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In vitro Susceptibility Testing of Clinical Zygomyceteous Species isolated in Cairo, Egypt against Ten Antifungal Agents by Broth Microdilution, Disk Diffusion and E-test Methods

Iman M. ElKholy[2], Sh.M. Zaki,[1] S.S. Mohamed[1], and Walaa M. Wasfy[1]

[1]Mycology Unit, Microbiology Department, Faculty of Science Ain Shams University and [2]Clinical Pathology Department, University Specialized Hospital, Cairo, Egypt.

THIS study was carried out on ten zygomycetous isolats belonging to 5 species namely, *Rhizopus oryzae* (3), *Lechtheimia corymbifera* (1), *Lechtheimia ramosa* (3), *Syncephalastrum racemosum* (2), *Rhizomucor pusillus* (1), isolated from clinical specimens in Cairo, Egypt. These isolates are classified in 3 families, *mucoraceae*, *lichtheimiaceae, syncephalastraceae* of order mucorales. They were tested for their susceptibility to ten antifungal drugs using broth microdilution, disk diffusion, and E-test methods. The antifungals used were amphotericin B, itraconazole, voriconazole, fluconazole, terbinafine, ketoconazole, griseofulvin, caspofungin, micafungin, and posaconazole. A good level of overall agreement between the disk diffusion and the broth microdilution methods was observed in this study, therefore disk diffusion method could be considered as a good alternative to the broth microdilution method. Members of the three families tested were fully susceptible to amphotericin B and itraconazole and completely resistant to voriconazole, griseofulvin, caspofungin, and micafungin. ketoconazole was active against lichtheimiaceae, and syncephalastraceae while it showed reduced activity to mucoraceae. Fluconazole showed reduced activity against all tested isolates. Terbinafine was inactive against mucoraceae and syncephalastraceae while it showed reduced activity against isolates of lichtheimiaceae. Although posaconazole was active against syncephalastraceae and showed reduced activity to lichtheimiaceae, it was inactive to mucoraceae. Variability of *in vitro* susceptibility was found in all mucorales genera tested therefore susceptibility profile of the isolated etiologic agents must be known before treatment of infections.

Keywords: Antifungal susceptibility, Zygomycetes, Broth microdilution, Disk diffusion, E-test, Egypt.

Zygomycosis is an infection caused by members of class zygomycetes that invade immunocompromised hosts and produce angioinvasive disease (Walsh *et al.*,2004). Patients at highest risk for zygomycosis are those with (i) immunosuppression related to neutropenia, corticosteroid use, hematologic malignancies, and solid-organ

* Corresponding author: Imankholy@yahoo.com

transplant, (ii) diabetes mellitus, especially those with ketoacidosis, (iii) conditions of iron overload with associated desferoxamine use, and (iv) skin disruption by trauma or other serious conditions, such as burns or heatstroke (Greenberg *et al.*,2006). The clinical presentations of zygomycosis include rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated and uncommon forms (Petrikkos *et al.,* 2012). *Rhizopus*, *Lichtheimia*, and *Mucor* are the most often zygomyceteous genera recovered from the clinical specimens (Gomes *et al.*, 2011).

In terms of systemic antifungal therapy, polyenes such as amphotericin B deoxycholate and its lipid derivatives remain the front-line agents used for zygomycosis treatment (Greenberg *et al.,* 2006 and Petrikkos *et al.,* 2012). Recently, a liposomal formulation of nystatin (polyene) has been developed and exhibited significant and strain-independent fungicidal activity against all zygomycetes isolates *in vitro* by all testing methods (Chamilos *et al.,* 2006). Azole drugs such as fluconazole, voriconazole, itraconazole, and posaconazole have a limited *in vitro* activity against zygomycetes (Goldani and Sugar, 1994 and Sugar and Liu, 2000). However, the echinocandins (caspofungin, micafungin and anidulafungin) have been reported as inactive *in vitro* against zygomycetes (Pfaller *et al.,* 1998 and Singh *et al.,* 2005). Few studies have analyzed the activity of terbinafine against zygomycetes and the results showed that terbinafine was less active than amphotericine B with a wide range of MICs as each strain had a different antifungal susceptibility profile (Dannaoui *et al.* 2002 and 2003).

Mucorales as major etiologic agents of zygomycosis cannot be considered as a single entity from an antifungal perspective because large differences in susceptibility could be detected between families, genera species, and strains. Molecular identification of the etiologic agent is therefore required unless the susceptibility profile of the strain is known (Vitale *et al.,* 2012).

In a previous work, we have reported 10 mucormycosis cases from one center in one year which is the first report concerning with the zygomycosis in Egypt and the cases was treated empirically by amphotericine B and itraconazole due to the lack of susceptibility profile of the isolated species (Zaki *et al.,* 2014).

The present study was conducted to test the *in vitro* antifungal susceptibility of isolated zygomycetes strains in Cairo, Egypt to ten antifungal agents available in the Egyptian market by using broth microdilution, disk diffusion and E-test methods in order to give clear information about the susceptibility profile of the known etiologic agents of zygomycosis in Egypt.

Materials and Methods

Zygomyceteous strains

A total of ten species of clinical origin were tested for their susceptibility to some selected antifungal agents. The species were identified by traditional and molecular methods (National Committee for Clinical Laboratory Standards (2002). They were deposited at Assuit University Mycological Center (AUMC), Assuit, Egypt. The test

species included 3 *Rhizopus oryzae*, 3 *Lechtheimia ramosa*, 1 *Lechtheimia corymbifera*, 2 *Syncephalastrum racemosum*, and 1 *Rhizomucor pusillus* (Table 1).

TABLE 1. List of zygomyceteous tested strains.

No.	Strain	Family	AUMC No.	GenBank accession No.
1	*Rhizopus* oryzae	Mucoraceae	7957	KC 117255
2	*Rhizopus* oryzae		7958	KC 117256
3	*Rhizopus* oryzae		7959	KC 117257
4	*Lichtheimia corymbifera*	Lichtheimiaceae	7960	KC 117258
5	*Lichtheimia ramosa*		7961	KC 117259
6	*Lichtheimia ramosa*		7962	KC 117260
7	*Lichtheimia ramosa*		7963	KC 117261
8	*Rhizomucor pusillus*		7966	KC 117252
9	*Syncephalastrum racemosum*	Syncephalastraceae	7964	KC 117253
10	*Syncephalastrum racemosum*		7965	KC 117254

AUMC : Assuit University Mycological Center.

Antifungal agents

The following drugs were obtained in powder form, Amphotericin B (Bristol Myers Squibb Woerden, The Netherlands), itraconazole (Apex pharma, Egypt), voriconazole (Pfizer, Egypt), fluconazole and terbinafine (Novartis, Egypt), ketoconazole (Ramedia, Egypt), griseofulvin (Kahira pharm & Chem. Ind. Co., Egypt), and caspofungin (Merck, Rahway, NJ, USA). Starting dose of 32 µg/ml for each of amphotericin B, itraconazole, voriconazole, ketoconazole and terbinafine antifungals were prepared by weighing 3.2 mg powder and dissolving in 1 ml dimethyl sulfoxide (DMSO). In case of griseofulvin and fluconazole 6.4 mg powder were weighed and dissolved individually in 1 ml DMSO to get 64 µg/ml starting dose. Caspofungin was prepared at a higher dose where a total of 25.6 mg powder was dissolved in 1 ml sterile water to give 256 µg/ml concentration. All prepared stock solutions were maintained at -20^{o}C until needed. A working solution of each drug was prepared by making 1:10 dilution in DMSO or sterile water as appropriate.

For disk diffusion method, four antifungal drugs were obtained as ready to use disks as follow: amphotericin B (100 µg), voriconazole (1 µg), fluconazole (25 µg), and ketoconazole (50 µg) (Biorad, USA). For the other four drugs a stock solution of each drug was prepared according to antifungal disks potency of Rosco Diagnostica Company (Neosensitabs, Denmark) using DMSO or sterile water for caspofungin, as follows: griseofulvin, 0.8 mg/ml; caspofungin 0.25 mg/ml, terbinafine, 50 µg/ml, itraconazole, 0.4 mg/ml. Taxo™ Blank paper disks (6 mm diameter) were loaded with 20 µl of the prepared stock solutions to obtain the desired drug concentration per disk (16 µg/disc for griseofulvin, 5 µg/disc for caspofungin, 1 µg/disc for terbinafine and 1 µg/disc, 8 µg/disc for itraconazole respectively) and allowed to air dry at room temperature. The air-dried disks were stored at 4°C.

For E-test method, two antifungal drugs were obtained as E-test strips. micafungin and posaconazole (BioMérieux SA, France).

Medium

Broth microdilution method was performed in RPMI 1640 medium (Sigma-Aldrich, Mississauga, Ontario, Canada) with L-glutamine but without sodium bicarbonate and buffered with 0.165M morpholinepropanesulfonic acid (MOPS) (Sigma-Aldrich) at pH 7.0 (Clinical and Laboratory Standards Institute (2008). The disk diffusion and E-test methods was performed on Mueller-Hinton (MH) 2% glucose agar medium (Remel, KS) (Espinel-Ingroff *et al* ., 2007).

Test procedure

Broth microdilution method: The CLSI M38-A2 guidelines were followed. Species were grown on Sabouraud dextrose agar for 3-5 days at 30°C while the quality control strain (*Candida albicans* NCPF 8154: QC species) was grown for 24hr before testing. The culture was gently swabbed with a cotton tip applicator to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile tube and the volume was adjusted to 5 ml with sterile saline solution and shaked well. The suspension was allowed to stand for a few minutes so that the hyphal segments settle down. Spores in the suspensions were counted with a hemocytometer and then diluted into RPMI 1640 broth medium to a concentration of 10^6 CFU /ml providing the most reproducible MIC data. The QC strain inoculum suspension was prepared by taking a swab from 24 h old culture into 5 mL of sterile distilled water and then adjusting the concentration using the 0.5 McFarland standard tubes to give a yeast suspension of 10^6 CFU /ml. A working suspension was prepared in sterile distilled water to yield 10^3 CFU/ml. A sterile microdilution plate (96-flat bottomed wells) was used for each species so that 100 µl of RPMI media was added to each well then another 100 µl of the working solution of each antifungal drug was added to a well in the 1st raw. Two-fold serial dilutions were made using the multichannel pipette so that rows 1-10 will contain the series of drug dilutions in 100 µl volumes. The final concentrations of the antifungal agents were 0.06-64 mg/L for griseofulvin and fluconazole, 0.25–256 mg/L for caspofungin and 0.03–32 mg/L for the rest of drugs. 100 µl of inoculum suspension was added to each well. The 11th row which served as the positive growth control contained 100 µl of inoculum suspension and 100 µl of drug free medium whereas the 12th row which served as negative control contained 200 µl of RPMI broth only. Microdilution trays were incubated at 35^{o}C. Minimum inhibitory concentrations (MICs) were read after 4-7 days. MIC was defined as the point at which there was 100 % inhibition of growth as compared with the growth control when read visually in the microtitre plates. Minimum effective concentration (MECs) values for caspofungin were determined visually and microscopically. The visual MEC was defined as the lowest drug concentration at which the fungus displayed growth retardation compared with the control while the microscopic MEC was defined as the lowest drug concentration at which the fungus displayed microscopic morphological changes.

Disk diffusion method: Inoculum suspensions were prepared as in broth microdilution method and adjusted to a concentration of 10^6 CFU/ml. A sterile cotton swab was moistened in each adjusted inoculum suspension. Swab loaded with fungal inoculum were individually streaked on the surface of MH agar plates in 4 different directions (at 90 degree angles) to cover the entire surface. The surfaces of MH agar plates were allowed to dry at 30 ^oC. Using a flamed sterilized forceps, disks loaded with the antifungal agent were applied onto the surface of the inoculated agar plates and pressed lightly to ensure complete contact with agar. Plates were incubated at 30^oC for 2-4 days. The inhibition zone diameters (IZDs) of growth were measured to the nearest millimeter at the point where there was a prominent reduction of growth (Espinel-Ingroff *et al.,* 2007).

E-test method: Inocula were prepared as in the above broth microdilution method and adjusted to a concentration of 10^6 CFU/ml. A sterile cotton swab was moistened in each adjusted inoculum suspension and excess moisture will be expressed by rolling the swab on the inside of tube above fluid level. The surface of MH agar plates were streaked in 4 different directions (at 90 degree angles) to cover the entire surface. The surfaces of MH agar plates were allowed to dry at 30 ^oC. Using a flamed sterilized forceps, the E-test strips were applied onto the surface of the inoculated agar plates and pressed lightly to ensure complete contact with agar. Plates were incubated at 30^oC for 2 - 4 days. The MIC values were the drug concentrations at which the border of the elliptical inhibition zone intersected the scale on the antifungal strip.

Quality control: The quality control strain (*Candida albicans* NCPF 8154) was included in each set of experiments for comparison.

Data analysis: MICs or MECs and IZDs breakpoints were analyzed according to the values presented in Tables 2 and 3. The categorical agreement between the results of broth microdilution method as a reference method and the disk diffusion assay was calculated, within the same pattern of susceptibility. Errors were ranked as very major (false-susceptible result by disc diffusion), major (false-resistance by disc diffusion) and minor (intermediate by disc diffusion, resistant or susceptible in broth microdilution method) (Espinel-Ingroff *et al.,* 2007).

TABLE 2. The MIC or MEC breakpoints of tested antifungal agents.

Antifungal agent	MIC or MEC			Reference
	S	I	R	
Amphotericin B	≤ 1 µg/ml	2 µg/ml	≥ 4 µg/ml	17
Itraconazole	≤ 1 µg/ml	2 µg/ml	≥ 4 µg/ml	17
Voriconazole	≤ 1 µg/ml	2 µg/ml	≥ 4 µg/ml	17
Caspofungin	≤ 1 µg/ml	2 µg/ml	≥ 4 µg/ml	17
Ketoconazole	< 4 µg/ml	-	4-16 µg/ml	32
Fluconazole	< 8 µg/ml	16-32 µg/ml	> 64 µg/ml	32
Terbinafine	< 1 µg/ml	1 µg/ml	> 1 µg/ml	33
Griseofulvin	< 2 µg/ml	2 µg/ml	> 2 µg/ml	34

S=Susceptible; I=Intermediate; R=Resistant.

TABLE 3. The IZDs breakpoints of tested antifungal agents.

Antifungal agent	IZDs			Reference
	S	I	R	
Amphotericin B	> 15 mm	13-14 mm	≤ 12 mm	17
Itraconazole	≥ 17 mm	14-16 mm	≤ 13 mm	17
Voriconazole	≥ 17 mm	14-16 mm	≤ 13 mm	17
Caspofungin	≥ 17 mm	14-16 mm	≤ 13 mm	17
Ketoconazole	≥ 30 mm	23-29 mm	≤ 22 mm	28
Fluconazole	≥ 21 mm	15-22 mm	≤ 14 mm	28
Terbinafine	≥ 20 mm	12-19 mm	≤ 11 mm	28
Griseofulvin	≥ 10 mm	-	0 mm	28

S=Susceptible; I=Intermediate; R=Resistant

Results

The minimum inhibitory concentration (MIC) results of the *in vitro* susceptibility testing of ten zygomyceteous strains against eight antifungal drugs are shown in Table 4. The results indicated that amphotericin B and itraconazole exhibited the best antifungal activities against all the tested strains giving MIC ranging from 0.004 – 1 µg/ml. ketoconazole comes next by showing activity against all strains with MICs ranging from 0.125µg/ml to 4µg/ml. Both fluconazole and terbinafine showed activity against some strains. voriconazole, griseofulvin and caspofungin showed no activity against all strains where the obtained MICs were higher than the breakpoints.

TABLE 4. In vitro susceptibility of zygomycetes strains to 8 antifungal drugs using broth microdilution method.

Strain	MIC (µg/ml) for antifungal agent							
	AMB	ITC	VRC	FLC	KTC	TRB	GRS	CAS
R. oryzae KC 117255	0.25	0.25	8	16	1	4	16	128
R. oryzae KC 117256	1	1	8	16	4	8	16	64
R. oryzae KC 117257	1	0.5	8	16	2	8	16	128
L. corymbifera KC 117258	0.004	0.004	8	64	0.125	8	16	64
L. ramosa KC 117259	0.004	0.06	8	64	0.125	1	32	64
L. ramose KC 117260	0.004	0.03	4	64	0.25	1	16	128
L. ramose KC 117261	0.125	0.06	8	64	0.25	8	16	64
S. racemosum KC 117253	0.5	0.25	8	64	1	8	16	64
S. racemosum KC 117254	1	0.125	8	16	2	8	16	64
Rh. pusillus KC 117252	0.125	0.125	8	16	0.5	1	16	64
MIC range	0.004 - 1	0.004 - 1	4 – 8	16-64	0.125-4	1- 8	16 - 32	64 -128
C. albicans (NCPF 8154)	0.5	0.06	0.5	0.125	0.06	16	16	0.5

MIC: Minimum Inhibitory Concentration; AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; FLC, fluconazole; KTC, ketoconazole; TRB, terbinafine; GRS, griseofulvin; CAS, caspofungin. NCPF: National Center for Pathogenic Fungi, England.

L. corymbifera strain and the three strains of *L. ramosa* were the most sensitive against amphotericin B, itraconazole and ketoconazole. *Rh. pusillus* strain comes next followed by the two strains of *S. racemosum* while the three strains of *R. oryzae* exhibited the lowest sensitivity. The three strains of *R. oryzae* and *S. racemosum* KC117254 strain showed dose-dependent susceptibility to fluconazole (MIC = 16 µg/ml) while the other tested strains were resistant. *R. pusillus* KC117252 and *L. ramosa* KC117259 and *L. ramosa* KC117260 strains showed intermediate sensitivity to terbinafine while the other tested strains were resistant. MIC result of *C. albicans* (control strain) was within the acceptable range for the drugs tested.

The inhibition zone diameter (IZDs) results of the susceptibility test of the ten species against the ten antifungal drugs are shown in Table 5. The results obtained indicated that amphotericin B exhibited the best activity against all tested species. Ketoconazole comes next by showing activity against all strains except for *R. oryzae* KC117256 strain followed by itraconazole showed activity against all tested strains except *R. oryzae* (KC117256 and KC117257) strains. Fluconazole showed reduced activity (IZD within intermediate range) against *S. racemosum* KC117254 and *R. pusillus* KC117252 respectively but had no activity against the other eight strains. Griseofulvin, terbinafine, caspofungin, and voriconazole had no activity against all tested strains (IZD within resistance range).

TABLE 5. *In vitro* susceptibility of zygomycetes strains to 8 antifungal drugs using disk diffusion method.

Strain	Mean value of inhibition zone diameter, IZDs (mm)							
	AMB 100 µg	**ITC 8 µg**	**VRC 1 µg**	**FLC 25 µg**	**KTC 50 µg**	**TRB 1 µg**	**GRS 16 µg**	**CAS 5 µg**
R. oryzae KC 117255	28.3	19	zero	zero	23	zero	zero	Zero
R. oryzae KC 117256	15	14	zero	zero	22	zero	zero	zero
R. oryzae KC 117257	15	14	zero	zero	25	zero	zero	zero
L. corymbifera KC 117258	26	25	zero	zero	39.3	zero	zero	zero
L. ramosa KC 117259	20	18	zero	zero	30	zero	zero	zero
L. ramosa KC 117260	20	19	11	zero	35	zero	zero	zero
L. ramosa KC 117261	22.3	19	zero	zero	34.6	zero	zero	zero
S. racemosum KC 117253	26.3	18	11.3	zero	38.3	zero	zero	zero
S. racemosum KC 117254	31.16	17	11.6	15.6	25	zero	zero	zero
Rh. pusillus KC 117252	27.6	25	zero	15	31.6	zero	zero	zero
MIC range	15 - 31.1	14 - 25	0 - 11.6	0 -15.6	22-39.3	zero	zero	zero
C. albicans (NCPF 8154)	16	25	35	35	40	zero	zero	23

IZD: Inhibition Zone Diameter; AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; FLC, fluconazole; KTC, ketoconazole; TRB, terbinafine; GRS, griseofulvin; CAS, caspofungin. NCPF: National Center for Pathogenic Fungi, England.

L. corymbifera strain and the three strains of *L. ramosa* were the most sensitive against amphotericin B, ketoconazole and itraconazole. The two strains of *S. racemosum* come next followed by *R. pusillus* strain. The three strains of *R. oryzae* exhibited the lowest sensitivity. *S. racemosum* KC117254 showed higher activity than *R. pusillus* KC117252 against fluconazole. The inhibition zone diameters of *C. albicans* (control strain) were within the established limits for the drugs tested.

The MICs results of the *in vitro* susceptibility testing of the ten strains to micafungin and posaconazole using E-test method indicated that posaconazole showed good activity against *L. ramosa* KC117259, *S. racemosum* KC 117253, and *S. racemosum* KC 117254 by MIC 0.25 µg/ml but had no activity against the other strains. Micafungin was not active against all tested strains.

In this study, a categorical agreement between the broth microdilution and disk diffusion methods was found for all antifungal agents tested. No major errors were detected, but only minor errors were observed, 2 (20%) for itraconazole, 3 (30%) for fluconazole and terbinafine. The minor error observed between the results of the two methods for itraconazole was due to the categorization of 2 *R. oryzae* strains as susceptible by the broth microdilution method and their categorization within the intermediate range by disk diffusion method. For fluconazole, 3 strains of *R. oryzae* were categorized as intermediate by broth microdilution method while as resistant by the disk diffusion method. The three minor errors detected for terbinafine included the 2 *L. ramosa* strains and *R. pusillus* were classified as intermediate by the broth microdilution method while as resistant by the disk diffusion method. The overall levels of agreement between the results of broth microdilution and the disk diffusion methods were 100% for amphotericin B, voriconazole, ketoconazole, griseofulvin, and caspofungin. 80% for itraconazole, 70% for terbinafine and fluconazole.

Discussion

Invasive zygomycosis is a devastating disease in immunocompromised individuals, inciting significant morbidity and mortality although several antifungal drugs have been licensed in the last years (Almyroudis *et al.,* 2007). The main reasons for this include intrinsic or acquired antifungal resistance, organ dysfunction preventing the use of some agents and drug interactions (Pasqualotto and Denning, 2008). Families mucoraceae, *lichtheimiaceae*, syncephalastraceae, cunninghamellaceae, and saksenaceae included the most frequently encountered etiologic agents of zygomycosis (Vitale *et al.*,2012). In Egypt we have reported 10 cases of mucormycosis caused by 3 strains of *Rhizopus* (Mucoraceae), 4 strains of *Lichtheimia* and 1 strain of *Rhizomucor* (*Lichtheimiaceae*), and 2 strains of *Syncephalastrum* (*Syncephalastraceae*) (Zaki *et al.*, 2014). This study, to our knowledge, represents the first report on the *in vitro* susceptibility testing of zygomyceteous strains of clinical origin to antifungal agents in Egypt.

Traditionally, amphotericin B and more recently, its lipid formulations are the front-line agents for the treatment of zygomycosis (Goldani and Sugar, 1994). Similarly in our study, amphotericin B was the most active antifungal drug against all the tested strains. Although amphotericin B is the agent of choice to treat zygomycosis, its nephrotoxicity, hepatotoxicity and cytotoxicity in addition to its poor oral bioavailability remains a problem and therefore alternative therapies are needed.

Azole drugs were considered ineffective against zygomycetes according to (Kwon-Chung and Bennett, 1992 and Sheehan *et al.,* 1999) but, some studies have shown that azole compounds may be active in animal models of zygomycosis (Goldani and Sugar, 1994 and Sugar and Liu 2000). However, both the echinocandins and the triazoles represent significant advances (Diekema *et al.* (2003). Itraconazole was the second most active agent against the tested strains after amphotericin B. It exhibited a good *in vitro* activity in agreement with earlier reports (Sun *et al.,* 2002, Alastruey-Izquierdo *et al.,* 2009) but different from (Gomez-Lopez *et al.,* 2001) whom stated that their isolates were resistant to itraconazole. Moreover, in agreement with the current results other *in vitro* results suggest that some zygomyceteous strains particularly, *Lichtheimia* strains are inhibited by relatively low concentrations of itraconazole (Johnson *et al.,* 1998 and Pakshir *et al.,* 2009) ketoconazole followed amphotericin B and itraconazole in activity although it showed higher MICs in some other studies (Espinel-Ingroff *et al.* and 2007). Ketoconazole was active against all strains except one *Rhizopus* strain and this result was similar to that reported by Dannoaui *et al.,* 2003 where they found that *Rhizopus* spp. were significantly less susceptible to azoles than *Lichtheimia and Rhizomucor* spp. Posaconazole showed strain dependent results where it showed good activity only against *L. ramosa* KC 117259 strain and the two *S. racemosum* strains only in contrast to reports of (Almyroudis *et al.,* 2007 and Sun *et al.,* 2002), who stated that posaconazole was active against all tested zygomycetes strains. Fluconazole showed poor to no activity against tested strains. In the other hand they were resistant to voriconazole and griseofulvin in accordance with the previous reports (Almyroudis *et al.,* 2007,Gomez-Lopez *et al.,* 2001).

In the other study, a range of MICs was obtained for terbinafine (1-8 µg/ml) which was slightly higher than the range obtained earlier (Dannaoui *et al.,* 2003 and Vitale *et al.,* 2012**)** The drug was slightly active against 2 *L. ramosa* strains and *Rh. pusillus* strain in broth microdilution but it had no activity against the other strains and it didn't show any inhibition zone diameters against all of them. This minor error might be attributed to the use of terbinafine 1 µg/disk instead of 30 µg/disk used in the other study of Pakshir et al. (Pakshir *et al.,*2009).

The echinocandins (caspofungin and micafungin) lacked the *in vitro* activity against zygomyceteous strains and these results were consistent with the earlier findings (Almyroudis *et al.,* 2007 and Sheehan *et al.,* 1999).

The current study revealed a categorical agreement between the broth microdilution and the disk diffusion methods in accordance with previous studies (Clinical and Laboratory Standards Institute 2008, Matar *et al.*, 2003, Karaca and Koc 2004 and Messer *et al.*, 2007).

In conclusion, members of the three families tested were fully susceptible to amphotericin B and itraconazole and completely resistant to voriconazole, griseofulvin, caspofungin, and micafungin. Mucoraceae members showed reduced susceptibility to fluconazole and ketoconazole and a lack of activity to terbinafine and posaconazole. Lichtheimiaceae members were characterized as susceptibile to ketoconazole and reduced susceptibility to fluconazole, terbinafine, and posaconazole. Species of family Syncephalastraceae were characterized as susceptibile to ketoconazole and posaconazole, but showed reduced susceptibility to fluconazole and resistance to terbinafine. Susceptibility profile of the isolated etiologic agents must be known before treatment of infections due to Mucorales. The agar disk diffusion method, simple, flexible and coast effective, could be a good alternative to the broth microdilution and E-test methods for use in clinical microbiology laboratories as no major diskrepancies were detected.

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حساسية اختبار أنواع الزيجومايستس المعزولة فى القاهرة ـ مصر ضد عشرة من المضادات الفطرية بطرق التخفيف الطفيف والقرص المنتشر واختبار الـ E-test

[٢]إيمان محمد الخولى، [١] شريف محمد زكى، [١] سمر سمير محمد، [١] ولاء محمد وصفى
[١]وحدة الفطريات، وحدة الميكروبيولوجى - جامعة عين شمس، كلية العلوم، [٢] قسم الباثولوجيا الإكلينيكية، مستشفى عين شمس التخصصى، جامعة عين شمس - القاهرة - مصر.

أجريت هذه الدراسة على عشرة من فصيلة الزايجومايستس ووجد أنهم ينتمون لخمس أنواع هى ريزوبسى أورزى (٣)، ليزسيميا كورمبيفرا (١)، ليزيسميا روزا (٣)، سنسفلسترم رسيموزيم (٢)، ريزوميوكريسي (١)، المعزولة من عينات سريرية فى القاهرة - مصر، وتصنف هذه العينات إلى (٣) عائلات ميكورس، ليزميزس، سنسفلسترسى من الميكورالس.

وقد تم اختبار العينات للمضادات الفطرية باستخدام التخفيف الطفيف واختبار القرص المنتشر وطرق الاختبار E-test. وكانت مضادات الفطريات المستخدمة هى الأمفترسين بى، الأتراكونازيول، الفوريكونازول، الفلوكونازول، تيربينافين، الكيتوكونازول، غريزيوفولفين، الميكافنجين، الكاسبوفنجين والبوساكونازول ولوحظ وجود مستوى جيد من الدراسة بين اختبار حساسية القرص المنتشر والتخفيف الطفيف وبالتالى يمكن اعتبار حساسية القرص المنتشر المضاد الفطرى بديل جيد لعمل حساسية الفطريات وكان أفراد الثلاث أسر التى تم اختبارهم حساسية إيجابية كاملة تجاه الأمفترسين بى. والأتراكونازول وحساسية مضادة كاملة تجاه الفوريكونازول، غريزيوفولفين، وكسبوفانجين والميكافنجين، والكيتوزوكونازول فعال ضد الليزوسيميزيسى والسنسفلستريزى بالرغم من أن له فعالية أقل تجاه الميكوريسى.

وجد أن الفلوكونازول له فعالية أقل تجاه كل العينات والتيرينافين غير فعال تجاه الميكوريسى والسنسفلسترسى بالرغم أنه ظهر أقل فعالية تجاه العينات من الليزوسيميزيسى مع أن البوساكونازول له فعالية تجاه السنسفلستريس وأقل فعالية تجاه الليزوسيميزيسى وغير فعال تجاه الميكوريسى.

وجد تنوع فى قابلية اختبار الحساسية فى كل رتبة الميكورالس لذلك لابد من معرفة حساسية المضادات الفطرية لكل العزلات قبل العلاج من الالتهابات.