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Prevalence of Geophilic Dermatophytic Fungi in Soil of Public Recreational Parks and Gardens in Abakaliki, Ebonyi State, Nigeria

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Abstract: Dermatophytosis is among the major common infections of human skin, hair, and nails, caused by a group of keratinophilic fungi known as geophilic dermatophytes. This study was aimed at the prevalence of geophilic dermatophytes from the soil samples within public recreational parks and gardens in Abakaliki metropolis. A total of 50 soil samples were randomly collected and analyzed for presence of geophilic dermatophytes. Antifungal susceptibility test was done using Kirby-Bauer disc diffusion method. The total fungal colonies counts were reported to range from 3.0 x 10^5 to 5.0 x 10^5 CFU/g, while the overall prevalence of geophilic dermatophytes was 23 (46.0%). Aspergillus species, Fusarium species, Penicillium species and Trichophyton rubrum were predominantly identified. Penicillium species had the highest prevalence of 47.8%, and the least prevalence was recorded on Fusarium species (8.7%). The antifungal susceptibility profile showed that Aspergillus flavus, Trychophyton rubrum, Penicillium species, Fusarium species and Aspergillus niger were 80.0%, 90.0%, 81.0%, 90.0%, 60.0% susceptible to ketoconazole respectively. The amphotericin-B and fluconazole showed least inhibitory effects on the isolates. Multiple antifungal resistance indexes were observed to range from 0.25 to 0.75. Ketoconazole was observed to be the most effective antifungal agent in the treatments of fungi infections. This study showed that recreational parks and garden can serve as reservoirs of geophilic dermatophytes. Therefore, periodical investigations are required for the efficient control and prevention of the disease caused by geophilic dermatophytes in the environment.

Keywords: Prevalence, geophilic dermatophytes, recreational parks, Abakaliki

Introduction

Dermatophyte is a common name for a group of morphologically related fungi that causes disease in human and animals. They cause infection of the skin, hair

and nails, obtaining nutrients from keratinized materials (Moskaluk and Vandewoude, 2022). Dermatophytes can affect the viable intact tissue of immunocompetent host and as such their growth is usually restricted to the nonliving cornified layer of the epidermis. They posses certain virulent factors which includes; proteases, elastases, and keratinase (Mercer and Stewarts, 2018; Manish and Neha, 2022). Geophilic dermatophyte resides in soil are rich in keratin with abundant supply of decaying debris, feathers, horns, hairs and dungs. The soil in public places such as gardens and parks is constantly influenced by the presence of both biotic and abiotic factors including plants, animals, temperature, humidity, climatic changes, light and soil pH (Sharon et al., 2002; Manish and Neha, 2022).

Direct or indirect contact with contaminated soil can lead to human infection. The dermatophytes grow on the dermis in a snake - like appearance termed ringworm or dermatophytosis. Toenail and fingernail infections termed onychomycosis is a common phenomenon. Occasionally, the organism can invade the subcutaneous tissues, resulting in keroin development (Kauffman, 2018; Jameson et al., 2018). However, success of infection in humans is hinged on the ability of the fungi to grow at a high temperature of or above 37°C, penetrate or circumvent host tissue barriers, and also overcome human immune system (Sharon et al., 2002; Burstein, 2020; Segal, 2021).

The treatment for ringworm depends on its location on the body and how serious the infection. Some superficial ringworm infections can usually be treated with non - prescription antifungal creams (topical therapy) such as clotrimazole for few weeks. However, the emergence of possible resistance among dermatophytes make antifungal combination approach interesting (Manjunath et al., 2023; Dogra, 2016). Undetected and untreated cases of dermatophytosis have been linked to recurrent infections (Verma and Madhu, 2017). Information on prevalence of geophilic dermatophytes in Abakaliki metropolis, Ebonyi State of Nigeria is limited to only a few reports. Hence this research is necessary to know the current prevalence of geophilic dermatophyte in soils of public recreational parks and gardens within Abakaliki.

Study area

This research was carried out at the major public recreational parks and gardens within Abakaliki metropolis. Abakaliki is the capital city of Ebonyi State in South Eastern Nigeria, located between 64 kilometers South East of Enugu. It is also located at the latitude of 6.3°E and longitude 8.1°N in the eastern part of the country. Climate is sub-humid tropical and bounded with the rainy season between early April and late October. Rainfall pattern is bimodal with two peaks in June and September. Maximum temperature is 26°C. The following study sites within the city were selected and sampled; Abakaliki township stadium, Ebonyi State University (College of Agricultural

Science and College of Health Science), Amusement Park, Fatilami and Dollarman Bar.

Sample collection

A total of 50 composite soil samples were randomly collected from different public recreational parks and gardens within Abakaliki metropolis for the purpose of this study. Exactly 10g of composite soil samples were collected in clean polyethylene bags by scooping up to a depth of 2-5 cm with the help of clean auger. Each bag was tightly packed and labeled indicating the date and site of collection. The samples were immediately taken to the Applied Microbiology Laboratory unit of Ebonyi State University Abakaliki for processing and fungal analysis.

Sample processing and analysis

Sterile petri dishes were half filled with homogenized soil samples. Hair baiting technique according to method described by Vanbeuseghem (1952) was used for isolating the dermatophytes. Briefly, sterile distilled water was added to the half filled soil according to the soil texture to obtain desired soil moisture and avoid dryness of sample. The fungi in the soil were then baited with short strands of sterilized human hair by spreading the hair uniformly as baits on the surface of the moisturized soil samples. The plates were incubated at 28 – 30°C for a period of 3 – 4 weeks. A duplicate of each sample was set up. The hair stripes were observed regularly for signs of fungal growth.

Identification of the fungal isolates

Samples of hair showing prominent fungal growth on their surfaces were aseptically collected with forceps and inoculated on Sabourauds dextrose agar (SDA) supplemented with chloramphenicol (0.05mg/ml) to knock off bacterial and saprophytic fungal growth. After an incubation of 24 hours, colonies were picked with a long sterile forceps and transferred to fresh slants of SDA medium repeatedly to obtain pure culture (Biedunkiewicz et al., 2016).

Microscopic examination

The fungi isolates were examined microscopically and identified according to their macro and micro morphological characteristics following the methods described by Roberts and Friedlander (2005).

Standardization of test organism

Colonies of fungi were selected from a 72hours agar plate culture. The top of each colony was touched with a sterile loop, and the growth was transferred into a tube containing 4ml of normal saline and compared with 0.5% McFarland standard (0.6ml of 1% barium chlorie solution to 99.4ml of 1% sulphuric acid). The turbidity was adjusted with sterile saline to obtain turbidity optically comparable to that of McFarland standard.

Antifungal drug susceptibility testing (disc diffusion method)

Within 15 minutes of adjusting the turbidity of the organism to McFarland standard, an aliquot of the test organism was plated onto a dried surface of a

sterile Mueller Hinton agar plates. The antifungal disc used for this study were commercially prepared (Oxoid Ltd) which includes; ketoconazole (10 µg), fluconazole (25 µg), amphotericin B (20 µg), and voriconazole (1 µg). The antifungal discs were placed onto the surface of the inoculated agar plate at a distance of 30 mm away from each other and 15 mm from the edge of the petri dishes. Each disc was pressed down to ensure its complete contact with the agar surface. The plates were incubated at 30°C and examined after 24hours of incubation. The zones of inhibition were measured in millimeters and results were interpreted using CLSI interpretative breakpoint for in vitro susceptibility testing (CLSI, 2017).

Determination of multiple antifungal resistance index (MARI)

The MARI value of the test isolates was carried out to establish the standard antifungal regimes for sequential in-vitro treatments. The MARI was calculated using the formula (MARI=x/b); Where x = the total number of resistances recorded and b = total number of antifungal tested (Ugbo et al., 2023).

Results

The total fungal counts of the soil samples obtained from public recreational parks and gardens revealed that highest fungal count of 5.0×10^5 was recorded from soil sample code ATSS3, followed by $4.9x10^5$ at FAT4 and CAS4 respectively. There was no growth at ATSS2, ATSS6, AMP2, AMP5, FAT3, FAT7, PRES4, CAS2, CAS7, DMB3, DMB4, DMB5, PSLG5 and PSLG8 (Table 1). Distribution of fungal isolates from soils of different public recreational parks and gardens showed that samples from stadium had the highest fungal isolates with total of seven (7) fungi isolates while dollarman bar had the least fungal isolates with total number of (1) fungal isolate (Table 2). The general percentage prevalence of geophilic dermatophytic fungi isolates in soil samples from public recreational parks and gardens within Abakaliki metropolis revealed that Penicillium species had the highest percentage prevalence of 48.0% while Fusarium species and Trichophyton rubrum had the lowest percentage prevalence of 9.0% respectively (Figure 1).

Table 1: Enumeration of total fungal counts from soil samples collected from different public recreational parks and gardens within Abakaliki metropolis.

Location	Sample code	**CFU /g**
Abakaliki township stadium	ATSS1	3.2×10^5
	ATSS2	No growth
	ATSS3	5.0×10^5
	ATSS4	3.0×10^5
	ATSS5	3.5×10^5
	ATSS6	No growth
	ATSS7	4.0×10^5
Amusement park	AMP1	3.6×10^5
	AMP2	No growth
	AMP3	3.1×10^5
	AMP4	4.0×10^5
	AMP5	No growth
	AMP6	3.6×10^5
	AMP7	4.5×10^5
Fatilami	FAT1	3.7×10^5
	FAT2	4.1×10^5
	FAT3	No growth
	FAT4	4.9×10^5
	FAT5	3.0×10^5
	FAT6	3.0×10^5
	FAT7	No growth
Presco love garden	PRES1	4.3×10^5
	PRES2	3.7×10^5
	PRES3	4.3×10^5
	PRES4	No growth
	PRES5	3.0×10^5
	PRES6	3.0×10^5
	PRES7	3.7×10^5
Cas Love garden	CAS1	4.0×10^5
	CAS2	No growth
	CAS3	3.8×10^5
	CAS4	4.9×10^5
	CAS5	3.0×10^5
	CAS6	3.1×10^5
	CAS7	No growth
Dollarman Bar	DMB1	3.0×10^5

	DMB2	3.2×10^5
	DMB3	No growth
	DMB4	No growth
	DMB5	No growth
	DMB6	3.5×10^5
	DMB7	3.1×10^5
Perm site love gardens	PSLG1	3.3×10^5
	PSLG2	4.0×10^5
	PSLG3	4.5×10^5
	PSLG4	3.0×10^5
	PSLG5	No growth
	PSLG6	3.7×10^5
	PSLG7	4.1×10^5
	PSLG8	No growth

Key: ATSS= Abakaliki Township Stadium, AMP=Amusement Park, FAT= Fatilami, PRES= Presco Love Garden, CAS= Cas Love Garden, DMB= Dollarman Bar, PSLG= Perm Site Love Garden.

Table 2: The distribution of geophilic dermatophytic fungal isolates in soil samples from different public recreational parks and gardens within Abakaliki metropolis

Fungal isolates	**Presco**	**Fatilami**	**Amusement park**	**Perm site**	**Dollarman Bar**	**Stadium**	**CAS**	**Total**	**%**
Aspergillus flavus	0	0	1	0	0	2	0	3	**13.0**
Aspergillus niger	1	1	0	0	0	2	1	5	**21.7**
Penicillium species	2	2	2	1	1	2	1	11	**47.8**
Fusarium species	0	1	0	0	0	1	0	2	**8.7**
Trychophyton rubrum	0	0	1	1	0	0	0	2	**8.7**
Total	**3**	**4**	**4**	**2**	**1**	**7**	**2**	**23/50**	**46.0**

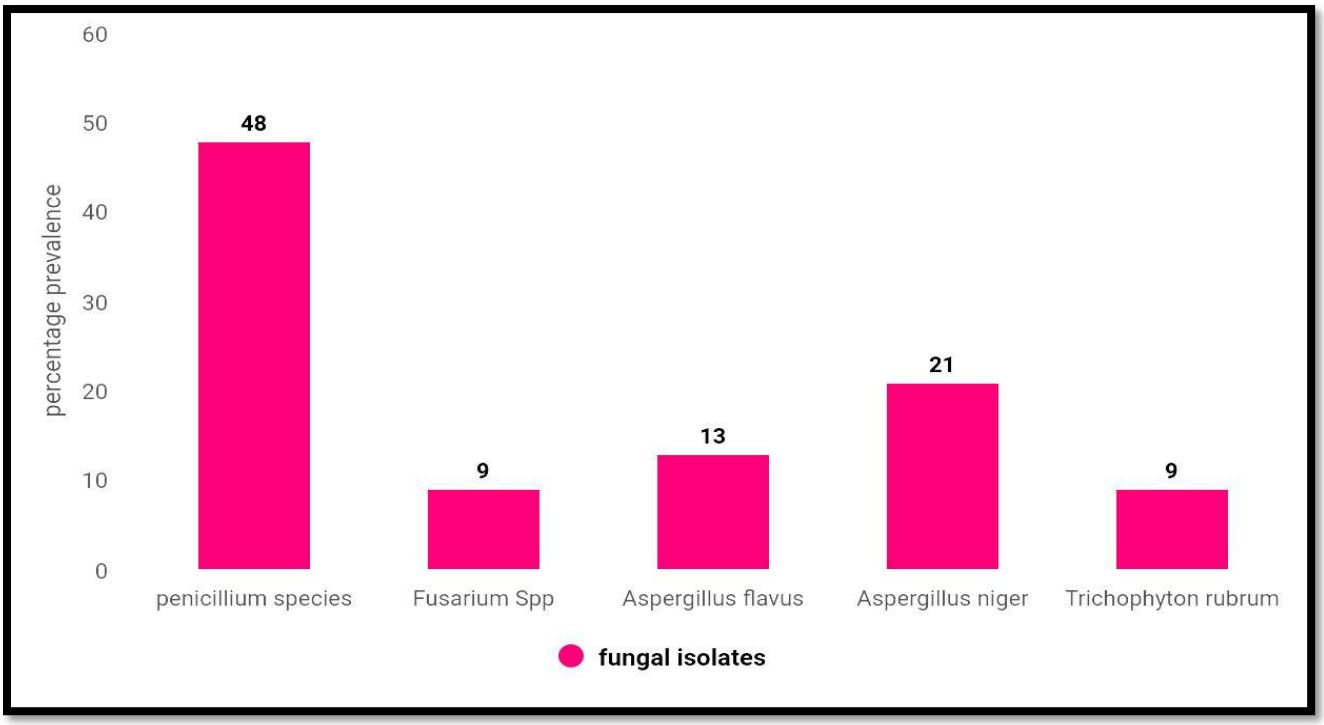

Figure 1: Overall percentage prevalence of geophilic dermatophytic fungi isolates in soil samples from public recreational parks and gardens within Abakaliki metropolis

The percentage antifungal susceptibility profile of dermatophytic fungal isolates in soil samples from public recreational parks and gardens within Abakaliki metropolis are shown that Trychophyton rubrum was 90.0%, 80.0% and 50.0% susceptible to ketoconazole, amphotericin B and voriconazole respectively. Aspergillus flavus was 80% susceptible to ketoconazole and 33.0% susceptible to voriconazole and amphotericin B respectively. Fusarium species was 90.0% susceptible to ketoconazole, voriconazole and fluconazole respectively, While, Penicillium species was 81.0% susceptible to ketoconazole, 64% susceptible to voriconazole and 36.0% susceptible to amphotericin B (Figure 2). Multiple antifungal resistance indexes revealed that the fungal isolates have varying MARI values from 0.25 to 0.75. It also showed that the average MARI values is 0.45 (Table 3).

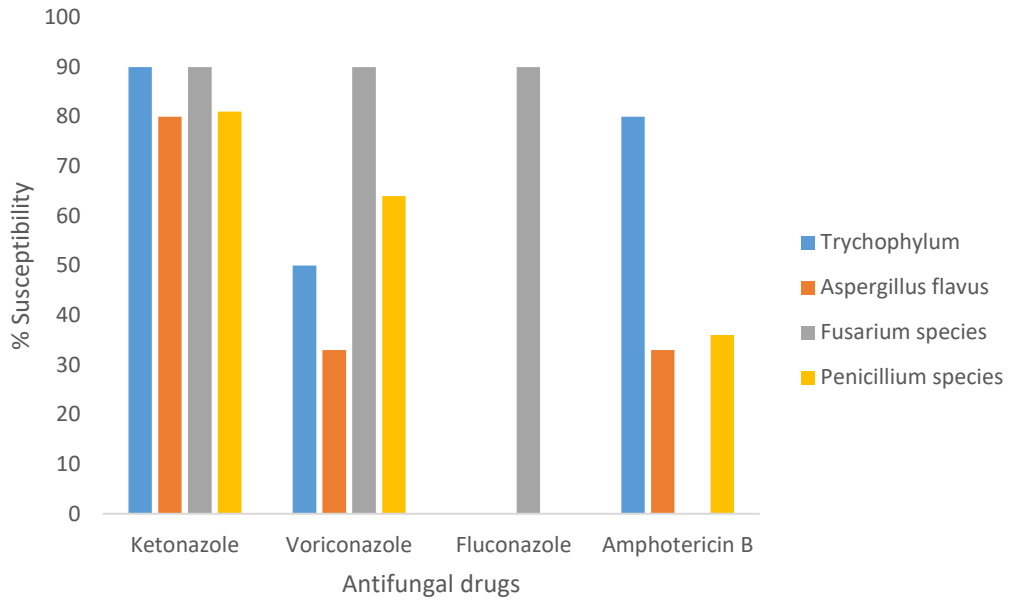

Figure 2: Percentage antifungal susceptibility profile of Fusarium species, Trichophylum rubium, Aspergillus niger and Penicillium species

Table 3: Multiple antifungal resistance index

S/N	**Fungal isolates**	**MARI**
1	Penicillium species	0.5
2	Aspergillus niger	0.25
3	Fusarium species	0.25
4	Aspergillus flavus	0.75
5	Trychophyton rubrum	0.5
Average MARI	**Five fungal isolates**	**2.25/5 = 0.45**

Discussion

Fungi are found in different environment with variable distribution patterns depending on various factors that may encourage their growth. Geophilic dermatophytes from soils of public recreational parks and gardens centre which are used by the visitors or children that play on the soli may pose a potential health risk. The total fungal counts from soil samples collected from different public recreational parks and gardens within Abakaliki metropolis ranged from 3.0 x 10^5 to 5.0 x 10^5CFU/g although some samples recorded no growth of geophilic dermatophytes. This studies showed that public recreational parks and gardens in Abakaliki harbored the presence of geophilic dermatophytes which includes; Aspergillus flavus, Aspergillus niger, Fusarium species, Penicillium species and Trichophyton rubrum. This is in line with the studied that was conducted at public recreational centre where

they find out that swimming pools and soil from the environment within the centre were contaminated with many species of fungi (Frenkel et al., 2020). Thus, public recreational parks and gardens can be a good carrier for transmission of fungal, parasitical, and bacterial diseases. The overall prevalence of geophilic dermatophytes from recreational centres in Abakaliki was 23 (46.0%) out of the 50 samples studied. However, the most predominant geophilic dermatophyte in this study was Penicillium species with prevalence rate of 47.8%, followed by Aspergillus niger (21.7%) and the least percentage prevalence was recorded on Fusarium species, Trichophyton rubrum with 8.7% each. A study in recreational centre demonstrated the presence of Aspergillus species and Penicillium species to be frequently isolated (Góralska et al., 2020). Łagowski et al. (2021) in their studies reported the prevalence of Trichophyton species to be 21.0 percent. Brandao et al. (2021) and Frenkel et al. (2020) from their research at recreational areas reported that the geophilic dermatophytes genera that are the most frequently found were Aspergillus spp, Candida spp, Fusarium spp and Cryptococcus spp both in sand and in water within their study areas.

According to other studies, the geophilic dermatophytes fungi from different genera which includes the Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Trichoderma, Mucor, and Rhizopus have often been detected in surface, soil from the ground, and tap water within and outside the recreational centers (Siqueira et al., 2011; Bandh et al., 2016; Novak-Babič et al., 2016). In this study, geophilic fungi from most of the above mentioned genera predominated, especially Aspergillus species and Penicillium species and are in agreement with the above observations and also with the report of (Góralska et al., 2020). The frequent occurrences of Aspergillus species and Penicillium species was also observed in various world regions (Baumgardner, 2017). The fungi that most frequently appeared in the samples from lakes were Aspergillus species as reported by Biedunkiewicz and Góralska, 2016 and they observation aligned with the report of the current studies. Dermatophytes are the most common fungal infections worldwide, which consists of a group of morphologically and physiologically related filamentous fungi with the capacity to penetrate keratinized tissues of humans and other animals and produce dermatophytosis (Jagadish, 2010).

Geophilic dermatophytes fungal infections may occur in different ways, such as exposure during sports and recreation, children playing with soil, drinking contaminated liquids, and from personal and home hygiene activities, including the inhalation of aerosols during sit-out at recreational centres. Geophilic fungal infections occurs during exposure to soil aerosols or contaminated soil which serves as reservoirs, primarily affects immune compromised people or those with trauma, including damaged skin who came in contact with the infected materials (Baumgardner, 2017). The soil

within the recreational parks contains many keratinophilic fungi closely related to the dermatophytes genera and are secondarily transmitted by animals to humans. These include Microsporum fulvum, Microsporum gypseum, Trichophyton ajelloi, Trichophyton terrestre, but most of these organisms are rarely isolated from human infections (Segal and Frenkel, 2015).

Another study done in Philadelphia revealed an increase in the number of fungal keratitis cases caused by Fusarium species especially among contact lens users; four of the 28 patients with keratitis reported specific water exposure: two from lakes and one each from well water exposure and swimming within recreational areas while wearing contact lenses (Baumgardner, 2017). Geophilic fungi from the genus Fusarium were sporadically isolated in Europe (NovakBabič et al., 2016) and it akin with the observation of 8.7% of Fusarium species in this present study. Additionally, recreational parks and gardens in Abakaliki can be a possible reservoir of dermatophytes, which are mainly zoophilic; Microsporum species, Trichophyton verrucosum,and Trychophyton rubrum and geophilic species; Microsporum species, that are highly transmittable between animal hosts or soil environment and people. However, it is important to note that Trichophyton spp. and Epidermophyton species which cause superficial fungal infections of the hair, fingernails, or skin, are the only fungal species considered as potential microbial hazards in the WHO guidelines for safe recreational parks and gardens (WHO, 2006). However, this study reported low prevalence rate of Trychophyton rubrum from recreational parks and gardens. Study has reported that among all the fungi described in the world, approximately 600 species are opportunistic pathogens, which are etiological agents of mycoses, mainly in immune compromised persons (Góralska et al., 2020). Fungal diseases affect an estimated 15% of the population at any point in time with approximately 70% getting fungal infections over their lifetime. Another study on onychomycosis from North West Greece (Lecha et al., 2020) observed that the most frequently isolated geophilic dermatophyte was Trichophyton rubrum (74.4%). These observations are in contrast with the findings of this present study which reported Penicillium species to be the most frequently isolated geophilic fungi followed by Aspergillus niger whereas Trichophyton rubrum had the least prevalence. The difference observations reported by the researchers could be as a result of the site and geographical location of the study areas.

The antifungal susceptibility profile of the geophilic fungi isolated from soil samples from public recreational parks and gardens within Abakaliki metropolis showed that Aspergillus flavus, Trychophyton rubrum, Penicillium species, Fusarium species and Aspergillus niger were 80.0%, 90.0%, 81.0%, 90.0%, 60.0% susceptible to ketoconazole. Lowest susceptibility was recorded on amphotericin B and fluconazole with susceptibility ranging from

27.0% to 50.0% among the different fungi isolates studied. Geophilic dermatophytes infections caused by Aspergillus species and Trychophyton rubrum are most prevalent strain responsible for dermatophytosis. The most common agents for the treatment of such infections are azoles such as clotrimazole, ketoconazole, miconazole, econazole, oxiconazole, tioconazole (Harvey et al., 2011). Serious infections caused by geophilic dermatophytes have been revealed as an increasing problem especially in immune-compromised patients. Out of many antifungal agents only very few substances are established as agents in the treatment of systemic fungal infections. Azoles, especially fluconazole, have been used as first-line antimycotics for the prophylaxis and treatment of fungal infections (Weintein and Berman, 2016). However, the number of patients with geophilic infections treated with fluconazole has been increase. Azole resistance has emerged as a major problem as a result of abuse and improper usage during treatments of such infections. This study reported high level of geophilic dermatophytes resistance to fluconazole. The emergence of clinically resistant geophilic fungi strains can occur by several mechanisms, including infection with intrinsically resistant organisms, selection of resistant organisms secondary to antifungal drug pressure, and development of resistance in a previously susceptible organism. Several mechanisms of resistance to azoles have been described expression of drug efflux pump genes, amino acid substitutions in the target enzyme Erg11p due to mutations in the ERG11 gene, as well as over expression of ERG11 (Błaszkowska and Wójcik,2012).

Conclusion

Soil samples from public recreational parks and gardens within Abakaliki metropolis has be proven to be a reservoir of geophilic dermatophytes which includes; Aspergillus flavus, Aspergillus niger, Fusarium species, Penicillium species and Trichophyton rubrum. Ketoconazole was observed to be the most effective antifungal agent in the treatments of infections caused by these fungi, whereas fluconazole was least effective. Therefore, periodical investigations on the epidemiological and distribution of geophilic dermatophyte that causes human infection are required for the efficient control and prevention of the disease caused by them in the environment. Encouragement of personal hygiene such as washing hands and taken bath after visiting recreational centers or garden is highly recommended.

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