal infections. Under favourable conditions, some of the bacteria isolated can establish infections through broken skin hence complicating or prolonging treatment of the skin infection.

Keywords: Fungi; bacteria; association; skin and soft tissue infection.

1. INTRODUCTION

The skin which is the largest organ of the body has the largest surface area when compared with other organs. It is also the most exposed organ and as such the most vulnerable. Diseases involving human skin, hair and nail diseases are common and may be caused by bacteria, fungi or viruses but fungal skin infections are more predominant than those of other microorganism [1,2] Human bacterial flora are approximately ten times more than human cells in the body. A great percentage of these are found on the skin [3]. Some of these skin floras have the ability to form spores that are so resistant to adverse conditions of dryness and temperature. In this form they may remain viable even after 50 years of dormancy [4]. Bacteria find it difficult to establish an infection in intact skin. They become opportunistic when given the right conditions like a crack, break or wound on the skin. Fungal infections are good creators of this situation, aiding bacterial skin flora to establish as opportunistic infections.

Early identification of Skin and soft tissue infections (SSTIs) help in their proper management and treatment [5]. Skin conditions that can predispose to SSTIs include eczema and psoriasis (which cause small fissures on the skin) and superficial fungal infections typified by tinea pedis and onychomycosis (which can cause changes in the affected skin that may lead to superimposed infection with bacteria). Venous stasis and lymphedema also can predispose patients to SSTIs [6]. The commonest primary bacterial skin and soft tissue infections are impetigo, folliculitis, furuncles and carbuncles, erysipelas and cellulitis.

Fungal skin infections are of public health concern as they can elicit social, economic and public health problems [7]. Heavily infected persons with visible lesions are often unconscious targets of social stigma of rejection/isolation because of their unsightly appearance. The cost of long term diagnosis and treatment can be over bearing for low income individuals who make up the bulk of developing countries where these infections occur in epidemic proportions due to poor hygiene. It therefore becomes more traumatic for such individuals when diagnosis or treatment becomes delayed or ineffective. Recently, it has been noted that complications have arisen in the diagnosis and treatment of fungal skin infection as a result of interference from opportunistic bacterial organisms which either prolong or reduce the efficiency/efficacy of diagnosis and treatment. This has necessitated the need to identify bacteria associated with these fungal skin infections in order to facilitate quicker and more efficient diagnosis and treatment. Hence, this study was initiated to identify

bacteria associated with SSTIs presumed to be of fungal origin amongst human subjects in Plateau State. This will aid in managing possible complications in treatment of ongoing fungal infections of subjects.

2. MATERIALS AND METHODS

2.1 Study Area/Location, Ethics, Sample Size and Sampling

The study was conducted at Dermatophilosis Research Centre, National Veterinary Research Institute Vom, Plateau State, Nigeria, which included patients referred from different hospitals and clinics in Plateau state. We carried out this study between September 2011 and December 2012. Nine hundred and forty (940) human subjects who were referred to the Dermatophilosis Research Centre for fungal diagnosis were enlisted after informed consent was obtained. Samples were collected from different infected sites of the body as indicated on the referral forms. Sample sites were classified as either moist and ulcerous or dry and scaly. Sampling was done by scraping suspected infected skin scales and nails of the body using a sterile scalpel blade (per site) into clean sample collection paper. Sampled sites were disinfected after sampling by soaking cotton wool in 70% alcohol and swabbing the scraped surface. All samples were divided into three parts and labeled properly.

2.2 Sample Processing, Analysis and Identification of Microbial Isolates

One part of each sample was processed by performing an initial wet mount preparation in 20% Potassium Hydroxide (KOH) for direct microscopy as described by [1]. The second part was seeded into Sabouraud dextrose agar (SDA) containing chloramphenicol at 16ug/ml using a straight inoculating wire. This was incubated at room temperature for three to four weeks. The third part was cultured in both nutrient and blood agar and incubated at 37^0C for 24hrs. Sub-culturing of isolates for purification and differentiation using various differential media was also done. Colonial morphology, microscopic examination, and biochemical reactions were used to indentify bacterial isolates as far as possible according to standard procedures [8]. The fungi cultures were identified by their colonial morphology, tease mount method and Biochemical tests [9].

3. RESULTS

The results of the study revealed that out of 940 samples, 892(94.9%) samples yielded 15 fungal species which include *Microsporum audouinii, Trichophyton mentagrophytes, T. rubrum, Aspergillus flavus A. niger, A. fumigates, Epidermophyton fluccosum, Candida albicans, Mucor sp., Rhizopus sp., Fusarium solani, Bipolaris sp., Sporothrix schenckii, Penicillium sp.* and *Curvularia sp* (Table 1).

All 940 (100%) samples yielded 8 bacterial species which include *Staphylococcus aureus* 125(13.3%), *Staphylococcus epidermidis* 145(15.8%), *Micrococcus luteus* 233(24.8%), *α-hemolytic Streptococci* 89(9.5%), *Escherichia coli* 59(6.3%), *Proteus mirabilis* 113(12%), *Bacillus subtilis* 78(8.3%) and *Klebsiella pneumonia* 98(10.4%) (Table 2).

The frequency of fungal elements identified with source of specimen showed that samples from the Head (scalp, face & neck) yielded *Microsporum audouinii* (4.8%); samples from the trunk yielded *Candida albicans* (3.4%); samples from the limbs yielded *Aspergillus fumigatus*

(60%); samples from nails yielded *Trichophyton rubrum* (6.4%) and samples from folds (elbow and knee) yielded *Candida albicans* (4.3%) as the most frequent isolates (Table 3).

Bacterial isolates distribution are as follows: *α-hemolytic Streptococci* (9.5%) from the head (scalp, face & neck), *Klebsiella pneumonia* (7.3%) from the trunk, *Micrococcus luteus* from the Limbs (6.6%), from nails (3.6%) and from folds (elbow and knee) (5.6%) respectively (Table 3).

Table 1. Frequency of fungal Isolates from skin infections

Fungal Isolates	Frequency of isolation	% of Frequency
Microsporum audouinii	45	4.8
Trichophyton mentagrophytes	81	8.7
Trichophyton rubrum	95	10.1
Aspergillus flavus	80	8.5
Aspergillus niger	25	2.7
Epidermophyton floccosum	32	3.4
Aspergillus fumigatus	111	11.8
Candida albicans	72	7.7
Mucor sp	141	15
Rhizopus sp	52	5.5
Fusarium solani	12	1.3
Bipolaris sp	23	2.4
Sporothrix schenckii	74	7.8
Penicillium sp	32	3.4
Curvularia sp	17	1.8
Total	**892**	**94.9%**

Table 2. Frequency of bacterial isolates from skin infection sites

Bacterial Isolates	Frequency of isolation	% of Frequency
Staphylococcus aureus	125	13.3%
Staphylococcus epidermidis	145	15.8%
Micrococcus luteus	233	24.8%
α-hemolytic Streptococci	89	9.5%
Escherichia coli	59	6.3%
Proteus mirabilis	113	12%
Bacillus subtilis	78	8.3%
Klebsiella pneumoniae	98	10.4%
Total	**940**	**100%**

Table 3. Distribution of fungal and bacterial isolates from sampled sites of the body and the nature of the sampled site

Sampled part of the body	Nature of sampled site	Fungal isolate	Frequency of isolation (%)	Nature of sampled site	Bacterial isolate	Frequency of isolation (%)
Head (scalp, face & neck)	A & B	*Microsporum audouinii*	45 (4.8)	A & B	*α-hemolytic Streptococci*	89 (9.5)
	A & B	*Trichophyton*	41 (4.4)	A & B	*Micrococcus luteus, Staphylococcus*	45 (4.8)
	B	*mentagrophytes,*	35 (3.7)	B	*aureus, Staphylococcus epidermidis*	16 (1.7)
	B	*Trichophyton rubrum,*	35 (3.7)	B		26 (2.8)
	B	*Aspergillus flavus*	25 (2.7)			
		Aspergillus niger				
Trunk	A&B	*Candida albicans*	32 (3.4)	B	*Staphylococcus aureus*	28 (3.0)
	A&B	*Mucor* sp	42 (4.5)	B	*Staphylococcus epidermidis,*	30 (3.2)
	A&B	*Rhizopus* sp	20 (2.1)	A	*Klebsiella pneumoniae, Bacillus*	69 (7.3)
	B	*Fusarium solani*	12 (1.3)	A	*subtilis,*	47 (5)
	B	*Epidermophyton*	11 (1.2)	A&B	*Micrococcus luteus*	39 (4.1)
	B	*fluccosum*	18 (1.9)			
		Aspergillus fumigatus				
Limbs	A	*Bipolaris* sp	23 (2.4)	B	*Staphylococcus aureus,*	32 (3.4)
	A&B	*Rhizopus* sp	32 (3.4)	B	*Staphylococcus epidermidis*	40 (4.3)
	A&B	*Mucor* sp	35 (3.7)	A	*Escherichia coli*	47 (5)
	A	*Sporothrix schenckii*	37 (3.9)	B	*Bacillus subtilis,*	31 (3.3)
	B	*Aspergillus flavus*	45 (4.8)	A&B	*Micrococcus luteus*	62 (6.6)
	A&B	*Aspergillus fumigatus,*	56 (60)			
Nails	A&B	*Trichophyton*	40(4.3)	B	*Escherichia coli*	12 (1.3)
	B	*mentagrophytes,*	32 (3.4)	A&B	*Proteus* spp	113 (12)
	B	*Penicillium* sp	29 (3.1)	B	*Staphylococcus aureus,*	12 (1.3)
	A&B	*Mucor* sp	60 (6.4)	B	*Staphylococcus epidermidis,*	20 (2.1)
		Trichophyton rubrum		A&B	*Micrococcus luteus*	34 (3.6)
Folds	A	*Epidermophyton*	21 (2.2)			
(elbow and	A&B	*fluccosum Sporothrix*	37 (3.9)	A	*Staphylococcus aureus,*	37 (3.9)
knee)	A	*schenckii,*	40 (4.3)	A	*Staphylococcus epidermidis*	29 (3.0)
	B	*Candida albicans*	35 (3.7)	A&B	*Micrococcus luteus,*	53 (5.6)
	B	*Mucor sp*	37 (3.9)	B	*Klebsiella pneumoniae*	29 (3.1)
	B	*Aspergillus fumigatus*	17 (1.8)			
		Curvularia sp				

key: A: Moist and ulcerous, and B: Dry and scaly

4. DISCUSSION

This study revealed that not all skin infections thought to be of fungal origin yielded fungi as expected (only 94.9% gave fungal isolates), yet all samples yielded bacterial growth of one type or another. These points to the fact that skin infections can sometimes be misdiagnosed to be of fungal cause rather than bacterial.

A wide range of fungi were isolated, confirming that the greatest causative agents of skin infections are the dermatophytes [10,1]. Dermatophytes identified include *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum audouinii and Epidermophyton floccosum.* Dermatophytes isolated from the most sampled sites co-habited with bacteria like *α-hemolytic Streptococci, Micrococcus luteus and Staphylococcus aureus.* If dermatophytes have the ability to break down the epidermis layer of the skin, identified subjects with dermatophyte-bacteria association have the probability of suffering from any of the afore mentioned bacterial infections of SSTIs. This could complicate their fungal disease status/treatment. Our findings agree with the findings of [11], that damaged skin which is defined by extensive cracking of skin surface, widespread reddening and bleeding has been found to be more frequently colonized by *Staphylococcus aureus*, gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*), *Enterococci* and *Candida*.

The study also revealed the type of bacteria most likely to be associated with fungal skin infection of moist and ulcerous nature as well as dry and scaly nature. *Escherichia coli, Proteus mirabilis, Bacillus subtilis* and *Klebsiella pneumonia* were isolated in greater frequency from moist ulcerous skin lesions. These bacteria are often isolated from moist intertriginous areas such as toe webs as increased moisture in this area encourages their easy adaptation and growth. This could account for *Bacillus subtilis* being one of the major causative agents of foot odor [12]. *Staphylococcus aureus* was isolated in high frequency from both moist and dry areas showing its high adaptability irrespective of moisture levels. *Micrococcus luteus* was isolated from all samples, both moist ulcerous and dry scaly lesions and the factors that may have contributed to this could be their presences in soil, dust, water and air, and as part of the normal flora of the mammalian skin. They colonize the human mouth, mucosa, or pharynx and upper respiratory tract and also degrade the compounds in sweat into ones producing unpleasant odors. The factor that probably makes *Micrococcus luteus* the most prevailing bacterium in this study is it's resistant to reduced water potential and their ability to tolerate drying and high salt concentrations [13], hence their occurrence in all samples of the study and in both moist ulcerous and dry scaly skin infections. *M. luteus* has been shown to survive in oligotrophic environments for extended periods of time [14]. It is also possible that *Micrococcus luteus* may have been misidentified as *Staphylococcus aureus* in the past and as such have not been given the attention it deserves. In order to confirm that *Micrococcus luteus* is not *Staphylococcus aureus*, a bacitracin susceptibility test should be performed which will show *Micrococcus luteus* to be susceptible and *Staphylococcus aureus*, to be resistant. Desiccation is a major factor preventing the multiplication of gram negative bacteria on dry intact skin, [15]. This could account for low levels of gram negative bacteria we isolated from such areas.

Bacterial notorious for skin infections infections are *Staph. aureus*, *Strep. pyogenes*, or both organisms together. In most cases, the severities of primary infections are mild to moderate [16]. Considering the high frequency at which we isolated these bacteria from all samples, we can speculate that our study subjects may be at high risk of developing impetigo, folliculitis, furuncles and carbuncles, erysipelas and cellulitis which could interfere with their current fungal infection making treatment prolonged or complicated and cost demanding.

5. CONCLUSION

The study has shown the presence of bacteria in high frequency in and around skin and soft tissue infection sites on the body. These calls for caution during diagnosis and treatment of such infections diagnosed to be of fungal cause as they could in actual fact be of bacterial origin or bacteria associated leading to misdiagnosis or delays in treatment and recovery of the infected individual.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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