Vol 10| Issue 2| 2020 | 274-279.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

# TO STUDY DIFFERENT FUNGAL SPECIES ASSOCIATED WITH DERMATOPHYTOSIS DIAGNOSIS AND TREATMENT IN SOUTHINDIANS

**Dr. Pranita Medhi[1], Dr. Ruplekha Goswami[2]***

[1]Assistant Professor of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences,Pondicherry. (Affiliated to Bharath University, Chennai), India.

[2]Associate Professor of Biochemistry, Sri Lakshmi Narayana Institute of Medical Sciences,Pondicherry. (Affiliated to Bharath University, Chennai), India.

**ABSTRACT**

Dermatophyte fungi are keratinophilic pathogenic fungi which lead to superficial mycotic infection of dermatophytosis. Although dermatophytes are not life threatening microbial agents but they are distributed around the world and cause mycotic infections with high morbidity . Dermatophytosis is a disease of global significance caused by pathogenic keratinolytic fungi called dermatophytes in both animals and humans. The recent taxonomy of dermatophytes classifies them into six pathogenic genera, namely Microsporum, Trichophyton, Epidermophyton, Nannizzia, Lophophyton and Arthroderma. It is because of the delayed diagnostic nature and low accuracy of dermatophyte detection by conventional methods that paved the path for the evolution of molecular diagnostic techniques, which provide the accurate and rapid diagnosis of dermatophytosis for an appropriate, timely antifungal therapy that prevents the nonspecific over-the-counter self-medication. The hot and humid climatic condition of central Karnataka promotes fungal infection. This study was done to determine the most predominant species of Dermatophyte causing infection in this region, which helps in treatment.The present cross-sectional observational study was conducted during September 2018 to February 2019.Samples were taken from 225 patients with clinically diagnosed dermatophytosis. Turn on the site of lesion, specimen collected from skin, hair or nails were taken. These samples were than examined phenotypic methods.Out of 225 patients, 65% samples were positive by Potassium Hydroxide (KOH) mount while 86% samples were positive by culture. Most frequent species of dermatophytes recognized was Trichophytonrubrum followed by Trichophytonmentagrophytes. dermatophytic infection mainly occurrence in Agricultural workers Males (61%) were more commonly affected than females (39%).Dermatophytosis is infections seen generally in people who work in hot and humid conditions and those who indulge in strenous work. Clothing patterns and personal hygiene also play an important role. By taking proper precautionary measures the incidence and disease burden can be minimized. Our study, tineacorporis was initiate to be the most frequent clinical type with T. rubrum being the commonest isolated species. Significant the resistance pattern of antifungal drugs will lead the family physicians and medical officers working in peripheral regions to choose the proper empirical therapy for better patient ending.

**Key words**: Dermatophytosis, Minimum Inhibitory Concentration, Superficial mycoses, KOH.

## INTRODUCTION

Dermatophytes are fungal agents of dermatophytoses Superficial mycoses of dermatophytoses are named after anatomic localization of the lesions Dermatophytosis (tinea or ringworm) is a general name for acute to mild and chronic lesions of the outer Layer of

Corresponding Author: **Dr.Ruplekha Goswami** Email: drvrvkk@gmail.com

keratinized tissues caused by dermatophytes. Dermatophytoses include Tinea barae, Tinea faciei, Tinea incognito, Tinea capitis, Tinea favosa, Tinea corporis, Tinea cruris, Tinea manuum, Tinea pedis, and Tinea unguium. Fungal culture medium Culture is an expensive and time consuming technique with low sensitivity (30%-35%) and higher specificity. In mycological methodology, it is important to have adequate amounts of samples to increase the accuracy of results. In traditional diagnostics, clinical samples including scale, hair and nail scraping must be used simultaneously for direct microscopy and culture medium. For positive result of culture technique usually takes two weeks while a negative result of culture method normally needs a period of 6 weeks. Sabouraud Dextrose Agar (SDA) is recommended for the most in culture medium technique

The dermatophytosis is generally chronic in nature, and its control requires proper identification of aetiological agents and its prevalence for the prescription of specific treatment. The rapid identification of aetiological agents from the clinical samples of dermatophytosis and route of infection is vital for specific antifungal therapy and for the prevention of further dissemination to human and/or other animals. The conventional diagnosis of dermatophytosis involves the easily available techniques of culture and morphological identification of the fungus or the detection of fungal elements by the direct microscopy of clinical specimens. Although the diagnosis by conventional culture and microscopy is suitable for fresh isolates, it is difficult to maintain and reproduce the reference strains for standardisation due to rapid degeneration on cultures. Furthermore, such conventional diagnostic techniques are highly time-consuming to arrive at a diagnosis, and the accuracy of the results depends on the expertise of the personnel. Thus, the need for polymerase chain reaction (PCR)-based techniques was felt to increase the sensitivity, specificity and the speed of diagnosis. The rapid diagnosis reduces the inconvenience caused to the patients due to delayed diagnosis and prolonged treatment course.

Dermatophytosis is one of the most common communicable human infectious diseases, often chronic in nature, causing considerable irreversible destruction to skin, hair and nails, if not detected and treated timely. The problem with the zoophilic dermatophytes is that the clinical lesions it produces in humans are more severe than those by the typical anthropophilic ones that are always transmitted by direct or indirect contact from person to person. The clinical presentations of dermatophytosis such as multifocal alopecia, scaling and circular lesions are often similar to those of other skin diseases, which make the treatment course highly uncertain. The isolation and identification of dermatophytes involved in infections are essential to prescribe species-specific treatment regimens.

Dermatophytes are the aetiological factors of a majority of superficial fungal infections. What distinguishes them from other pathogenic filamentous fungi is their unique ability to degrade keratin. The remarkable ability of this group of fungi to survive in different ecosystems results from their morphological and ecological diversity as well as high adaptability to changing environmental conditions. Paradoxically, despite the progress in medicine, the prevalence of dermatophyte infections is increasing from year to year. At the beginning of the third millennium, practical diagnostic and therapeutic options are still very limited. Our aim is to determine the incidence of different fungal species associated with dermatophytosis, to estimate the possible organization of dissimilar clinical parameters with fungal species, if any,to find out the connection between the site of involvement and the causative agent,to investigate minimum inhibitory concentration for antifungal agent

**MATERIAL AND METHODS:**

The present study was A hospital based cross-sectional will be approved by a Department of Dermatology, Sri Bhaarath Medical College and Hospital , during the period September 2018 to February 2019 to study socio-demographic outline of clinically diagnosed case of dermatophytosis and associate site of infection and causative Dermatophytes. The data was collected in prescribed proforma and later analysed.

**Inclusion Criteria**

Patients of all ages and of both the sexes who are clinically suspected with dermatophytic infection of skin, hair or nails and who are not using any antifungal treatment for at least one week. Patients who gave informed consent for required investigations.

**Exclusion Criteria**

- Who are used with antifungals or topical steroids in the recent past.
- Those have superficial fungal infections other than dermatophytes, such as pityriasisversicolor and Candidiasis and secondary bacterial infection.
- Patients with subcutaneous and deep fungal infection.
- Patients with Diabetes, chronic diseases and immunocompromised and immuno suppressive etc.

**Sample Collection:**

Samples were collected from skin scraping, hair and nail on the site on the lesion of fungi. Skin scraping: swabbed affected area with 70% alcohol and allowed to dry and collected by scrapping the active margin of the lesion with help of blunt edge of sterile scalpel. Hair: Hair was plucked with sterile forceps from basal portion of the hair where fungus is usually found. Nail: cleansed with 70% alcohol on the affected area of nail and clippings and scrapings beneath the nail.

These samples were screened for the presence of fungal element and keep into the *potassium hydroxide*

*(KOH) for wet mount* preparation of various concentrations (10%, 20%, & 40%) depending upon the type of clinical specimen likes skin,hair,nail respectively. Sabouraud's dextrose agar(SDA)contain 0.05% chloramphenicol and 0.5% cycloheximide and add dermatophyte test medium (3 test tubes).The first two test tubes are incubated at $28^0$ C for 2-4 weeks and was observed periodically for growth. If no growth was found after 4 weeks, it's negative. The third test tube will be incubated at $28^0$ C for up to ten days and observed for colour change. Fungal isolates were identified based on colony morphology, pigmentation, growth rate and microscopy (LPCB mount) CMA was used to differentiate *Trichophytonrubrum*from *Trichophytonmentagrophytes*based on pigment production on the media. In addition, hair perforation studies were accepted out to distinguish between these two species(Larone DH. Dermatophytes.2002, Padhye AA, Weitzman I. 1998).Identification of the organisms was done by growth of fungal colony on culture plate and microscopic appearance of organism by using Lactophenol Cotton Blue (LCB) and slide culture method.

**Preparation of inoculum:**

Seven to eightdays old grown of dermatophytes species on potato dextrose agar slants at 27°C were used to prepare inoculums. The clear suspension of inoculum having conidia was transferred to fresh tube, and its optical density was set equal to 0.5 McFarland standards. The final inoculum was set from $1 \times 10^3$ to $3 \times 10^3$ colony forming units per ml which was used in the sensitivity testing.

Here we are performed Antifungal susceptibility test broth microdilution method as per Clinical Laboratory Standards Institute (CLSI) approved standard M38-A2 guidelines suggested for molds.[14] Quality control isolates *Aspergillusflavus*ATCC 204304, *Candida parapsilosis*ATCC 22019 and *Candida krusei*ATCC 6258 were included. MIC50 and MIC90 values for isolates were also recorded.

In present study we used antifungal agents were fluconazole, itraconazole, ketoconazole, and terbinafine in powdered form. Stock solutions of itraconazole, ketoconazole and terbinafine were prepared in dimethyl sulfoxide, and fluconazole was dissolved in distilled water. Two fold dilutions of stock solution were further prepared in RPMI 1640 with L-glutamine without sodium bicarbonate and were buffered at pH of 7.0 ± 0.1 with 0.165M 3(N-morpholino) propanesulfonic buffer along with 1N NaOH. Concentration used for fluconazole was from 0.125-64 μg/ml, and for other drugs was 0.03-16 μg/ml.

**RESULTS:**

**Tables 1: Age and gender distribution of various cases**

Sample analysis shows that most common age group was 21-30 years (38.6%) followed by 31-40% (20.4%) with mean of 28 years. Male: female ratio was 3:2

**Table 2: To determine the KOH and culture positive of clinical samples of dermatophytes**

A total of 225 specimens were collected from patients with clinically suspected tinea infection out of which 175 were from skin, 26 from nails and 24 were hair samples. Out of them 187 (83.1%) samples were positive by KOH mount

**Table 3: Incidence of different species of dermatophytes and its isolation from different clinical samples**.

Sample analysis shows that most common age group was 21-30 years (46.5%) followed by 31-40% (23.5%) with mean of 28 years. Male: female ratio was 3:2

In the present study 225 dermatophyte species isolates96cultures were *T.rubrum* (42.6%) 45 isolates were *T. mentagrophytes*(20%)39 isolates were *E. flocosum*(17.3%)and 12 were *T. tonsurans*(5.3%). Most common isolate from hair was *T.tonsurans*although from nail and skin was *T. rubrum*. All the three cases of *Fusarium*were isolated form nails

**Table: 4 Table showing number of isolates as per cut-off value**

Our study showed that isolates with MIC values of >2 μg/ml for fluconazole and >1 μg/ml for itraconazole, ketoconazole and terbinafine were classified as resistant.(Ghannoum MA,*et al*. 2004)Isolates resistant to fluconazole and itraconazole were 52.8% and 51.5%, respectively. While isolates which were sensitive to fluconazole and terbinafine were 47.1% and 48.4%, respectively. MIC values for itraconazole and ketoconazole were <1 μg/ml for 100% of isolates.

**Table 1: Age and gender distribution of various cases**

| Age group | Number | Male | Female |
|---|---|---|---|
| 0-10 | 11(4.8%) | 5 | 6 |
| 11-20 | 46(21.3%) | 28 | 18 |
| 21-30 | 87(38.6%) | 56 | 31 |
| 31-40 | 46(20.4%) | 27 | 19 |
| 41-50 | 18(8%) | 12 | 6 |
| 51-60 | 10(4.4%) | 7 | 3 |

| | | | |
|---|---|---|---|
| >60 | 7(3.1%) | 4 | 3 |

**Table 2: To determine the KOH and culture positive of clinical samples of dermatophytes**

| Site | No of cases | KOH | Culture positive | Both KOH and culture positive | Culture positive |
|---|---|---|---|---|---|
| Skin | 175 | 25(14.2%) | 09(5.14%) | 68(38.8) | 73(41.7) |
| Nail | 26 | 10(38.4%) | 0 | 6(23.0) | 10(38.4) |
| Hair | 24 | 6(25%) | 3(12.5%) | 4(16.6%) | 11(45.8) |
| Total | 225 | 41(18.2%) | 12(5.3%) | 78(34.6) | 94(41.7%) |

**Table 3: Incidence of different species of dermatophytes and its isolation from different clinical samples..**

| Species | No of cases (%) | Skin | Hair | Nail |
|---|---|---|---|---|
| ***T. rubrum*** | 96(42.6%) | 56 | 12 | 23 |
| ***T.mentagrophytes*** | 45(20%) | 41 | - | 4 |
| ***T.tonsurans*** | 12(5.3%) | 5 | 8 | - |
| ***M.canis*** | 7(3.1%) | 7 | - | - |
| ***M.gypseum*** | 9(4%) | 6 | 3 | - |
| ***E.flocosum*** | 39(17.3%) | 29 | | 10 |
| ***Candida albicans*** | 10(4.4%) | 6 | - | 4 |
| ***fusarium*** | 7(3.1%) | - | - | 7 |
| ***Total*** | 225 | 150 | 23 | 48 |

**Table 4: Table showing number of isolates as per cut-off value**

| Anti fungal | No. of isolates below cut-off value | No. of isolates above cut-off value |
|---|---|---|
| Fluconazole | 106(47.1%) | 119(52.8%) |
| Itraconazole | 225(100%) | 0 |
| ketaconazole | 225(100%) | 0 |
| Terbinafine | 109(48.4%) | 116(51.5%) |

**DISCUSSION:**

There are two treatment methods comprising topical or systematic antifungal agents for dermatophyte infections. To achieve a definitive and successful pharmacotherapy of dermatophytosis, there is an essential need for an accurate identification of pathogenic agent at species level . According to different reports, several types of Tinea show insufficient response to topical medication. Thus, there are many kinds of Tinea which must be treated with systemic antifungal drugs. Amphotericin, azole antifungal agents including clotrimazole, fluconazole, itraconazole, ketoconazole, miconazole, voriconazole), grizeofulvin, and terbinafine are predominant good choices with satisfied effective antifungal activities, the objective and reproducible species identification for the specific treatment, monitoring and control of dermatophytosis. The sudy have a significant edge in terms of sensitivity and specificity over the conventional methods of culture and microscopy for dermatophytes identification. However, it is equally important to take into consideration the time required, economics, complexity and range of species spectrum detection while selecting an appropriate molecular technique.

Our study, it was showed that 38.6% cases of dermatophytosis were in the age group 21-30 years while 20.4%cases were in the age group 31-40 years. Which is correlated with (Dhayagude S et al. 2014), also observed that the frequent age group concerned in dermatophytosis was 21-40 years. The present observation similarity with previous publications by (Phudang RT et al.2014, Konda C et al.2013, Sudha M et al. 2012). Commonest (40.76%) age group was between 30-40 years. Usually adults in the age group of 20 -40 years are most physically active resulting in increased perspiration. Because of a hot, humid, environment in the body, favouring the growth of dermatophytes. In present study, the male: female ratio was 3:2 which correlates with other studies by (Dhayagude S et al. 2014, Sudha M et al. 2012, Doddamani PV et al., 2013). Higher prevalence in males might be as a result of greater physical and outdoor activity. In the present study, 88% cases were agricultural workers and labourers working outdoors leading to profuse sweating which in turn resulted in increased dermatophyte infection.

Our study showed T. rubrumwas the predominant 42.6%isolate followed by T. mentagrophytes20% E. floccosum17.3%and T.tonsurans (5.3%) between all culture confirmed cases of dermatophytosis correlated with (Dukare A et al.2013, Jain N et al 2014) studies also establish the T. rubrumas the most widespread isolate (Moto JN, et al. 2014,Wayne PA. 2018, Ghannoum MA, et al. 2004, Dhayagude S, et al. 2012). This may be due to adaptability to survive in varying climatic condition,

overcrowding and unhygienic conditions (Dhayagude S, et al. 2012). Some research showed (Guruprasad KY, et al. 2012, Phudang RT, et al. 2011), obseverdT. mentagrophytesas the most regular species.

In the present study, Itraconazole and ketoconazole had lower MIC for all species of dermatophytes, which indicates that these drugs could be the better choice for successful treatment of dermatophytic infections. (Pathania S, et al. 2014,Aktas AE, et al. 2011) have reported similar findings with itraconazole and ketoconazole.

119isolates (fifty two.Eight%) confirmed higher MIC against fluconazole (i.E. Reduce-off MIC > 2 μg/ml) and 116 isolates (51.Five%) in opposition to terbinafine (i.E. Reduce off MIC >1 μg/ml). Patients with those isolates were switched over to itraconazole, as it carried fewer unfavorable consequences as compared to others. No patient turned into switched over to ketoconazole. Patients with isolates having decrease MIC values for fluconazole or terbinafine have been suggested to hold equal remedy and were suggested to maintain employees hygiene and affected vicinity dry. With implementation of above strategies all remedy failure cases of dermatophytosis have been treated successfully.

**CONCLUSION:**

Dermatophytosis is a familiar superficial mycotic infection. Males are more frequently infected by dermatophytes. Middle age group especially 3rd decade is more vulnerable to Dermatophytosis.In present study most common isolate being *T. rubrum.* Dermatophytoses are not easy to be detected and identified only via clinical demonstrations. Therefore, it is necessary to utilize suitable diagnostic techniques for an appropriate diagnosis and treatment.

Today, there are two particular mycological laboratory diagnostics including traditional and advanced molecular techniques. As mentioned through the text, advanced molecular diagnostic tools are preferable because of their rapidity, accuracy, sensitivity, specificity; but sometimes, they are not available or may be expensive. Thus, direct KOH microscopy is an acceptable diagnostic method yet. Simultaneous application of direct microscopy and culture are used as a best choice in some countries worldwide. We purpose that, the use of direct microscopy, culture medium and molecular diagnostics. simultaneously is the best choice until now. In addition, by detection of fungal elements via direct microscopy and molecular diagnostic approaches, pharmacotherapy must be started and the respond of culture medium is a confirmation test for an accurate diagnosis. Although different types of antifungal drugs are available today; we believe that topical pharmacotherapy is the first choice. Negative respond to topical treatment, may be a good evidence for administering systematic antifungal drugs.

**REFERENCE:**


1. Aktas AE, Yigit N, Aktas A, Gozubuyuk SG. Investigation of in vitro activity of five antifungal drugs against dermatophytes species isolated from clinical samples using the E-test method. *Eurasian J Med* 46, 2011, 26‑31
2. Asticcioli S, Di Silverio A, Sacco L, Fusi I, Vincenti L, Romero E. Dermatophyte infections in patients attending a tertiary care hospital in northern Italy. *New Microbiologica*. 31(4), 2008, 543-48.
3. Bishnoi A, Vinay K, Dogra S. Emergence of recalcitrant dermatophytosis in India.*Lancet Infect Dis* 18, 2013, 250–1.
4. DeiCas E, Vernes A. Parasitic adaptation of pathogenic fungi to mammalian hosts. *Crit Rev Microbiol*. 13(2), 1986, 173-218.
5. Dhayagude S, Arjunwadkar V, Chavan R, Bharadwaj R, Kagal A. Clinicomycological study of tinea infections in and around Pune. *Int J Res Dermatol*. 5(3), 2012, 598-602.
6. Dhayagude S, Arjunwadkar V, Chavan R, Bharadwaj R, Kagal A. Clinicomycological study of tinea infections in and around Pune. *Int J Res Dermatol*. 5(3), 2014, 598-602.
7. Doddamani PV, Harshan KH, Kanta RC, Gangane R, Sunil KB. Isolation identification and prevalence of dermatophytes in tertiary care Hospital in Gulbarga District. *PJSR*. 6(2), 2013, 10-13.
8. Doddamani PV, Harshan KH, Kanta RC, Gangane R, Sunil KB. Isolation identification and prevalence of dermatophytes in tertiary care Hospital in Gulbarga District. *PJSR*. 6(2), 2013, 10-13.
9. Dukare A, Khadse R, Boyar S, Chavan S, Raut S. Mycological study on Trichophytoninterdigitale isolated from clinically diagnosed cases of dermatophyte infection. *WJPMR*. 3(10), 2013, 297-301.
10. European Committee on Antimicrobial Susceptibility Testing Antifungal Agents Breakpoint tables for interpretation of MICs. 2014.
11. Ghannoum MA, Chaturvedi V, Espinel‑Ingroff A, Pfaller MA, Rinaldi MG, Lee‑Yang W. Intra‑ and interlaboratory study of a method for testing the antifungal susceptibilities of dermatophytes. *J ClinMicrobiol* 42, 2004, 2977‑9.
12. Guruprasad KY, Javed MW, Roopa C, Ansari H, Takalkar AA. Clinico-epidemiological study of dermatophytosis in teaching hospital of North Karnataka.*Int J Res Dermatol*.5(1), 2012, 106-09.
13. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *J Invest Dermatol* 134, 2014, 1527–34.

14. Jain N, Sharma M, Sharma M, Saxena VN. Spectrum of dermatophytoses in Jaipur, India.*African J Microbiol Res.* 8(3), 2014, 237-43.
15. Konda C, Surekha JK, Jahnavi I, Madhuri DS, Nagamani K. Isolation and identification of dermatophytes in a tertiary care hospital. *Int J CurrMicrobiol App Sci*. 6(12), 2013, 4088-101
16. Larone DH. Dermatophytes. In: Medically Important Fungi: A Guide to Identification. 4th ed. Washington DC: American Society for Microbiology (ASM) Press, 2002, 229-53.
17. Moto JN, Maingi JM, Nyamache AK, *et al*. Prevalence of tineacapitis in school going [6] children from Mathare, informal settlement in Nairobi, Kenya. *BMC Res Notes*.8(1), 2014, 274.
18. Padhye AA, Weitzman I. The Dermatophytes. In: Topley& Wilson's Microbiology and [8] MicrobialInfection (Medical Mycology;vol. IV) L Ajello, RJ Hay, L Collier, A Balows, M Sussman (Eds.); 9th Edn.; Arnold publication, Great Britain, 1998, 215-25.
19. Pathania S, Rudramurthy SM, Narang T, Saikia UN, Dogra S. A prospective study of the epidemiological and clinical patterns of recurrent dermatophytosis at a tertiary care hospital in India.*Indian J Dermatology VenereolLeprol* 84, 2014, 678‑84.
20. Phudang RT, Vasant PB, Jayanthi SS. Clinico-mycological study of dermatophytosis and dermatomycosis in tertiary care hospital.*Int J CurrMicrobiol App Sci*. 8(1), 2014, 1297-306.
21. Poluri LV, Indugula JP, Kondapaneni SL. Clinicomycological study of dermatophytosis in South India.*J Lab Physicians.* 7(2), 2011, 84-89.
22. Rouzaud C, Chosidow O, Brocard A, Fraitag S, Scemla A, Anglicheau D. Severe dermatophytosis in solid organ transplant recipients: A French retrospective series and literature review. *Transpl Infect Dis.* 20, 2014 e12799.
23. Rouzaud C, Chosidow O, Brocard A, Fraitag S, Scemla A, Anglicheau D. Severe dermatophytosis in solid organ transplant recipients: A French retrospective series and literature review. *Transpl Infect Dis*. 20, 2014, e12799.
24. Rouzaud C, Hay R, Chosidow O, Dupin N, Puel A, Lortholary O. Severe Dermatophytosis and Acquired or Innate Immunodeficiency: A Review. *J Fungi Basel*.2, 2014, 4.
25. Sudha M, Ramani C, Anandan H. Prevalence of dermatophytosis in patients in a tertiary care centre. *Int J Contemp Med Res.* 3(8), 2013, 2399-401.
26. Sudha M, Ramani C, Anandan H. Prevalence of dermatophytosis in patients in a tertiary care centre. *Int J Contemp Med Res.* 3(8), 2012, 2399-401.
27. Verma SB. Emergence of recalcitrant dermatophytosis in India. *Lancet Infect Dis.* 18, 2013, 718–9.
28. Wayne PA. CLSI.Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi.2nd ed. CLSI standard M38.Clinical and Laboratory Standard Institute, 2008.