STUDIES ON THE EFFICACY OF VARIOUS ANTIMYCOTIC DRUGS ON EMERGING AND REEMERGING, SUPERFICIAL, CUTANEOUS AND SUBCUTANEOUS MYCOTIC INFECTIONS

Abstract

Introduction. Studies on efficacy of various antimycotic drugs on dermatomycosis that affect the superficial layers of the skin, nails, foot, and hair were conducted with 180 patients to test the efficacy of 5 systemic and Topical antifungal agents namely Voriconazole, clotrimazol,beclometasone Itraconazole and Fluconazole

Methods Specimen collection, processing, microscopy and culture were carried out and antifungal susceptibility testing was carried out with E-test method. Automated Vitek 2 was utilized for confirmation of the Candida species and for testing their susceptibility to Voriconazole and Fluconazole.

Results. Final strain identification revealed 41(69.49%) dermatophytes, 11(18.64%) non dermatophytic molds (NDM) and 7 (11.87%) yeasts (candida). Candida was the commonest species identified among nondermatophytes. The most common species isolated in Tinea corporis, T.cruris, T.capitis and T.faciei was Trichophyton rubrum. All the strains of dermatophytes showed most susceptibility to beclometasone and clotrimazole (MIC range of 0.04 – 0.64) but uniform resistance to Fluconazole (i.e. MIC ≥32 μg/ml) when tested by the E strips.

Conclusion.Variation in species distribution with respect to clinical presentation was found to be statistically significant ($p = 0.001$).

Keywords: Dermatomycosis, Antifungal Etest, VITEK-2 vericonazole itraconazole, Clomatrizole, beclometasone **and Fluconazole.**

Introduction Superficial fungal infections (Dermatomycosis) are among the most common dermatological diseases. High temperature,, poor personal hygiene, poor nutrition, tight clothing, overcrowding, debilitating systemic diseases like diabetes, drug resistance, immune

compromised states like HIV infection etc. have combined to make these infections common [1].

Mycoses, (Disease caused by Fungi) can be classified either as superficial, deep, or systemic. Dermatophytes cause superficial mycosis. The lesions appear in circular patterns, with desquamation and erythema of the edges. The dermatophytes can attack keratinized tissue (skin, hair and nails) of humans as well as other animals to cause an infection known as dermatophytosis.. Mycoses are a major cause of morbidity and mortality even with the latest advances in diagnosis and treatment. . Timely commencement of the appropriate therapy have a direct positive impact on the patient's recovery. **[2]** Azole antifungals are commonly used to treat fungal infections. These include Itraconazole, Fluconazole, Voriconazole, Posaconazole, Isavuconazole, clotrimazole and beclometasone. [3] The recent availability of new anti mycotic drugs has given rise to more treatment options, as well as prophylactic or preemptive purposes..

Increased inappropriate use of anti mycotic drugs has induced resistance on fungal strains, Resistance has emerged either by: several species developing secondary resistance or susceptible species being replaced by resistant ones, hence changing the epidemiology of mycotic infections. [4] Available Antifungal susceptibility testing methods can detect antifungal resistance and also arrive at the best treatment approach for a specific fungus. **[5]**

VITEK-2 yeast susceptibility test is an automated method of yeast species identification and antifungal susceptibility testing through the analysis of yeast growth.

The system is a small version of the broth dilution method that integrates a software program which validates and interprets susceptibility test results according to CLSI clinical breakpoints based on the drug MIC values.

Methods

A two year prospective study was conducted in ten selected hospitals in Orlu Local Government Area of Imo State, Nigeria. A total of 180 patients were involved. An approval from the institutional ethics committee was taken. Data was collected in a predesigned format. For patients with sufficient scales, specimen collection, processing, microscopy and culture were done and antifungal susceptibility testing was carried out as per E-test. E-Test: Isolates of

dermatophytes were tested along with Trichophyton rubrum ATCC 28188 and Trichophyton mentagrophytes ATCC 9533 as control. Dermatophytes were subcultured on Potato Dextrose Agar (PDA) & incubated at 28°C for 7 days to enhance sporulation. The growth was harvested in sterile saline & the conidial and hyphal suspension was adjusted to 1x10 6 /ml using a haemocytometer. Plates of Mueller Hinton Agar (MHA) were inoculated using a swab dipped in the inoculum suspension. The inoculated plates were then dried before applying the E-strips. Commercially available E-strips (HIMEDIA) were used to detect the susceptibility of various dermatophytes isolated, to Fluconazole, Itraconazole and Voriconazole. Sterile disks were also impregnated with 10 l of 1:100 dilution of DMSO to serve as control. The Estrips for the aforementioned 3 drugs were applied to each inoculated & dried plate & then incubated at 28°C for up to 16 hours or longer for filamentous fungi depending on the fungus' genus for the E-strips. When growth took place, the size of zones of inhibition were measured for each antifungal agent as was done by in their study.6 VITEK-2: Automated Vitek 2 was utilized for confirmation of the Candida species and for testing their susceptibility to Voriconazole and Fluconazole., beclometasone and clotrimazole,

Ethical Clearance.

Ethical clearance was obtained from the Imo State Ministry of Health via a letter referenced IMMH/20/08/27,

Data Analysis: MIC range was obtained and compared with all the isolates examined. Lower MIC range implies a higher susceptibility.

Results and Discussion A total of 180 clinically suspected cases of superficial fungal infections who visited the hospitals were selected for microbiological diagnosis.. Distribution of samples collected showed that 104 (57.78%) of samples were obtained from skin, 36 (20.0%) from skin, and 40(22.22%) from nails. Total number of positive cultures was 89 while final strain identification showed 51(57.39%) dermatophytes, 21(23.6%) non dermatophytic molds (NDM) and 17 (19.1%) yeasts (candida). The commonest species identified among nondermatophytes was Candida, , similar to the results of the study carried out in the past [6] **[7]**

The various species were identified by direct microscopy and culture. While 37 (20.55%) samples were positive on both microscopic examination and culture , 52 (28.89%) samples were

positive only on culture. A total of 49 (27.7%) samples were positive only on microscopy while 42 (23.33%) samples were found to be negative on both. The 91 culture negative samples could not be processed for antifungal susceptibility testing. The total culture positivity rate was only 49.44%.

Table 1: Most common species in various clinical presentations

Clinical presentation	Most common species
Tinea corporis & cruris	**T.mentagrophytes (65%)**
Tinea corporis	**T.rubrum (58.8%)**
Tinea cruris	**T.rubrum (60.7%)**
Onychomycosis	**T.mentagrophytes (34.4%), T.rubrum (34.4%)**
Candidal intertrigo	**Candida sp (100%)**
Candidal vulvovaginitis	**Candida sp (100%)**
Tinea pedis	**T.mentagrophytes (52%)**
Tinea capitis	**T.rubrum (63%)**
Tinea faciei	**T.rubrum (63%)**

The variation in the distribution of species based on clinical presentation was found to be statistically significant ($p = 0.001$). In this study, strains of dermatophytes isolated were tested for their antifungal sensitivity to Voriconazole, Fluconazole Itraconazole, clotrimazole and beclometasone using the E-test method.

Table 2: E-test result for Voriconazole, Itraconazole, Fluconazole, clotrimazole and beclometasone for 3 species of dermatophyte

Drug Name	Strain	MICRange (µg/ml)
Voriconazole	**T.mentagrophytes**	**0.031 – 0.064**
	T.rubrum	**0.007 – 0.017**
	M.gypseum	**0.010- 0.017**

Itraconazole	**T.mentagrophytes** **T.rubrum** **M.gypseum**	**0.046 – 0.064** **0.015 – 0.064** **0.017- 0.019**
Fluconazole	**T.mentagrophytes** **T.rubrum** **M.gypseum**	**≥32** **≥32** **≥32**
Clotrimazole,	**T.mentagrophytes (n = 20)** **T.rubrum (n = 10)** **M.gypseum (n = 1)**	**0.010 – 0.064** **0.005 – 0.018** **0.009 – 0.064**
Beclometasone	**T.mentagrophytes (n = 20)** **T.rubrum (n = 10)** **M.gypseum (n = 1)**	**0.008 – 0.064** **0.004 – 0.018** **0.010 – 0.064**

As shown in table 2, the MIC of Voriconazole ranged from 0.007 µg/ml to 0.064 µg/ml. All the strains of dermatophytes exhibited same resistance to Fluconazole with MIC ≥32 µg/ml after being tested by the E strips.

The seeming poor susceptibility of dermatophytes to Fluconazole by E-test method , (Uniform MIC ≥32 µg/ml) is conformity with findings of studies done by Favre et al(2003), Santos et al,(2006), Barros et al(2010) and Sarifakioglu et al.(2007). Rampant usage and easy accessibility of Fluconazole at pharmacies, coupled with self medication by patients due to its over the counter (OTC) status could have led to development of Fluconazole resistance by the strains.

In this study, Itraconazole had MIC range from 0.015 µg/ml to 0.064 µg/ml. [8] in their study, got MIC range of 0.038–1.5 µg/ml for Itraconazole. Comparable results were also found by [4] in their study .on the evaluation of E-test for dermatophytes. The three dermatophyte species were most susceptible to Beclometasone, with MIC range of 0.004 to 0.064 µg/ml, followed by Clotrimazole, with MIC range of 0.005 to 0.064 µg/ml.

Table 3: Various species of Candida isolated and their respective clotrimazole and beclometasone MICs

Candida Specie	Clotrimazole MIC (µg/ml)	Beclometasone MICs

C.tropicali	**≤ 1**	**≤0.13**
C.albicans	**≤ 1**	**≤0.13**
C.parapsilosis	**≤ 1**	**≤0.13**
C.parapsilos	**≤ 1**	**≤0.13**
C.albicans	**≤ 1**	**≤0.13**

The Candida species were discovered to be evenly sensitive to clotrimazole (MIC≤ 1µg/ml) and to beclometasone (MIC≤0.13µg/ml). The finding of high sensitivity of Candida species to beclometasone (MIC≤0.13 µg/ml) in this study seems to agree with the findings of [2] In this study, differences between the MIC s of antifungal drugs on the various species was not statistically significant. ($p > 0.05$).

Conclusion. Treatment of dermatophytic infections should be dictated ideally by a culture sensitivity repor. On the bases of their MIC ranges, beclometasone and clotrimazol are most appropriate treatment options.

However, they must be exclusively reserved for resistant and difficult to treat infections so as to forestall quick development of drug resistance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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