# Isolation and identification of pathogenic important fungi from the moth fly *Clogmia albipunctata* (Diptera: *Psychodinae*) in teaching hospitals, Babol, Iran.

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**Abstract**

**Some arthropods, such as the house fly, cockroach and moth flies, could act as mechanical vectors for pathogenic agents. The objective of this study was identification of pathogenic fungi on the cuticular surface and alimentary tract of the moth fly collected from four teaching hospitals in Babol, north of Iran. Flies were collected in different areas of four teaching hospitals. Alive Adult flies were immediately transported to the entomology and mycology laboratory. All Flies were recognized based on the taxonomic keys. Fungi isolates were identified on the sabouraud dextrose petri dish according to the standard microbiological tests. All moth flies were identified as *Clogmia albipunctata* (Diptera: *Psychodinae*). Totally, 242 samples were cultured from 146 adult moth flies. Seven genus of fungi were identified. 51.12%, 28.09% and 7.30% of fungi isolates were related to the *Aspergillus* spp, *Cladosporium* spp and *Penicillium* spp, respectively. The present study has shown that moth fly carries pathogenic fungi in the hospital environments. Therefore, control of C. *albipunctata* in these places is essential for prevention of fungal infections.**

**Keywords**: Moth fly, *Clogmia albipunctata*, Fungi, Hospital environments.

*Accepted on November 09, 2020*

## Introduction

The existence of continuous insect infestations of health services and hospital poses is a possible health threat because of their potential role in transmission of Healthcare-associated infections (HCAIs). *Clogmia albipunctata* belong to the family of *Psychodidae*, which are non-biting moth flies recognized as sewerage, and washroom or filter flies [1]. They are worldwide distribution between latitudes 42°N and 40°S. The adult flies are frequently seen in humid places, particularly bathrooms and toilets [2]. The clinical role of C. *albipunctata* is low significance, but, have been involved in inhalant allergy in many cases in South Africa [3]. So, its act as a mechanical pathogen vectors for HCAIs [4]. Certain human pathogens could be lived inside and/or outside of the insect body [5]. There are several human pathogens such as yeast and filamentous fungi, which cause invasive and non-invasive infections, on the insects. The relation of pathogenic fungi and insects has been confirmed by several studies [6-10]. Faulde, showed that the moth fly C. *albipunctata* is among the medically important insects in hospital environments that cause serious public health problems. They have been found to harbor a number of pathogenic, potentially pathogenic and opportunistic microorganisms which were carried either on the cuticle or in the gut [10]. A rare data is available about the role of moth flies to transmission of pathogenic fungi in hospitals in Iran. So, the aim of the current study was the isolation of pathogenic important fungi in the hospital environment which transmitted by moth fly C. *albipunctata*.

## Methods

### Collection of C. *albipunctata*

This study was performed in the several teaching hospitals in the Babol, north of Iran, in a period of 5-months from

May 2016 to September 2017. A total of, 146 moth flies samples were collected randomly from different sites of teaching hospitals. Then, each fly was placed in a sterile tube separately and samples alive transferred to the entomology and mycology laboratories of Babol university of medical sciences. The moth fles were identified at species level using taxonomic keys [11,12].

## Fungi Isolation

All C. *albipunctata* were placed separately in a sterile tube and anesthetized by freezing at 0°C for 5 min. In order to rinse down fungi colonizing at the cuticular surface, 2 ml of phosphate-buffered saline (PBS; pH=7.0) was added and vortexed for 20 s. To further characterize the fungi flora colonizing the alimentary tract, each C. *albipunctata* was placed in a new sterile tube containing 70% alcohol (to decontaminate external surfaces) for 2 min, and allowed to dry at 25°C under sterile condition. The moth flies were then washed in PBS for 4–5 min to eliminate traces of alcohol. The alimentary tract of the adult files was suspended in PBS and these solutions were centrifuged at 2000 rpm for 5 minutes. 0.1 ml of the internal and external solutions were harvested and transferred to the sabouraud dextrose agar medium supplemented with chloramphenicol (SC) (Merck, Darmstadt, Germany) [13,14]. The plates were incubated at 25°C and 37°C for at least 4 weeks. The fungal colonies were identified by macroscopic and microscopic criteria. Methylene blue staining was performed in order to direct examinations of colonies.

## Results

In this cross-sectional study, 146 moth flies were collected and all of them identified as C. *albipunctata* (Insecta: Diptera: *Psychodinae*). Totally, 242 samples including, 146 external and 96 internal specimens were obtained. 73.6% (n=178) of samples were positive for fungi culture. The frequency of fungi were 68.8% (n=66) and 76.7 % (n=112) of internal and external samples, respectively (Table 1). As shown in table 2, the prevalence of fungi from alimentary tract and cuticle surface was 82.1% (n=87), 71.9% (n=41), 74.3% (n=26) and 54.5% (n=24) in Rohani, Shahid Yahyanejad, Shahid Rajaee and Shahid Beheshti hospitals, respectively. Seven fungi genus were obtained from cuticular surface and alimentary tract. The prevalence of isolated fungi was as follows: *Aspergillus* spp, (n=91, 51.12%), *Cladosporium* spp, (n=50, 28.09%) and *Penicillium* spp, (n=13, 7.30%) (Table 3).

***Table 1:*** *The prevalence of fungi at the internal and external samples of C. albipunctata.*

| Criteria | Internal | External | Total |
|---|---|---|---|
| Positive | 66 (68.8%) | 112 (76.7%) | 178 (73.6%) |
| Negative | 30 (31.2%) | 34 (23.3%) | 64 (26.4%) |
| Total | 96 (100%) | 146 (100%) | 242 (100%) |

***Table 2:*** *The prevalence of fungi isolated from C.albipunctata in four hospitals.*

| Hospital name | Positive or negative | Internal | External | Total |
|---|---|---|---|---|
| Rohani | Positive | 25(78.1%) | 62(83.8%) | 87(82.1%) |
| | Negative | 7(21.9%) | 12(16.2%) | 19(17.9%) |
| | Total | 32(100%) | 74(100%) | 106(100%) |
| Shahid Yahyanejad | Positive | 19(70.4%) | 22(73.3%) | 41(71.9%) |
| | Negative | 8(29.6%) | 8(26.7%) | 16(28.1%) |
| | Total | 27(100%) | 30(100%) | 57(100%) |
| Shahid Rajaee | Positive | 6(40%) | 20(100%) | 26(74.3%) |
| | Negative | 9(60%) | 0 (0%) | 9(25.7%) |
| | Total | 15(100%) | 20(100%) | 35(100%) |
| Shahid beheshtic | Positive | 16(72.7%) | 8(36.4%) | 24(54.5%) |
| | Negative | 6(27.3%) | 14(63.6%) | 20(45.5%) |
| | Total | 22(100%) | 22(100%) | 44(100%) |
| Total | Positive | 66(68.8%) | 112(76.7%) | 178(73.6%) |
| | Negative | 30(31.2%) | 34(23.3%) | 64(26.4%) |
| | Total | 96(100%) | 146(100%) | 242(100%) |

***Table 3:*** *Fungi Isolation from the C. albipunctata sample.*

| Fungi | Internal | External | Total |
|---|---|---|---|
| *Aspergillus spp* | 56(84.85%) | 35(31.25%) | 91(51.12%) |
| *Cladosporium spp* | 4(6.05%) | 46(41.07%) | 50(28.09%) |
| *Penicillium spp* | 0 (0%) | 13(11.61%) | 13(7.30%) |
| *Fusarium spp* | 1(1.5%) | 9(8.04%) | 10(5.62%) |
| *Mucor spp* | 0 (0%) | 4(3.57%) | 4(2.25%) |
| *Nigrspora spp* | 0 (0%) | 1(0.89%) | 1(0.56%) |
| *Mold* | 0 (0%) | 3(2.68%) | 3(1.69%) |
| *Candida spp* | 5(7.6%) | 1(0. 89%) | 6(3.37%) |
| Total | 66 (100%) | 112 (100%) | 178 (100%) |

## Discussion

Potentially pathogenic fungi can be transmitted by hospital care workers or medical devices to patient in hospitals [15]. However, vectors such as insect can carry human pathogenic fungi in environments such as hospitals [16]. Several studies showed that fungi such as *Candida* spp, *Aspergillus* spp, and *Penicillium* spp, have been isolated from external surface of insect vectors in hospital [8,16,17]. C. *albipunctata* is a potential mechanical vector of bacterial human pathogens related to the hospital acquired infections [4]. In addition, C. *albipunctata* has been described to harbour *Nocardia* spp, in a hospital of Brazil, and to rarely cause of intestinal and nasopharyngeal myiasis [18-20]. C. *albipunctata* had a cuticle covers with a highly density of hair that results in hydrophobic properties that permit the fly to escape from water even after submerging or shaking, and in some fungi, cell-wall-specific composition hydrophobicity indexes may play an significant role for this affinity [4,21]. However, its likely role in mechanical transition of human fungal pathogens, particularly in hospital environments, is not clear. In the present study, all 73.6% (n=178) moth flies were found to

carry the 7 genera of medically important fungi. Saichua, (2008) reported that 13 genera of medically important fungi in forty five cockroaches were caught from Thammasat Chalermprakiat Hospital, Thailand [16].

Our data showed that the prevalence of *Aspergillus* spp, was significantly higher than other genus which is agreement with Davari, [13] but disagree with Saichua [16]. This conflict may be related to the geographical distance (Thailand compared to Iran), host (cockroaches compared to moth fly), climates and level of hygiene. The opportunistic fungal infection due to *Aspergillus* spp, is a serious invasive hospital infection [22]. In comparison with this study, Okafor, showed a higher prevalence rate of *Candida* spp, (C. *tropicalis* and C. *parapsilosis*) in the intestinal tracts of cockroaches [23]. This disagreement may be related to the diversity of the host microbial flora (cockroaches compared to moth fly) and ecology (such as, habitat, diet). So, the filamentous fungi including, *Penicillium* spp, *Cladosporium* spp, *Fusarium* spp., and *Mucor* spp, were identified from the C. *albipunctata* samples. Infections caused by filamentous fungi is a serious concern, because its can occurred in the solid organ transplants, uncontrolled diabetes, and malignancies such as lymphomas and leukemias patients [24]. Our results indicated that isolation of these fungi from insects such as C. *albipunctata* in a hospital environment is a alarming, particularly immunocompromised patients.

## Conclusion

C. *albipunctata* is a potential mechanical vector for opportunistic fungi associated with nosocomial infections. Thus, eliminate their breeding places in the hospital environment is an important in order to infection prevention and control.

## Acknowledgment

This study was approved by the Ethics Committee of Babol University of Medical Sciences (No. 9542832).

## Author contribution

MK, RR and SMO managed the project, performed the isolation and identification of the flies and fungi, analyzed the data, and wrote the first draft of the manuscript AH and JA participation in the collection of specimens M T A and A P contributed in editing the article.

## Conflicts of interest

None declared.

## Financial disclosure

The authors declare no financial interests related to the materials of this study.

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