PRESENCE OF PATHOGENIC YEASTS AND FUNGI ON SCHOOLS PLAYING-GROUNDS: DANGER POSED TO PUBLIC HEALTH

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ABSTRACT

One hundred and twenty (120) soil samples collected from National Veterinary Research Institute Staff School, St. Joseph Primary School and St. Andrew Primary School, all in Vom, were processed and cultured on Sabouraud Dextrose Agar (SDA) for isolation and identification of yeasts and fungi. One hundred and sixteen (116) of the 120 samples (96.67%) yielded positive cultures. Four species of yeasts and 5 fungal organisms were identified as ; *Candida albicans* 2(1.72%); *C.stellatoidea* 3(2.59%) *C. tropicalis* 4(3.45%); *Cryptococcus neoformans* 3(25.59%): *Aspergillus niger* 5(4.31%); *Penicillium species* 2(1.72%) *and Mucor spp* 2(1.72%). The significance of these isolates and their relationship to human health especially as regards to school children and adults with immune suppression are discussed.

KEY WORDS: Fungi, yeasts, pathogenic, immune suppression.

INTRODUCTION

The soil is one of the natural ecological niches for yeasts and fungi. Some of the pathogenic fungi found in the soil have been shown to have association with droppings of animals and birds (Emmons, 1955; Ajello, 1956). The spores of fungi reside in the soil and become disseminated into the air by wind currents. While on the soil the spores are the major sources of dissemination to man through contact especially children who play on such contaminated grounds.

According to Cruickshank *et al* (1968) and Ballows (1991) diseases caused by fungi and yeasts have common clinical features and children of school age are the most affected because of their frequent contact with environments likely to harbor spores of these organisms. Also, Jawetz *et al* (1995), highlighted on the ubiquitous nature of yeasts especially *Candida* species which can cause localized, invasive or dissemination disease in both normal and immune suppressed hosts. Earlier, Guguani (1982) indicated that mycotic infection is alarming in tropical countries including Nigeria. Mycotic (fungal and yeasts) diseases can be grouped into:

(i) Superficial infective (mycoses) in which the keratinized tissues, the skin, hair and nail, are affected.

(ii) Subcutaneous mycoses which result from inoculation of the cutaneous and subcutaneous tissues by fungi growing as saprophytes in soil and decaying vegetation. They are referred to as mycoses of implantation because they are acquired when the pathogen are inoculated through the skin cuts, scratches or splinter wounds.

(iii) Systemic or deep mycoses which are usually caused by soil fungi which produce large numbers of air-borne spores which if inhaled may eventually penetrate deep into the respiratory system and initiate primary pulmonary disease.

(iv) Opportunistic mycoses which are fungal infection caused by some saprophytic fungi which ordinarily are not pathogenic but become so when the host's defensive mechanisms are impaired.

Two factors prompted this work; first, the fact that animals, both domestic and wild life parade the schools playing grounds and deposit their droppings and secondly, the fact that most children who attend the schools in Vom are from relatively affluent and enlightened parents yet most of them still come down with these fungal infections. The findings will therefore help to develop epidemiologic tools to control mycoses among the populace.

MATERIALS AND METHODS

Study Site: Playing grounds of the 3 primary schools, where animals frequent and deposit their droppings.

Sample Collection: Soil samples were collected four times during the warmest period of the year March to June, (March-April dry season, and May-June raining season). A total of 120 soil samples. 40 from each school were collected in sterile polytene containers/bags. The samples was taken 5cm below the soil surface and taken to laboratory for processing.

Media and Reagents: Media used included Sabouraud's, Dextrose Agar (SDA), and Sugar Assimilation Agar (SSA). Chloramphenicol and streptomycin were incorporated to all media to inhibit bacterial growth as recommended by Cheesbrough (1984). The media were prepared in the Bacteriology lab of Federal College of Veterinary and Medical Laboratory Technology (FCVMLT), Vom, according to manufacturers instructions. The reagents used include lactophenol cotton blue, Grams Staining reagents, 2% sugar solution (glucose, sucrose, maltose, galactose, lactose), sterile normal saline, sterile distilled water and sterile phosphate buffer saline (pH 7.2).

Processing of Sample: One gram of each soil sample was dissolved in 5ml of Phosphate Buffer Saline (PBS) and left at room temperature for 24 hr. The mixtures were shaken thoroughly before culture.

Culture: Sabouraud Dextrose Agar plates were properly dried in the incubator at 37^{oC}. The specimen mixtures were re-suspended before inoculation using sterile wire loop. The plates were incubated aerobically at 37^{oC} for 48 hrs after which further incubation was carried out at room temperature for 2-3 weeks while the plates were examined daily for growth as recommended by Duguid *et al* (1978).

Identification: After appropriate incubation, plates that contain creamy to white tan pasty colonies were noted and sub cultured onto new SDA slopes for purification.

Microscopy: All the colonies which appeared creamy to white or orange in colour were stained by Grams method and examined under the microscope using x 100 magnification. Gram +ve oval ellipsoidal or spherical shaped, budding cells were noted; 2-4mm in diameter with or without pseudohyphae were considered as yeast or yeast-like organisms. These were then sub cultured onto fresh SDA for further purification and identification.

INDIAN INK STAINS FOR CAPSULES (COLLEE *et al* 1989).

A loopful of Indian ink stain and a colony were homogenized and a cover slip placed over the suspension. This was then examined under microscope using x 100 and x 40 objectives in reduced light for the presence or absence of capsules. If present the size of the capsule is noted. The shape of any yeast cell present was also noted. Refractile outline indicates positive for capsules.

Other tests: Other tests carried out to achieve identification included urease test as described by Cheesbrough (1984) using *C. albicans* as positive control, needle mount staining techniques, biochemical tests which include both sugar fermentation and sugar assimilation tests as described by Collee *et al* (1989). With all these tests all the isolates were able to be identified to the level shown below (tables 1 and 2).

RESULTS

The results obtained from this study showed that both pathogenic fungi and yeasts are present in the three school playing grounds. One hundred and sixteen (96.7%) soil samples out of the 120 samples collected yielded isolates.

As shown in table 1, isolates obtained during the dry season (March-April) showed predominance of yeasts while fungi predominated during the rainy season (May-June).

Table 2 shows the different fungal and yeast isolate, obtained from the 116 positive samples: *Trichophytom mentagraphyte* 27(23.28%), *T. rubrum* 30(25.86%); *Mucur* 40 (34.48%); *Aspergillus niger* 5(4.31%); *Penicillium* 2(1.72%) *Candida albicans* 2(1.72%); *C. stellatoidea* 3(2.59%). *S. tropicalis* 4(3.45%) and *Cryptococcus neoformans* 3(2.59%).

Table 1: Distribution of Isolates in the three Schools in Vom.

ISOLATES	NVRI SCHOOL		ST. JOSEPH SCH		ST. ANDREW SCH.	
	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season
Tricophyton mentagraphyte	7	4	3	2	5	6
T. rubrum	8	5	7	4	3	3
Mucor	5	10	4	6	11	4
Aspergillus niger	3	-	-	-	-	2
Pencillium	-	-	-	2	-	-
Candida albicans	2	-	-	-	-	-
C. stellatoidea	-	-	3	-	-	-
C. tropicalis	-	-	2	-	2	-
Cryptococcus	-	-	-	-	3	
Total	**25**	**19**	**19**	**14**	**21**	**18**

Table 2: DIFFERENT FUNGAL AND YEAST ISOLATES OBTAINED FROM THE SOIL SAMPLE FROM 3 SCHOOLS IN VOM.

ISOLATES	NUMBER POSITIVE	PERCENTAGE
Tricophyton mentagraphyte	27	23.28
T. rubrum	30	25.86
Mucor	40	34.48
Aspergillus niger	5	4.31
Pencillium	2	1.72
Candida albicans	2	1.72
C. stellatoidea	3	2.59
C. tropicalis	4	3.45
Cryptococcus neoformans	3	2.59

DISCUSSION

From the results obtained it is apparent that fungi colonize the school play grounds more than the yeasts at all seasons. However, there is a higher incidence of yeasts during the dry season than in the rainy season. This could be because the dry season is a more favourable period when birds and animals are on free range and frequent the playing grounds more and consequently deposit their droppings.

The isolation of *Cyptococcus neoformans* only from St. Andrew Primary School located in the village part of Vom where birds especially pigeons frequent more than the other schools confirmed the earlier study of Emmons (1955) who asserted that birds droppings by virtue of their chemical constituents serve as a natural selective medium for this yeast. There are always plenty of pigeons and other domestic birds hovering and perching on St. Andrew Primary School playing ground. *C. neoformans* is a potential pathogen and the causative agent of cryptococcosis. *C. neoformans* has a predilection for the brain and meningenes but also invades the skin, lungs and other parts (Jawetz *et al*, 1995).

The other yeasts-like organisms isolated are also of public health importance especially *C. albicans* which can cause havoc particularly in individuals suffering from diabetes mellitus, general debility or immunosuppressed or those under corticosteroid therapy. In addition to being one of the agents of deep mycoses candidal organisms are involved in candidal infections of children such as mouth thrush and vulvovaginits which can pose serious problems.

Among the fungi isolated, two species *Trichoplyton mentagrophyte* and *T. rubrum* are important agents of dermatophytoses (superficial and subcutaneous mycoses). These are among the agents of *Tinea pedis* (Athletes foot), Tinea capitis and *Tinea barbae*. *Tinea barbae* causes a menace in adults just as *Tinea capitis* causes "ring worms" in children. *Tinea pedis* affects both children and adult and often very difficult to treat. *Asperpillus niger* is one of the fungal agents of opportunistic mycoses. *A. niger* ordinarily is non pathogenic but may become pathogenic when the hosts defensive mechanisms are impaired. Also *Mucor species,* which were isolate in highest number ordinarily, are saprophytes but when inhale by individuals whose defensive mechanisms have been compromised mucormycosis sets in. This is a fungal disease caused by any of Phycomycetes *Absidia, Mucor and Rhizopus.* This disease can be systemic and fatal especially in persons suffering from diabetes mellitus.

The finding of these potential pathogens in the playing grounds of these schools is hazardous and poses a public health problem to the youngsters in particular and to the entire public in general.

RECOMMENDATION

Children should be educated to always play with shoes on as most of them remove their shoes and play bare footed.

There is need to always water the playing ground before children come out to play especially during the dry season to reduce raising of dusts and air borne spores which could be inhaled and subsequently lead to infection especially in debilitated children and adults.

Maintenance of optimum personal hygiene is an adjunct to preventing some spores remaining on the skin and thus poses danger of infection.

Regular study of fungal contamination of an environment is desirable.

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