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Susceptibility of Some Antifungal Drugs Against Selected Fungal Species Isolated From Indoor Public Swimming Pools in Duhok City, Iraq

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Abstract

The widespread use of antifungal often causes an increase in the fungal resistance to drugs and causes many side effects in humans. This study aimed to assess the *in vitro* sensitivity of three antifungal drugs, namely Fluconazole, Itraconazole and Terbinafine against 15 fungal species isolated from indoor swimming pools in Duhok city. Antifungal drugs were dissolved with DMSO (dimethyl sulfoxide) and used for evaluation of antifungal sensitivity by agar well diffusion method. Filamentous fungi were resistant to Fluconazole, while *Candida* species were inhibited at a minimum inhibitory concentration (MIC) value 25 and 100 µg/ml. The MIC value for Itraconazole against filamentous fungi was 6.25 µg/ml, while for Yeasts 1.65 µg/ml. Regarding the effect of Terbinafine, the tested filamentous fungi had a MIC value of 6.25 µg/ml, while all tested Yeasts were resistant except *Candida glabrata*, which had a MIC value 100 µg/ml. In conclusion, antifungal sensitivity testing reveals that fungal isolates were most sensitive to Itraconazole and Terbinafine and least susceptible to Fluconazole. Swimming pools are a suitable environment for fungal growth; hence surveillances are essential to control and reduce the fungal growth.

Keywords: *Antifungal, Swimming Pool, Fungi, Duhok*

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I. INTRODUCTION

Fungi are eukaryotic, heterotrophic, having a rigid cell wall and lacking chlorophyll organisms that include molds, yeasts, yeast-like fungi and dimorphic fungi. Fungi grow very easily and reproduce by forming spores and mycotoxins in damp and wet conditions, this leads to inducing acute health effects such as inflammation, allergy, or infection with symptoms like respiratory problems, nasal congestion, eye irritation, headaches, cough, nose or throat irritation and skin rashes. Fungal infections have increased dramatically during the last two decades, mainly due to increased use of invasive and immunosuppressive medical procedures; this was followed by the more widespread use of antifungal agents (Dismukes *et al*., 2003).

Swimming are one of the most common and useful activity for individuals. This sport is recomended as the appropriate way to keep human healthy. The pathogens such as bacteria, fungi, viruses and protozoa may contaminate the swimming pools through the skin, saliva, urine and feces of the pool users; those agents threaten the pool user's health (Robins and Morell, 2007).

Antifungal drugs are classified according to their targets in fungi. Until now, only three targets have been exploited, namely plasma membrane sterols, nucleic acid synthesis and cell wall constituents (Fridkin and Jarvis, 1996; Kaplan *et al*., 2000). The performance of an antifungal susceptibility test has become very necessary due to the recognition of antifungal resistance among strains of particular pathogens. Therefore, it is essential to know how to choose the most appropriate drug for the individual organism (Odd and Bernaesrt, 1994). This work aimed to study the *in vitro* sensitivity of three antifungal drugs: Fluconazole, Itraconazole and Terbinafine against 15 fungal species isolated from indoor swimming pools in Duhok city.

II. MATERIALS AND METHODS

A. Fungal Isolates

Nine filamentous fungal isolates (molds) (*Aspergillus niger, Aspergillus fumigatus*,

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Scopulariopsis sp., *Rhizomucor* sp., *Bipolaris* sp., *Phialophora* sp., *Exophiala* sp., *Fusarium* sp. and *Alternaria* sp.) and six yeast species (*Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis, Geotrichum* sp. and *Rhodotorula* sp.) were tested for their *in vitro* sensitivity to selected antifungal drugs. The species used in present study were previously isolated and identified (Abdullah, 2015).

B. Antifungal Drugs

Three commonly used antifungal drugs were chosen, namely: Fluconazole (flu.) (Fluconazole 150 mg capsule, Bristol, UK), Itraconazole (Itr.) (Conazole, Itraconazole 100mg, 4 capsules, JOSWE medical, Jordan) and Terbinafine (Ter.) (Terbinafine, LAMIFEN 250mg, 14 tablets, Jamjoom pharma, Jeddah, Saudi Arabia).

C. Antifungal Solution Preparation

The stock solution of each of the three antifungal drugs was prepared by dissolving 50 mg of each of them with 5 ml DMSO (dimethyl sulfoxide) in sterile screw-capped glass vials to obtain a concentration of 10000 µg/ml stock solution. Two ml from 10000 µg/ml stock solutions were transferred to another sterile screw-capped glass vial containing 18 ml sterile distilled water (SDW) to obtain a concentration of 1000 µg/ml working solution. A serial dilution (0.05 - 100 µg/ml) of each antifungal was prepared from the working solution.

D. Well Diffusion Method

The well diffusion method was used to test the susceptibility of fungi toward different drugs based on Magaldi *et al*., (2004). This method is cheap, simple, and easy to handle. Six plates of Potato Dextrose Agar (for molds isolates) and six plates of Sabouraud's Dextrose Agar (for yeast isolates) were used. 0.1 ml of each isolate was spread, by cotton swab, over the plates. Then, three wells (6 mm) were made by using a cork borer. After that, 0.15 ml of the desired antifungal was added to each well. The plates were then incubated for 24-48 hrs. at 25^0 C (for molds) and 37^0C (for yeasts). After the incubation period, the minimum inhibitory concentration (MIC) was determined as the least concentration of the antifungal drug used which inhibit the fungal growth.

III. Results

This study was achieved to conduct the sensitivity of three antifungal drugs, namely Fluconazole, Itraconazole and Terbinafine against nine filamentous fungal isolates (molds) and six yeast species (Table 1). The *in vitro* sensitivity testing was done by agar well diffusion technique to obtain MIC of each drug.

Yeasts species were more sensitive to Fluconazole than molds; all filamentous fungi were resistant to Fluconazole while some yeast species were inhibited at a minimum inhibitory concentration value 25 and 100 µg/ml. The MIC value of Itraconazole against filamentous fungi was 6.25, 25 and 100 µg/ml) while for Yeasts 1.65 µg/ml. All tested yeasts were resistant to Terbinafine except *Candida glabrata*, which had a MIC value of 100 µg/ml (Figure 1). At the same time, some filamentous fungi were inhibited by Terbinafine with different MIC values (6.25, 25 and 100 µg/ml). The results of the antifungal activity of the Fluconazole, Itraconazole and Terbinafine to both filamentous fungi and yeasts were shown in table 1.

Table 1. The MIC of antifungal drugs against selected fungal species.

No.	Fungal isolates	MIC (µg /ml)		
		Flu.	Itr.	Ter.
1	*Aspergillus niger*	_	6.25	25
2	*A. fumigatus*	_	25	6.25
3	*Scopulariopsis* sp.	_	_	25
4	*Rhizomucor* sp.	_	_	_
5	*Bipolaris* sp.	_	_	_
6	*Phialophora* sp.	_	6.25	_
7	*Exophiala* sp.	_	100	100
8	*Fusarium* sp.	_	_	100
9	*Alternaria* sp.	_	100	_
10	*Candida albicans*	100	_	_
11	*C. tropicalis*	100	_	_
12	*C. krusei*	25	1.65	_
13	*C. glabrata*	25	1.65	100
14	*Geotrichum* sp.	_	_	_
15	*Rhodotorula* sp.	_	_	_

IV. Discussion

Fluconazole is a first-generation triazole antifungal medication. It differs from azoles antifungal, such as ketoconazole, in that its structure contains a triazole ring instead of an imidazole ring. Fluconazole and certain other triazole antifungal are preferred because of their safety and predictable absorption when administrated orally (Ford and Roach, 2014).

All filamentous isolates showed resistance to this antifungal, the percentage resistance is 100%, which is the same result found by Davey and Blaxter (2011) and Yang *et al*., (1993).

Regarding the effect of Fluconazole on yeasts, *Candida albicans* and *Candida tropicalis* were inhibited at the concentration of 100 µg /ml. In comparison, *Candida krusei* and *Candida glabrata* were inhibited at the concentration of 25 µg /ml. These results were in agreement with Therese *et al*., (2006).

Fluconazole is being used for candidiasis, urinary tract infection and peritonitis. The high tolerance of *Aspergillus* sp. to Fluconazole cannot be attributed to the low solubility of the Fluconazole in water, as it has been reported that solubility of Fluconazole in water is high (Sheppard and Lampiris, 1998). The same result was declared by Bossche (1997) who found the MIC of pathogenic species of *Aspergillus fumigatus* to be higher than 100 µg /ml. Fair *et al*., (2001) found that *Candida glabrata* was difficult to treat because of its increased resistance to Fluconazole and other azoles antifungal drugs.

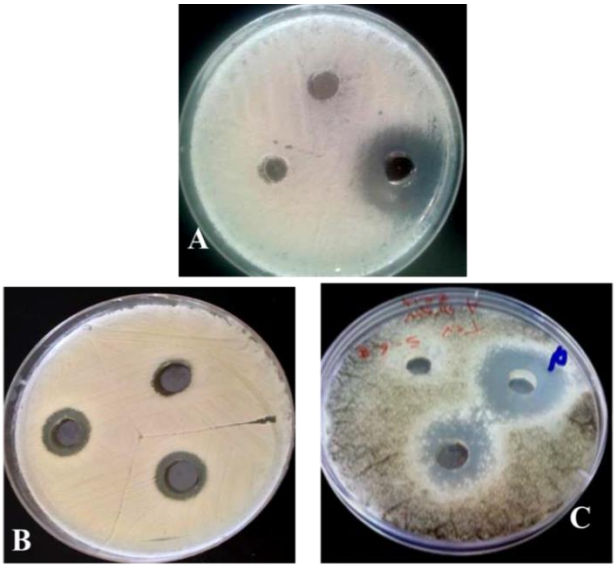

Figure 1. The inhibition zones by three antifungal drugs with concentrations 6.25, 25 and 100 µg /ml: A. *Candida glabrata* (Flu.) B. *Candida krusei* (Itr.) and C. *Aspergillus niger* (Ter.)

Itraconazole is a broad-spectrum, triazole antifungal drug that is used for the treatment of some fungal infection such as invasive mycoses (Lestner and Hope, 2013). Regarding the effect of Itraconazole on molds, *Aspergillus niger* and *Phialophora* sp. inhibited at the concentrations 6.25 µg /ml while *Aspergillus fumigatus* inhibited at 25 µg /ml. *Exophiala* sp and *Alternaria* sp. inhibited at 100 µg /ml. These results are in line with Mohammed (2005).

All isolates of yeasts were resistant except *Candida krusei* and *Candida glabrata* that were inhibited at concentrations 1.65 µg /ml. These results are in line with Sabaly (2014) who found that *Candida tropicalis* was resistant to Itraconazole. Liu *et al*. (2008) stated that the antifungal susceptibility test of Itraconazole against *Candida albicans* shows resistance to it. Bossche (1997) and Moore *et al*., (2000) reported that *Aspergillus* sp. and *Candida* sp. possess various active mechanisms that enhance the efflux of triazoles outside the fungus cell, keeping their internal concentration low. Moreover, Bossche (1997) reported that these mechanisms were more efficient in effluxing Fluconazole than Itraconazole. This might be the reason why Itraconazole is more used (among triazole) in combating systemic mycosis, in addition to its broad antifungal spectrum (Mycek *et al*., 2000).

Terbinafine is an allylamine, soluble in methanol and slightly soluble in water, act by inhibiting fungal ability to build sterols that are an important part of the cell membrane. It is used to treat fungus infection of the scalp, body, groin (Jock itch), feet (athlete's foot), finger, nail and toenails (Ford and Roach, 2014).

Regarding the effect of Terbinafine on selected fungi, *Aspergillus fumigatus* had MIC at concentrations 6.25µg/ml, while *Aspergillus niger* and *Scopulariopsis* sp. inhibited at 25 µg /ml. *Fusarium* sp. and *Exophiala* sp. inhibited at 100 µg /ml. All yeast isolates were resistant except *Candida glabrata* which had MIC at the concentration of 100 µg /ml.

Terbinafine is basically a fungistatic drug enters the cell via energy-dependent rout. Strains resistant to this drug should presumably occur through mutations in this route (Papich *et al*., 2001).

V. Conclusion

It was concluded that swimming pools are appropriate sites for fungal growth; also, it was observed that most of the opportunistic fungi isolated revealed resistance to the antifungal drug used in the present study; this means that they have more ability to infect people especially those with low immunity. Continuous surveillances and guides are necessary to control and reduce the fungal growth in swimming pools.

Conflict of Interest

We declare no conflict of interest

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