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Screening for Azole Resistance among *Aspergillus* spp isolated from Soil of Hospitals and a University Campus in Gorgan, Iran

Original Article

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ABSTRACT

Background and objectives: Aspergillosis is a widely distributed infectious disease, which is difficult to manage. According to recent studies, the prevalence of resistant *Aspergillus fumigatus* has increased from 3.3% to 6.6%. Acquired triazole resistance in *Aspergillus* species is an evolving global health challenge, which has made the control of diseases caused by *Aspergillus* a concern. This study was performed to investigate prevalence of azole resistance in *Aspergillus* isolates from environmental samples.

Methods: In this study, 316 soil samples were collected from three hospitals and a university campus in Gorgan (Iran) from July to September 2017. Two grams of each sample were suspended in 5 ml of 0.2M NaCl with 1% Tween 20. Then, 100 µl of the suspension was plated on sabouraud dextrose agar (SDA) supplemented with chloramphenicol, SDA supplemented with chloramphenicol and voriconazole (VOR, 1 mg/L) and SDA supplemented with chloramphenicol and itraconazole (ITC, 4 mg/L). The plates were incubated at 37 °C and examined for growth after 24, 48 and 72 hours.

Results: We detected *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* isolates in 187(59.2%), 84(26.6%), 147(46.5%) and 65(20.6%) samples, respectively. We found no VOR resistant isolate. However, 21 (25%) *A. flavus* and 16 (8.6%) *A. fumigatus* isolates were intermediate for VOR. In addition, seven (8.3%) *A. flavus*, 68 (36.4%) *A. fumigatus*, 41 (27.9%) *A. niger* and three (4.5%) *A. nidulans* isolates were resistant to ITC.

Conclusion: We were able to detect *A.fumigatus*, *A. flavus*, *A. niger* from all four sampling sites in Gorgan, North of Iran. *A. fumigatus* is the most prevalent and most resistant isolate in the studied area. History of previous agriculture activity and use of pesticides in the proximity of sampling sites may have affected the rate of ITC resistance.

Keywords: *Aspergillus Fumigatus*, *Aspergillus Flavus*, Azole Resistance, Voriconazole, Iran

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INTRODUCTION

Aspergillosis is a infectious disease (1) caused by *Aspergillus*, a genus of saprophytic fungi that can cause invasive and pulmonary infections (2). These fungi have important roles in the carbon and nitrogen recycling. They are commonly found in soil and decaying vegetables but can also be found in different environments. Inhaling air contaminated with *Aspergillus* spores can lead to aspergillosis (1, 3), especially in immunosuppressed patients, bone marrow recipients and broad spectrum corticosteroid consumers. Invasive aspergillosis (IA) is difficult to diagnose and therefore considered as a problem in mycology (4, 5). It is responsible for 10% of all cases of chronic pulmonary aspergillosis and affects about 3 million people annually (6). Drugs including voriconazole (VOR), posaconazole (POS) and itraconazole (ITC) were approved as first-line therapy for treatment of aspergillosis. *Aspergillus fumigatus* is generally susceptible to all these antifungal agents (2, 7, 8).

Azoles are antifungal agents that are used in both agriculture and medicine. Environmental exposure to this fungicides may lead to increased resistance (8). Resistance of *Aspergillus* spp. has been reported against various triazoles including itraconazole (9), VOR (10), ravuconazole, POS (9), propiconazole and tebuconazole (11). According to recent studies, the prevalence of resistant *A. fumigatus* has increased from 3.3% to 6.6% (12). Acquired triazole resistance in *Aspergillus* is not common, but increasing rate of azole resistance has been reported from the Netherlands, Italy, Turkey, Spain, Australia, Iran, Belgium, Denmark, China, India, United Kingdom, France, United States, Germany, Taiwan, Kuwait, Poland, Colombia and Tanzania (3, 13). In this screening study, we identified and detected resistant *Aspergillus* isolates from Gorgan, North of Iran.

MATERIALS AND METHODS

Environmental samples were collected from three hospitals and a university campus in Gorgan, Iran. The study received approval from the ethics committee of Golestan University of Medical Sciences (Ethics code: 1396.113). Overall, 316 soil samples were obtained from three hospitals and a university campus in Gorgan, Iran. Two grams of each sample were suspended in 5 ml of 0.2M NaCl with 1% Tween 20 (Merck, Germany). After homogenization (for about 1 min), the samples were incubated at room temperature for 15 minutes. Then, 100 µl of the suspension were plated on sabouraud dextrose agar (SDA, Conda, Europe) supplemented with chloramphenicol (Merck, Germany), SDA supplemented with chloramphenicol and 1 mg/L VOR (Hakim, Iran) and SDA supplemented with chloramphenicol and 4 mg/L ITC (Sigma, Germany). The plates were incubated at 37 °C and examined microscopically and macroscopically for growth at 24, 48 and 72 hours.

Data analysis was performed using SPSS Statistic (version 23) and at significance of 0.01. The chi-square test was used to determine statistically significant differences.

RESULTS

Aspergillus was isolated from 316 soil samples. Of these isolates, 87 (59.2%), 84 (26.6%), 147 (46.5%) and 65 (20.6%) were *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* species, respectively. The highest and lowest rate of ITC resistance was observed against *A. fumigatus* (21.5%) and *A. nidulans* (1.3%). All isolates were susceptible to VOR (Table 1). In addition, 17 (5.4%) *A. fumigatus* and 21(6.6%) *A. flavus* isolates were VOR intermediate (Table 2). We also detected one *A. terreus* in samples collected from hospital No. 2.

Table 1. Frequency of *Aspergillus* isolates in different sampling sites

	Aspergillus spp.								
Sampling site	***A. flavus***		***A. fumigatus***		***A. niger***		***A. nidulance***		
	Number	%	Number	%	Number	%	Number	%	**Total**
Hospital 1	18	20.9	28	32.6	43	50	6	7	**95**
Hospital 2	57	29.4	133	68.6	89	45.9	57	29.4	**336**
Hospital 3	6	24	19	76	11	44	2	8%	**38**
University campus	3	27.3	7	63.6	4	36.4	0	0	**14**
Total	84	26.6	187	59.2	147	46.7	65	20.6	**483**

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The detection of azole resistant *Aspergillus* isolates was based on the use of an azole-containing medium (Figure 1). In this study, 21 (25%) *A. flavus* and 16 (8.6%) *A. fumigates* Isolates were VOR intermediate. In addition, ITC resistance was observed in 7 (8.3%) *A. flavus*, 68 (36.4%) *A. fumigatus*, 41 (27.9%) *A. niger* and three (4.5%) *A. nidulans* isolates. Moreover, the highest rate of azole resistance was observed against *A. fumigates* (Table 2).

Table 2. Frequency and pattern of azole resistance among *Aspergillus* isolates according to the culture-based method

Sampling site	*A. flavus*				*A. fumigatus*				*A. niger*				*A. nidulance*			
	ITC		VOR		ITC		VOR		ITC		VOR		ITC		VOR	
	R	S	I	S	R	S	I	S	R	S	I	S	R	S	I	S
1	1(5.6%)	17(94.4%)	9 50%	9 (50%)	17(60.7%)	11(39.3%)	2(7.1%)	26(92.9%)	12(27.9%)	31(72.1)	O	43	1 (16.7%)	5 (83.3%)	0	**6 (100%)**
2	6 (10.5%)	51 (89.5%)	12 (21.1%)	45 (78.9%)	43(32.3%)	90(67.7%)	13(9.8%)	120(90.2%)	25 (28.1%)	64 (71.9)	O	89	2 (3.5%)	55 (96.5%)	0	**57 (100%)**
3	0	6 (100%)	0	6 (100%)	8 (42.1%)	11 (579%)	1(5.3%)	18(94.7%)	4 (36.4%)	7 (63.6%)	0	11	2 (100%)	O	0	**2 (100%)**
4	0	3 (100%)	0	3 (100%)	0	7 (100%)	0	7(100%)	0	4(100%)	0	4	0	0	0	**0**
	7 (8.3%)	77 (91.7%)	21 (25%)	63 (75%)	68 (36.4%)	119 (63.6%)	16 (8.6%)	171 (91.4%)	41 (27.9%)	106 (72.1)	0		3 (4.5%)	62 (95.4%)	0	**65 (100%)**

DISCUSSION

Azoles including ITC, VOR and POS are the first-line treatment for IA (14). Therefore, the emergence of azole resistant *Aspergillus* strains is of concern. We performed this study to fill the gap in the knowledge about frequency of azole-resistant environmental *Aspergillus* isolates. We collected 316 soil samples from three hospitals and campus of the Golestan University of Medical Sciences in Gorgan, Iran. The most frequent isolates were *A. fumigatus* (59.2%) and *A. flavus* (26.6%). According to the results, the frequency of azole resistance was 21.5% in *A. fumigatus* and 2.2% in *A. flavus* isolates. The highest rate of ITC resistance was observed against *A. fumigatus* (21.5%) and *A. niger* (1.3%). In Austria *A. terreus* was found in seven samples from places with a high proportion of patient with IA (2). We found one *A. terreus* isolate in samples collected from hospital No. 2.

We also detected three ITC-resistant isolates: *A. fumigatus* (n=2) and *A. flavus* (n=1). However, confirming these results with the microdilution method could be beneficial. Based on the results, VOR therapy may be effective in controlling ITC-resistant isolates. The present of the intermediate resistant isolates increase the risk of emergence of multidrug resistant strains.

Although we collected our samples from one city, the prevalence of resistant *Aspergillus* spp. varied widely from nil in the University campus to 36.56% in hospital No. 2. It is well-established that the geographic location influences the prevalence of resistant isolates (15). Moreover, previous use of azole-based fungicide and agricultural activity around the hospital No. 2 might be responsible for the high rate of azole-resistant. We categorized the isolates into resistant, intermediate and susceptible based on growth. We detected 17 *A. fumigatus* and 21 *A. flavus* isolates that

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were intermediate for VOR and resistant to ITC.

In a study by White et al., 671 soil samples from urban and rural area and 44 air samples were collected from three major hospitals. The mentioned study reported that the overall prevalence of triazole-resistant and -intermediate *A. fumigatus* as 6% and 18.1% in the environmental samples, respectively (15). In our study, hospital No. 2 had the highest rate of contamination with *A. flavus*, *A. fomigatus*, *A. niger*, *A. nidolance* and *A. terreus*.

Screening for azole resistance in *Aspergillus* species is based on growth when exposed to ITC (4 mg/L) and VOR (1 mg/L) (2). Then, resistant isolates are retested using azole-containing agar. It has been proposed that exposure to azoles may induce mutations in the isolates (2). The increased rate of resistant strains is an important health concern due to limited number of antifungal agents and accessibility to these agents.

CONCLUSION

We were able to detect *A.fumigatus*, *A. flavus*, *A. niger* from all four sampling sites in Gorgan, North of Iran. *A. fumigatus* is the most prevalent and most resistant isolate in the studied area. History of previous agriculture activity and use of pesticides in the proximity of sampling sites may have affected the rate of ITC resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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