International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-8, Issue-10; Oct, 2021
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.810.38

Antifungal Susceptibility Pattern against Dermatophytic Strains Isolated from Humans in Anambra State, Nigeria

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Received: 11 Sep 2021,

Received in revised form: 08 Oct 2021,

Accepted: 21 Oct 2021,

Available online: 31 Oct 2021

***Keywords*— Antifungal drugs, Dermatophytic strains, Profile, Susceptibility**

***Abstract*—** *Dermatophytosis are fungal infection that involve only superficial keratinized tissue of the body, skin, hair and nails. Out of the 1000 cultured samples on Sabroud dextrose agar, 320(32%) isolates of dermatophytes were isolated. Susceptibility testing was performed using sterilized discs (6mm) prepared from whatman No. 1 filter paper, impregnated with different concentrations (25mg, 50mg, 100mg and 200mg) of terbinafine, itraconazole, ketoconazole, fluconazole and griseofulvin dissolved in 2% dimethylsuphuroxide (DMSO).These dermatophytes tested were susceptible to the five antifungal drugs used. The MIC of the quality control strains was within established ranges. However no resistance was recorded among the isolates. The isolates were less susceptible to griseofulvin and fluconazole with MICs ranging from 0.1 – 4.00 and 0.007 – 0.500ug/ml respectively. The MIC range for them respectively is between 0.06 – 0.125ug/ml for griseofulvin and 4.0 – 16.0ug/ml for fluconazole. Most dermatophytes 320(32%) were susceptible to terbinafine, since the MIC range was between 4.0 – 16.0 ug/ml. The study showed that there is no abuse of antifungal drugs in this area whose samples were used for the study.*

I. INTRODUCTION

Dermatophytes are the fungal pathogens of human and animals infecting the keratinized tissues of the body namely skin, hair, nails. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors. (1). Dermatophyte infections are common disorders worldwide, and dermatophytes represent the prevailing type of fungi that cause infection of the skin and nails (2). These infections lead to a variety of clinical manifestations including *Tineacapitis*, *Tineapedis*, *Tineacorporis*, *Tineacruris*, and Majocchi's granuloma. These are typically superficial, involving the epidermis.

Dermatophytosis routinely affect individuals who are otherwise healthy, but people with compromised immune systems are particularly susceptible. Bizarre presentations and failure to respond to treatment should alert care providers to the possibility of an underlying immunologic problem (3).

In the last 50 years numerous drugs have been introduced for the treatment of superficial infections. The choice of

treatment is determined by the site and extent of the infection, the species involved as well as the efficacy, safety profile and kinetics of the drugs available. For localized non-extensive lesions caused by dermatophytes, topical therapies with an imidazole, allylamines, tolnaftate, morpholine derivates is generally used.

According to the World Health Organization (WHO) survey on the incidence of dermatophytic infection, about 20% of the people worldwide present with cutaneous infections (4) and affect people of all ages (5).

With very rare to no exception, oral antifungal therapy is needed to eradicate dermatophytoses. Griseofulvin remains a very effective treatment for many cases of *Tinea* infections caused by both *Trichophyton spp* and *Microsporum spp*, provided an adequate daily dose is administered and an appropriate duration of therapy completed which is commensurated with what is needed in each individual case. Unlike the newer oral antifungal agents, which include the allylamine agent, terbinafine, and the triconazoles, fluconazole and itraconazole, griseofulvin does not persist in cutaneous tissue for a prolonged time period after discontinuation, often necessitating a longer duration of therapy in many cases in order to achieve complete cure, Importantly, although use of oral griseofulvin in children was initially plagued by exaggerated fears of major side effects, such as hepatotoxicity and haematological disturbances, such side effects have proven to be very rare in both adults and children

Griseofulvin, terbinafine, fluconazole, and itraconazole, appear in the literature, including in approved product labelling, and may serve as a guide to the clinician. Importantly, PDA approval status in pediatric patients for *Tinea* infections with available oral antifungal agents does not necessarily encompass all clinical situations that the clinician may encounter in clinical practice. The risk of hepatocellular injury or haematological reactions with these agents is low in Overall, oral antifungal therapy has been safe and well tolerated in children with a variety of superficial and systemic fungal infections, including infants with dermatophytosis and other mycotic infections in some analyses and case reports. As with any other therapy, especially with a systemic agent, patient monitoring to assess both efficacy and safety is vital to the success of treatment and allows for adjustments in therapy if needed based on clinical response and/or suspicion of adverse reactions.

The decision to perform baseline and repeat serum transaminase testing during treatment of *Tinea* infections with fluconazole or itraconazole in children and infants is ultimately left to the decision of the clinician along with the patient (or parent legal guardian when applicable) on a case-by-case basis after discussion of the benefits versus risks of oral antifungal therapy. Additionally, in the presence of underlying major medical disorders of concern, the clinician may elect to monitor more closely. Therefore the purpose of the present work was to carry out invitro activities of antifungal drugs tested against dermatophytic strains isolated from Anambra State, Nigeria.

II. MATERIALS AND METHODS

2.1 STUDY POPULATION AND STUDY DESIGN

The study design is randomized cohort selected schools were randomly chosen to represent the L.G.As investigated. The study population was primary school pupils and staff members. All were examined and screened for the presence of lesions. Only pupils and staff members who had lesions were further examined and samples collected from the observable dermatophytic lesions. From the population, age group of 1 to 20 were 820 and age group of 21 and above were 180 . then 186 were male and 134 were female.

2.2 ANTIFUNGAL AGENTS TESTED

Antifungal agents used in this study included five (5) antifungal agents namely itraconazole (ITR) ketoconazole (KET) (Jansen), fluconazole (FLU) (Pfizer), terbinafine (TER) (Novertis) and griseofulvin (GRI) (Schering plough).

Fluconazole and ketoconazole were dissolved in sterile distilled water while the rest were dissolved in 100% dimethylsulpuroxide (DMSO) (Sigma-Aldrich). They were subsequently prepared as stock solution and stored at 25 ^{0}C temperature.

2.3 Collection of scalp scrapping

The scalp area with lesion were swabbed with 70% ethanol to remove surface contaminants. The scalp scrapping was taken from the border areas of lesions with the help of sterile scapel and placed between two clean microscopic slides in clean envelopes and transported to the laboratory. Moist exudates present on the lesions were also collected and examined. This was collected in clean envelopes and taken to the laboratory for examination.The lesions were scrapped with a sterile blunt scapel as well as the stubs of broken hairs were pulled with tweezers.

2.4 Collection of nail samples

Scrappings of infected nails or clippings of nail were collected by cutting nails that have been cleaned with 70%

ethanol. The scrapings were taken from the proximal to the distal end of the nail (6). The samples collected were labelled with the patients identification number, Age, Sex, Date of collection, Code of patient and location. Seventy (70%) ethanol, sterile uricol (Himedia) sterile scalpel/tweezers, L – shaped needle, Bunsen burner, cover slip, culture plates, glass slide, sterile razor blades, epilator forceps.

2.5 DIRECT MICROSCOPY

A potassium hydroxide mount was prepared and few drops of 10% potassium hydroxide was placed on a clean glass slide. The specimen was placed in the solution and allowed to stand for 30 minutes. A gentle heat was applied through a bunsen flame to facilitate softening and clearing of the keratin found in specimen.

2.6 CULTURE

Petri dishes containing dermatophyte test medium (Jinhua Noke Biotechnology Co., Ltd) were inoculated with scalp scraping and hair samples collected from infected pupils. This medium is a selective and chromogenic medium that permits the growth of dermatophytes which impacts reddish colouration on the medium. Plates were incubated at room temperature of 25 – 27 ^{0}C for up to 10 days during which the plates were observed for growth. Each fungal growth was sub-cultured on SDA to obtain a pure culture which was then stored in agar slants for further studies.

IDENTIFICATION: Colonial morphology on dermatophyte test medium was used for preliminary identification of the dermatophytes. Pure fungal colonies were also subjected to lactophenol blue staining for microscopy observation of their specialized hyphae and the morphology of their macronidia, micro conidia and chlamydospores.

2.7 SUSCEPTIBILITY PROFILE OF DERMATOPHYTES

2.7.1 STANDARDIZATION OF DERMATOPHYTES

Isolates were inoculated onto SDA plates and incubated at 25 ^{0}C for 7 – 10 days to obtain young actively growing cultures consisting of mycelia and conidia. A mycelial disc, 5 mm in diameter, cut from the periphery of the 7 – 10 days old cultures, was aseptically inoculated into tubes containing Sabouraud dextrose broth. The tubes were incubated at 25^{0}C for 48 – 72 hours. After incubation, the tubes were placed on a vortexing machine and vortexed for about 15 – 20 minutes to properly disperse the cells in the broth. The concentration of organisms in the tubes was standardized by adjusting to a concentration of about 10^4 CFU/ml, from 10^8 CFU/ml already prepared by Macfarland standard. Dimethylsulphoxide used as control in time kill kinetics, do not have any dermatophytic organism in it.

2.8 DETERMINATION OF ANTI DERMATOPHYTE ACTIVITY

Sterilized discs (6 mm) prepared from Whatman No 1 filter paper were impregnated with different concentrations (25 mg, 50 mg, 100 mg, 200 mg) of anti-fungal agent dissolved in 2% dimethylsuphuroxide (DMSO) and sterile distilled water. (7).

The discs of different concentrations of antifungals were placed on SDA plates seeded with 10^4 CFU/ml dilutions of inoculum preparation. The plates were prepared in duplicates, incubated at room temperatures for 7 days and average diameter zone of inhibition recorded. Discs impregnated with 2% DMSO and 100 ul/disc of clotrimazole were included as negative and positive controls respectively.

2.8.1 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

MIC: Two hundred milligrams/millilitre of the DMSO are serially diluted in sterile water. Different tubes containing different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of the antifungals were inoculated with 0.1ml of 10^{-4} dilution of the test dermatophytes and incubated at room temperature for 7 days. These were done in duplicate and broth medium containing no antifungal was used as control.

MIC was recorded as the tube with the lowest concentration of antifungal that failed to show any visible macroscopic growth.

MFC: Loopful from tubes of MIC and the preceding tubes were inoculated on sterile SDA plates without drug supplements. The plates prepared in duplicates, were incubated for 7 days at room temperature and observed for growth. The lowest concentration of the tube dilutions that showed no visible growth on SDA plates was considered as the MFC as described by *(*7).

DATA ANALYSIS

SPSS version 21 was used for the statistical analysis of the data generated. Bar chart, tables, ANOVA, Chi – square and student t test were used in the representation of the data generated.

III. RESULTS

Out of the total of 1000 cultured samples on SDA, 320(32%) were dermatophytes. Out of this, 120(12%) isolates were *Trichophyton tonsurans*, 26(2.6%) *Trichophyton violaceum*, 18(1.8%) *Trichophyton verrucosum*, 73(7.3%) *Trichophyton rubrum*, 13(1.3%)

Trichophyton mentagrophytes, 6(0.6%) *Epidermophyton floccosum*, 56(5.6%) *Microsporum audounii* and 12(1.2%) *Microsporum canis*. *T. tonsurans* is the most prevalent in the Local Government Areas, with percentage of (37.5%).

Susceptibility pattern of dermatophytes against various antifungal agents and their MIC (ug/ml) are as shown on Table 1. The in vitro susceptibilities of 320 isolates of dermatophytes to ketoconazole, fluconazole, itraconazole, terbinafine and griseofulvin are summarized in Table 1. All the 320 isolates of dermatophytes tested were susceptible to the five antifungal drugs used in the study. The MIC 90 microgram per ml are shown, the data are presented as MIC ranges and were appropriate as the drug concentrations required to inhibit 90% of the isolate of each species (MIC 90). The MIC of all the quality control strains used was within established range.

Majority of the isolates had luxirant growth in five days but the result reflect readings recorded at 25^0C room temp. The various dermatophytic isolates were less susceptible to griseofulvin and fluconazole with MICs ranging from 0.01 – 4.00 and 0.007 – 0.500 ug/ml. The MIC 90 range for them respectively is between 0.03 – 0.125 ug/ml for griseofulvin and 0.06 – 0.125 ug/ml for fluconazole. Terbinafine was the most effective drug against all isolates of dermatophytes since MIC 90 range was between 4.0 to 16.0 ug/ml.

Fig 1 shows the susceptibility pattern of dermatophytic isolates against different concentration of the antifungal drugs.

All the 320 isolates of dermatophytes tested were susceptible to the five antifungal agents used. The isolates were less susceptible to griseofulvin and fluconazole. The order of in vitro activity is therefore terbinafine > itraconazole > ketoconazole > griseofulvin > fluconazole.

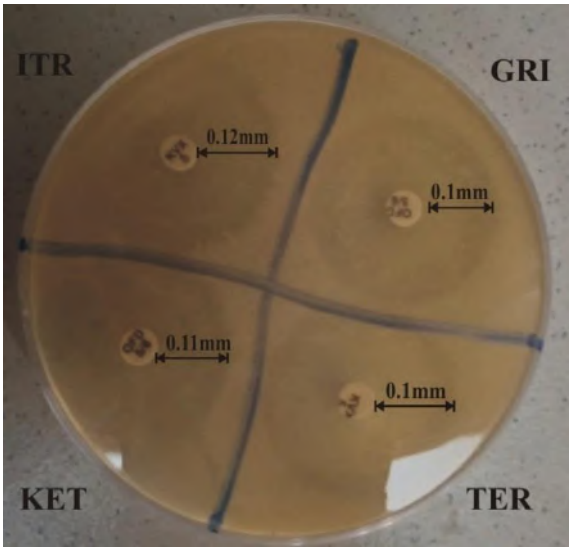
Table 1: Minimum inhibitory concentration (ug/ml) of the various antifungal drugs on dermatophytic isolate.

			MIC (ug/ml)	
Species (n^2)	**Antifungal drugs**	**Range (mm)**	**MIC**	**MIC 90**
T. tonsurans (120)	GRI	0.06 – 0.5000	0.01	0.03
	TER	0.25 – 16.00	1.00	4.00
	FLU	0.007 – 0.125	0.07	0.125
	KET	0.125 – 1.00	0.125	0.25
	ITR	0.50 – 16.00	2.00	8.0
T. violaceum (36)	GRI	0.01 – 1.00	0.01	–
	TER	1.00 – 8.00	1.00	–
	FLU	0.007 – 0.125	0.01	–
	KET	0.03 – 0.25	0.25	–
	ITR	0.25 – 8.00	0.50	–
T. verrucosum (18)	GRI	0.125 – 1.00	0.125	–
	TER	4.00 – 16.00	4.00	–
	FLU	0.007 – 0.125	0.007	–
	KET	1.00 – 16.00	2.00	–
	ITR	0.50 – 4.00	4.00	–
T. rubrum (60)	GRI	0.03 – 1.00	0.03	–
	TER	1.00 – 8.00	2.00	–

	FLU	0.03 – 0.25	0.007	–
	KET	0.125 – 4.00	0.125	–
	ITR	0.50 – 4.00	4.00	–
T. mentagrophytes (14)	GRI	0.01 – 4.00	0.01	–
	TER	0.06 – 64.00	2.00	–
	FLU	0.007 – 0.500	0.07	–
	KET	0.06 – 4.00	0.05	–
	ITR	0.125 – 4.00	0.50	–
M. audounii (54)	GRI	0.01 -0.125	–	–
	TER	0.50 – 2.00	–	–
	FLU	0.01 – 0.02	–	–
	KET	0.01 – 0.50	–	–
	ITR	0.25 – 1.00	–	–
M. canis (10)	GRI	0.03 – 0.125	0.03	0.125
	TER	8.00 – 32.00	8.00	16.0
	FLU	0.01 – 0.25	0.01	0.06
	KET	1.00 – 16.00	1.00	2.00
	ITR	2.00 – 16.00	2.00	8.00
E. floccosum (8)	GRI	0.01 – 0.50	–	–
	TER	0.50 – 2.00	–	–
	FLU	0.01 – 0.125	–	–
	KET	0.01 – 1.00	–	–
	ITR	1.00 – 2.00	–	–

NOTE: n^2 = (320 number of isolates tested); B± MIC for 50% of the isolates tested

KEY: GRI = Griseofulvin TER = Terbinafine FLU = Fluconazole KET = Ketoconazole ITR = Itraconazole



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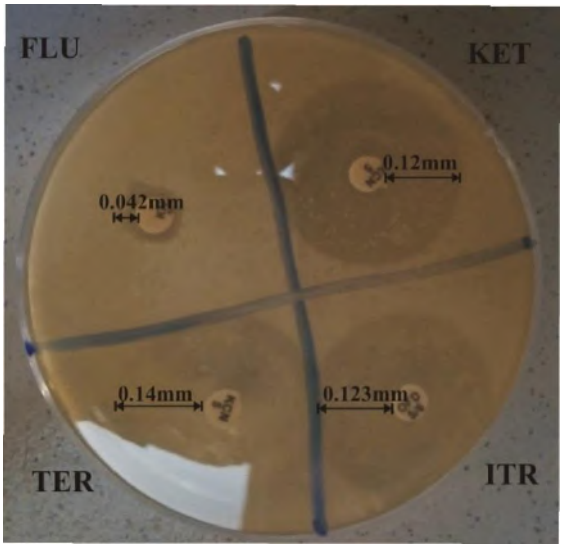

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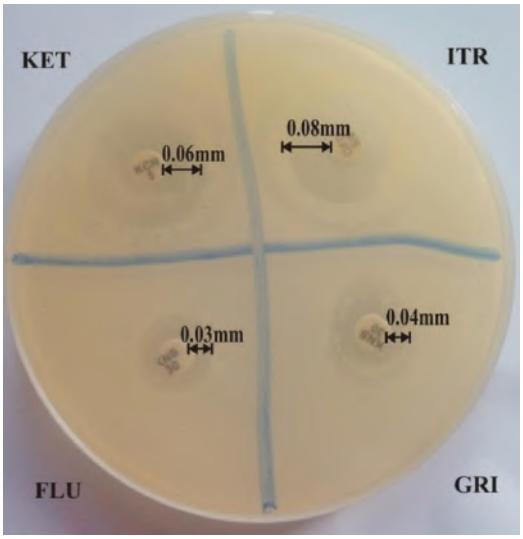

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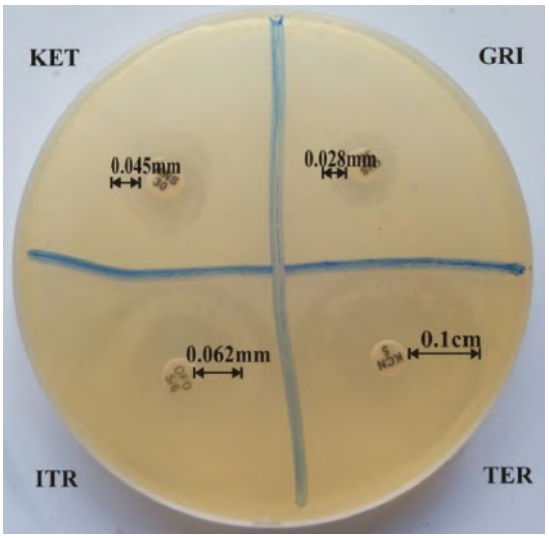

d.

KEY: ITR – Itraconazole TER – Terbinafine GRI – Griseofulvin KET – Ketoconzaole
FLU – Fluconazole
Zone of inhibition

Plate 1: Zone of inhibition exhibited by various antifungal agent the dermatophytic isolates

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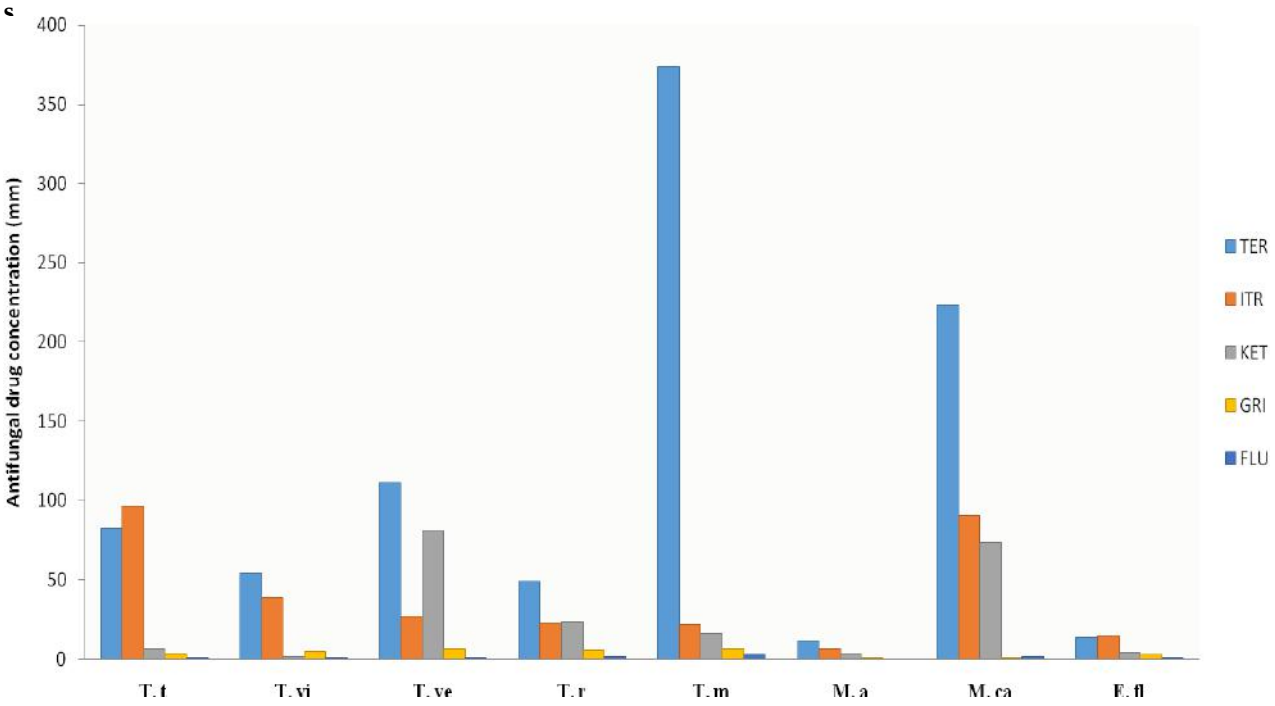

KEY

Dermatophyte species: T. t = *T. tonsurans*, T. vi = *T. violaceum*, T. ve = *T. verrucossum*, T. r = *T. rubrum* T. m= *T. mentragrophytes*, M. a = *M. audounii*, M. ca = *M. canis*, E. fl = *E. floccosum*

Drugs: TER = Terbinafine**,** ITR = Itraconazole**,** KET = Ketoconazole, GRI = Griseofulvin, FLU = Fluconazole

Fig.1: Susceptibility pattern of dermatophytic isolates against antifungal drugs tested

IV. DISCUSSION

The susceptibility pattern of the dermatophyic isolates in relation to antifungals used showed that Terbinafine was the most effective drug followed by Itraconazole. This also agrees with the work of Nweze (8)

Antimicrobial resistance monitoring is useful in tracking and detection of resistance trends by microorganisms, it also gives clues to emerging new resistance. This serves among other things, in assessing interventional efforts and empirical treatments recommendations. Previous authors like (9)., (8)., (10) and(11) have performed in vitro susceptibility tests on various strains of dermatophytes. Therefore, this is an in-vitro susceptibility profile of dermatophytes obtained in the Local Government Areas where the study was conducted revealed that there was no major difference in the MIC by incubating at 30 ^{0}C or 35 ^{0}C. Information available in the literature from other authors indicated that four days of incubation were sufficient to observe noticeable growth in the control wells. We therefore recorded our MIC values after four days of incubation. In the study of (12) tested 508 strains belonging to 24 species of dermatophytes against conventional (Itraconazole and Fluconazole) and some newer antifungal agents like Vericonazole and UR – 9825, with similar results. This seems to support the fact that incubating for four or seven days does not have a significant effect on the MIC readings.

Nevertheless, the result on terbinafine which was the most active agent for example agrees with the observation from previous authors (13) and (5). These antifungal agents used in the susceptibility testing were effective on the dermatophytic isolates. This antimycotic showed an excellent in-vitro potency and broad-spectrum activity against all the tested species. This suggested that terbinafine can be used to treat a majority of dermatophytic infections especially those showing high MIC values on the azoles, such as fluconazole. Although the newer antifungals were not included in the study, such as posaconaloe, voriconazole, and literatures had shown their promising antifungal activities. In Nigeria these antifungals are relatively new and are not readily available, affordable and not widely used as the ones tested during this study.

It will be of interest to state that all isolates used in this study were obtained from school children, teachers and other staff members in all the schools sampled. Interestingly, there was no record of resistance in this study even though some agents recorded high MIC values than others. From the data, Ketoconazole appeared to be the next choice in terms of in vitro activity after terbinafine and itraconazole.

V. CONCLUSION

Tinea capitis is still a problem of childhood, particularly among those living in unhygienic crowded conditions and mostly in riverine areas. This showed that *T. tonsurans, T. violaceum, T. verrucosum, T. rubrum, T. mentagrophytes, M. audounii, M. canis* and *E. floccosum* are dermatophytres found in public school pupils in the Local Government Area of Anambra State, Nigeria in which *T. tonsurans* predominates.

This showed that there is no abuse of antifungal drugs in this area which further affirmed non affordability of these drugs by the individuals whose samples were used for the study.

ACKNOWLEDGEMENT

We want to sincerely appreciate the Headmasters and Headmistresses of all the schools that gave us permission to use their schools for this project. May God bless you all Amen.

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