JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2016. Vol. **10**(1), p. 81-88

Occurrence of Important Mucormycosis Agents in the Soil of Populous Areas of Isfahan and Their Pathogenicity in Immunocompromised Patients

Ardeshir Ziaee[1], Mohammadali Zia[2]*, Mansour Bayat[1] and Jamal Hashemi[3]

[1]Department of Medical and Veterinary Mycology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Basic Sciences, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran.
[3]Department of Medical Parasitology and Mycology, School of Public Health &Institute of public Health Research, Tehran university of Medical Sciences, Tehran, Iran.

(Received: 01 November 2015; accepted: 04 December 2015)

Soils are the main habitat of saprophytic and pathogenic fungi. Zygomycetes are one of the most parts of soil fungi and certain members are among opportunistic fungi and can cause systemic fungal infections in immunocompromised patients. The majority of human and animal infections are caused by members of the genera *Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*, *Cunninghamella*, *Mortierella*. The objective of this research was to isolate and identify the main genera of Zygomycetes, especially family Mucoraceae. A total of 340 soil samples were collected from seven different public parks and 14 municipality districts in Isfahan. All samples were cultured on appropriate media and incubated at 25-30° C for 2 weeks, examined daily for visible fungal growth. Five hundred fungal colonies belonging to various classes of filamentous fungi were obtained. Finally 400 pure colonies belonging to six genera of Zygomycetes including *Absidia*, *Rhizopus, Rhizomucor, Mucor, Cunninghamella* and *Mortierella* were identified. The genus *Rhizopus* (35.5%) was the most frequent isolate, followed by *Mucor* (32.25%) and *Rhizomucor* (27.5%). These finding may help us to understand about the importance of opportunistic fungi in public areas and the risk of exposure with immuno-compromised persons.

Key words: Soil-borne fungi; Zygomycetes**;** Immuno-compromised patients; *Mucoraceae***.**

Mucormycosis is rapidly progressing and fatal infection that is caused by members of the Mucorales order. The importance of mucormycosis has grown in recent year as the number of patients with predisposing factors has increased dramatically. Zygomycosis the term used previously has become accurate based on a recent taxonomic reclassification that abolished Zygomycetes as a class. Mucormycetes are ubiquitous filamentous fungi in nature, belonging to the subphylum Mucormycotina[1-3], and are increasingly becoming recognized as opportunistic pathogens in immuncompromised and immunosuppresed patients[4, 5]. Invasive fungal Infection (IFI) is a life threatening complication in immunocompromised hosts and a cause of mortality in such patients[4, 6, 7].

The overall mortality associated with IFI is high and ranges from 40 to 60%, but can approach 100% in the presence of some pathogens such as Zygomycetes molds[8].

The term mucormycosis is used to refer to infections due to molds belonging to the order mucorales[9]. Several members of this order are among important agents of several human infections[10].

These organisms can cause rhinocerebral, pulmonary, gastrointestinal, cutaneous or

* To whom all correspondence should be addressed.
Mob.: +989133094852; Fax: +983135354033;
E-mail: Zia.mohammadali@gmail.com

disseminated infections in predisposed individuals. The severity of the disease depends on the interaction between the fungus and the host immune defenses[9].

Mucormycosis many advance rapidly, leading to fatality, particularly in patients with underlying conditions[11]. Host risk factors include diabetes mellitus, neutropenia, sustained immunosuppressive therapy, chronic prednisone use, iron chelation therapy, broad spectrum antibiotic use, severe malnutrition and primary break down in the integrity of the cutaneous barrier such as trauma, surgical wounds, needle sticks, or burns[12].

Mucormycosis is the third most common IFI following aspergillosis and candidiasis. Several species of mucorales involved in different types of infectious in immunecompromised individuals[13]. The true incidence of mucormycosis is not known and is probably underestimated owing to difficulties in antemortem diagnosis and the low autopsy rates. Nevertheless, a rise of infections has been observed over the last years, which is attributed to rising numbers of immunocompromised hosts[3].

Predominant the most common isolated genera recovered from clinical samples are *Rhizopus*, *Absidia*, *Cunninghamella*, *Mucor*, *Rhizomucor* and other genera are rare[4,13].

The majority of human pathogenic fungi are soil inhabiting saprophytes. Mucorales are generally saprophytic fungi and grow on organic matter such as dead plant or animal material within soil[14]. They act either as opportunistic pathogens which take advantage of susceptible individuals, such as those who are immunocompromised. These organisms may be survived within the soil for a long time before infecting humans who come into contact with contaminated soil[9].

There is no known relationship between species distribution and environment[15]. The quantity and type of microorganisms in a particular portion of soil is related to some factors such as sunlight, temperature, moisture, soil pH, nutrients, and redox potential[16]. Fungi are one of the most widely distributed groups of microorganisms in soil which have important roles in the soil ecosystem and also soil-borne fungal diseases. It is now well established that soil can be a reservoir of most pathogenic and opportunistic fungi. They can transfer to human by different activities especially gardening and agricultural operations[17-20].

Soil-borne pathogenic fungi may enter humans via direct inoculation into wounds, direct ingestion of soil (geophagia) or indirect ingestion via contaminated food. Fungal spores can be dispersed in different environments via dusts or mud particles from soil disturbances and introduced into respiratory tract. Soil minerals may suppress local host defenses and help to promotion of infection[16]. The hyphae invade blood vessels, causing tissue infraction and necrosis[5,13].

The systemic fungi are largely acquired via inhalation from contaminated soil and near-soil environments. These fungal infections are particularly life-threatening in those with compromised immune systems[16].

Six families including *Cunninghamellaceae, Lichtheimiaceae, Mucoraceae, Saksenaeaceae, Syncephalastraceae,* and *Thamnidiaceae* have been described as causing human infections. *Rhizopus* species is the most common known cause and *Mucor* species or *Absidia* is listed as a distant second, dependent on the patient group affected[2].

The Mucorales are characterized by aseptate (coenocytic) hyaline hyphae, sexual reproduction with the formation of zygospores, and asexual reproduction with nonmotile sporangiospores[21].

Hence, the members of Mucorales are among the most important fungal infection agents in immuno-compromised persons, the main objective of this study was to investigate for diversity of them in soil of public parks and municipality districts of Isfahan, Iran.

MATERIALS AND METHODS

During a five months period from August 2014 to December 2014, three hundred forty soil samples were taken from different sites in seven public parks and street-side gardens located on fourteen municipality districts in Isfahan. First, all debris present on the surface of soil were removed and then approximately 400 g of soil to the depth of 5 to 10 cm were collected from the surface layer and stored in sterile bags using a sterile stainless steel spoon. All samples were transferred to laboratory for the next processing step as soon as possible.

The samples were crushed in a mortar under sterile conditions and then homogenized. Four methods were applied for culturing soil samples in sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) medium supplemented with chloramphenicol:

1. Five grams of each soil sample were suspended in 20 ml of sterile double distilled water and was shaken for 5 minutes to make a soil suspension. Approximately 0.5 ml of the suspension was poured on the bottom of Petri dish and then molten cooled agar medium was added to it and mixed well.
2. 0.5 ml of the soil suspension was spread on the surface of Petri dishes containing media under sterile conditions.
3. Approximately 0.1g of each soil sample was poured on the bottom of Petri dish, and then molten cooled agar medium was added to it and was shaken gently to disperse the soil particles in the medium.
4. Approximately 0.1g of each soil sample was scattered on the surface of Petri dishes containing media under sterile conditions.

All samples were incubated at 25 to 37º C for 2 to 4 days and were observed daily in terms of fungal colony growing. The identification of the Mucormycetes was performed on the basis of macroscopic and microscopic features, and growth ability at different temperatures.

On media, colonies typically are floccose and dense and rapidly fill the entire Petri dish with abundant intertwining aerial mycelium, which looks rather like gray cotton candy. Pigmentation of the isolates varies somewhat with the genus isolated, but pigments are variations of white, gray, brown, or beige.

In microscopic examination, the hyphae are predominantly a septate or very sparsely septate and wider (average 10 µm) than the hyphae of most other fungi isolated from human infections. The ribbon-like hyaline hyphae are thin walled and irregular width and frequently intertwined. The features that are most useful for distinguishing among Mucorales are the presence of the rhizoids, the shape of sporangium, the length of sporangiophore, and the shape of columella, the presence or absence of apophysis and collarette, and the organization and branching of stolons[3, 12].

RESULTS

A total of 393 and 7 pure colonies belonging to order Mucorales and Mortierellales were obtained respectively. The results obtained from preliminary analysis of these cultures are summarized in table1**.** Six genera and nine species were identified based on macroscopic and

Table 1. Distribution of Mucormycetes in soil of public parks and municipality districts

Genus	species	No (%)	Total (%)
Mucor	*circinelloides*	3 (0.75%)	129 (32.25%)
	racemosus	4 (1%)	
	plumbeus	2 (0.5%)	
	Mucor sp	120 (30%)	
Rhizomucor	*pusillus*	6 (1.5%)	110 (27.5%)
	Rhizomucor sp	104 (26%)	
Rhizopus	*oryzae*	10 (2.5%)	142 (35.5%)
	stolonifer	12 (3%)	
	Rhizopus sp	120 (30%)	
Absidia	*corymbifera*	2 (0.5%)	10 (2.5%)
	Absidia sp	8 (2%)	
Cunninghamella	*bertholletiae*	2 (0.5%)	2 (0.5%)
Mortierella	*wolfii*	1 (0.25%)	7 (1.75%)
	Mortierella sp	6 (1.5%)	
Total (%)		400 (100%)	400 (100 %)

Table 2. Distribution of Mucormycetes in soil of public parks

Genus	Park NoSpecies	1	2	3	4	5	6	7	No (%)	Total(%)
Mucor	*circinelloides*	-	-	-	1	-	1	-	2 (1.20)	2313.86))
	racemosus	1	-	-	-	-	-	-	1 (0.60)	
	Mucor sp	2	9	1	2	1	3	2	20 (12.06)	
Rhizomucor	*pusillus*	-	1	-	-	1	-	-	2 (1.20)	47(28.31)
	Rhizomucor sp	12	20	3	3	4	-	3	45 (27.11)	
Rhizopus	*oryzae*	1		1	1	-	1	1	5 (3.01)	86(51.81)
	stolonifer	1	2	1		-	3	2	9 (5.42)	
	Rhizopus sp	20	1	2	17	-	30	2	72 (43.38)	
Absidia	*corymbifera*	1	-	-	-	-	-	-	1 (0.60)	5(3.01)
	Absidia sp	2	-	-	-	1	1	-	4 (2.41)	
Cunninghamella	*bertholletiae*	1	-	-	-	-	-	-	1 (0.60)	1(0.60)
Mortierella	*Mortierella* sp	1	1	1	-	-	1	-	4 (2.41)	4(2.41)
Total		42	34	9	24	7	40	10	166	166
(%)		(25.30)	(20.48)	(5.42)	(14.46)	(4.22)	(24.10)	(6.02)	(100)	(100)

Table 3. Distribution of Mucormycetes in soil of municipality districts

Genus	District NoSpecies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No (%)	Total(%)
Mucor	*circinelloids*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1 (0.43)	106(45.30)
	racemosus	1	-	-	-	-	-	1	-	-	-	-	-	1	-	3 (1.28)	
	plumbeus	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2 (0.85)	
	Mucor sp	5	13	22	5	-	4	21	2	6	3	7	3	2	7	100 (42.74)	
Rhizomucor	*pusillus*	-	2	-	-	-	-	-	-	-	-	-	1	-	1	4 (1.71)	63(26.92)
	Rhizomucor sp	7	8	10	2	1	1	3	10	5	2	1	3	5	1	59 (25.21)	
Rhizopus	*oryzae*	-	-	-	-	1	-	-	1	-	1	-	-	1	1	5 (2.14)	56(23.93)
	stolonifer	-	-	-	1	-	1	-	-	-	-	-	1	-	-	3 (1.28)	
	Rhizopus sp	4	5	9	6	-	4	12	-	1	-	3	2	-	2	48 (20.51)	
Absidia	*corymbifera*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1 (0.43)	5(2.14)
	Absidia sp	3	-	-	-	-	-	-	-	-	-	-	1	-	-	4 (1.71)	
Cunninghamella	*bertholletiae*	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1 (0.43)	1(0.43)
Mortierella	*wolfii*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1 (0.43)	3(1.28)
	Mortierella sp	-	-	-	-	-	-	-	-	1	1	-	-	-	-	2 (0.85)	
Total (%)		20	28	43	14	3	11	37	13	13	7	11	11	9	14	234	234
(100)		(8.55)	(11.96)	(18.38)	(5.98)	(1.2)	(4.7)	(15.81)	(5.56)	(5.56)	(2.99)	(4.70)	(4.70)	(3.85)	(5.98)	(100)	

microscopic examinations. Genus *Rhizopus* was the most frequent species with frequency rate of 35.5%, followed by *Mucor* (32.25%), *Rhizomucor* (27.5%), *Absidia* (2.5%), *Mortierella* (1.75%) and *Cunninghamella* (0.5%) (Table 1).

Also 51.81% of isolates in public parks soil samples was related to the genus *Rhizopus* which followed by *Rhizomucor* with frequency rate of 28.31%, while in municipality districts soil samples, the genus *Mucor* with frequency rate of 45.30% was the most frequent isolate and followed by *Rhizomucor* (26.92%) (Table 2 and 3).

DISCUSSION

The saprophytic and pathogenic soil-borne fungi are one of the most important components of soil mycoflora that they are transmitted with wind and can cause many infectious diseases and different degrees of allergy in animals and human[20].

Most medically important Mucormycetes species grow rapidly on virtually any carbohydrate substrate and commonly found in soil and in decaying organic matter[5, 22, 23]. In addition, inhalation of sporangiospores in dust has been linked to outbreaks of rhinocerebral or pulmonary zygomycosis due to excavation, construction, or contaminated air-conditioning filters[9].

Mucormycosis is a rare fulminating fungal infection caused by fungi belonging to Mucorales order. The infection usually starts in the middle or inferior nasal meatus and then spreads to the paranasal sinuses and the orbit[24].

The main aim of this study was to investigate the diversity of Mucormycetes in the soil of different public places and populated areas of municipality districts in Isfahan. Identification of fungi was carried out based on standard mycological methods.

The Mucorales are thermo-tolerant moulds that are widely found on organic substrates and soils. The optimum environmental conditions for the growth and sporulation of zygomycetes on these substrates are 27º C and high humidity. These are some thermophilic species, most of which have been isolated from composting plant materials[9].

Most cases of mucormycosis are caused by members of the *Mucoraceae*. These include the genera *Absidia*, *Mucor*, *Rhizomucor* and *Rhizopus*[9].

In our study, the genera of *Rhizopus*, *Mucor*, and *Rhizomucor* were dominant genera with frequency rate of 35.5%, 32.25%, and 27.5% respectively (Table 1). These organisms are ubiquitous saprophytes in nature rarely infecting organisms with intact immune system[7]. Members of the genus *Rhizopus* are the most common isolates recovered from clinical samples of mucormycosis and members of the genus *Mucor* are second to *Rhizopus* in terms of frequency[13].

The occurrence of mucormycosis in immunocompromised patients is increasing, and physicians who treat patients in intensive care units must be aware of these fatal emerging infections[22].

The commonest cause of mucormycosis is *Rhizopus arrhizus* (*oryzae*). Other less frequent aetiological agents include *Absidia corymbifera, Apophysomyces elegans*, *Cunninghmlla bertholletiae*, *Rhizomucor pusilus* and *Saksenea vasiformis*[9].

In our study *Rhizopus arrhizus (oryzae), Rhizomucor pusilus, Absidia corymbifera,* and *Cunninghmlla bertholletiae* were isolated from soil samples of public parks and municipality districts and the genus *Rhizopus* was the most frequent isolate (Table 2 and 3). However, *Rhizopus* spp. remains as the major cause of most mucormycosis cases, being responsible for almost 80% of the infections. Also a considerable number of mucormycosis cases have been associated with *M. circinelloides*[25].

In our study, *M. circinelloides* and *M. racemosus* were isolated from soil samples of public parks and municipality districts and *M. plumbeus* was just isolated from two municipality district samples (Table 1, 2, and 3). *M. plumbeus* is extensively used for research purposes in biotransformation of natural products, but there have not yet been any mycosis cases associated with this species[25].

In our study *Absidia* sp and *Absidia corymbifera* were isolated from parks and municipality districts soil samples (Table 1, 2, and 3). The genus *Absidia* consists of fungal species, which are ubiquitous soil inhabitants and represent important causative agents of mucormycosis in human and animals[26].

Malek *et al*. (2013) isolated 17 different fungal genera from park soils in Gorgan, which 36% of all isolates were related to Zygomycetes and the genus *Mucor* was the most frequent isolate among other genera of Zygomycetes[27]. In our study, the genus *Rhizopus* with frequency rate of 51.81% was the most frequent isolate from parks soils, followed by *Rhizomucor* (28.31%), *Mucor* (13.86%), *Absidia* (3.01%), *Mortierella* (2.41%) and *Cunninghamella* (0.60%) respectively (Table 2). It can be because of temperature and humidity differences, organic content and plant diversity of each area.

The *Mortierellaceae* may be differentiated *Mucoraceae* by virtue of their very delicate features. Sporangia are small and have few or no columella. The mycelium is dichotomously branched. These delicate features provided the original grounds for their placement in a separate family from the *Mucoraceae*[12].

Some published papers are available about the isolation of the genus *Mortierella* from the soil[28]. In our study, *Mortierella wolfii* and *Mortierella* sp were isolated from the soil of parks and municipality districts with frequency rate of 2.41% and 1.28% respectively (Table 2 and 3). *Mortierella wolfii* is probably the only pathogenic species being an important causal agent of bovine mycotic abortion, pneumonia and systemic mycosis[12].

Yazdanparast *et al*. (2014) reported the isolation of some saprophytic and keratinophilic fungi from soil samples of parks and municipality districts of Tehran. They were isolated the genera *Cunninghamella, Mucor* and *Rhizopus* from soil samples of several parks. The isolation rate of *Cunninghamella* was more than the others[29].

Up to now, some researchers have isolated many different fungi from various types of soils. Agamirian *et al*. (2013) investigated the prevalence of fungi in soil of Qazvin, Iran, and reported 14 genera which were included *Rhizopus* and *Mucor*[20]. Although our study focuses on Mucormycetes, but the genera belonging to the other classes of fungi were isolated which were included *Fusarium, Caldosporium, Aspergillus, Penicillium, Trichophyton mentagrophytis, Chrysosporium,* and *Scopulariopsis.*

Hedayati *et al*. (2004) published a paper in which they isolated *Mucor* spp and *Rhizopus* spp from soil samples of potted plants in hospitals of Sari, Iran. Other isolated genera were included *Acromunium, Penicillium, Caldosporium, Paecilomyces, Chrysosporium, Altrenaria, Aspergillus, Verticillium, Geotrichum* and yeast[30], which is almost in agreement with isolated fungi in our study.

Rhinocerebral mucormycosis was diagnosed in a man with diabetes mellitus in Isfahan; the isolate was identified as *Rhizopus oryzae* and the patient[31] and it shows that the debilitated persons are predisposed to mucormycosis.

CONCLUSION

In conclusion, fungal abundance in different soils is related to some factors such; percentage of total carbon and soil moisture and these factors are present in soil of parks and street side gardens of Isfahan. Finally, 13 genera of saprophytic and pathogenic fungi were isolated in this study in which 6 genera were belonged to Mucormycetes. These opportunistic fungi can be dispersed via wind, insects or other agents and reach to immunocompromized persons.

ACKNOWLEDGEMENTS

Authors wish to thank responsible of Mycology lab Ahmadreza Ahmadi for his technical assistance and valuable helps.

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