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Mortuary: The Inevitable Evil: Mortuary Staff is a Victim of the Sudden Death Caused by the Invisible Mycoburden oAf Human Cadavers

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Abstract
Working in a mortuary is an extremely stressful and lethal experience, as repeated exposure to different fungal spores, makes the staff as victims of sudden death. The presence of invisible mycoburden on human cadavers and rule out their effect on mortuary staff health was the aim of this research. A total of 20 cadavers along with 79 samples from different surfaces were collected in the city of Sana'a, Yemen, from governmental and private owned hospitals. After submitting to conventional mycological procedures, *Aspergillus* spp., *Penicillium* spp. and *Candida* spp. were the main fungal isolates in both bloated and putrefied stage, while each of *Eurotium* spp. and *Mucor* spp. predominated the skeletonized stage. Massive fungal load was detected on different mortuary surfaces, except for draining boards and necropsy tables. Among the identified species in both of cadavers and surfaces; *Cladosporium cladosporioides, Histoplasma* sp. and *P. marneffei* are classified in risk group 3, *A. flavus, A. fumigatus* and *Candida albicans* in risk group 2, which pose an allergic potential risk, while others are listed in the risk group 1, even if they may not found significant, it obviously represent serious risk for personnel working there especially those with open scare causing uncommon human disease. Safe working conditions for handling cadavers are recommended along with proper education, use of protective clothing and practice of hygiene measures.
Keywords: Mortuary, Victims, Invisible, Cadavers, Decomposition stages, Risk group.

INTRODUCTION

The mortuary is a place of mystery, sadness, grief or repulsion and we all hope, while alive, that we will never need to visit such a place. For families who have lost a loved one to a sudden death, this becomes a reality (Brysiewicz, 2007).

Like all other occupations, working in a mortuary has its own risks; the potential of infection hazard of human cadavers is one of them (Yaragalla and Rajput, 2017). This basic information is completely absent in the dictionary of the hospital's owners , as a result mortuary is the most neglected place in almost all of Yemeni hospitals as well as medical colleges. It doesn't have even basic facilities for the departed souls, public and officials working there.

Cadavers remain a teaching tool for students. However, they still may pose infection hazards to forensic medicine personnel dealing with them directly or indirectly, including pathologists, mortuary attendants, embalmers, funeral directors and members of the emergency services (Weed and Baggenstoss, 1951). All of them are at continuous risk of acquiring various kinds of infections including severe fungal infections, which are not restricted to the mortuary personnel only, but they further pass to others existent outside this field. This makes them a timing bomb moving freely between all departments of hospitals spreading the infectious agents by their aerosols, clothes, boots…etc. they shed during their activities.

Although the presence of fungi on the surfaces of cadavers has been recognized for some time by forensic pathologists, the association between their presence and potential risk for human has still not received any attention by researches and even their habitat haven't been reported yet in Yemen. When we observed that the previous studies in this field focused on the visible fungal growth on cadavers (van de Voorde and van Dijck, 1982; Wiltshire, 2005; Hitosugi *et al.,* 2006; Ishii *et al.,* 2006; Wiltshire, 2006a,b; Simón *et al.,* 2011; Hawksworth, 2013; Wiltshire *et al.,* 2015; Schwarz *et al.,* 2015; Tranchida *et al.,* 2018), we directed our attention to invisible fungal growth that could be of great risk on human health.

It was so important to draw attention to the infective agents that can be detected in cadavers and different surfaces in mortuary to suggest safety guidelines precautions for the protection of all those who deal with cadavers, especially when no vaccines are useful against fungal infections, as no special safety precautions are taken into account during handling cadavers when fungal growth is noted, how about when the growth is invisible!!. The aim of this study was to determine the presence of invisible mycoburden on human cadavers and rule out their effect on mortuary staff health.

MATERIALS AND METHODS

Ethical statement and biosafety

All the studies related to the animals were conferring to the Committee for animal Ethics of Sana'a University, Yemen. To preserve the integrity of the material collected and to protect the researchers' health, biological masks and disposable coats and gloves were worn at all times.
All the experiments were performed in replicates to show the biological and measurement variability, respectively.

Cadavers and surfaces sampling

Swabs were taken from human cadaveric skin (head, thorax, abdomen, and thighs), and exposed bones in the last stage of decomposition: bloating stage (*n*=5), putrefaction stage (*n*= 10), skeletonization stage (*n*=5). All cadavers were collected from four selected hospitals (government and private owned hospitals), they were all males between 18 and 70 at death, victims of unnatural death-homicide, suicide, and from battlefront.

The fungal samples were taken by standard microbiological techniques using sterile swabs from cadavers and different surfaces such as necropsy tables (*n*=2), draining boards (*n*=9), refrigerator handles (*n*=19), walls (*n*=39), and trays (*n*=10), then cultured on Sabourad Dextrose Agar (SDA) containing streptomycin and chloramphenicol to inhibit bacterial growth.

The collected samples were incubated at 25°C for up to 7 days with daily inspection until fungal growth was detected. The resulting mycelium was then transferred to a new dish with medium, in order to maintain the axenity of the isolate. The new cultures were maintained in slants until fungal identification.

Fungal identification

The fungi were identified by phenotypical analyses, comprising macromorphology (texture, surface and diameter of the colony as well as the presence of pigmentation), micromorphology (size, surface and pigmentation of conidia and morphology of conidiogenic cells) according to (De Hoog *et al*., 2000).

Determination of risk groups

For the definition of risk groups, the Directive 2000/54/EC of the European Parliament was used (Table 1).

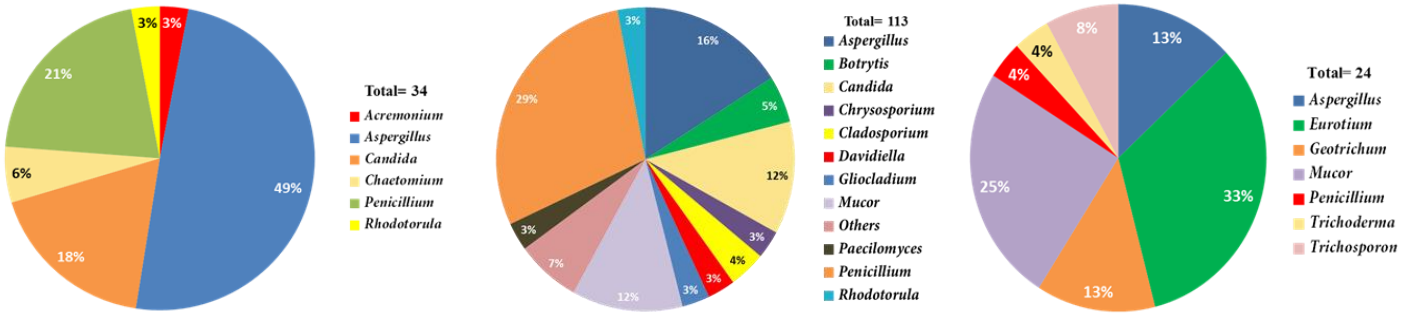
Table 1. Risk group definition according to the directive 2000/54/EC of the European Parliament

Risk group[a]	Definition
1	A biological agent that is most unlikely to cause human disease.
2	A biological agent that may cause human disease and may be a hazard to laboratory workers, but is unlikely to spread in the community. Laboratory exposure rarely produces infection and effective prophylaxis or treatment is available.
3	A biological agent that may cause severe human disease and presents a serious hazard to laboratory workers. It may present a risk of spread in the community, but there is usually effective prophylaxis or treatment.
4	A biological agent that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread in the community, and there is usually no effective prophylaxis or treatment.

[a] No fungi are currently classified as risk group 4 (Schwarz *et al.,* 2015).

RESULTS

A total of 168 fungal strains belonging to 41 species and 22 genera were isolated from 80% of the sampled cadavers, while the other 20% of the cases were fungi-free (Table 2). The relative abundance of all isolated strains according to genera and the different postmortem stages is shown in Figure 1. It was obvious that *Penicillium* was the genus with the highest number of isolated species (*P. brevicompactum, P. citrinum, P. crustosum, P. implicatum, P. oxalicum, P. pinophilum, P. rubens, P. rugulosum, P. vulpinum,* and *P.* sp.), followed by *Aspergillus* (*A. awamori, A. flavus, A. fumigatus, A. jenseni, A. niger, A. tamarii* and *A. versicolor*). Less frequent genera detected were *Acremonium, Allophoma, Botrytis, Byssochlamys, Candida, Chaetomium, Chrysosporium, Cladosporium, Davidiella, Emericella, Eurotium, Fusarium, Geotrichum, Gliodadium, Mucor, Paecilomyces, Rhodotorula, Trichoderma* and *Trichosporon.* This study contributes to the previous findings in forensic mycology, marking the first isolation of the following species *Allophoma nicaraguensis, A. tamarii, A. versicolor, Chrysosporium keratinophilum, Davidiella* sp., *Emericella heterothallica, Fusarium crookwellense, P. vulpinum, Trichoderma cremeum,* and *T. lingorum*. In both of bloated and putrefied stages, *Aspergillus, Penicillium* and *Candida* predominated, while *Eurotium* and *Aspergillus* were prevalent in skeletonized stage. The greatest number of isolates was obtained from the putrefied stage (*n*= 113), compared with the bloated (*n*= 34) and skeletonized (*n*= 24) stages.



(a) (b) (c)

Fig. 1. Relative abundance of isolated fungi from cadavers according to genera (a) in bloated stage, (b) in putrefied stage, and (c) in skeletonized stage.

Table 2. Species and number of fungi isolated from the skin and bones of cadavers in the bloated, putrefied and skeletonized stages and their risk group according to literature research

Fungi	Decomposition stages of cadavers			R.G*	References
	Bloated (*n*=5)	Putrefied (*n*=10)	Skeletonized (*n*=5)		
Acremonium sp.	1	2	-	1	Dosa, 1955 & Fincher *et al*., 1991
Allophoma nicaraguensis	-	1	-	1	Chen and Cai, 2015
Aspergillus awamori	6	1	-	≤2	Lopez-Martinez *et al.,* 2007& HSC, 2013
Aspergillus flavus	4	5	2	2	Harley *et al.,* 1995; Mori *et al.,* 1998 & Vollmer *et al.,* 2008
Aspergillus fumigatus	2	4	-	2	2000/54/EC & Cavka *et al.,* 2010
Aspergillus jenseni	-	2	-	≤2	HSC, 2013
Aspergillus niger	5	3	1	2	Nakagawa *et al.,* 1999 & HSC, 2013
Aspergillus tamarii	-	2	-	≤2	HSC, 2013
Aspergillus versicolor	-	1	-	≤2	De Amicis, 1950; Torres-Rodriguez *et al.,* 1998 & Rotoli *et al.,* 2001
Botrytis cineria	-	6	-	2	Kauffman *et al.*, 1987
Byssochlamys nivea	-	2	-	1	Wickes, 2014
Candida albicans	2	5	-	≤2	Tumbarello *et al.,* 1996 & Levy *et al.,* 2006
Candida lipolytica	4	9	-	≤2	Tumbarello *et al.,* 1996 & Gouba *et al.,* 2014
Chaetomium sp.	2	-	-	1	Friedman, 1998; Guppy *et al.,* 1998 & Haselwandter and Ebner, 1994
Chrysosporium keratinophilum	-	3	-	1	Lacey, 1981 & Cavka *et al*., 2010
Cladosporium cladosporioides	-	4	-	3	van de Voorde and van Dijck, 1982; Collier *et al.,* 1998 & Lagier *et al.,* 2017
Davidiella sp.	-	3	-	-	Gouba *et al.,* 2014 & Lagier *et al.,* 2017
Emericella heterothallica	-	1	-	≤2	Honoer *et al.,* 1995
Eurotium repens	-	-	7	2	Honoer *et al.,* 1995 & Wickes, 2014
Eurotium rubrum	-	-	1	2	Honoer *et al.,* 1995 & Wickes, 2014
Fusarium crookwellense	-	1	-	2	Hawksworth and Wiltshire, 2011& HSC, 2013
Geotrichum candidum	-	-	3	2	Buchta and Otcenasek, 1988; Lopez-Martinez *et al*., 2007 & Gouba *et al.,* 2014
Gliocladium sp.	-	3	-	1	Ishii *et al.,* 2006
Mucor circinelloides	-	6	-	1	Aderka *et al.,* 1983& Schwarz *et al.,* 2015
Mucor hiemalis	-	-	6	1	Caraveo *et al.,* 1977; Lopez-Martinez *et al*., 2007; Vollmer *et al.,* 2008 & Hawksworth and Wiltshire, 2011
Mucor racemosus	-	7	-	1	Pickles *et al.,* 1994; Lopez-Martinez *et al*., 2007 & Schwarz *et al.,* 2015
Paecilomyces variotii	-	3	-	1	McClellan *et al.,* 1976; Byrd *et al.,* 1992; Dhindsa *et al.,* 1995; Cohen-Aboo and Edwards, 1995;Groll and Walsh, 2001 & Schwarz *et al.,* 2014
Penicillium brevicompactum	-	1	-	1	Schwarz *et al.,* 2015 & Lagier *et al.,* 2017
Penicillium citrinum	-	2	-	1	Dosa, 1955; Hitosugi *et al.,* 2006; Lopez-Martinez *et al*., 2007 & Cavka *et al.,* 2010
Penicillium crustosum	1	7	-	1	Schwarz *et al.,* 2015
Penicillium implicatum	-	3	-	1	Dosa, 1955; Hitosugi *et al.,* 2006; Lopez-Martinez *et al*., 2007; Cavka *et al.,* 2010 & Gouba *et al.,* 2014
Penicillium oxalicum	1	5	-	1	
Penicillium pinophilum	-	4	-	1	
Penicillium rubens	1	-	-	1	
Penicillium rugulosum	-	1	-	1	
Penicillium vulpinum	4	10	-	1	
Penicillium sp.	-	-	1	≤2	
Rhodotorula sp.	1	3	-	1	Naveh *et al.,* 1975; Pore and Chen, 1976; Petrocheilou-Paschou *et al.,* 2001; Lopez-Martinez *et al*., 2007 & Gouba *et al.,* 2014
Trichoderma cremeum	-	-	1	1	Lopez-Martinez *et al*., 2007
Trichoderma lingorum	-	1	-	1	
Trichosporon sp.	-	2	2	≤2	Walsh, 1989; Walsh *et al.,* 1993; Gueho *et al.,* 1994; Nagai *et al.,* 1999; Kustimur *et al.,* 2002; Lopez-Martinez *et al*., 2007 & Gouba *et al.,* 2014
Total fungal strains (*n*= 171)	**34**	**113**	**24**	-	-

* R.G: Risk group

In the second part of this research, mycoflora of the mortuary surfaces was investigated, which consisted of 290 strains belonging to 54 species and 31 genera (Table 3), their relative abundance according to genera and different surfaces is shown in Figure (2).

It is well known that the higher the hygiene level, the fewer microbes were allowed on surfaces. This idea was reinforced by our findings where both of operating tables and draining boards were almost fungi-free except for the presence of *A. awamori, Cladosporium cladosporioides, P. mallochii* and *P. rugulosum* in the former and *P. oxalicum* and *Ulocladium atrum* in the latter theater, respectively, in contrary to others at the same place i.e. refrigerator walls, trays and handles.

During this study, refrigerator walls showed the highest level of contamination with 147 strain, followed by refrigerator handles (*n*= 83), refrigerator trays (*n*= 46), and finally necropsy tables (*n*= 11) and draining boards (*n*=3) (Table 3).

In such surfaces, some of fungal species isolates were resemble to cadavers, while others are completely different.

Table 3. Species and number of fungi isolated from different surfaces in mortuary and their risk group according to literature research

Fungi	D.B[a] (*n*=9)	R.W[b] (*n*=39)	R. H[c] (*n*=19)	R.T[d] (*n*=10)	N.T[e] (*n*=2)	R.G	References
Abasidia corympfira	-	-	-	2	-	2	HSC, 2013 & Wickes, 2014
Acremonium strictum	-	-	1	-	-	1	Dosa, 1955 & Fincher *et al*., 1991
Alternaria infectoria	-	-	-	1	-	1	Dosa, 1955; Garau *et al.,* 1977; Pujol *et al.,* 2000 & Cavka *et al.,* 2010
Aspergillus awamori	-	12	-	-	4	2	Lopez-Martinez *et al.,* 2007& HSC, 2013
Aspergillus candidus	-	-	-	2	-	≤2	Dosa, 1955 & HSC, 2013
Aspergillus flavus	-	5	-	1	-	2	Dosa, 1955; Harley *et al.,* 1995; Mori *et al.,* 1998; Vollmer *et al.,* 2008 & HSC, 2013
Aspergillus fumigatus	-	3	4	-	-	2	Dosa, 1955; Vollmer *et al.,* 2008; Cavka *et al.,* 2010 & 2000/54/EC
Aspergillus glaucus	-	-	-	1	-	≤2	HSC, 2013
Aspergillus niger	-	17	3	-	-	2	Dosa, 1955; Nakagawa *et al.,* 1999; Vollmer *et al.,* 2008 & HSC, 2013
Aspergillus sp.	-	-	5	-	-	≤2	HSC, 2013
Aspergillus terreus	-	1	4	-	-	≤2	Walsh *et al.,* 2003; Graybil *et al.,* 2004; Steinbach *et al.,* 2004; Hitosugi *et al.*,2006; Warnock, 2007 & HSC, 2013
Aspergillus ustus	-	-	2	-	-	≤2	Carrizosa *et al.,* 1974; Ricci *et al.,* 1998; Verweij *et al.,* 1999; Azzola *et al.,* 2004; Lopez-Martinez *et al.*, 2007 & HSC, 2013
Beauveria brassiana	-	2	3	-	-	1	Lopez-Martinez *et al.*, 2007 & Lagier *et al.,* 2017
Bipolaris sp.	-	-	4	-	-	1	Lake *et al.,* 1991; Pingree *et al.,* 1992 & Lopez-Martinez *et al.*, 2007
Boeremia exigua	-	-	2	-	-	2	Zaitz *et al.,* 1997; De Hoog *et al.,* 2000 & Balis *et al.,* 2006
Botrytis cineria	-	-	4	1	-	2	Kauffman *et al.*, 1987 & Horner *et al.,* 1995
Botrytis elliptica	-	-	-	-	-	2	
Candida albicans	-	14	-	2	-	2	Tumbarello *et al.,* 1996, 2000/54/EC, Levy *et al.,* 2006; Vollmer *et al.,* 2008; HSC, 2013 & Gouba *et al.,* 2014
Chrysosporium keratinophilum	-	-	1	-	-	1	Lacey, 1981; Lopez-Martinez *et al*., 2007 & Cavka *et al*., 2010
Circinella muscae	-	-	1	-	-	1	Kaul and Sumbali, 2000
Cladosporium cladosorioides	-	5	5	3	2	3	van de Voorde and van Dijck, 1982; Collier *et al.,* 1998 & Lagier *et al.,* 2017
Cochliobolus lunatus	-	-	-	1	-	1	Yau *et al.,* 1994; Kirk and Dan, 2001
Cunninghamella elegans	-	1	1	-	-	1	Weitzman, 1984
Curvularia hominis	-	2	-	-	-	1	Rinaldi *et al.,* 1987; Ujhelyi *et al.,* 1990; Lake *et al.,* 1991; Travis *et al.,* 1991 & Lopez-Martinez *et al*., 2007
Eurotium amstelodami	-	-	-	1	-	≤2	Honoer *et al.,* 1995
Fusarium pseudonygami	-	-	3	-	-	2	van de Voorde and van Dijck, 1982; Lopez-Martinez *et al*., 2007; Hawksworth and Wiltshire, 2011& HSC, 2013
Fusarium solani	-	-	1	1	-	2	
Geotrichum candidum	-	5	-	10	-	2	Buchta and Otcenasek, 1988; Lopez-Martinez *et al*., 2007; Hawksworth and Wiltshire, 2011; HSC, 2013 & Gouba *et al.,* 2014
Histoplasma sp.	-	2	-	-	-	3	2000/54/EC & HSC, 2013
Microsporum gypsum	-	-	2	-	-	2	2000/54/EC & Schwarz *et al.,* 2015
Mucor circinelloides	-	-	1	-	-	1	Aderka *et al.,* 1983; Lopez-Martinez *et al*., 2007; Hawksworth and Wiltshire, 2011 & Schwarz *et al.,* 2015
Mucor racemosus	-	-	1	-	-	1	Pickles *et al.,* 1994; Lopez-Martinez *et al*., 2007 & Schwarz *et al.,* 2015
Paecilomyces variotii	-	12	5	2	-	1	McClellan *et al.,* 1976; Byrd *et al.,* 1992; Dhindsa *et al.,* 1995; Cohen-Aboo and Edwards, 1995; Groll and Walsh, 2001 & Schwarz *et al.,* 2015
Penicillium chrysogenum	-	1	-	-	-	1	van de Voorde and van Dijck, 1982 & Gouba *et al.,* 2014
Penicillium crustosum	-	-	2	-	-	1	Schwarz *et al.,* 2015
Penicillium griseofulvum	-	9	1	-	-	1	Dosa, 1955; Hitosugi *et al.,* 2006; Lopez-Martinez *et al*., 2007 & Cavka *et al.,* 2010
Penicillium implicatum	-	28	4	1	-	1	
Penicillium mallochii	-	-	1	-	4	1	

Penicillium marneffei	-	-	-	2	-	3	Deng *et al*., 1988; Singh *et al*., 1999; 2000/54/EC; HSC, 2013; Kohler *et al.,* 2015 & Seyedmousavi *et al.,* 2015
Penicillium oxalicam	2	-	11	3	-	1	Dosa, 1955; Hitosugi *et al.,* 2006; Lopez-Martinez *et al*., 2007 & Cavka *et al.,* 2010
Penicillium piceum	-	-	-	2	-	1	
Penicillium ruben	-	2	-	1	-	1	
Penicillium rugulosum	-	2	-	2	1	1	
Penicillium sp.	-	-	1	-	-	≤3	
Penicillium vulpinum	-	24	-	3	-	1	
Piedraia hortae	-	-	-	1	-	2	Adam *et al.,* 1977; Coimbra and Santos, 1989; Venugopal and Venugopal, 1992; Gip, 1994 & Figueras and Guarro, 1997
Pythium volutum	-	-	-	-	-	2	Hawksworth and Wiltshire, 2011
Rhodotorula sp.	-	-	-	1	-	1	Naveh *et al.,* 1975; Pore and Chen, 1976; Petrocheilou-Paschou *et al.,* 2001; Lopez-Martinez *et al*., 2007 & Gouba *et al.,* 2014
Scedosporium prolificans	-	-	-	1	-	2	Berenguer *et al.,* 1997; 2000/54/EC; Vollmer *et al.,* 2008 & HSC, 2013
Scopulariopsis brevicualis	-	-	1	-	-	2	Cox and irving, 1993; Dosa, 1955; Migrino *et al.,* 1995; Tosti *et al.,* 1996; Bruynzeel and Starink, 1998; Lopez-Martinez *et al*., 2007 & HSC, 2013
Sporothrix schenckii	-	-	2	-	-	2	Kwon-Chung, 1979; Travassos *et al.,* 1980; Lesperance *et al.,* 1988; Castrejon *et al.,* 1995; Kwon-Chung and Bennett, 1992; 2000/54/EC & HSC, 2013
Trichophyton rubrum	-	-	2	-	-	2	Bronson *et al.,* 1983; 2000/54/EC; Vollmer *et al.,* 2008 & HSC, 2013
Trichophyton tonsurans	-	-	5	-	-	2	
Ulocladium atrum	1	-	-	1	-	1	Horner *et al.,* 1995
Total fungal strains (*n*= 290)	**3**	**147**	**83**	**46**	**11**	**-**	**-**

(a) Draining boards, (b): Refrigerator walls, (c): Refrigerator handles, (d): Refrigerator trays and (e): Necropsy tables.

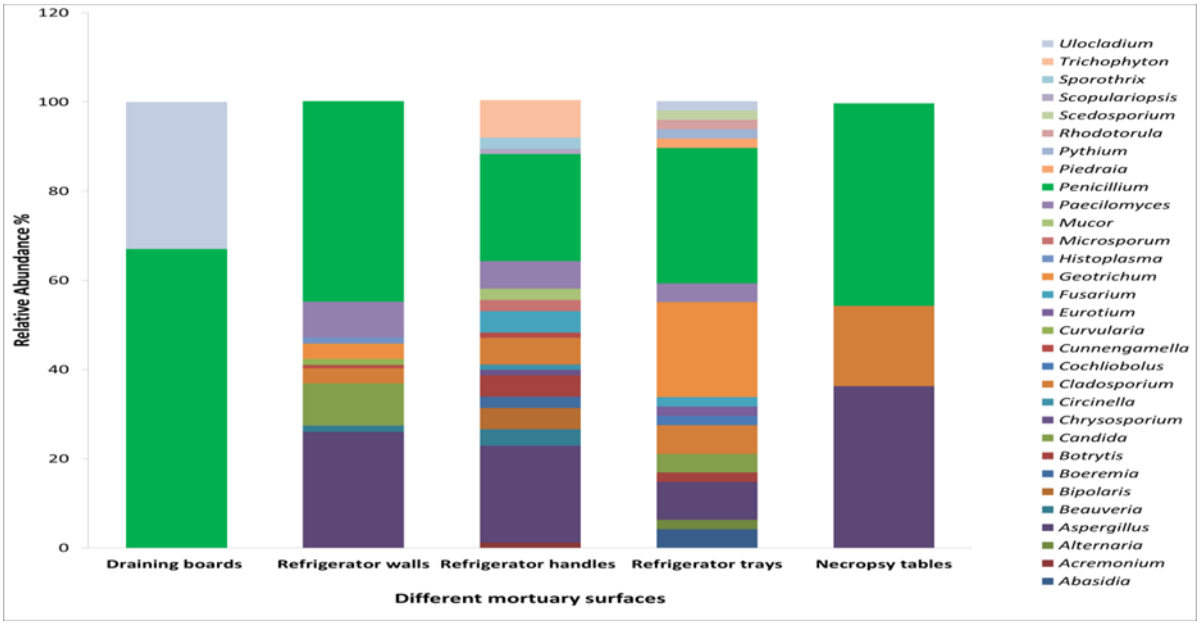


Fig. 2. Relative abundance of isolated fungi from different mortuary surfaces

DISCUSSION

The invisible presence of pathogenic fungi on decomposing human cadavers in Yemen is never considered; the workers there are often unaware that such presence may be of a high risk on their health as the inhalation of the spores may lead to pulmonary infections and is a well-known cause of allergies and asthma (Horner *et al.,* 1995), especially for persons subjected to repeated exposure. In fact, our findings call a second look at the ignorance of all those related to mortuary.

When tracing the fungal species in all investigations reported so far, *Penicillium* and *Aspergillus* always come at the front, suggesting that both genera are widely spread in nature, and many of them are airborne strains and can easily grow on practically any substrate including cadavers at different stages of decomposition (Sharma, 1988). Such species can be the first to settle on the body and are still able to be present at further stages of decomposition (Tranchida *et al.,* 2018).

Interestingly, numerous species which were detected in the human cadavers subjected to sampling during this investigation have previously been isolated by other authors (Dosa, 1955; Van de Voorde and Van Dijck, 1982; Ishii *et al.,* 2006; Wiltshire, 2006a,b; Hitosugi *et al.,* 2006; Sidrim *et al.,* 2010; Martínez-Ramírez *et al.,* 2013; Schwarz *et al.,* 2015; Tranchida *et al.,* 2018), Most of fungal species recovered during this investigation, other than *Aspergillus* and *Penicillium,* can be considered as a natural mycoflora of the gastrointestinal tract (Cohen *et al.,* 1969). Others are facultative pathogenic members and may invade the cadavers after death because a cadaver is a plentiful source of organic materials and these fungi present in the place where the corpse is found (Carter and Tibbett, 2003; Lakchayapakorn *et al.,* 2008), begin a colonization process of different body tissue, mentioning that the fly larva activity, might also have been expected to have had a key role in enabling the fungi to colonize so quickly (Hawksworth and Wiltshire, 2015).

Unlike our findings which clearly indicated that the greatest number of isolates was obtained from the putrefied stage (*n*= 113), compared with the bloated (*n*= 34) and skeletonized (*n*= 24) stages, the findings of Sidrim *et al.,* (2010) revealed the recovery of 134 isolate in the bloated stages, 12 and 26 isolates in putrefied and skeletonized stages, respectively. It is not surprising that there are differences between this report and others available to forensic mycology researchers, as fungi on human remains may respond differently under different environmental circumstances and individual characteristics of cadavers (Fromtling *et al.,* 2003), in addition, the rate of degradation turns faster in putrefaction stage resulting of rupture of skin integration opens extra access point for microorganisms, arthropods and scavengers (Shkrum and Ramsay, 2007).

The number of fungal isolates recovered from bloated stage (*n*= 34) was much more than the number of fungi obtained from skeletonized stage (*n*= 24), this is reasonable as in the latter stage the dehydration of body fluids allows the preservation of the cadaver (Carter *et al.,* 2007; Janaway *et al.,* 2009) resulting from inadequate moisture content for microorganisms activity of tissue degradation causing decomposition to be hindered (Powers, 2005). But still, the frequently held assumption that the older a dead body is, the less danger there is for the examiner, doesn't hold for the spore-forming filamentous fungi as reinforced by our findings leading us to recommend the use of respiratory protection masks during handling cadavers even when the fungal growth isn't noted, because not seeing them doesn't mean that they are not exist.

In response to the differences in the results, we believe that refinement of the isolation method employed can have a great impact on the recovery of some fungal species as in the case of using benomyl in the isolation media, that capable of inhibiting the *Aspergillus* sp. (Luz *et al.,* 2007) to permit the less competitive power species to show up, or even by incubating of the materials at a temperature of 37°C, because of the heat sensitivity of various species of *Penicillium* spp. (De Hoog *et al.,* 2000), which explains the predominantly of both genera in the tested samples.

Possible environmental contamination is an issue to be considered, but in our investigation, this idea is largely to be excluded, because if so, the same fungi in different samples of the same case would have detected. However, this wasn't observed. Nonetheless, still further studies on cadavers in the three stages of decomposition are needed to decide if the species detected are characteristics of each stage and can be used as a forensic tools or not. As we believe that the present study is only a starting point in this field.

In the second part of this research, some of the fungal species of the mortuary surfaces isolates were resemble to those of cadavers, while others are completely different. Indicating the reuse of refrigerator chambers without disinfection procedures between each use, which in turn bring different mycoflora inside, as the history of each cadaver, differs from the other (Nolte *et al*., 2002; Vij and Krishan, 2003; Sharma and Reader, 2005; Burton, 2003; Sharma *et al*., 2004). Gloves and clothes are another source of different fungi not related to cadavers.

Among all fungal strains recovered during this study, *Cladosporium cladosporioides, Histoplasma* sp. and *P. marneffei* are classified in risk group 3, being able to cause severe human disease, other fungi species belonged to risk group 2, from which *A. flavus* and *A. fumigatus* are the most causative agents of invasive aspergillosis along with *A. terreus, A. niger* and *A. nidulans* (Warnock, 2007). In addition to *Candida albicans* which is the most important cause of candidemia worldwide (Colombo *et al.,* 2006). While the others which are listed in the risk group 1, even if they may not found significant, they are obviously represent serious risk for personnel working their especially those with open scare causing uncommon human disease (Walsh *et al.,* 2004; Warnock, 2007). Strict procedures, including cleaning and disinfection practices, should be followed at all times besides the entry of such room should be restricted except for the experts and workers who are trained in handling the infected materials, this should be reinforced at periodic intervals.

CONCLUSION

The findings of the current investigation permit tracing out a horizon for deeper understanding of the mycoflora related to human cadavers in different decomposition stages and their impact on the health of mortuary staff and others dealing with dead. As they may be exposed to a wide variety of infectious agents. Still in Yemen, any sort of regularity and standardization eludes autopsy work as no standard design or guidelines are outlined for construction of mortuary complexes. It is therefore prudent to consider all the dead bodies to be potential carriers of infection and follow the universal precautions, while handling them. Gloves, clothes and similar materials should be treated as standard hospital red bag waste and incinerated. Because of lack of awareness in handling dead, besides no vaccination for human pathogenic fungi are licensed, much more researches with larger samples will be necessary to ratify the necessity of dealing with each cadaver potentially high risk case. Safe working conditions for handling cadavers can be provided through proper education, use of protective clothing and practice of hygiene measures.

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CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

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