Journal of Science and Technology, Vol. 9 No. 4 (2017) p. 64-69

Identification of Fungi Isolated from Clinical Wastes

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Received 30 September 2017; accepted 30 November 2017; available online 28 December 2017

Abstract: The distrubition of fungi in the hospitals wastes are coming from the clinical wastes specimens used for the disgnistic process. The aim of the present study was to identify the fungal isolates obtained from clinical wastes based on phenotype method. The fungal isolates were obtained by the direct plate method on Potato Dextrose Agar (PDA) medium and incubated at 28◦C for 7 to 14 days, therafter purified by single spore isolation. The cultural characteristics of the fungal isolates were described on different media, while the morphologies were observed using a light microscope. Eight fungal species from five genera were identified and included *Curvularia Trichoderma* spp. *Rhizopus* sp. *Fusarium* spp. *Oidiodendron* sp. These results indicated that the clinical wastes have a diversity of fungi which might possess health risk to humans if these fungi have not inactivated in the clinical wastes before the final disposal into the environment.

Keywords: Clinical wastes; *Curvularia* spp.; *Trichoderma* spp.; *Rhizopus* sp.; *Fusarium* spp.; *Oidiodendron* sp.; Fungi

1. Introduction

The high increases in the total populations and healthcare facilities have associated with production huge amounts of the medical wastes. In Malaysia, the total numbers of the medical wastes generated from the hospitals and clinics have increased to 18,055 tonnes in 2012 and expected to reach 33,000 tonnes per year by 2020 [1,2].

Healthcare wastes are general terms used to define the wastes generated from healthcare facilities. In some references these wastes are defined as clinical wastes, medical waste, bio-medical wastes, hospital wastes, hazards and bio-hazards wastes. These wastes contains blood or human body fluids as well as heavily infectious loads [3].

The presence of fungi in the clinical wastes are related to the high contents of organic matter as well as pH which support the fungal growth [4]. Among several fungal species isolated from the clinical wastes are *Fusarium* sp., *Mucor* sp., *Scopulariopsis* sp., *Paecilomyces* sp., *Aspergillus* spp. *Cladosporium* spp., *Penicillium* spp., *Basipetospora* sp., *Curvularia* sp. *Aureobasidium* sp., *Scytalidium* sp. and *Alternaria* sp. *Acremonium* spp. and *Alterneria* spp. [5-7].

A very few studies have been performed on the fungi from the clinical wastes in Malaysia. Noman et al. [8] found that *A. fumigatus*, *A. niger*, *T. harzianum* and *P. chrysosporium* were the most common [8]. However, more studies are required due to the high importance of these wastes on the human health and environment. Therefore, the present study aimed to investigate the presence of fungi in the clinical wastes to best understand the fungal load in these wastes.

2. Materials and Methods

2.1 Recovering and purifying of fungi

The medical waste samples were obatined from a Wellness Centre at Universiti Sains Malaysia (USM) during the period between

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January and Jun 2014. The collected samples included tissue papers, gloves, cotton, gauze, pasture pipette, needles, urine strips, kits, serum containers, blood wastes, ACCU-CHEK Safe-T-Pro Plus lancets, strips of glucose test lancets, microscopic slides, yellow tips, HB cuvettes and wood sticks. The fungi were recovered on Potato Dextrose Agar (PDA) medium using direct plate technique and purified based on the single spore technique [2]. For the fungi from air a new media was prepared and left in the storage room for 12 hours to allow for fungal spore to colonize the medium.

2.2. Fungal identification

Fungal isolates were identified based on their growth characteristics on the selective culture medium included; Czapek-Dox Agar (CZ); V8 juice agar (V8A); Malt Extract Agar (MEA); Czapek Yeast Extract Agar (CYA); Sabouraud dextrose agar (SDA) and Potato Dextrose Agar (PDA). The following references were used in the identification process Rifal [9], Ellis and Martin [10], Barnett and Hunter [11], Watanabe [12], and Samson et al. [13]. The colony size (diameter, mm), texture and surface of the fungal growth were recorded in the cultured media incubated for seven days at 28˚C [1]. The sporulation were also recorded based on the spores occurrence in the culture. The shape and size of fungal spores were determined under light microscope. The spore size of 25 spores was determined by using cell Sens imaging programme/software.

3. Results and Discussion

Eight fungal species from five genera were identified and included *Curvularia* spp. *Trichoderma* spp. *Rhizopus* sp. *Fusarium* spp. *Oidiodendron* sp. (Table 1).

Table 1 Fungal species isolated from clinical wastes

Genus	Species
Curvularia	*C. lunata*
	C. clavata
	C. brachyspira
Trichoderma	*T. viride*
	T.longibrachiatum
Rhizopus	*R. stolonifer*
Fusarium	*F. beomiforme*
Oidiodendron	*Oidiodendron* sp.

Fungal isolates belonging to *Trichoderma* spp., *Curvularia* spp., *Rhizopus* spp., *Fusarium* spp. and *Oidiodendron* sp., exhibited clear morphological characteristics based on their growth in the culture. Further characterization under light microscope confirmed the species (Table 2). It was noted that the fungal species exhibited different characteristics on the selective culture media. *C. clavata* occurred more sporulation than *C. brachyspira*. In contrast, *F. Beomiforme* showed more spores on V8A and PDA than other culture media used in the study. *T. Viride* produced spores on all culture media while *T. longibrachiatum* sporulation was detected on CZ and MEA. These differences indicated the role of culture media in the induction of fungal sporulation.

Table 2 Culture characteristics of *Curvularia* spp., *Fusarium* sp., *Oidiodendron* sp., *Rhizopus* sp. and *Trichoderma* spp. on different culture media after seven days at 28˚C

Fungus	Media type	Colony diameter (mm)	Colony character			Zonation (Margin)	Sporulation
			Source of isolation	Texture	Surface		
Curvularia brachyspira	V8A	31±3.4	Cotton and gloves wastes obtained from hematology section	thick floccose	white to light grey	grey	low
	CZ	34±4.1		amaranthine	grey	white	low
	CYA	25±5.2		thin floccose	grey	white	low
	MEA	41±5.3		amaranthine	grey	white	low
	PDA	25±3		thick floccose	light brown	white	low
	SDA	33±2.1		thick floccose	grey	white	low

C. clavata	V8A	65±3.4	Pester pipette and yellow tipswastes resulted from hematology Section	velvety/sulcate	dark green/grey	white to grey	moderate
	CZ	53.5±1.5		velvety	dark brown/black	dark brown/black	moderate
	CYA	53±6.2		velvety	black	grey	moderate
	MEA	70±2.9		velvety	black/grey	grey	moderate
	PDA	66±1.8		velvety	grey	beige	moderate
	SDA	72±1.3		velvety	black	grey	moderate
C. lunata	V8A	55±3.8	Cotton, gloves wastes Gauze, kits, pester pipette and urine strip wastes obtained from urine Section, Gloves wastes obtained from labeling Section	thick floccose	grey	greenish	moderate
	CZ	42±4.1		floccose	dark grey	grey	low
	CYA	49±6.7		floccose	dark grey/black	grey	moderate
	MEA	48±2.5		wrinkled	white to creamy	grey to greenish	moderate
	PDA	47±11		floccose	dark grey/black	grey	low
	SDA	54±1.5		floccose/ wrinkled edge	white	grey	low
Fusarium beomiforme	V8A	50±3.8	Glucose lancet wastes from hematology Section Tissue paper of emergency Section	lumbar growth	white to orange	white	high
	CZ	50±4		lumbar growth	white to yellowish	yellowish	moderate
	CYA	55±5.4		lumbar growth/radially edge	white	white	moderate
	MEA	55±6.2		lumbar growth/radially edge	white	white	moderate
	PDA	46±2.9		floccose	white/ orange	yellowish	high
	SDA	67±2.8		lumbar growth	orange	yellowish	moderate
Oidiodendron sp.	V8A	79±8	Air of the storage room	velvety	white to creamy	white	high
	CZ	12±2.4		velvety	white to transparent	white	low
	CYA	13±3.9		creamy growth	white	white	moderate
	MEA	64±7		velvety	light yellow	yellowish	high
	PDA	75±8.3		velvety	yellow/ creamy	white	high
	SDA	8.3±1.3		creamy	creamy	creamy	low
Rhizopus stolonifer	V8A	80±0.0	Tissue paper wastes from hematology and emergency Section Air of the storage room	floccose crisp	white grey	grey	high
	CZ	80±0.0		thin floccose	white	grey	low
	CYA	70±3.5		floccose crisp	grey/white	grey	high
	MEA	73±4.4		floccose	grey/white	white	high
	PDA	80±0.0		floccose	grey/white	black	high
	SDA	80±0.0		thick floccose	black	dark green	high
Trichoderma longibrachiatum	V8A	80±0.0	Wood stick wastes collected from hematology Section	floccose	yellow	white	Moderate
	CZ	62.6±3.8		granules	green	green	high
	CYA	70±2.5		radially/floccose	white	white	Moderate
	MEA	74.9±4.2		granules	green	green	high
	PDA	66.2±2.8		floccose	white to yellow	white yellow	Moderate
	SDA	80±0.0		velvety	white	white	low
T. viride	V8A	80±0.0	HB cuvette wastes resulted	amaranthine/floccose	Green/ yellowish	white	high

CZ	25±7.8	from hematology Section	granules	dark green	white	high
CYA	50±10		granules/thick mass	dark green	yellow	high
MEA	63±6.7	Sharps wastes from emergency Section	granules/thick mass	dark green	yellow	high
PDA	51±4.9		granules/thick mass	dark green	white	high
SDA	80±0.0		granules/thick mass	dark green	white	high

V8 juice agar (V8A); Czapek-Dox Agar (CZ); Czapek Yeast Extract Agar (CYA); Malt Extract Agar (MEA); Potato Dextrose Agar (PDA); Sabouraud dextrose agar (SDA)

In a view for the Microscopic morphology of fungal isolates (Table 3 and Fig. 1 and 2), it was noted that *C. lunata* has a large spores size (25.1 µm), while *C. clavata* has the smallest spore diameter s (12 µm). *T. longibrachiatum* has a large spore size (9 µm) than *T. viride* (4 µm) as determined under light microscope using cell Sens imaging programme/software.

Table 3 Microscopic morphology of *Curvularia* spp., *Trichoderma* spp., *Fusarium* sp., *Oidiodendron* sp. And *Rhizopus* sp. spores as observed using a light microscope with 100X of magnification

No	Fungal genus	Fungal species	Conidia diameter (µm)			Spore shape
			mean	max	min	
1	*Curvularia*	*C. brachyspira*	13.5	19	9	Curved shape with two-three septa
		C. clavata	9.3	12	6.5	Elongated shape with three septa
		C. lunata	19.7	25.1	11	Curved shape with three septa
2	*Trichoderma*	*T. longibrachiatum*	6.2	9	4.8	Ellipsoidal-sub-cylindrical with smooth surface
		T. viride	3.2	4	2.9	Globose to broadly ovoid with rough surface
3	*Fusarium*	*F. beomiforme*	4.9	9.5	1.9	Curved shape for macro-conidia with two-three septa and elongated shape of micro-conidia
4	*Oidiodendron*	*Oidiodendron* sp.	6.6	12.2	4.2	Globular, sub-globular, globose to broadly ovoid, some spores have lemon shape. All spores have smooth surface
5	*Rhizopus*	*R. stolonifer*	6.8	11.5	4.6	Vary in their shape ranged from globular to ovoid shape. The spore has thick well with filamentous structures on the surface.

Fig. 1 Microscopic morphology of *Curvularia spores, A) C. lunata* B) *C. brachyspira;* C) *C. clavata.* The morphology characteristics were determined using a light microscope with 100X of magnification.

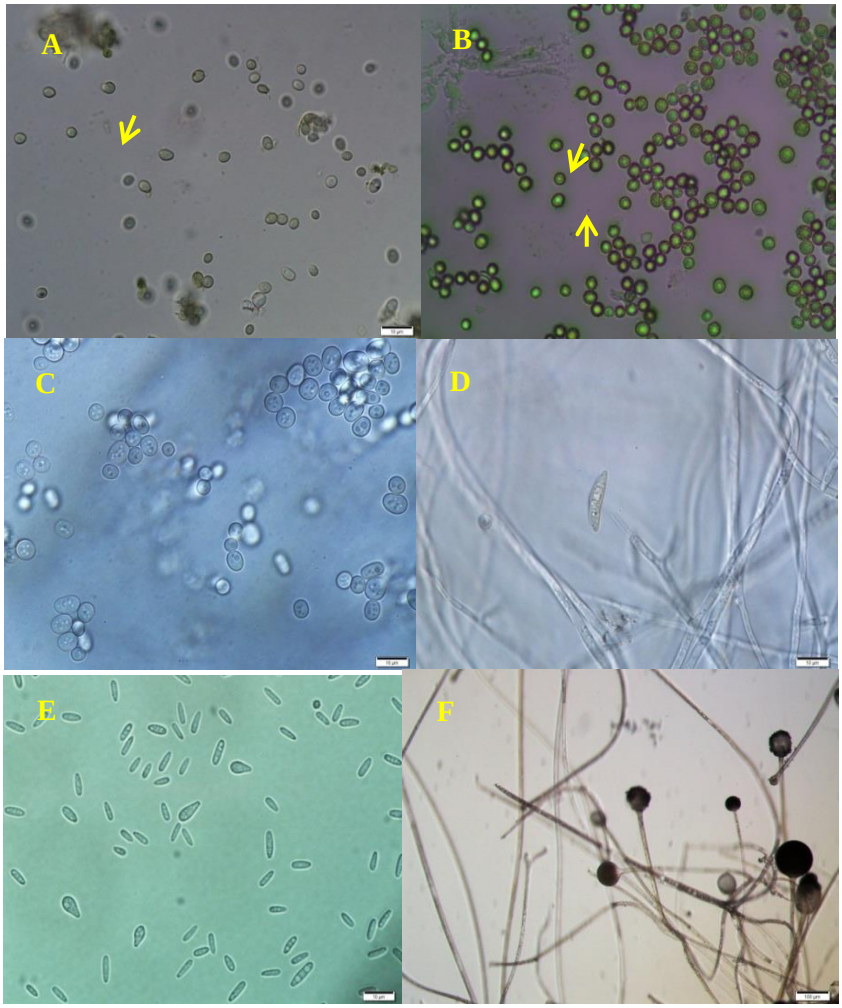

Fig. 2 Microscopic morphology of different fungal spores; A) *T. longibrachiatum* spores B) *T. viride; C) Oidiodendron* sp. *spores D) F. beomiforme* macro-conidia; E) *F. beomiforme* micro-conidia; *F) R. Stolonifer*. The morphology characteristics were determined using a light microscope at 100X magnification.

Pencillium marneffei, *Candida* spp., *Cryptococcus neoformans* are reported as the most common species as invasive fungal infections (IFIs) in Malaysia. Morever, *P. lilacinus*, *Fusarium* spp. and *Curvularia* spp., have also reported in the laboratory diagnostic process [14]. The presence of *Candida* spp. among 3837 of clinical specimens have been detected by Abdul-Rahman et al. [15] in a study conducted at Hospital of USM (2001 to 2006). *Candida* spp. has reported as a predominant species in blood culture specimens, urine specimens and genital specimens. The present study revealed different species of the fungi available in the medical wastes, which should be considered before their disposal into the environment. The health risk concerns related clinical wastes lie in the potential of the pathogens for the regrowth or persistence and then their transmission into the food chain [16]. It has to mention that the fungal species obtained here are those have the ability to grow in the culture medium, while non-culturable fungi are not investigated in the study.

4. Conclusion

Healthcare wastes contain different species of the fungi and thus represent a biohazards wastes with adverse effects on the human and environmental health. This work revealed a potential health risk for the clinical wastes and suggested that these wastes should be managed safely to prevent the distribution of infectious agents.

Acknowledgements

The authors gratefully acknowledge the research project financial support under FRGS 1574. The authors wish also to thank the Ministry of Science Technology and Innovation (MOSTI) for supporting this research under Science Fund vot S029 and also the Office of the Research, Innovation and Commercialization Centre (ORICC) UTHM for providing grant U682 for this research.

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