Comparison of Broth Micro Dilution and Disk Diffusion Methods for Susceptibility Testing of Dermatophytes

Manal Abd El Alim, Rania M Abdel Halim, Sara A Habib
Clinical Pathology Department, Ain Shams University, Cairo, Egypt

ABSTRACT

Background: Dermatophytes are responsible for the majority of the fungal infections involving skin, hair and nails. There has been a remarkable increase in the number of fungal infections especially in those people whose immune system is compromised by aging, HIV infection, organ transplantation or cancer therapy.

Objective: The aim of this study was to compare both broth microdilution method & disk diffusion method for in-vitro activity of some antifungal drugs (Terbinafine, Fluconazole, Itraconazole) against different species of dermatophytes.

Patients and Method: This study was performed on 50 dermatophyte isolates recovered from various clinical specimens (skin, hair and nail) collected from dermatology outpatient clinic of Ain Shams University Hospital. All samples were cultured on sabarouds. Isolates recovered from SDA 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 0.5 macfarland. Then antifungal susceptibility was done using: Disk diffusion (DD) method and Broth micro dilution (BMD) method.

Results: There was **a highly significant** agreement between the antifungal susceptibility testing of fluconazole, itraconazole and terbinafine by disk diffusion method and Broth micro-dilution method. In our study agreement between both methods for itraconazole was 1.00 (kappa), for terbinafine was 0.947, and for fluconazole was 0.878. The factors that may affect the results of BMD or DD are type and size of inoculum, composition of the media, temperature and duration of incubation and disc strength.

Conclusion: There was **a highly significant** agreement between the antifungal susceptibility testing of fluconazole, itraconazole and terbinafine by disk diffusion method and Broth micro-dilution method.

Keywords: Dermatophytes, Disk diffusion, Broth micro-dilution.

INTRODUCTION

Dermatophytes are responsible for the majority of the fungal infections involving skin, hair and nails[1].

There has been a remarkable increase in the number of fungal infections especially in those people whose immune system is compromised by aging, HIV infection, organ transplantation or cancer therapy[2].

The organisms are transmitted either by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, marching, excessive temperature and humidity[3].

Clinically, dermatophytes (ringworm) can be classified depending on the site involved. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea cruris (groin), Tinea pedis (foot) or athlete's foot and Tinea barbae or barber's itch (bearded areas of the face and neck). Favus is a chronic type of ringworm involving the hair follicles[4].

According to the genera, dermatophytes can be classified into Trichophyton which affect mainly the skin and nails, Microsporum which affect mainly the hair and Epidermophyton which affect mainly the skin[5]. Trichophyton rubrum, Trichophyton tonsurans and Trichophyton mentagrophytes are the most common dermatophytes. Trichophyton rubrum affect face, trunk, beard area, nails, feet and groin area infection. Trichophyton mentagrophytes affect the surface of the hair (large spore ectothrix) which manifest clinically as kerion. Trichophyton tonsurans invade the hair shaft (endothrix) which manifest clinically as black dot infection. Another common dermatophyte is Microsporum canis, which is transmitted from animals such as cats and dogs to humans causing small spore ectothrix which manifest clinically as scally ringworm[6].

Received: 12 / 6 /2017
Accepted:21 /6 /2017
DOI : 10.12816/0040624

Though there are several antifungal drugs used to treat dermatophytosis, some infections respond well to topical antifungal therapy, whereas others like tinea capitis, tinea unguium (nail infection), and more extensive or severe types may require systemic therapy[7].

The concurrent increase in fungal infections with increase in the use of antifungal drugs mostly for prolonged periods has led to development of resistance to antifungal drugs[8].

Antifungal susceptibility testing is performed to provide information for clinicians to select appropriate antifungal agents useful for treating a particular fungal infection. For a definitive therapy also, it is essential to evaluate the resistant dermatophytes using a standardized, simple and reproducible in-vitro assay to determine the antifungal activity of drugs against isolates. In vitro antifungal susceptibility tests are now mainly used for epidemiological surveys, determination of the degree of antifungal activity, and the prediction of clinical outcome based upon an optimization of antifungal therapy[7].

Various methods, such as broth macro and microdilutions, agar dilution, E-test, Sensititre colorimetric microdilution panels and disk diffusion have been used for determining the susceptibility of dermatophytes to antifungal agents[9].

PATIENTS AND METHODS

The present study was conducted on 55 clinical isolates of dermatophytes retrieved from patients suffering from dermatophytic infection of skin, hair and nail attending dermatology outpatient clinic of Ain Shams University Hospital in the period from August 2016 till March 2017.

Demographic data were taken from patients. Collection of specimen: skin, hair and nail scrapings were collected in sterile disposable 9 mm petri dishes.

All specimens were subjected to: Culture on sabaroud dextrose agar (SDA) containg chloramphenicol and cycloheximide then isolates recovered from SDA are subcultured on potato dextrose agar (PDA) and incubated at 28ºC for 7 days to enhance sporulation, microspopic examination using scotch tape technique was done, then the growth was harvested in sterile saline and the conidial and hyphal suspension was adjusted to 0.5 macfarland.

Then antifungal susceptibility was done using:

1. Disk diffusion (DD) method : Inoculum suspension was prepared from colonies grown on potato dextrose agar (PDA) at 30-35^{o}C. Mueller-Hinton agar was evenly streaked using a sterile cotton swab that was dipped into the inoculum suspension.

Antifungal discs of Fluconazole, Itraconazole, Gresiofulvin and Terbinafine were applied. Plates of Mueller-Hinton agar were incubated at 28^{o}C for 5 days. When growth took place, the size of zones of inhibition was measured for each antifungal agent. Interpretation of zone diameters was done according to CLSI 2009 guidelines.

2. Broth micro dilution (BMD) method: The test was performed in microtitre plastic plates (96 well). For each drug six dilutions were used. Drugs were prepared by adding the powder to Muller Hinton Broth. One hundred microlitre of muller Hilton broth containing the powder of the drugs with two-fold drug serial dilutions were placed in wells with a multichannel pipette to yield twice the final strength required for the test i.e. 4-128 µg/ml for FLC, 0.125-4.0 µg/ml for ITR and 0.015-0.50 µg/ml for TER. The plate was then inoculated with 100 µl of the inoculum suspension that is equivalent to 0.5 macfarland thus bringing the final dilution of drugs to 2.0-64.0 µg/ml for FLC, 0.062-2.0 µg/ml for ITR and 0.015-0.5 µg/ml for TER respectively. The plates were incubated at 28°C and checked every day for 5 days. Minimum inhibitory concentration (MIC) was determined for each antifungal agent by detecting the turbidity of the broth.

3. Broth microdilution method and rezasurin: Fifty µl of Muller Hinton broth and 50µl of risazurin dye were introduced on the same previous six dilutions of the drugs. The plate was then inoculated with 100 µl of the diluted inoculum suspension that is equivalent to 0.5 macfarland. The plates were incubated at 28°C and read visually everyday for 5 days for colour change. Minimum inhibitory concentration (MIC) was determined for each antifungal agent by detecting color change of rezasurin.

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

In tinea capitis 21 (55.3%) patients were T. tonsurans, 10 (26.3%) patients were M. canis, and 7 (18.4%) patients were T. mentegrophytes.

In tinea circinata 3 (25%) patients were T. tonsurans, 3 (25%) patients were M. audonii, 2 (16.7%) patients were T. mentegrophytes, 2 (16.7%) patients were T. rubrum, 1 (8.3%) patient was M. gypsum, and 1 (8.3%) patient was E. floccosum, all onychomycosis cases (5) revealed candida species **(Table 1).**

(Table 1): Distribution of dermatophyte species among clinically diagnosed cases

		Diagnosis			
		Tinea capitis		Tinea circinate	
Strains of organism	Trichophyton tonsurans (n %)	21	55.3%	3	25%
	Microsporum canis (n %)	10	26.3%	0	0%
	Trichophyton mentegrophytes (n	7	18.4%	2	16.7%
	Microsporum audonii (n %)	0	0%	3	25%
	Trichophyton rubrum (n %)	0	0%	2	16.7%
	Microsporum gypsum (n %)	0	0%	1	8.3%
	Epidermophyton floccosum (n	0	0%	1	8.3%

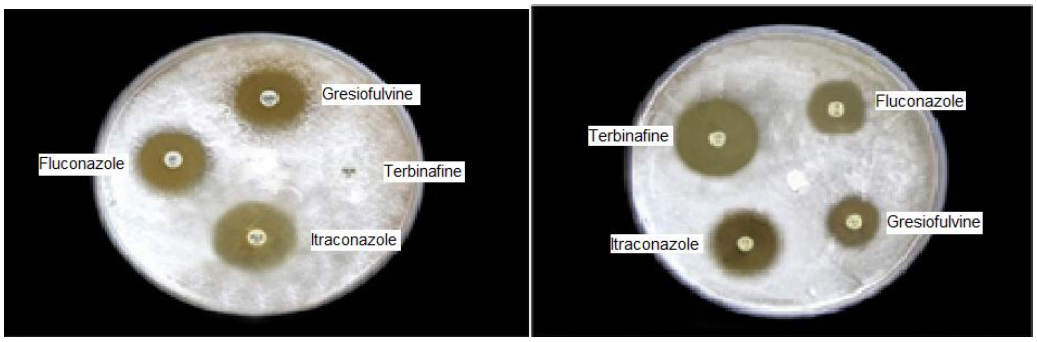
Sensitivity testing by disc diffusion method using:

1. fluconazole disk revealed 5 (10%) strains of dermatophytes were sensitive, while 45 (90%) strains of dermatophytes were resistant.

2. Itraconazole disk revealed, 10 (20%) strains of dermatophytes were sensitive, while 40 (80%) strains of dermatophytes were resistant.

3. Terbinafine disk revealed, 13 (26%) strains of dermatophytes were sensitive, while 37 (74%) strains of dermatophytes were resistant.

Gresiofulvin disk revealed, 8 (16%) strains of dermatophytes were sensitive, while 42 (84%) strains of dermatophytes were resistant **(Figure 1).**

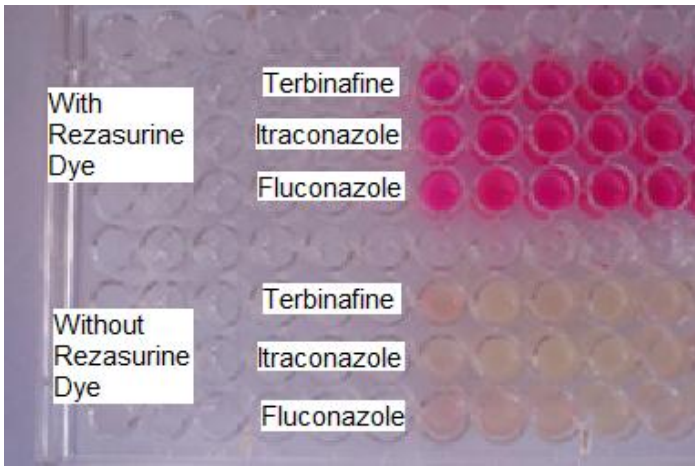


(Figure 1):

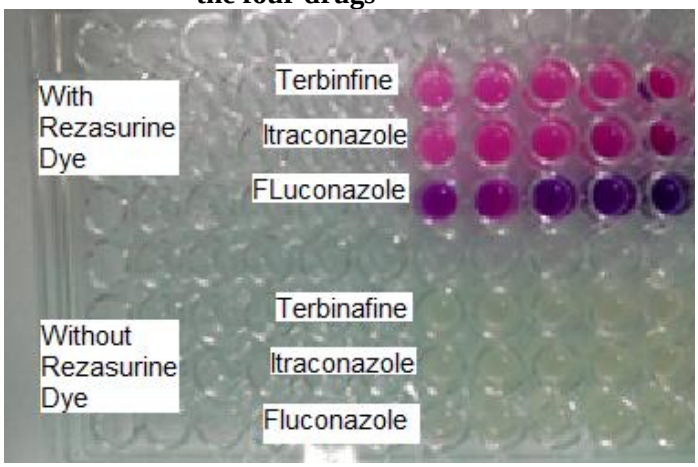
(A)	**(B)**
T. Rubrum: Sensitive to fluconazole, itraconazole and gresiofulvin, Resistant to terbinafine.	**T. Mentegrophytes: Sensitive to fluconazole, terbinafine, itraconazole and gresiofulvin**

MIC broth micro-dilution method was done with and without resazurin dye but both gave similar results but detection was more rapid with resazurin (detected after 24 hours) in relation to MIC broth microdilution method without resazurin (detected after 48-72 hours). Also, resazurin method was easier in visual interpretation.

1. Fluconazole revealed: 4 (8%) strains of dermatophytes were sensitive, while 46 (92%) strains of dermatophytes were resistant.
2. Itraconazole revealed: 10 (20%) strains of dermatophytes were sensitive, while 40 (80%) strains of dermatophytes were resistant.
3. Terbinafine revealed: 12 (24%) strains of dermatophytes were sensitive, while 38 (76%) strains of dermatophytes were resistant **(Figure 2).**



(Figure 2):
(A) Trichophyton Tonsurans: Resistant to the four drugs



(B) Trichophyton Tonsurans: sensitive to fluconazole, resistant to itraconazole and terbinafine with rezasurin dye: Pink: resistant / Purple: sensitive

1. Fluconazole: Out of 50 strains, four (80% of sensitive strains) of dermatophytes were found to be sensitive to fluconazole in both methods, while 1 strain of dermatophytes (20% of sensitive strains) was found to be sensitive to fluconazole in disk diffusion method but resistant in broth micro-dilution method. Fourty five (100% of resistant strains) of dermatophytes were found to be resistant in both methods **(Table 2).**

2. **Itraconazole:** Out of 50 strains, ten (100% of sensitive strains) of dermatophytes were found to be sensitive in both methods. Fourty (100% of resistant strains) of dermatophytes were found to be resistant in both methods **(Table 3).**

3. **Terbinafine:** Out of 50 strains, twelve (92.3% of sensitive strains) of dermatophytes were found to be sensitive in both methods, while 1 (7.7% of sensitive strains) of dermatophytes was found to be sensitive in disk diffusion method but resistant in broth micro-dilution method. Thirty-seven (100% of resistant strains) of dermatophytes were found to be resistant in both methods **(Table 4).**

There was **a highly significant** agreement between the antifungal susceptibility testing by disk diffusion method and Broth micro-dilution method with Kappa= 1 for itraconazole, Kappa= 0.947 for terbinafine, and Kappa= 0.878 for fluconazole.

(Table 2): Agreement between Disk diffusion method and Broth micro-dilution sensitivity method for fluconazole

			DD Fluconazole			kappa	P	Sig
			S		R			
		N	%	N	%			
MIC Fluconazole	S	4	80.0%	0	0%	0.878	0.0001	HS
	R	1	20.0%	45	100.0%			

*kappa statistics

(Table 3): Agreement between Disk diffusion method and Broth micro-dilution sensitivity method for Itraconazole

		DD Itraconazole				Kappa	P	Sig
		S		R				
		N	%	N	%			
MIC Itraconazole	S	10	100.0%	0	.0%	1.00	.0001	HS
	R	0	.0%	40	100.0%			

*kappa statistics

(Table 4): Agreement between Disk diffusion method and Broth micro-dilution sensitivity method for terbinafine

		DD Terbinafine				Kappa	P	Sig
		S		R				
		N	%	N	%			
MIC Terbinafine	S	12	92.3%	0	.0%	0.947	.0001	HS
	R	1	7.7%	37	100.0%			

*kappa statistics

Sensitivity of disk diffusion method in relation to broth micro-dilution method for fluconazole was 80%, for itraconazole was 100% and for terbinafine was 92.3%. Specificity for fluconazole was 97.9%, for itraconazole was 100% and for terbinafine was 97.4%. Positive predictive value for fluconazole was 80%, for itraconazole was 100% and for terbinafine was 92.3%. Negative predictive value for fluconazole was 97.9%, for itraconazole was 100% and for terbinafine was 97.4% **(Table 5).**

(Table 5): Sensitivity and specificity results of disk diffusion method in relation to MIC broth mircro-dilution method for each antifungal drug

	Sensitivity	Specificity	PPV	NPV
Fluconazole	80%	97.7%	80%	97.99
Itraconazole	100%	100%	100%	100%
Terbinafine	92.3%	97.4%	92.3%	97.4%

PPV= Positive predictive value
NPV= Negative predictive value

DISCUSSION

In the current study, there was a statistical significant difference between young age (ranged from 3-18 years) and dermatophytic infections. This was concordant that found by **Abdel-Rahman *et al.*[10]** who studied genes of dermatophyte virulence factors on 50 patients of dermatophytic infections and showed that tinea infection is predominantly a disease of preadolescent children and typical age of onset is between five and ten years.

In our study, 60% of patients had positive risk factors for dermatophytic infections, Also, **Cafarchia *et al.*[11]** stated that 62%-65% of patients had positive risk factors for dermatophytic infections, which can explain the role of immunosuppression, personal hygiene and environmental factor in the incidence of dermatophytic infections.

We found that patients with history of previous systemic antifungal intake (38%) are more resistant to antifungal treatment**, Ghannoum and Rice [12]** studied the mechanism of resistance of antifungal agents and stated that with increased use and availability of different classes of antifungal agents, it is anticipated that an increasing number and variety of fungal species resistant to these agents will be seen.

In our study, we found that the most common dermatophyte species was T. tonsurans (48%) followed by M. canis (20%), T. mentegrophytes (18%), M. audonii (6%), T. rubrum (4%), M. gypsum (2%) and E. floccosum (2%), this conclusion disagree with that found by **Gupta *et al.*[13]** in India who stated that the most common dermatophyte species was Trichophyton rubrum (46.5%), followed by T. mentegrophytes (39.6%), M. gypsum (6.8%), T. tonsurans (3.4%), M. oudonii (1.7%) and M. ferrugineum (1.7%), which can be explained by different environmental fungal population.

In the present study, the most common organism causing tinea capitis was T. tonsurans (55.3%). This conclusion concur that found by **Nasir *et al.*[14]** on 391 children with suspected tinea capitis who found that T. tonsurans accounts for greater than 90% of cases of infection, followed by M. canis (1.1%) then T. mentegrophytes (0.7%). Whereas, in tinea circinata both T. tonsurans and M. audonii were equally recovered from our species (25%) for each. A study carried out by **Hryncewicz-Gwózdz *et al.*[15]** found that T. tonsurans is the most common dermatophyte to cause tinea capitis (95.8%), and people with an anthropophilic tinea capitis infection are more likely to develop associated tinea corporis. In other study made by **Ravenscroft *et al.*[16]** on incidence of T. tonsurans in both tinea capitis and corporis, found that T. tonsurans has emerged as a major cause of tinea capitis and tinea corporis.

By using disc diffusion method for dermatophytes, we found that fluconazole was 10% sensitive and 90% resistant, to itraconazole was 20% sensitive and 80% resistant, to terbinafine was 26% sensitive and 74% resistant and to gresiofulvin was 16% sensitive and 84% resistant. This observation disagree with that remarked by **Gupta *et al.* [13]** in a study who performed both disk diffusion and broth microdilution methods on 58 clinical isolates of dermatophytes using four antifungals (fluconazole, itraconazole, terbinafine and gresiofulvin). They showed only six strains resistant to FLC, five resistant to TER and five, four and three strains were found intermediate sensitive to FLC, ITR and GRI respectively. All other strains in that study were sensitive to all the four antimycotic agents, High resistance to antifungal drugs in our isolates could be explained by the significant previous history of systemic antifungal intake (38%).

By using MIC broth microdilution method for dermatophytes, we found that fluconazole was 8% sensitive and 92% resistant, to itraconazole, 20% sensitive and 80% resistant and to terbinafine, 24% sensitive and 76% resistant. **Santos and Hamdan[17]** studied broth microdilution method for testing susceptibilities of dermatophytes to antifungals on fifty clinical isolates of Trichophyton rubrum using five antifungals (ketoconazole, fluconazole, itraconazole, gresiofulvin and terbinafine) found that the evaluation of in vitro activities of tested drugs revealed that terbinafine was the most potent active drug that observed in the present study. **Gupta *et al.*[13]** studied both disk diffusion and broth microdilution methods on 58 clinical isolates of dermatophytes on four antifungals (fluconazole, itraconazole, terbinafine and gresiofulvin) and found that Fluconazole was found to be the least effective drug similar to our study.

In this study, rezasurin was used as a color indicator for metabolic activity in broth micro dilution method. Resazurin is a blue, non-fluorescent dye that is converted to pink and fluorescent resurfin in the presence of a respiring organism, it's an easy method to read because of clear-cut end points, the use of resazurin dye test in antifungal susceptibility testing of biofilm-forming cells has benefits like simplicity, low cost, lack of toxicity, and easy determination of end points[18].

We found that results of rezasurin MIC broth microdilution method were the same as that of traditional MIC for all samples. However, it was more rapid as it started to change its color after 12 hours and complete change was achieved after 24 hours of incubation, while MIC without resazurin gave results in 48-72 hours that proves that with resazurin is more rapid and give faster results to the physicians in order to take a rapid action in treatment of the patient.

Daniel *et al.*[19] studied the application of resazurin for estimating abundance of contaminant-degrading micro-organisms on 24 clinical isolates found that resazurin changed color from blue to pink after overnight incubation. On the other hand, **Tiballi *et al.*[20]** studied the comparison of a photometric method using rezasurin dye with standardized methods of antifungal susceptibility testing of yeasts on 101 clinical isolates have also shown the usefulness of resazurin as a cell viability indicator in antifungal susceptibility testing.

There was **a highly significant** relation between the antifungal susceptibility testing of fluconazole, itraconazole and terbinafine by disk diffusion method and Broth micro-dilution method, in our study the relation between both methods for itraconazole was 1.00 (kappa), for terbinafine was 0.947, and for fluconazole was 0.878.

In a study of evaluation of microdilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes made by both **singh *et al.*[21] and Mendez *et al.*[22]** on in-vitro testing of itraconazole, fluconazole and voriconazole against dermatophytes had reported contrary results with poor or no correlation at between broth micro dilution and disk diffusion, they justified their results by the use of dermasel agar medium which is unacceptable for antifungal susceptibility testing. **Fernández Torres *et al.*[23]** stated that the factors that may affect the results of BMD or DD are type and size of inoculum, composition of the media, temperature and duration of incubation and disc strength.

In our study, disk diffusion method was highly sensitive and specific in relation to broth micro-dilution method and sensitivity for fluconazole was 80%, for itraconazole was 100% and for terbinafine was 92.3%, specificity for fluconazole was 97.9%, for itraconazole was 100% and for terbinafine was 97.4%. Positive predictive value for fluconazole was 80%, for itraconazole was 100% and for terbinafine was 92.3%. Negative predictive value for fluconazole was 97.9%, for itraconazole was 100% and for terbinafine was 97.4%. This conclusion agrees with that found by **Gupta *et al.*[13]** who performed both disk diffusion and broth microdilution methods on 58 clinical isolates of dermatophytes using four antifungals (fluconazole, itraconazole, terbinafine and gresiofulvin). They found broth microdilution (BMD) is the standard method for testing antifungal susceptibility of dermatophytes and there was high sensitivity and specificity of disk diffusion method in relation to broth microdilution method with sensitivity for fluconazole was 98.1%, for itraconazole was 96.6% and for terbinafine was 94.3%. Whereas specificity for fluconazole was 87.5%, for itraconazole was 75% and for terbinafine was 72.7%.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCE

1. Chinelli P, Sofiatti A, Nunes R and Martins J (2003): Dermatophyte agents in the city of São Paulo, from 1992 to 2002 Revista do Instituto de Medicina Tropical de São Paulo., 45: 259-263.

2. Kannan P, Janaki C and Selvi G (2006): Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal infections Indian J. Med. Microbiol., 24(3): 212 15.

3. Bokhari M (2009): Antifungal activity of some medicinal plants used in Jeddah Saudi Arabia, Mycopath., 7(1):51-57.

4. Ananthanarayan R and Paniker C (2009): Medical mycology Textbook of Microbiology; 8th

edition. Hyderabad, India: Universities Press Private Limited,Pp: 604-607.

5. El-Gohary M, van Zuuren E, Fedorowicz Z *et al.* (2014): Topical antifungal treatments for tinea cruris and tinea corporis. Cochrane Database Syst Rev., 89(2)*:*259-64.

6. Parija S (2011): Mycology. Textbook of Practical Microbiology, 1st edition. New Delhi, India, Aph Ahuja Publishing House, Pp:211-237.

7. Pakshir K, Bahaedinie L and Rezaei Z (2009): In vitro activity of six antifungal drugs against clinically important dermatophytes IJMM., 2(4):158-163.

8. Jain N, Sharma M and Saxena V (2008): Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal infection Indian J. Dermatol. Venerol. Leprol., 74(3): 274 75.

9. Perrins N, Howell S, Moore M and Bond R (2005): Inhibition of the growth in vitro of Trichophyton mentagrophytes, Trichophyton erinacei and Microsporum persicolor by miconazole and chlorhexidine. Veter Dermatol., 16: 330-333.

10. Abdel-Rahman S, Farrand N, Schuenemann E *et al.* (2010): The prevalence of infections with Trichophyton tonsurans in school children: the CAPITIS study. Ped., 125 (5):966-73.

11. Cafarchia C, camarda A, Coccioli C *et al.* (2010): Epidemiology and risk factors for dermatophytoses in rabbit farms. Med Mycol., 48, 975–980.

12. Ghannoum M and Rice L (1999): Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev., 12(4): 501–517.

13. Gupta S, Kumar R, Mittal G *et al.* (2015): Comparison of Broth Micro Dilution and Disk Diffusion Method for Susceptibility Testing of Dermatophytes. Int J Curr Microbiol App Sci., 4(5): 24-33.

14. Nasir S, Ralph N, O'Neill C *et al*.(2013): Trends in Tinea Capitis in an Irish Pediatric Population and a Comparison of Scalp Brushings Versus Scalp Scrapings as Methods of Investigation. Pediatr Dermatol., (5**):**622-3.

15. Hryncewicz-Gwózdz A, Beck-Jendroschek V, Brasch J, Kalinowska K and Jagielski T (2011): Tinea capitis and tinea corporis with a severe inflammatory response due to Trichophyton tonsurans. Acta Derm Venereol., 91(6):708-10.

16. Ravenscroft J, Goodfield M and Evans E (2000): Tricophyton tonsurans Tinea capitis and tinea corporis: treatment and follow up of four affected family members. Pediatr. Dermatol., 17(5): 407-409.

17. Santos D and Hamdan J (2005): Evaluation of broth microdilution antifungal susceptibility testing conditions for Trichophyton rubrum. J. Clin. Microbiol., 43: 1917-1920.

18. Tizzard, A, Bergsma J and Lloyd-Jones G (2006): A resazurin-based biosensor for organic pollutants. Biosens Bio electron., 22:759–763.

19. Daniel M, Kai H, Serge R, et al., (2010): Screening for antifungal peptides and their modes of action in aspergillus nidulans, J. applied and envir. microbiol., 7102–7108.76-21.

20. Tiballi R, Zarins T, Revankar S and Kauffman C (1995): Use of a Calorimetric system for yeast susceptibility testing. J Clin Microbiol., 33:915-7.

21. Singh J, Zaman M and Gupta K (2007): Evaluation of micro dilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes. Med. Mycol., 45: 595-602.

22. Mendez C, Serrano, M, Valverde A, et al., (2008): Comparison of E-test disk diffusion and a modified CLSI broth micro dilution (M 38-A) method for in-vitro testing of itraconazole, fluconazole and voriconazole against dermatophytes. Med. Mycol., 46(2): 119 23.

23. Fernández-Torres B, Cabañes F, Carrillo-Munõz A *et al.* (2002): Collaborative evaluation of optimal antifungal susceptibility testing condition for dermatophytes. J Clin Microbiol., 40: 3999-4003.