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Microbial contamination in local markets refrigerators in Babylon province

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Abstract :

Local markets refrigerators regard as source of food contamination which can cause food poisoning and disease occurrence , that because of these refrigerators have been opened by a huge number of customers so it be best way for microbial infections and disease transport between customer. In this study we collect 35 samples from different local markets refrigerators in Babylon province , result revealed that there are bacterial and fungal growth can isolate from inside refrigerator (shelves) and outside it (door hand) , by using three culture media for bacterial isolation which are (MSA –S S Agar- EMB) and (SDA – PDA) for fungal isolation .Inside refrigerators revealed 31+ve samples for *Staphylococcus aureus* , 8+ve samples of *Klebsiella* , 6+ ve samples of *Escherichia coli* , 8+ ve samples of *Shigella* , 1+ ve sample of *Salmonella* ,while isolation fungi inside refrigerator on PDA were : *Cladosporium* (41.7%), *Aspergillus niger* (23.1%) , *Penicillium sp*.(16.6%) , *Ulocladium* (8.3%), *Aspergillus flavus* (6.5%) , *Aspergillus nidulans*(1.9%) *finally Alternaria* (1.9%) , isolation fungi on SDA were : *Cladosporium* (16.3%) , *Penicillium sp.*(30 %) , *Aspergillus niger* (28.1%) , *Ulocladium* (15.5%) , *Aspergillus flavus* (8.2%) , *Alternaria* (9%) finally *Aspergillus fumigatus* (9%) . Either from outside refrigerator revealed : 24+ve samples for *Staphylococcus aureus* , 8+ve samples of *Klebsiella* , 8+ ve samples of *Escherichia coli* , 6+ ve samples of *Shigella* and 2+ ve samples of *Salmonella,* while isolation fungi outside refrigerator on PDA were : *Cladosporium* (45.4%) , *Penicillium sp.*(15.5%) , *Aspergillus niger* (14.4%) , *Ulocladium* (10.3%) , *Aspergillus flavus* (8.2%) , *Alternaria* (4.1%) finally *Aspergillus terrus* (2.1%),on other hand fungi isolation outside refrigerator on SDA were : *Aspergillus niger* (28.4%) , *Ulocladium* (22.1%) , *Penicillium sp.(*21.1%) , *Cladosporium* (14.7%) , *Aspergillus flavus* (10.5%) , *Alternaria* (2.1%) finally *Aspergillus terrus* (1.1%) in addition to yeast with high ratio .

Literature review & introduction :

Microorganisms are organisms that are scattered everywhere in nature where nutrients, moisture and heat are present to grow and multiply (Green wood,2014) , Since these conditions are suitable for microorganisms are also suitable for humans so we live among thousands of microorganisms, they are in the air when breathing and in the food we eat and on the surfaces of our bodies and their presence may be harmful or useful (Out- Bassey, 2017) The refrigerator is an important appliance in the kitchen, markets or stores and is important in storing and keeping food for as long as possible. It allows control of the proportion of chemicals and enzymes in foods fixed until they are used and also controls the effectiveness of microorganisms in food (Out-Bassey,2017) Therefore, the temperature of the refrigerator must be controlled to prevent microbial growth because its temperature changes lead to the activity of these organisms and the production of metabolic products and cause the corruption of food consumed, making these refrigerators a source of transmission among users, and while these refrigerators in the markets open by a large number Of consumers because they do not know their health level and how much they care about hygiene when they are in these markets

(American Chemisty Council,2010) Common types of genera in refrigerators are the psychrophilic, which have the potential to grow at low temperatures and they are found in refrigerators because may be The foods stored inside them are contaminated before they are stored in the refrigerator or the temperature of the refrigerator is not the required grade , the genera common in the refrigerator are : Coliforms , *pseudomonas, Listeria, Penicillium ,Cladosporium* , Which are harmful to human health, although some genera are not harmful to the human in itself, but that its presence in food will lead to the secretion of toxins that are affecting human health which cause (Food borne illnesses) such as: poisoning - diarrhea - dysentery - meningitis - gastritis - urinary tract infection, in addition to fungal toxins that are carcinogenic carcinogenic agents (Jackson *et al.* , 2007) .

methods & materials :

1. Culture media :

1. **Mannitol salt agar (MSI) :**

Preparation according to instructions on the Hi Media Laboratories By dissolving (111gm) in (1L) and sterilizing by autoclave , it was used to diagnose *Staph.aureus* bacteria.

2. **Eosin methylene blue (EMB) :**

Preparation according to instructions on the Hi Media Laboratories by dissolving (35.96) gm in (1L) and sterilizing by autoclave, it was used to diagnose *Klebsiella* , *E. coli*.

3. **Salmonella-Shigella agar(S-Sagar) :**

Preparation according to instructions on the Hi Media Laboratories by dissolving (63) gm in (1L) and sterilizing by autoclave, it was used to diagnose Salmonella and Shigella .

4. **Potato Dextrose agar (PDA) :**

Preperation according to instructions on the Hi Media Laboratories by dissolving (39) gm in (1L) and sterilizing by autoclave , after adding Chloramphenicol , use to isolate fungi from samples .

5. **Sabouraud dextrose agar (SDA) :**

Preparation according to instructions on the Hi Media Laboratories by dissolving (65) gm in (1L) and sterilizing by autoclave , after adding Chloramphenicol , use to isolate fungi & yeast from samples .

6-Peptone water :

Preparation according to instructions on the Hi Media Laboratories by dissolving (15) gm in (1L) and sterilizing by autoclave , It was used to collect bacteria samples from refrigerators until they were transferred to the laboratory and cultured .

7- yeast extract+ Peptone water :

Preparation according to instructions on the Hi Media Laboratories by dissolving (23+15) gm in (1L) and sterilizing by autoclave , use to isolate all fungi from the refrigerators and transfer them to the laboratory until cultured .

2.Collect samples :

Samples were collected from refrigerators in the local markets in Babylon governorate for the period from 15/10/2017 to 25/12/2017. The number of refrigerators was 35 from markets different in terms of location and level of hygiene. Samples were taken by special swabs for bacteria and fungi , Where two samples of bacteria were taken from inside the refrigerator shelves and from the handles of the refrigerators, as well as to the fungus, and transferred to the laboratory and cultured on the prepared media in the Microbiology Laboratory in the Department of Biology at the Faculty of Science for Women / Babylon University.

3. Diagnosis of isolated microorganisms :

1 - Diagnosis isolated bacteria: The media used as a differential and diagnostic of bacteria , MSA is used to isolate *Staph*. aureus bacteria, EMB to diagnose *E. coli, klebsiella* and S-S agar to diagnose *Salmonella* and *Shigella* bacteria.

2 - Diagnosis of fungi: Was diagnosed depending on a appearance of macroscopic characters (color - texture - appearance - the diameter of the colony) and microscopic characters (spores and mycillium) (Barnett & Hunter,1972) .

Result and discussion :

After the completion of the collection and cultivation of the samples on the culture media, the results were recorded in the tables (1,2,3), where it was noted that the number of positive samples of bacteria *Staph.aureus* Is the highest among isolated bacteria inside refrigerators or their handles (31 inside refrigerators and 24 handles) followed by *Klebsilla*, and the number of positive samples (8 inside and outside) , *E.coli* (6 inside and 8 from outside) , *Shigella* (8 inside and 6 outside) and finally *Salmonella* (1 inside and 2 outside), as shown in the table (1).

Table (1) shows the bacterial species isolated from inside and outside of refrigerators on the media (MSA, EMB,S S agar).

Bacteria	Isolated place	Positive samples	Media used	+
Staph . aureus	Inside ref.	31	MSA	4
Staph . aureus	Outside ref.	24	MSA	11
Klebsiella	Inside ref.	8	EMB	27
Klebsiella	Outside ref.	8	EMB	27
E. coli	Inside ref.	6	EMB	29
E.coli	Outside ref.	8	EMB	27
Shigella	Inside ref.	8	S S agar	27
Shigella	Outside ref.	6	S S agar	29
Salmonella	Inside ref.	1	S S agar	34
Salmonella	Outside ref.	2	S S agar	33

It is clear that *staph*ylococcus the highest in terms of the number of positive samples compared with other types of bacteria, Which is what call it Staphylococcal food borne disease (SFD) It is one of the most common diseases arising from food, which is caused by food poisoning with toxins *Staph.aureus* enterotoxin , as a result of the transfer of cooked food or non-cooked meat such as hands to the refrigerators and open from the handles without constantly cleaning these refrigerators, so these refrigerators become sources of transmission and dangerous to the health of consumers if not cleaned continuously and the symptoms of infection : Vomiting - nausea - cramps in the abdominal cavity may be accompanied by diarrhea, as a result of toxins (SES) Which are produced by bacteria *staph.* , one of the nine types of persistent heat toxins (heat Sable enterotoxin) As these toxins are resistant to heat and freezing and to digest by the stomach enzymes Pepsin, Trepsin and acidity low pH (kadariya ,et al., 2015) .

The presence of *klebsiella* and *E.coli* is evidence of lack of health awareness and lack of interest in the cleanliness of the refrigerator and its use by a large number of consumers(Agnes,et al.,2008) , This bacteria is one type of hospital infection and is transmitted from one person to another by contact with hands and thus move into the refrigerator or outside, these bacteria produce toxins Extra cellular complex (ETC) which is considered fatal and causes lung injuries (Agnes,et al.2008) .

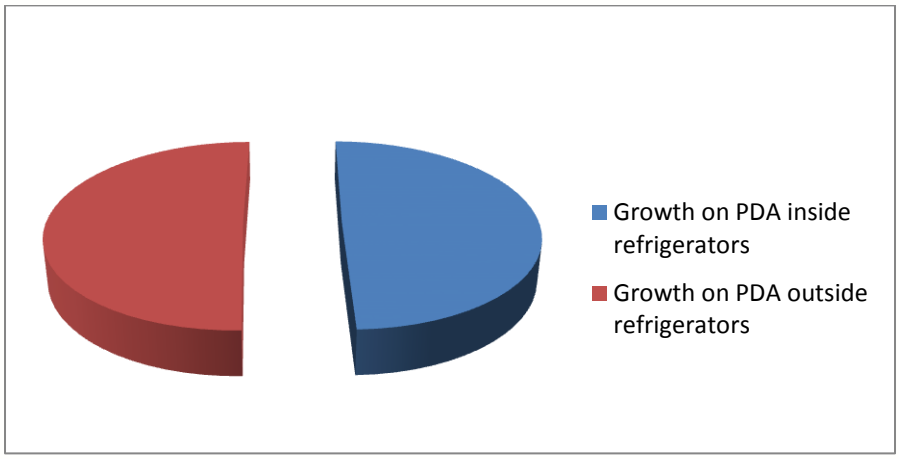
E.coli is really harmless and is an essential part of the intestinal health of the human intestine. However, increasing them leads to diarrhea, urinary tract infections, respiratory and other diseases as they release the toxins of the Shiga toxin and the arrival of these bacteria to the refrigerators as a result of their migration from the products of sheep, cows and chickens during the preparation and marketing, And milk and its products with poor treatment (Agnes,et al.2008) .

Salmonella bacteria were the least hesitant for other types of isolated bacteria and this does not indicate that their presence is not dangerous, but they were less hesitant than others , It is responsible for food poisoning where endo toxins are produced, *Shigella* bacteria have been shown between positive samples and are considered to be dangerous and cause of dysentery, one of the major causes of diarrhea in the world (james & George, 2004) .

Table (2,3) shows the number of fungal isolates and their isolated frequency from inside and outside the refrigerators using the SDA-PDA media.

Table (2) shows the fungal species isolated from inside refrigerators on the PDA and SDA media.

Fungi isolated on PDA agar			**Fungi isolated on SDA agar**		
Fungal species	**No. of colony**	**Percentage**	**Fungal species**	**No. of colony**	**Percentage**
Aspergillus niger	25	23.1	*Aspergillus niger*	31	28.1
Penicillium sp.	18	16.6	*Penicillium sp.*	33	30
Cladosporium	45	41.7	*Cladosporium*	18	16.3
Aspergillus flavus	7	6.5	*Alternaria*	1	0.9
Alternaria	2	1.9	*Ulocladium*	17	15.5
Aspergillus nidulans	2	1.9	*Aspergillus flavus*	9	8.2
Ulocladium	9	8.3	*Aspergillus fumigatus*	1	0.9
Total	108	100	Total	110	100



The above diagram shows the fungal growth on the PDA and SDA medium from inside the refrigerator where the growth on the mean was (108 ,110) isolation. This indicates that the two mediums have the same efficiency in isolation. The two mediums contain components very close to the natural habitat in which they grow.

Table (3) shows the fungal species isolated from outside refrigerators on PDA and SDA media.

Fungi isolated on PDA agar			**Fungi isolated on SDA agar**		
Fungal species	**No. of colony**	**Percentage**	**Fungal species**	**No. of colony**	**Percentage**
Aspergillus niger	14	14.4	*Aspergillus niger*	27	28.4
Penicillium sp.	15	15.5	*Penicillium sp.*	20	21.1
Cladosporium	44	45.4	*Cladosporium*	14	14.7
Aspergillus flavus	8	8.2	*Alternaria*	2	2.1
Alternaria	4	4.1	*Ulocladium*	21	22.1
Aspergillus terrus	2	2.1	*Aspergillus flavus*	10	10.5
Ulocladium	10	10.3	*Aspergillus terrus*	1	1.1
Total	97	100	Total	95	100

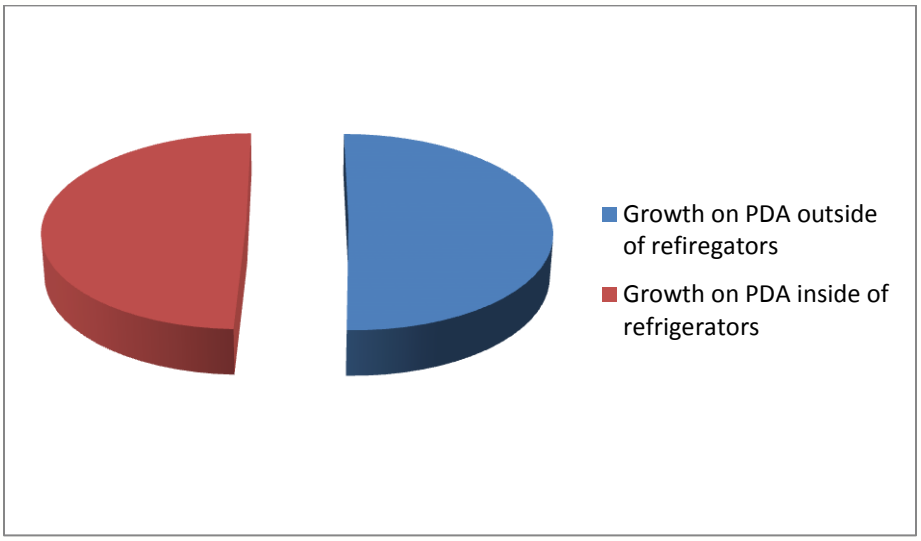


Diagram (2) shows the growth of fungi on PDA and SDA media for samples from outside the refrigerator.

It is noted from the above diagram that the two mediums had the same efficiency in isolating fungi from outside the refrigerator, because these media are considered the best media and their components are very close to the natural environment. Where the presence in the refrigerators can be the result of the spread of fungi spores with dust or soil and the environment in which grow vegetables and fruits used in the manufacture of food and beverages, which is contaminated by the preparation and before keeping in the refrigerators and move between consumers and the conditions of the refrigerator and moisture inside is able to survive And the secretion of toxins to Mycotoxins, which is considered a toxic substance harmful to human health , *Aspergillus*, especially *As.flavus*, is responsible for the production of aflatoxins and the survival of food in the refrigerator allows it to become carcinogenic agents if consumed by humans (Out-Bassey,et al,2017) . We see these fungal growths on the shelves of refrigerators in the markets.

Recommendations :

Despite human attempts to maintain public health in all ways, refrigerators are a source of contagion and keeping them clean is one of the most important means of reducing transmission. This study showed that microorganisms can grow inside and outside refrigerators on food stored inside. Therefore, the spread, growth and survival of food pathogens can be controlled in proper ways to store food, clean these refrigerators constantly and control the temperature of the refrigerator within the required degree to prevent the growth of these microbes.

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