

# Isolation and Identification of fungi located in refrigerator and the effects on the preservation food

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## ABSTRACT

Household refrigerators are a potential source of pathogen contamination for foods leading to food spoilage and food borne diseases. This study was carried out to isolate and identification of fungi located in household refrigerators in Baghdad /Iraq. Fifty samples collected randomly. The interior of each refrigerator was swabbed using a sterile swab stick that had been pre-soaked in peptone diluents. Each sample collected was plated out on culture media Potato Dextrose agar. Results showed that all refrigerators were found to be contaminated. Fungi isolated from the refrigerators were *Aspergillus* (*Aspergillus niger*, *A. paracitcus*, *A. penicilloides*). *Mucor* were isolated including (*M. hemalis*, *M. racemosum*) and two species belong to the genus (*Penicillium verrucosum*, *Penicillium aurantiogriseu*). As well as one fungi species were isolated from different genus, including (*Alternaria alternata*, *Cladosporium cladosporioides*, *Ulocladium chartarum* and *yeast*).

**Keywords:** psychrophilic fungi, contamination of food stored in refrigerators.

## 1. INTRODUCTION

Various kinds of food store in refrigerator at an unsuitable temperature which could give rise to food borne diseases or illness. In the developed countries food borne pathogens are responsible for millions of cases of infections such as gastrointestinal diseases each year (1). New food borne pathogens and food borne diseases are likely to emerge due to factors such as pathogen evolution, changes in Agriculture and food manufacturing practices and changes in the human host status (2).

One of the most important kitchen appliances found in homes are refrigerators which used in storing and keeping food products so as to prolong the shelf-life of the foods. Refrigeration is employed to control the rate of certain chemical and enzymatic reactions as well as rate of growth of food microorganisms (3). Lower temperature in a confined volume lowers the reproduction rate of bacteria and fungi, so the refrigerator reduces the rate of spoilage. Cooling is a popular food storage technique in many countries and

works by decreasing the reproduction rate of bacteria and fungi (5).

Foods are stored in the refrigerators for preservation so as to be available for future use. When there is contamination with microbes, this can cause food spoilage (change in characteristics of food rendering it unacceptable or unsafe for consumption) and food borne diseases (defined by WHO, as any disease of an infectious or toxin nature caused by or thought to be caused by the consumption of food or water).

Many studies have shown that perishable food will deteriorate, even at refrigeration temperature, due to spoilage because of microorganisms, enzymes and oxidation (6). Type of container or wrapping material they are also important factors that influence the type of microbial growth, toxicity and spoilage of food during refrigerated storage. Refrigerated foods are therefore subjected to spoilage by moulds, yeasts and bacteria (4).

In 2005 alone reported that, 1.8 million people died from diarrheic diseases (7). Fungi from unwashed raw foods, leaking packages, unclean hands, and unclean container surfaces introduced into domestic refrigerators can directly contaminate other stored foods and persist on internal refrigerator surfaces. This in turn creates the risk of indirect long-term contamination during subsequent food preparation (8). Poor refrigeration has been reported to be a contributory factor to food borne illness.

The most common problem in Nigeria is keeping foods in the danger zone of 4°C to 60°C. This is due to the fact that electricity supply is erratic like in Iraq.

The aim of this study investigation of fungi present in refrigerators, houses in Baghdad / Iraq and how they are dangerous on foods stored on it.

## 2. MATERIALS AND METHODS

### 2.1. Samples collection

A total of 50 household refrigerators were sampled in Baghdad /Iraq . Refrigerator was swabbed using a sterile swab stick that had been soaked in normal saline diluents. The swab sticks were transported back to the laboratory under chilled conditions (4°C ± 1.0). Each sample collected was plated out on the following commercially available culture media in the laboratory after appropriate serial dilution was done.

### 2.2 Isolation and identification of fungi isolates

#### 2.2.1 Plating technique:

Serial dilutions up to 10<sup>-6</sup> were done. One ml aliquots of (10<sup>-1</sup> – 10<sup>-6</sup>) dilutions was pipette out into sterilized petridishes and about 15 ml of Potato dextrose agar medium was pour plated in duplicate. The petridishes were then rotated clockwise and anti-clockwise for uniform distribution of the samples. The solidified plates were incubated at 28°C in an incubator from (5) days.

#### 2.2.2 Single hyphal tip method for isolates purification

Czapek-Dox agar was prepared and dispensed onto sterile petridishes. After solidification, peripheral mycelia from the slants were carefully lifted, and

stabbing on to CzA plates and incubated at 28°C for 5 days. After incubation, the colonies were observed for hyphal developments. The peripheral tip of the mycelial growth was taken from the plates, reinoculated onto CzA medium and incubated at 28 °C for 5 days.

#### 2.2.3 Identification of isolates

The grown colonies were initially divided into groups on the basis of their gross morphology. For characterization of the morphology of fungal isolates, slides stained with lactophenol cotton blue stain and examined by a compound microscope. Identification was based on morphological characteristics such as (growth pattern, hyphae, color of colony and medium, surface texture, margin character, aerial mycelium) by using standard procedures and taxonomic keys. The references were used as classification and identification keys for the fungal isolates were: (9-12).

### 2.3 Occurrence and frequency of fungal isolates

Occurrence %= No. of the samples in which the species occurs/No. of the total samples ×100.

Frequency %= No. of the isolates of each species /No. of the isolates of all genera ×100 (13).

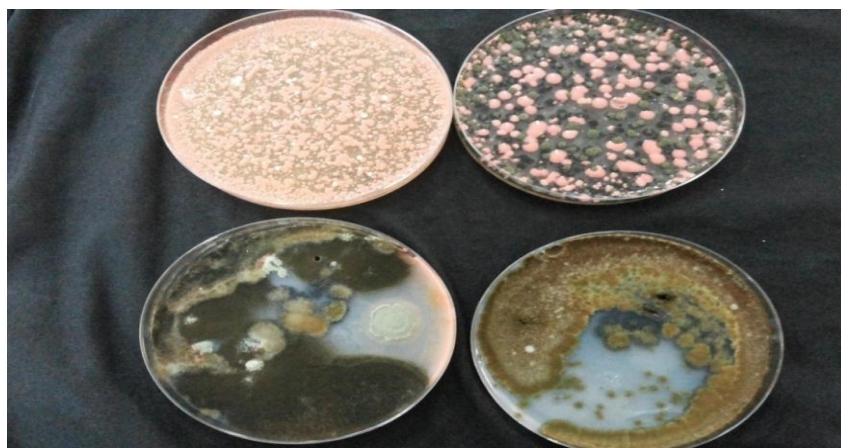
### 2.4 Fungi effects on the stored foods

For study the fungi effect on the stored food in refrigerators, various types of food were incubated in the refrigerator at 4°C for three days, during this time has been cut power to the refrigerator for 4 hours per day.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation of fungi from house refrigerators

Different colonies of fungi were observed on PDA media at 28°C after 5 days with different Colonies color and features, such as (dense, dark green, compact white or yellow colonies, at first white to pale yellowish, creamish – yellow in daylight, more grayish in darkness, Light greyish, colony pale and brownish-grey). Colony smooth radial furrows, whitish to cream-colored or greyish–brown as shown in figure (1).









**Figure 1:** Different types of fungi colonies isolated from house refrigerators.

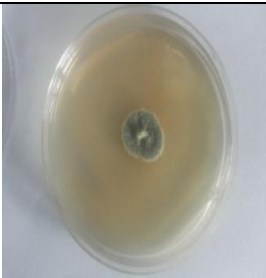




### 3.2 Identification of fungal isolates

The isolates were identified by compound microscope according to (9-12). The results found that three fungi species belong to the genus *Aspergillus* (*Aspergillus niger*, *A. paracitcus*, *A. penicilloides*). Whereas two species of *Mucor* were isolated including (*M. hemalis*, *M. racemosum*) and two species belong to the genus

(*Penicillium verrucosum*, *Penicillium aurantiogriseu*). As well as one fungi species were isolated from different genus, including (*Alternaria alternate*, *Cladosporium cladosporiodes*, *Ulocladium chartarum* and *yeast*). The macroscopic features of isolated fungi species in the present study were as follows in the table (1):

**Table 1:** Isolated fungi located in house refrigerators with characterization.

Isolates	characterization	Colony figure
<i>Aspergillus niger</i>	Colonies compact felt with dark brown to black conidiophores. Distinguished by the flattened conidia.	
<i>A. paracitcus</i>	Colonies are dense felt green conidiophores. Conidial heads radiate.	
<i>A. penicilloides</i>	Colony sporulating well, consisting of a dense felt of dark green conidiophores, arising from the agar or from the scanty aerial mucelium	
<i>Mucor hiemalis</i>	Colony creamish-yellow more grayish in darkness.	
<i>Mucor racemosus</i>	Colony-White, brownish-gray with age -Sporangiophore branched	
<i>Penicillium verrucosum</i>	Colony yellow green, dark-green, grey green or blue-green rarely white or ochraceous.	

<b><i>Penicillium aurantiogriseu</i></b>	Colony grey green to dull blue green. vegetative aerial mycelium mostly absent .Exudate mostly present in fresh isolate as colorless droplets .Odour strong	
<b><i>Alternaria alternata</i></b>	Colony black or alliaceous or greyish isolate from colder regions at 0 °C to -2 °C	
<b><i>Cladosporium cladosporiodes</i></b>	Colonies are olive-green to olive-brown and appear velvety or powdery. The edges of the colony can be olive-grey to white, and feathery The colonies are diffuse and the mycelia form mats and rarely grow upwards from the surface of the colony.	
<b><i>Ulocladium chartarum</i></b>	Colonies <i>Ulocladium</i> grow moderately rapidly. The growing colonies are woolly to cottony. From both the front and the reverse, the colour is olive-brown to black	
<b>Yeast</b>	Single-celled growth habit, can be contrasted with molds, which grow hyphae. Fungal species that can take both forms (depending on temperature or other conditions)	

### 3.3 Occurrence and frequency of fungal isolates

According to the observed data for fungi exist in the house refrigerators, the results showed *Penicillium verrucosum* was the highest ratio of occurrence and frequency 62 % and 37.5 % respectively , followed

by *P. aurantiogriseu* occurrence 60% and frequency 32.5% and respectively. Whereas *Mucor hiemalis*, *Mucor racemosus* and *Alternaria alternate* recorded the lowest results of occurrence and frequency 4%, 2.5% respectively, as clerical in table (2).



**Table 2:** Number of fungal isolates, occurrence and frequency in house refrigerators.

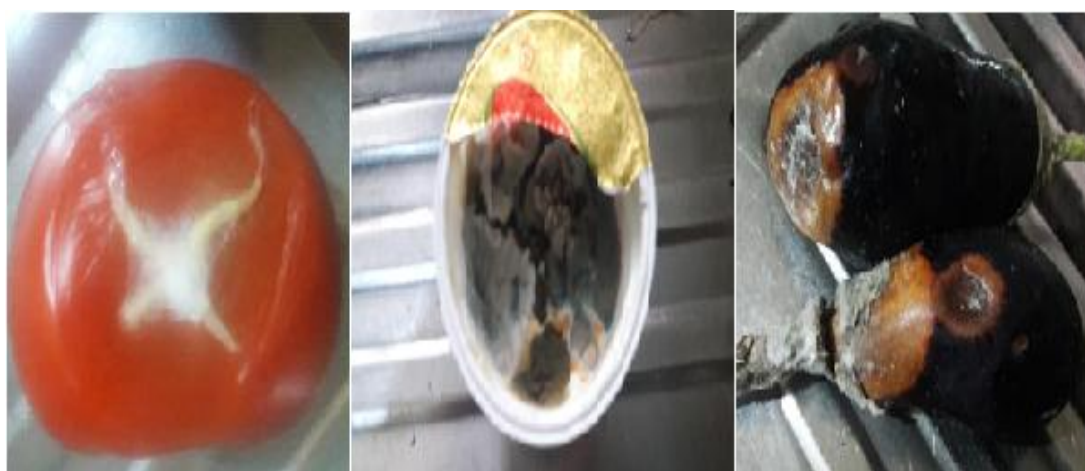
Fungal Species	No. of isolates	Occurrence% No. of isolates /50	Frequency% No. of Frequencies /40
<i>Aspergillus niger</i>	11	22	10
<i>A. paracitcus</i>	7	14	5
<i>A. penicilloides</i>	24	48	12.5
<i>Mucor hiemalis</i>	2	4	2.5
<i>Mucor racemosus</i>	2	4	2.5
<i>Penicillium verrucosum</i>	31	62	37.5
<i>Penicillium aurantiogriseu</i>	30	60	32.5
<i>Alternaria alternate</i>	2	4	2.5
<i>Cladosporium cladosporiodes</i>	15	30	25
<i>Ulocladium chartarum</i>	22	44	20
Yeasts	11	22	10

### 3.4 Fungi effects on the stored foods

The results showed in (figure 2), the growth of fungi on the food stored in the refrigerator and then leading to contamination food which become dangerous and unfit to eat.

Most refrigerators are not functioning optimally due to power failure or erratic power supply, resulting change in the refrigerator temperature to greater than 4°C which will allow growth of mesophilic microorganisms which can be pathogenic and therefore increases the

risk of food borne disease (14). As important as electricity is to powering the refrigerator, none of the householders do have electricity round the clock. Some householders reported having 6-9 hours of electricity, others reported 3 hours daily while others do not have any defined pattern of electricity (erratic). These findings show that most refrigerators do not work optimally because temperature will change when there is no adequate supply of power to the refrigerators (15).



**Figure 2:** Different type of food contaminated by fungi located in the refrigerator.

The total viable count contamination levels observed in this study show that 11 isolate out of 50 refrigerators had fungi the highest recorder *Penicillium verrucosum* which producer of the nephrotoxin och atoxin A and *P. aurantiogriseu*.

The levels of contamination observed in domestic refrigerators are likely to be influenced by a range of

factors including the nature and levels of initial contamination introduced on contaminated foods, the presence and absence of effective packaging, the hygiene of those preparing and placing foods into the refrigerators, and the efficiency and frequency of refrigerator maintenance and cleaning (16).

The presence of microorganisms in the refrigerator affects the microbial quality of foods kept in it. During the course of this study observed that the level of cleanliness and general knowledge of food safety greatly accounted for the presence of spoilage and pathogenic microorganisms inside the refrigerator.

#### 4. REFERENCES

1. Chan, Z and Jiang, X .(2004) .Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A review. *Agric* 4: 1-29.
2. Fratamico ,P.M. and Bayles, D.O. (2005). *Foodborne pathogens: Microbiology and Molecular Biology*. Caister Academic Press.
3. Srivastava ,R.P. and Kumar, S .(2006) .*Fruit and Vegetable Preservation: Principles and Practices*, ( 3<sup>rd</sup> edn), Lucknow: International.Book Distributing Co. India.
4. Roday ,N. (2002) .*Food Hygiene and Sanitation*, Tata McGraw Hill Publishing Co. Ltd., New Delhi.
5. Godwin ,S.; Chen ,F.C.and Chambers, E. (2006) . Correlation of visual perceptions of cleanliness and reported cleaning practices with measures of microbial contamination in home refrigerators. *Food Prot Trends* 26: 474-480.
6. Jay ,M.J. (2000). *Food Analysis: Theory and Practices*, (3<sup>rd</sup>edn), CBS Publishers and Distributors, New Delhi.
7. Hendriksen ,R.S.; Vieira ,A.R.; Karlsmose , S.L.; Wong , D.M.;jensen , A.B. (2011). Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog Dis* 8: 887-900.
8. Michaels B, Ayers T, Celis M, Gangar V (2001) Inactivation of refrigerator biofilm bacteria for application in the food service environment. *Food Service Technol* 1:169–179.
9. Raper, K.B. and Fennell , D.I .(1965) *The genus Aspergillus*. Williams and Wilkins, Baltimore, USA.
10. Ellis, M.B .(1971) . *Dematiaceous hyphomycetes*. Kew,Surrey, ,Commonwealth Mycological Institute, U.K.
11. Robert, A.S. and Ellen, S. H . (1988) *Introduction to food – borne fungi*. Baarn; Delft : Centraalbureau voor Schimmelcultures, 3<sup>rd</sup> ed. Royal Netherlands Academy and sciences .
12. John, I. P. and Ailsa, D. H.( 2009 ). *Fungi and food spoilage* springer , Science Business Media , LLC. Third edition. Dordrecht Heidelberg ,New York,USA.
13. Choi, Y.W.; Hyde, K.D. and HO,W.H.(1999) .Single spore isolation of fungi .*Fungal Diversity* ,3:29-38.
14. James ,S.J.; Evans, J.and James, C. (2008). A review of the performance of domestic refrigerators. *J Food Eng* 87: 2-10.
15. Parry ,S.M.; Slader, J; Humphrey ,T.; Holmes ,B. and Guildea ,Z . (2005) .A case-control study of domestic kitchen microbiology and sporadic Salmonella infection. *Epidemiol Infect* 133: 829-835.
16. Flynn ,O.M.; Blair, I .M. and Dowell ,D. (2006). The efficiency and consumer operation of domestic refrigerators. *Intl J Refrig* 15: 307-312.
17. Karthikeyan, K; Nanthakumar,K ; Suriyanarayanan, S. and Lakshmanaperumalsamy , P.(2012). Screening of Textile Dye Impacted Soils for Novel Dye Decolorizing Fungi Employing Various Nutrient Media, India. *Advances in Biological Research*, 6 (1): 44-51.

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