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Isolation and Identification of Psychotropic Fungal Contamination in Food Storage Refrigerators in Ahvaz City Restaurants

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Abstract

Background: Food-borne pathogens are one of the most important problems in less developed and developed countries. Commercial refrigerators are a potential source of fungal contamination that causes food spoilage, food-borne intestinal infectious diseases or mycotoxin diseases.
Objectives: The purpose of this study was to determine the distribution of psychotropic fungi in food storage refrigerators (n = 50) with temperatures above and below 0°C at selected restaurants (n = 25) located in the city of Ahvaz, Iran.
Methods: Samples were collected from the surfaces of the refrigerators by using sterile swab sticks pre-moistened with sterile distilled water, and then each collected sample was plated out on Sabouraud dextrose agar medium. Finally, all fungi were counted and identified based on macroscopic and microscopic characteristics.
Results: According to our results, 100% of the sample refrigerators showed fungal contamination. *Cladosporium sp.* (42.34%) and *Mucor sp.* (0.06%) had the highest and the lowest frequency of fungi recovered from our research, respectively. Furthermore, the P value calculated indicated a significant correlation between refrigerators above 0°C and refrigerators below 0°C ($P < 0.00001$).
Conclusions: The presence of fungi in commercial refrigerators could be an indicator of a potential source of food spoilage and food-borne diseases. Therefore increased education, and proper packaging and refrigerator management are recommended since regular cleaning of these refrigerators is important in order to prevent food-borne diseases and other health risks.

Keywords: Fungi, Ahvaz, Commercial Refrigerators, Food-Borne Diseases

1. Background

Every year, millions of people in the world suffer from health problems associated with the consumption of contaminated food, and this is one of the health problems of today (1). Many types of microorganisms can be found in food, including gram-negative bacteria, gram-positive bacteria, and fungi (yeasts and molds). Fungi are one of the most important causes of food degradation and corruption. With growth and creation of mycelium, these microorganisms damage the texture and appearance of food, and by producing different toxins, they endanger the consumer's health (2, 3). Filamentous fungi produce spores in an unfavorable condition to resist drought and cold. These spores can be dispersed in the refrigerators as the main source of storage space of food items, and then as soon as conditions are favorable (temperature and humidity), they quickly transform from the spore state and convert to the active form. Yeasts typically break down sugars to carbon dioxide and alcohol. Some yeasts can degrade and change food and thus reduce the quality of the food (4, 5). Spores of fungi can be transmitted in various ways, such as air, utensils, improper packaging, hands, and contaminated food to the refrigerator. The previous studies reported that the filamentous fungi isolated from food belonged mainly to the genera *Alternaria, Aspergillus, Botrytis, Cladosporium, Fusarium, Geotrichum, Aureobasidium, Trichothecium, Mortierella, Mucor, Neurospora, Penicillium, Rhizopus, Thamnidium*, and *Manoscus*. Among the yeast genera involved are *Candida, Cryptococcus, Rhodotorula, Schizosaccharomyces*, and *Trichosporon*. Nevertheless, these fungi are most often found on meat and poultry, but they can also be found in many other food items (6, 7).

Although the cold led to a reduction of the growth of fungi, a number of fungal agents are able to grow at low temperatures, even below -10°C, in the refrigerator. These fungi can be the cause of the corruption of a wide range of food ingredients in refrigerators (8) Therefore, identification of types and the presence of fungal agents at various surfaces of refrigerators especially those that are pathogenic to humans is an important part of any hygiene monitoring program (9). Moreover, this study was undertaken to isolate and identify psychotropic fungal contamination in food storage refrigerators in Ahvaz city restaurants.

2. Objectives

The present study aimed to investigate the fungal type and burden in commercial food-storage refrigerators (meat and poultry products, appetizers, dairy products, vegetables, and fruit) with temperatures above and below 0°C using a swab from different surfaces of refrigerators in Ahvaz city restaurants.

3. Methods

3.1. Study Area, Sample Collection and Culture Media

The study was conducted in the city of Ahvaz. The Ahvaz area is located in southwestern Iran, which has a subtropical hot desert climate with many sandstorms and dust storms common during the summer period. Fifty samples were obtained from 50 commercial refrigerators (25 with a temperature above 0°C and 25 with a temperature below 0°C) from 25 restaurants located in eastern and western regions of Ahwaz city during the period from April to July 2017. To achieve normalization of colony counts, standard surface sampling was done with a selected area of 25×10 cm from the surface of the refrigerators using sterile swab sticks pre-moistened with sterile distilled water. All swabs were aseptically inoculated and rotationally cultivated on the entire surface of Sabouraud Dextrose Agar (SDA) media (10). Moreover, we used 9-cm diameter Petri dishes to normalize and standardize the results to CFU m^{-2} for the count (11).

3.2. Fungal Identification and Counting

All Petri dishes were incubated at 25°C for 7 - 10 days and checked daily for fungal growth. The number of cultured colonies on SDA was counted and reported as colony-forming units per square meter (CFU m^{-2}). Fungal isolates were identified at the genus and/or species level according to their culture (colony's appearance including color and texture) and morphological features such as sporulation apparatus and conidium. Slide cultures to confirm some fungi were prepared, and finally, they were identified by referring to mycological atlases (10, 12).

3.3. Statistical Analysis

All data such as frequency and mean were analyzed using SPSS statistical software (version 22), and also to test significance, we used chi-square (χ^2) statistical test, and differences were considered significant at $P < 0.05$.

4. Results

In this research, different fungi were isolated from commercial refrigerators. Fungi were present in all refrigerators. Specifically, 1313 fungal colonies (1139 mold and 174 yeast) were isolated from all refrigerators. Mold fungi included 571 dematiaceous fungi and 742 hyaline hyphomycetes. A total of thirteen fungal species belonging to ten different genera were isolated, identified. Finally, fungi recovered in this study were reported as colony-forming units per square meter and frequencies which are revealed in detail in Table 1 for both groups of commercial refrigerators above 0°C and below 0°C. Genera of fungi isolated in descending order of frequency (percent) including *Cladosporium specie (sp.)* (42.34), *Penicillium sp.* (41.50), *Schizosaccharomyces sp.* (12.10), *Trichosporon sp.* (1.14), *Aspergillus sp.* (0.91), *Alternaria sp.* (0.83), *Rhizopus sp.* (0.60), *Botrytis sp.* (0.30), *Fusarium sp.* (0.15) and *Mucor sp.* (0.07). Among the isolated fungi, only a genus of *Cladosporium, Penicillium*, and *Schizosaccharomyces* from both groups of commercial refrigerators was isolated (Figure 1).

In the present study, refrigerators above 0°C had the highest mean value of fungi (805944 CFU m^{-2}) and the lowest mean value was found in refrigerators below 0°C (325264 CFU m^{-2}) (Table 2) There was a significant correlation between refrigerators above 0°C and refrigerators below 0°C ($P < 0.00001$).

5. Discussion

Food-borne diseases and food poisoning are a global problem. Food spoilage by microorganisms can be reduced by storage at a low temperature (chilling, freezing) (13). Low temperatures are used to minimize the chemical and enzymatic activity in order to slow or stop the growth and activity of microorganisms. However, some psychrotrophic fungi can grow at low temperatures (14).

Frozen food can increase the risk of food-borne disease outbreaks. Restaurants are the place where the highest rates of a food-borne disease outbreak could be observed (9, 15). Other studies indicate food-borne diseases can be

Table 1. Frequency of Fungal Isolates in the Different Sides of Refrigerator

Fungus	Number of Fungi (%) Based on the Temperature of the Refrigerator		
	> 0	< 0	Total, No. (%)
Penicillium sp.	290 (31.14)	255 (66.75)	545 (41.50)
Cladosporium sp.	517 (55.53)	39 (10.20)	556 (42.34)
Mucor sp.	-	1 (0.26)	1 (0.07)
Aspergillus niger	-	3 (0.7)	3 (0.22)
Aspergillus terreus	6 (0.64)	-	6 (0.45)
Aspergillus flavus	-	1 (0.26)	1 (0.07)
Aspergillus ochraceus	2 (0.21)	-	2 (0.15)
Schizosaccharomyces sp.	93 (9.98)	66 (17.27)	159 (12.10)
Rhizopus sp.	8 (0.85)	-	8 (0.60)
Alternaria sp.	11 (1.18)	-	11 (0.83)
Fusarium sp.	-	2 (0.52)	2 (0.15)
Trichosporon sp.	-	15 (3.92)	15 (1.14)
Botrytis sp.	4 (0.42)	-	4 (0.30)
Total, No. (%)	931 (70.90)	382 (29.09)	1313 (100)

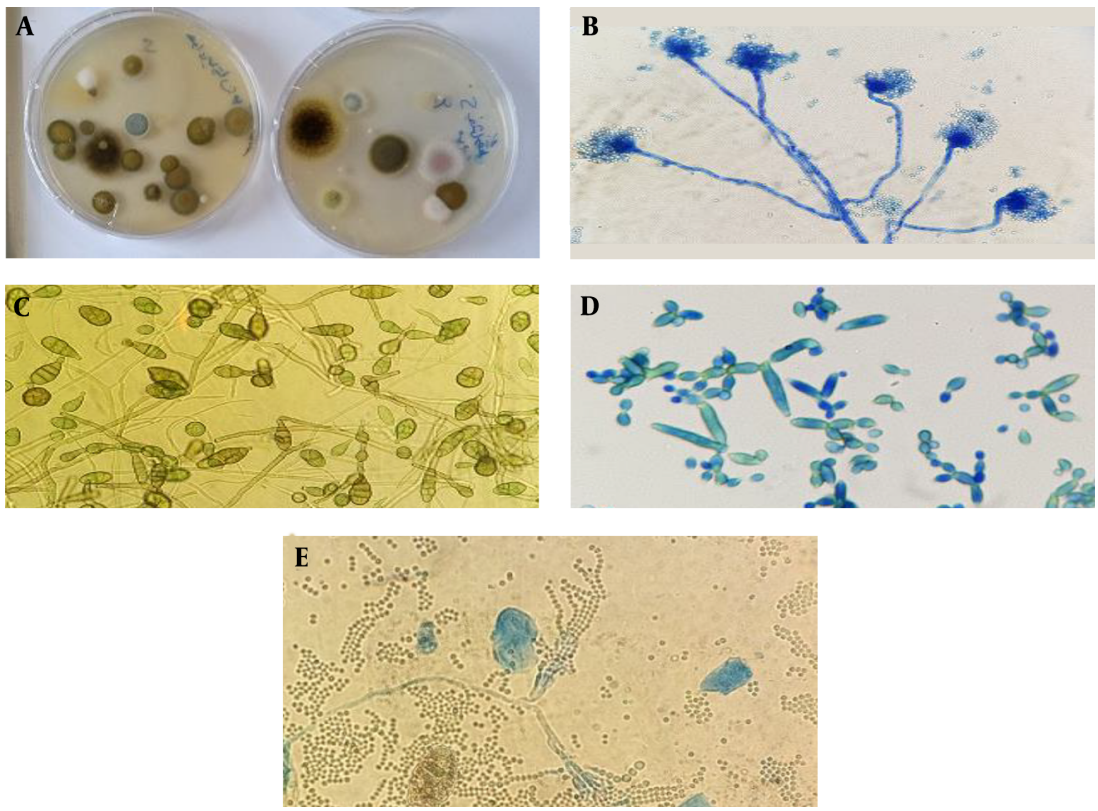

Figure 1. The macroscopic and microscopic filamentous fungi recovered from refrigerators above 0°C and refrigerators below 0°C. A: Growth of fungi on SDA medium; B: *Aspergillus flavus*; C: *Alternaria sp.*; D: *Cladosporium sp.*; E: *Penicillium sp.*

caused by inappropriate food storage. For example, the lack of periodic refrigerator cleaning and management may promote the risk of food-borne diseases (16, 17). Fungal agents from various sources including dirty hands, un-

Table 2. Mean Value of Fungi Per Square Meter of Surfaces

Point of Sampling	Mean (CFU cm^{-2}) (Min - Max)
< 0	325264 (42462 - 785562)
> 0	805944 (63694 - 2802547)

clean refrigerator surfaces, lack of cleaning raw materials, leakage packed food (meats, eggs, and milk), refrigerator door left open, temperature fluctuations, unwashed or rotten vegetables, and fruits can enter and spread to other refrigerator surfaces, causing contamination of other food items (18).

In this study for the first time, a total of 50 samples from fifty commercial refrigerators in restaurants of Ahwaz were analyzed to isolate and identify psychrotrophic fungi. All of the 50 (100%) refrigerators showed fungal contamination. These results indicate poor hygienic conditions and mismanagement of commercial refrigerators, which can be a threat to the consumer's health. This finding could be a warning for further monitoring of food storage environments such as commercial refrigerators. Control measures including appropriate training for staff employed in the restaurant, food storage design on shelves of refrigerators, paying attention to the point of order of food items, and checking the actual temperature of the refrigerator are recommended.

Ten fungal genera were isolated and identified. This finding is, to some extent, different from the results of other researchers, since the fungi isolated in this study are more diverse and have different concentrations (9, 18). This difference could be attributed to the geographical conditions of this area, Ahwaz, which is in the dry climate group, experiencing dust storms, or it can be due to differences in seasons and sampling time.

Cladosporium and *Penicillium* were the main isolated molds from commercial refrigerators in Ahwaz and were recovered from 80% of the 50 examined refrigerators. Reports indicate that *Cladosporium* and *Penicillium*, are always present in the refrigerator sides, and can continue to live and reproduce at low temperatures. In previous studies, it was noted that meat can be spoilt by mold growth (19-21). According to the reports of Gill, various species of fungi, including *Cladosporium* and *Penicillium*, are capable of producing black spot colonies on meat and fat tissue in the refrigerator. In addition, during storage in refrigerator, the above-mentioned fungi produce poisonous compounds (mycotoxin), for a variety of reasons such as inappropriate packing of food items (22). Some of these compounds such as aflatoxin cause toxicity and carcinogenicity in humans, while other mold metabolites like ochratoxin, citrinin, fumonisins, deoxynivalenol, and zearalenone have potential various adverse effects to consumers too, such as impairing normal digestion and reproductive, mutagenic effect, neurologic or immunologic dysfunction (17, 23). In addition, some members of *Cladosporium* spoil butter and margarine, and some cause limited rot of stone fruits and black rot of grapes. In addition to the production of mycotoxin and black spots on meat, some species of *Penicillium* are the causative agent of blue and green mold rots of citrus fruits and blue mold rot of apples, grapes, pears, and stone fruits (4, 24, 25). After *Cladosporium* and *Penicillium*, the most common fungi isolated from all samples were yeasts, such as *Schizosaccharomyces* and *Trichosporon*. Some species of *Schizosaccharomyces* are osmophilic and resistant to some chemical preservatives. Some species of *trichosporon* are involved in the fermentation of cacao beans, and some of them are recovered from fresh shrimp, ground beef, poultry, frozen lamb, and other foods (25). *Mucor* 1(0.07%) was the lowest fungus isolated from refrigerators below 0°C. *Mucor* is often produced in cottony colonies. The conditions described as "whiskers" of beef and "black spot" of frozen mutton are caused by some kinds. It is found in fermented foods and many vegetables (25).

The four *Aspergillus sp.* are agents isolated from commercial refrigerators that can cause food-borne mycotoxins for consumers. Aspergillus species produce aflatoxins such as Aflatoxin B1 that is one of the most important mycotoxins, which are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. In 2017, the International Agency for Research on Cancer (IARC) designated aflatoxins as Group 1 carcinogens (26).

The results of this study showed that fungi causing food spoilage can survive on commercial refrigerators surfaces and potentially lead to the contamination of other food items, and subsequently produce toxins and pose a health risk to consumers. A number of potential food-related fungal pathogens including *Penicillium sp., Cladosporium sp., Mucor sp., Aspergillus sp., Schizosaccharomyces sp., Rhizopus sp., Alternaria sp., Fusarium sp., Trichosporon sp., Botrytis sp.* were isolated from refrigerators in the Ahwaz restaurants.

This study also showed that refrigerators above 0°C have more fungal contamination than refrigerators at temperatures below 0°C. This finding agrees with the previous study because they reported, increasing temperature led to more microbial population growth (9). These results demonstrate the importance of hygiene monitoring programs in commercial refrigerators especially the refrigerators with temperatures above 0°C. One reason for this may be the constant opening of the door of these refrigerators by kitchen workers and thus warming up the internal temperature or letting more fungal bioaerosols in. Therefore, to prevent food-borne intestinal infectious disease, it

is necessary to maintain normal refrigerator temperature in order to reduce the growth, reproduction and spread of these fungal bioaerosols on food. The fungi found in commercial refrigerators could cause food spoilage or produce toxins and cause adverse health effects for consumers, especially immunocompromised patients. The authors recommend further studies on bacterial sampling from the refrigerator and also surveying the effects of a variety of disinfectants at different times on the microbial load of surfaces in the refrigerator to achieve the best hygiene management.

In this study, the presence fungi in commercial refrigerators could be an indicator of a potential source of food spoilage and food-borne diseases, thus one way food-borne diseases could be controlled is by education or training about kitchen hygiene with a focus on refrigeration and proper packaging and other health risks. Therefore, the types and persistence of fungi in environmental food storage such as commercial refrigerators, are an important part of hygiene monitoring programs.

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Footnotes

Authors' Contribution: Study concept and design: M.F. and N.K.; analysis and interpretation of data: A. N. and N.K.; drafting of the manuscript: S.A.; critical revision of the manuscript for important intellectual content: M.F., N.K., and S.A.; statistical analysis: A.N.

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