



ORIGINAL ARTICLE

Isolation of medically important fungi from cockroaches trapped at hospitals of Sari, Iran

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ABSTRACT

*This study was done to evaluate the presence of medical important fungi on the external surface of cockroaches collected from the public and residential areas of three hospitals of Sari, Iran. A total of 38 cockroaches were caught from staff resting rooms / working areas, the ward floors and patient rooms, during two consecutive days at three hospitals between December and November, 2013. The frequency of *Blattellagermanica* and *Periplaneta Americana* among 38 trapped cockroaches were 84.2 and 15.8 percent, respectively. *Candida* spp. was the most yeast isolated (94.7%) on external surfaces of cockroaches and *Rhodotrula* spp. (57.9%) was the following. Also, *Aspergillus* spp. (84.2%), *Fusarium* spp. (15.8%), *Penicillium* spp. (10.6%) and *Geotrichum* spp. (10.6%) were the most molds appeared on external surfaces of cockroaches. Among 36 (94.7%) cockroaches, 4 species of *Candida* were identified by mycological examinations. *C. glabrata* (52.8%) and *C. albicans* (38.8%) were the highest species isolated from cockroaches. *A. niger* (50%) was the most species that was isolated from cockroaches. In conclusion, cockroaches are vectors of microbial agents such as fungi, yeast, etc than can cause nosocomial infection. Thus, public centers such as hospitals should have definite plan to combat with these pest insects.*

Key Words: Fungi, Cockroach, hospital, nosocomial infection

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INTRODUCTION

A hospital-acquired infection or nosocomial infection (NI) is an infection acquired by a patient who receives care a disease in a hospital. Some infections including fungal and bacterial infections are worsening by the reduced resistance of individual patients [1]. The process of infection is like a chain and each factor can be considered as one of its links. One of the links or ways to defeat hospital infections is to destroy the reservoirs of organism (for instance, body tissues and the wastes of humans, contaminated food and water, and insects). As microorganisms in hospitals can develop a resistance to antibiotics, they make the infection more difficult and expensive to treat. So a NI may be more dangerous than other infections [2]. Infection can be transmitted by direct or indirect contact with vectors. Some vectors in hospital may be insects such as flies, mosquitoes and cockroaches capable of harboring and spreading the infectious (3 & 5). Cockroaches can be found in a wide range of environments around the world and they are one of the most commonly noted household pest insects. Cockroaches are insects of the order Blattaria or Blattodea, of which about 30 species out of them are joined in human habitats. Among them

are the American cockroach, *Periplaneta americana*, which is about 30 mm (1.2 in) long and the German cockroach, *Blattella germanica*, about 15 mm (0.59 in) long [6, 7]. They feed on human and pet food and can leave an offensive odor (8). Cockroaches may be found inside wards, kitchens, medicine stores and patient rooms in hospitals [9, 11]. They can transport microbes on their body surfaces which include the infection that are potentially dangerous to humans, particularly in hospitals environments [9, 10]. They can carry organisms such as virus, bacteria, parasites and fungi in hospitals. Certain fungi have the capacity to spread through insect carriers such as flies, mites and cockroaches [12, 14]. It makes them ideal carriers for transmitting several medically important fungi and may be factors to spread nosocomial fungal infection. *Candida spp.*, *Aspergillus spp.* and other species of fungi have been isolated from cockroaches recovered in several health care sectors of the hospitals [13, 15]. *Candida spp.* is the fourth most common organism recovered from cultures of blood from hospitalized patients (16). *Aspergillo*sis is common in selected populations, such as patients with lung disorders and bone marrow transplant recipients [17, 19]. *Aspergillus spp.* has been isolated in 36% of these patients and it caused high mortality rates among them [20, 21]. Although some researchers worked on spread of pathogenic bacteria by cockroaches in Iran [22,23], information on the carriage of pathogenic fungi is scanty. In this study, we evaluated the presence of medical important fungi on the external surface of cockroaches collected from the public and residential areas of three hospitals of Sari, Iran in 2013.

MATERIALS AND METHODS

Samples collection: The study is descriptive laboratory research. At the onset of the investigation, different parts of three educational hospitals of Sari (Mazandaran Province, Iran) were evaluated for the presence of cockroaches. The cockroaches were caught from staff resting rooms / working areas, the ward floors and patient rooms, during two consecutive days at three hospitals between December and November, 2013. Thirty eight cockroaches (*X Periplaneta americana* and *Y Blattella germanica*) were trapped and collected in sterile test tubes and transported to the Mycology Laboratory of Faculty of Medicine, Mazandaran University of Medical Sciences, for mycological studies. Diagnosis was done by the modulus taxonomic keys. The cockroaches were immobilized by fridgidity at 0°C for 5 minutes.

Isolation and identification of medically important fungi from external surfaces of cockroaches: Each cockroach was washed in 5 ml of sterile normal saline (0/9%) in a test tube by shaking thoroughly for 2 minutes and then the liquid was transferred to a secondary sterile tube. 1 ml of the washing liquid was cultured on Sabouraud's dextrose agar with 0.05% chloramphenicol and incubated at 30°C for 3 weeks. The various filamentous and yeast colonies were identified by macroscopic and microscopic examinations according to medical mycology text books [24-26]. Yeast were identified by germ tube test, the presence of chlamydoconidia on Corn meal plus Tween 80 agar (Oxoid, United Kingdom) and color of colony on CHROM agar *Candida* (France).

Statistical analysis

A comparison of isolation rates and fungal species from external surface of trapped cockroaches and the difference between different wards of hospitals were made by using t-test. Level of significance was set at $P < 0.05$.

RESULTS

A total of 38 cockroaches that were caught from different places at 3 hospitals is shown in Table 1. The cockroaches commonly were caught from department of nutrition and toilets located in the admission and medical records office. Out of 3 hospitals only 3 cockroaches were trapped from emergency, radiology and corridor wards (1 cockroach from each ward). We could not trap cockroaches in medicine, surgical, pediatrics and clinical pathology wards in the hospitals. However, in patient rooms, only 1 American cockroach was trapped from medical intensive care unit (ICU) and 1 German cockroach was caught from emergency ward. Among 38 trapped cockroaches, 32 (84.2%) were *Blattella germanica* and 6 (15.8 %) were *Periplaneta americana*. Although several stages of *B. germanica* were caught from three hospitals, nymphs showed the higher infestation rate in all departments surveyed in 3 hospitals. All samples (100%) carried one or more species of medically important yeast on their external surfaces and 32 (84.2%) had one or more species of medically important mold on external surfaces. In this study, *Candida spp.* was the most yeast isolated (94.7%) on external surfaces of cockroaches and *Rhodotrula spp.* (57.9%) was the next. *Aspergillus spp.* (84.2%), *Fusarium spp.* (15.8%), *Penicillium spp.* (10.6%) and *Geotrichum spp.* (10.6%) were the most molds appeared on external surfaces of cockroaches. Other medically important mold, *Alternaria spp.*, *Cladosporium spp.*, *Trichoderma spp.*, *Mucor spp.* and *Chrysosporium spp.* were rarely isolated from a few cockroaches (Table 2). Among 36 (94.7%) cockroaches, 4 species of *Candida* were identified by mycological examinations. *C. glabrata* (52.8%) and *C. albicans* (38.8%) were the highest species isolated from cockroaches. *C. parapsilosis*, and *C.*

guilliermondii were found on the external surfaces in a few cockroaches. The percentage of isolated *Candida* species is showed in Fig. 1. Also, among 26 (68.4%) cockroaches, 3 species of *Aspergillus* were identified. *A. niger* (50%) was the most species that was isolated from cockroaches. *A. fumigatus* and *A. flavus* were found on external surface in a few ones. A total of 6 samples were found to carry two species of *Aspergillus* on their external surfaces. The frequency of colonies of *Aspergillus* species was shown in Fig. 2.

DISCUSSION

The presence of cockroaches is considered as a health problem in hospitals. As these insects move freely from areas within and around hospitals and they may harbor pathogenic organisms, they can be considered as factors for nosocomial infection. In recent years, a number of studies have been carried out to determine the population dynamic of cockroaches in some hospitals and apartments, e.g. in Egypt [27], Iran [22, 23, 28, 29], Turkey [30] and Korea [31, 32]. These studies revealed that cockroaches successfully can found in different wards of hospitals, particularly, where food or waste materials can be found easily and the temperature and humidity are suitable, such as department of nutrition and toilets. The results of the present study showed that two cockroach species inhabit in three hospitals. Thus, *B. germanica* was the predominant cockroach species that were caught in hospitals. This result was similar to some previous studies [31, 32]. This species is the most common worldwide pest species due to small size, nutritional habits and specific behavior [33]. Our results showed that the most cockroaches were caught from the department of nutrition and admissions and medical records office toilets. These results were similar to a number of studies [23, 30, 31] except the study done by Dong-Kyu [32], who caught the highest rates of German cockroaches from patient rooms. Although the population density of cockroaches in hospital correlated with pest control program [32], *B. germanica* still has a high population density in hospitals. It is necessary to continue work on effective insecticides to control for this species. This study confirmed that cockroaches in different wards of hospitals were contaminated with known fungal pathogens. Although the direct involvement of these insects in disease transmission was not investigated in this study, the isolation of medically important fungi including *Candida* spp, and *Aspergillus* spp. shows a serious concern for possible nosocomial transmission. Also, we concluded in our study that the common nosocomial pathogens may well survive or persist on surfaces of cockroaches and can thereby be a continuous source of transmission if no regular prevention disinfection of hospital environment is performed. Some researchers [22, 23, 34, 35] noted that the cockroaches were involved in the transmission of pathogens in health care environments.

Overall, 2 yeast and 9 filamentous species of fungi which are known to cause of nosocomial fungal infections were isolated from the cockroach specimens. The average of 100% isolation of yeast and 84.2% mold from external surface of trapped cockroaches indicated that an important concern about cockroach problems should be raised. No significant difference was found between the percentages of *B. germanica* and *P. americana* carrying medically important fungi ($P > 0.05$).

The results from this study about medically important fungi isolated from external surface of cockroaches in hospitals are agreed with the findings of some workers [34, 36]. Several studies had previously reported that some of these fungal species were isolated from cockroaches [22-23, 30, 35]. Nosocomial fungal infections are considered important causes of morbidity in immune compromised patients, particularly in those who have stayed in hospital for a long time [37]. The overall burden of disease caused by nosocomial fungal infections is substantial. Exposure with medically important fungi within the hospital environment may cause outbreaks of nosocomial mycosis. *Candida* spp. and *Aspergillus* spp. were the most frequently fungi causing serious health care-associated infections, especially in patients admitted to intensive care units (ICUs) [38, 40]. *Candida* spp. and *Rhodotorula* were yeast isolated from both cockroach species. Certain *Candida* spp, especially *Candida albicans* are a part of the human microbial flora. However these yeasts may spread from the health care environment [41]. Among *Candida* species, *C. albicans* the most commonly isolated and responsible for the majority of nosocomial infections. However, many non-*albicans* species, such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. guilliermondii* have recently emerged as important pathogens in suitably debilitated individuals [42 & 43]. *C. glabrata* seems to be more frequently isolated from older patients [44], patients with cancer [45] and patients treated with vancomycin [46]. *C. parapsilosis* has emerged as an important cause of candidemia in the neonatal population [47-48] and transplant recipients [49]. *C. parapsilosis* is the most common *Candida* spp isolated from the hands of health care workers [50]. Emerging *Candida* spp that are relatively resistant to fluconazole, such as *C. guilliermondii* [51] has also been associated with nosocomial outbreaks, such as in patient with intravascular catheters. *Rhodotorula* species have been reported as nosocomial endophthalmitis and meningitis, especially in human immunodeficiency virus- (HIV) infected persons [52-54]. At present, there is no uniform definition of what constitutes nosocomial mold infection

[55]. One definition that is frequently used is considering nosocomial mold infection as invasive mold fungal disease that occurs after 1 week of hospitalization or within 2 weeks of hospital discharge [56]. Although most hospital outbreaks mold infection has been caused by *Aspergillus* spp, other molds that isolated from external surfaces of cockroaches have also been implicated in nosocomial infection. *Aspergillus fumigatus* is the species most often associated with disease [57, 58], although other species, including *Aspergillus flavus* [59 & 60] and *Aspergillus niger* [61, 62] have also been isolated from patients with invasive disease. However, many other mold species isolated from external surface of cockroaches, such as *Fusarium* spp. [63, 64], *Penicillium* spp. [65, 66], *Geotrichum* spp. [67, 68], *Alternaria* spp. [69, 70], *Cladosporium* spp. [71, 72], *Trichoderma* spp. [73, 74], *Mucor* spp. [75] and *Chrysosporium* spp. [76] have recently emerged as important pathogens in suitably debilitated individuals.

Table 1. The number of cockroaches in different place at 3 hospitals of Sari

Place	Staff resting room/working area			Patient room		
	No. of cockroach	No. of cockroach	No. of cockroach	No. of cockroach	No. of cockroach	No. of cockroach
		positive mold	positive yeast		positive mold	positive yeast
Emergency ward	1	1	1	1	1	1
Medicine ward	0	0	0	0	0	0
Surgical ward	0	0	0	0	0	0
Pediatrics ward	0	0	0	0	0	0
Comidor ward	1	0	1	0	0	0
Medical Intensive care unit ward	0	0	0	1	0	1
Department of Nutrition*	20	17	20	-	-	-
Clinical Pathology ward*	0	0	0	-	-	-
Radiology ward*	1	1	1	-	-	-
Admissions and medical records office *	13	9	13	-	-	-
Total	36	28	36	2	2	2

* Have not patient room.

Table 2. The medically important fungi from the external surfaces in each cockroach

Fungi	<i>B. germanica</i>	<i>P. Americana</i>	Total
	(n=32)	(n=6)	(n=38)
	No. (%)	No. (%)	No. (%)
Yeast			
<i>Candida</i> spp.	30 (93.7)	6 (100)	36 (94.7)
<i>Rhodotrula</i> spp.	18 (56.4)	4 (66.7)	22 (57.9)
Mold			
<i>Aspergillus</i> spp.	26 (81.3)	6 (100)	32 (84.2)
<i>Penicillium</i> spp.	4 (12.5)	0 (0)	4 (10.6)
<i>Fusarium</i> spp.	6 (18.7)	0 (0)	6 (15.8)
<i>Geotrichum</i> spp.	3 (9.4)	1 (16.7)	4 (10.6)
<i>Alternaria</i> spp.	3 (9.4)	0 (0)	3 (7.9)
<i>Cladosporium</i> spp.	3 (9.4)	0 (0)	3 (7.9)
<i>Trichoderma</i> spp.	0 (0)	2 (33.3)	2 (5.3)
<i>Mucor</i> spp.	2 (6.2)	0 (0)	2 (5.3)
<i>Chrysosporium</i> spp.	1 (3.1)	0 (0)	1 (2.6)

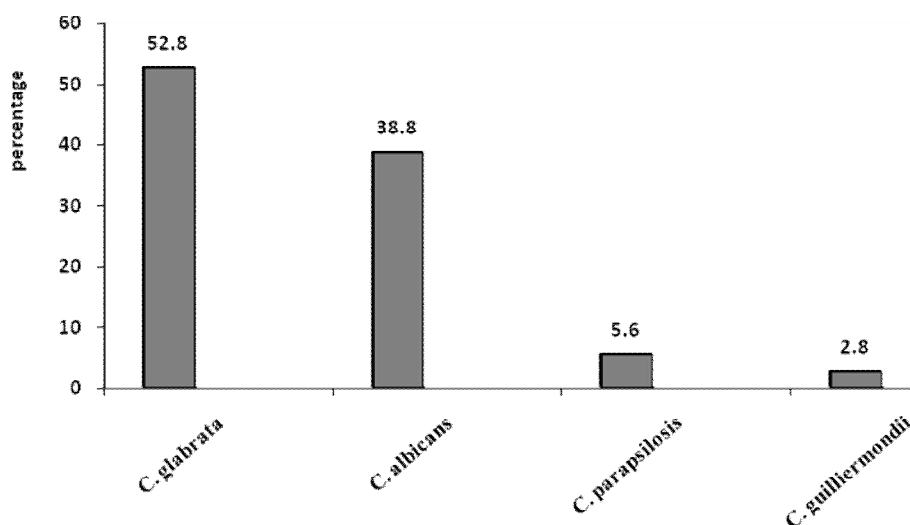


Figure 1. The percentage of *Candida* species isolated from the external surfaces of 36 cockroaches

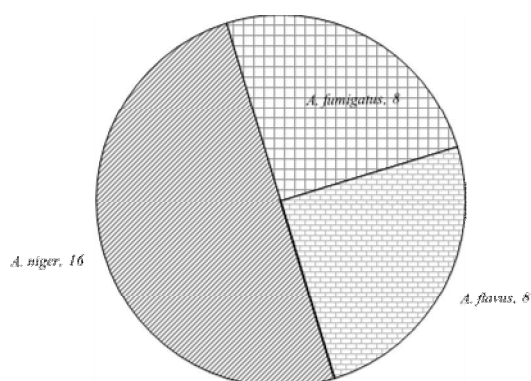


Figure 2. The frequency of colonies of *Aspergillus* species on the external surfaces of 26 cockroaches

CONCLUSION

Cockroaches interfere in the transmission of pathogenic agents to humans and these insects often carry microorganisms that are important in nosocomial infections. Efforts should be made to ensure effective infection control practices. Thus, surfaces in hospitals should be cleaned and disinfected regularly to remove cockroaches and fungi from the hospital environments. This finding suggests the probability that almost all cockroaches in health and medical service centers can carry medically important fungi and biosecurity plans has priority in these centers.

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