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Study of Pattern of Emerging Antifungal Resistance in A Zonal Hospital in North-East India.

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Abstract:
Introduction: *Superficial fungal infections of skin are common worldwide. The etiological agents are varied with emerging resistance pattern especially among azoles. The present study aims to demonstrate the current trend of anti fungal resistance among superficial mycoses using the method of disk diffusion in a peripheral hospital.*
Materials and Methods: *This prospective study was done at a Government zonal hospital from Nov 2015 to Nov 2016. 135 consecutive OPD patients of the department of Dermatology clinically diagnosed to have a fungal infection of skin and then confirmed on KOH mount were included. Skin scrapings were cultured on SDA and differential media. Isolate and identified species were further subjected to anti fungal sensitivity test using Itraconazole, Fluconazole, Ketoconazole and Voriconazole by the Disk diffusion method.*
Results: *Both dermatophyte and non dermatophyte fungi were isolated and identified in the cultures. Antifungal resistance was marked for Fluconazole and Ketoconazole.*
Conclusion: *Epidemiological variance found in etiological agents is in resonance with the geographical location of our study. Resistance was rampant to both Fluconazole and Ketoconazole which are commonly used as first line anti fungal drugs. Itraconazole and Voriconazole had lesser degree of resistance. Culture and identification of causative organism is a must in superficial mycoses. Disk diffusion as an accepted method of antifungal sensitivity test must be encouraged. The relative ease of the method coupled with its reliability and cost effectiveness would go a long way in establishing anti fungal resistance testing as a routinely done investigation even in remote areas.*
Keywords: *Superficial mycoses, Antifungal sensitivity test, emerging resistance, Ketoconazole, Fluconazole, Voriconazole, Itraconazole, Disk diffusion method.*

I. Introduction

Superficial fungal infections of skin are amongst the commonest dermatological diseases worldwide. The morbidity from fungal diseases remains very high despite the availability of plethora of antifungals and new therapeutic strategies. The present study was undertaken to report the current antifungal resistance pattern among cases of common superficial fungal infections in a secondary care hospital in North-East India. There is no such study undertaken previously in this remote tropical region.

II. Background

Fungal infections of skin are among the commonest dermatological diseases worldwide. The morbidity and mortality from fungal diseases remains very high despite the availability of plethora of antifungals and new therapeutic strategies.[1] There are various studies on resistance of invasive fungal infections however there is a paucity of studies on resistance of superficial fungal infections to common antifungal drugs and none from this region. The present study was undertaken to report the current antifungal resistance pattern among cases of superficial fungal infections of skin isolated in a secondary care hospital in North East India.

III. Methods

A prospective hospital based study conducted in the department of Dermatology of this hospital from Nov 2015 to Nov 2016.

Study population:

Patients enrolled in the study were 135 consecutive OPD patients of the department of dermatology who fulfilled the laid down inclusion and exclusion criteria:

Inclusion criteria:
I. Patient aged between 10 and 60 yrs of age.
II. Patient clinically diagnosed to have a fungal infection and then confirmed on KOH mount.

Exclusion criteria:
I. Patients on any oral immunosuppressant
II. Patients with poorly controlled diabetes

IV. Materials And Methods

Skin scrapings from the edge of the lesion were taken after wiping with alcohol swab and sent to the hospital laboratory in a sterile container.

The samples were processed according to Clinical and Laboratory Standards Institute (CLSI) document M-54A. The samples were cultured in plates and tubes of Sabourauds dextrose agar (SDA) plain and SDA with cycloheximide. These were incubated at 37°C and 25°C for a maximum period of 3 weeks. The gross colony features including colony morphology and pigment production were examined. Slide cultures were used for microscopic examination and identification of species.

Inoculum was prepared according to M51-A and M38-A2 CLSI guidelines. Isolates were subcultured on potato dextrose agar for hyphal fungi. For inoculum preparation colonies were suspended in 5 ml of sterile normal saline with turbidity adjusted visually to 0.5 McFarland standard. Mueller- Hinton Agar plates were inoculated with cotton swab to cover the entire plate. Commercially available discs of fluconazole (25 µg/disk), itraconazole (8 µg/disk), ketoconazole (15 µg/disk) and voriconazole (1 µg/disk) were used. The plates were incubated at 35°C and were read after 24h and 48h. Inhibition zone diameters (IZD) were measured in millimeters. In case of Itraconazole (Itr), they were considered sensitive if IZD ≥12 mm. For voriconazole (Vor) IZD≥23 mm was taken as cutoff. IZD≥15 mm and IZD≥23 mm were considered sensitive in case of Fluconazole (Flu) and Ketoconazole (Ket) respectively.[2]

V. Results

The study included 77 Males and 58 females with an age range of 12 to 59. Of a total of 135 Skin scrapings sent, 86 (63.7%) were culture positive. Average number of days for growth was 5.1 days. Earliest growth seen was at 3 days and maximum period to show growth was 9 days, although conidial growth and sub culturing took longer. Dominant species grown was *Trichophyton rubrum* (39%) amongst dermatophytes and Candida (11%) amongst non-dermatophytes (Table 1).

Table 1

Fungal species	T. rubrum	T. mentagrophytes	Candida Sps	Aspergilus Sps	Mucor Sps	Rhizopus Sps	Others
No.	34	15	10	8	7	6	6
% of total culture	39.5%	17.5%	11.5%	9.3%	8%	7%	7%

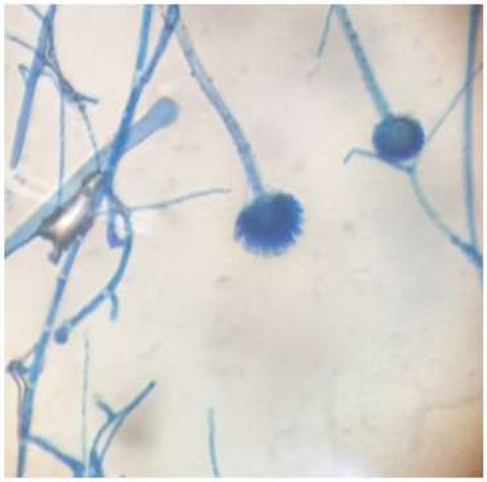

Figure 1. Aspergillus Sps,

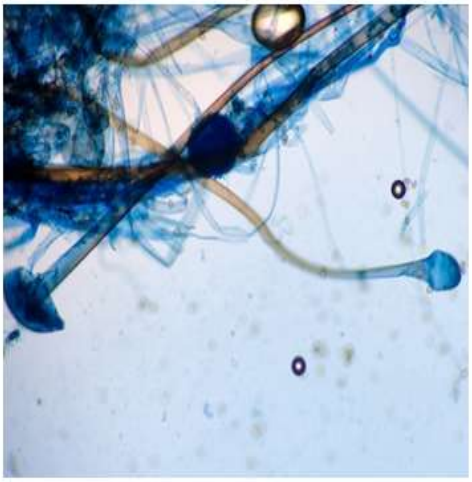

Figure 2. Rhizopus Sps

Figure 3. *Trichyophyton mentagrophyte* Culture on MHA sensitive to all four drugs

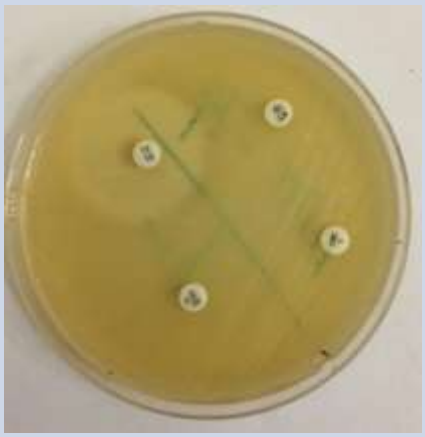

Figure 4. *Trichyphyton rubrum* culture on MHA sensitive only to Itraconazole

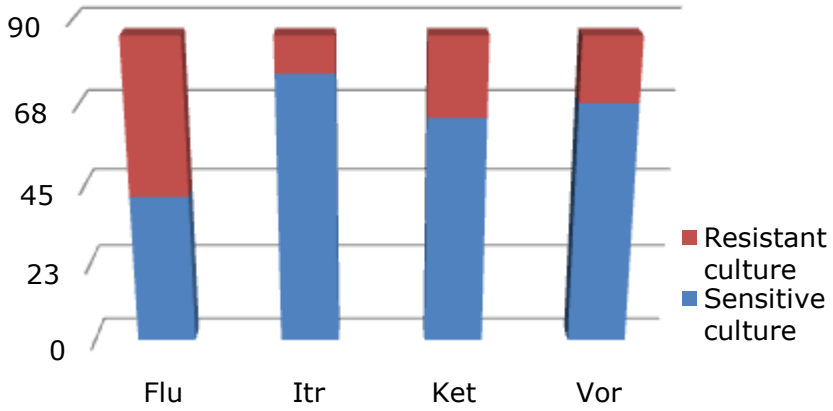

Figure 5. Resistance and susceptibility pattern

Out of 86 culture positive cases, only 17 (19.77%) cultures were sensitive to all four drugs and 69 (80.23%) were resistant to at least one of the four drugs. 6 cultures were resistant to all four drugs. Maximum resistance was seen to Fluconazole (73.3%) and least resistance was to Itraconazole (16.6%) (Fig5).

VI. Discussion

Since the late 1960s when antibiotic therapies were developed, a drastic rise in fungal infections has been observed, and they currently represent a global health threat, affecting millions every year.[1, 3]The high morbidity and mortality of invasive fungal infections have ensured adequate research and studies in that field, however there is paucity of studies for superficial fungal infections. Therefore this study was undertaken in this remote tropical region. In view of the increasing number of fungal infections, rampant use of antifungal agents, and emergence of antifungal resistance as an important clinical problem, it is imperative to have standardized and reproducible antifungal susceptibility testing (AST).[4] This rampant use of antifungal agents without culture and AST has promoted antifungal resistance and deprived the clinician of a precision therapy; however the prolonged and often cumbersome methods of culture have kept clinicians at bay and standardized culture methods have mostly been fraternized by the researchers only.[5]

Micro titre dilution tests are currently the most accepted form of testing anti fungal resistance but these methods are difficult to be used in most laboratories.[2] We have used disk diffusion-based assay for evaluating the antifungal susceptibility of dermatophytes because of the ease of use, reproducibility, accuracy, and low cost.[6, 7] The easy availability of commercially prepared anti fungal disks and familiarity with the Kirby-Bauer method makes the procedure readily acceptable even in a remote geographical setting. Our experience suggests that technical acumen notwithstanding, the disk diffusion method deserves international standardization for all pathogenic fungal species.

Our findings in epidemiology are in consonance with previous studies in this region.[8,9,10] The male predominance is consistent with most of the earlier studies, although it could also be because of this being a service hospital.[8,10] The predominance of dermatophytes as causative agents of skin infections is a well documented phenomenon in this part of the country.[9,10] Previous studies have also recorded a healthy proportion of non dermatophytic hyphal fungi in similar cases.[10] The dominant species found in our study - *T. rubrum, T. mentagrophytes and Candida Sps* also match those in previous studies in this region.[9,10]

Azole resistance is a known phenomenon worldwide most especially in *Aspergillus Sps* and *Candida Sps*.[11,12] Fluconazole resistance of 73.3% in our study is consistent with most studies although documented resistance varies widely with some studies getting even 100% resistance to fluconazole.[13, 14]

Voriconazole and Itraconazole were the most effective antifungals in our study across all species. This is in line with previous studies[15]. Many earlier studies have quoted a resistance rate of 1.7% to 6% to Itraconazole.[16] The rising resistance to Itraconazole is a major concern. Our own study has shown the Itraconazole resistance of 16.6 %. In spite of this, azoles remain the most widely available anti fungal agents. It is therefore recommended that use of combination drugs in previously treated cases should be considered to counteract the rapid emergence of resistance to azole derivatives.

VII. Conclusion

The common superficial fungal skin infections are set to get more difficult to treat with tenacious organisms exhibiting rampant resistance to almost all common oral antifungal agents. Empirical use of antifungal agents will only add to this increasing resistance. Isolation and identification of fungi are a must in this scenario. The cheer in this gloom is that as seen in our experience, contrary to the common opinion, fungal culture does not take much time. We further recommend the Disk diffusion method as an easy, affordable, quick and reliable method to detect in-vitro resistance and guide precise therapy especially in peripheral centers.

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