Isolation and Identification of Pathogenic Bacterial Species from Refrigerators in Basrah City, South of Iraq

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Abstract

Objective: To isolate and identificate bacterial species from refrigerators in Basrah city.

Method: A total of 37 household refrigerators were sampled in Basrah city. With the consent of householders swabs moistened soaked with Buffered Peptone Water were collected from the base, shelves and sides of the refrigerator and were transported to laboratory under chilled conditions (4°C ± 1.0)**.** Each sample collected was plated out on many culture media**.**

Results: *Citrobacterfreundi* was the most frequently isolated pathogen in this study, being recovered from 31.57 % of refrigerators. *Listeria monocytogenes* and *Pseudomonas spp*. were recovered from 17.55 % of refrigerator surfaces, *Salmonella spp*. from 14.03 %. *Escherichia coli* from 10.63% and *Listeriainnocua* from 8.77%.

Conclosions: This study concludes that most of the refrigerators under study were contaminated with some bacterial species and some of these are pathogenic and lead to diseases such as *Listeriaand Salmonella*. Continuous cleaning and using disinfectant agents reduces bacterial contamination.

Keywords: *Isolation, Bacteria, Contamination, Basrah.*

Introduction

Food-borne diseases which caused by ingesting contaminated foodstuffs are widespread and growing public health problem. The global incidence of food-borne disease is difficult to estimate, but it has been reported that in 2005 alone, 1.8 million people died from diarrhoeic diseases[1]. Stored foods in domestic refrigerators may be contaminated when introduce contaminated foods, leaking packages, hands, and surfaces which may attach to the internal surface of the refrigerator leading to indirect longer term contamination during activities of subsequent food preparation[2]. Most studies focused on the contamination of surfaces in kitchens and rest rooms by bacteria[3,4]. Some pathogenic bacteria can survive on the surfaces in these environments for some time, and contaminate food leading to illness. Because refrigerators are used to store food, Microbial contaminations of refrigerators have been studied[5,6]. Moisture and nutrients in refrigerators provide favorable growth conditions for contaminating bacteria[5].

Refrigeration is used to control the rate of certain chemical and enzymatic reactions and the rate of growth of food microorganisms[7]. The refrigerator reduces the rate of spoilage by reducing the reproduction rate of bacteria[8]. 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).In the light of the above, the present study aimed to isolation and identification of bacterial species from refrigerators in Basrah city.

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Materials and Method

Sampling procedure: A total of 37 household refrigerators were sampled in Basrah city. With the

consent of householders swabsmoistened soaked with Buffered Peptone Water (BPW, Oxoid) were collected from the base, shelves and sides of the refrigerator and were transported to the laboratory under chilled conditions (4°C ± 1.0)[9]. Each sample collected was plated out on the following commercially available culture media in the laboratory listed in Table1.

Table 1: Media used for isolation of bacteria (origin - Paris, France) from refrigerators

Culture media	Isolated bacteria
Listeriachromagar	Listeria monocytogenes and Listeria innocua
E. coli chromagar	Escherichia coli and other gram negative bacteria
Salmonellachromagar	Salmonella spp.
Pseudomonaschromagar	Pseudomonas aeruginosa

Study protocol (questionnaire distribution):

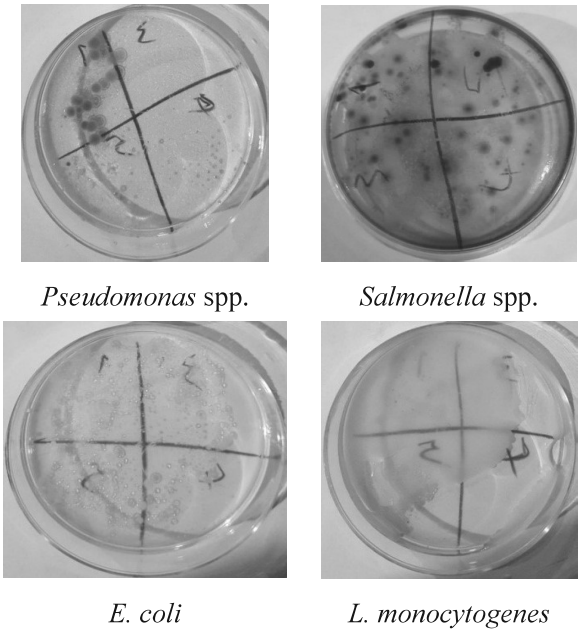
Thirty seven households participated in the study that randomly selected were participated in the evaluation of their knowledge in relation to the use of refrigerator by giving an Oluwafemiet al.[10] with modifying questionnaires consisting of questions covering social demographics, refrigeration practices, refrigerator maintenance practices, etc. to each householder[10].

Results

Citrobacterfreundi was the most frequently isolated pathogen in this study, being recovered from 31.57 % of refrigerators.*Listeriamonocytogenes* and *Pseudomonas spp*. were recovered from 17.55 % of refrigerator surfaces, *Salmonella spp*.from 14.03 %. *Escherichia coli* from 10.63% and *Listeria innocua* from 8.77% (Table 2).The use of chromo agar media provide easy and rapid diagnosis of bacteria as shown in Figure (1).

Table 2: Frequency of bacterial species isolates from refrigerators

Bacterial isolates	Frequency	Percent
Citrobacterfreundi	18	31.57 %
Listeria monocytogenes	10	17.55 %
Pseudomonas spp.	10	17.55 %
Salmonella spp.	8	14.03 %
Escherichia coli	6	10.63 %
Listeriainnocua	5	8.77 %
Total	49	100%



Pseudomonas spp. *Salmonella* spp.

E. coli *L. monocytogenes*

Figure 1: Bacterial isolates on chromagar media

The social demographicresult shows, refrigerator maintenance practices and food safety knowledge of the householders participated in the study. Educational levels of respondents ranging from primary school to doctorate degree.67.6% of respondents did not clean vegetables and fruits before keeping in refrigerators. Another factor that could have affected the microbial quality of refrigerators was the cleaning regimes and the type of cleaning. Most respondents reported varying degrees of cleaning regimes. 45% of respondents reported cleaning their refrigerators weekly while only 8.1 % of respondents reported cleaning their refrigerators daily. 18.9% of householders only used disinfectants in cleaning their refrigerators while most of them (43.2%) used water and soap.Many of respondents (72.9%) have knowledge about food borne diseases from different sources.

Fifteen refrigerators (40.5%) provided no bacterial growth, the owners of most of them cleaned them weekly and often did not store leftovers for more than 24 hours (table 3).

Table 3: Frequency of refrigerators, according to bacterial isolates

Bacterial type	Frequency	Percent
No bacterial isolate	15	40.5
C. freundii	5	13.5
L. monocytogenes	1	2.7
Pseudomonas spp.	1	2.7
More than one bacterial type	15	40.5
Total	**37**	**100.0**

As shown in Table 4, about 45.8% of samples collected from refrigerators from urban areas provided no bacterial growth compared with 38.5% samples from rural areas.

Table 4: Relation between bacteria present and area of samples

Area	Bacteria absent	Pathogenic bacteria	Non-pathogenic bacteria	Mixed bacteria	Total
Urban	11 (45.8%)	2 (8.3%)	5 (20.8%)	6 (25.1%)	24 (100.0%)
Rural	5 (38.5%)	1 (7.7%)	2 (15.4%)	5 (38.4%)	13 (100.0%)
Total	16 (43.2%)	3 (8.1%)	7 (18.9%)	11 (29.8%)	37 (100.0%)

Chi-Square =.757df =3

Discussion

Citrobacterfreundi was the most frequently isolated bacteria in this study. *C. freundii* is a common component of the gut microbiome of healthy humans[11]. While most strains are beneficial, Some rare strains of *C. freundii* have been associated with opportunisticnosocomialinfections of the respiratory tract, urinary tract, blood, and many other normally sterile sites in immunocompromised patients.*Listeria monocytogenes* and *Pseudomonas spp*. were the most frequently pathogens in this study and were recovered from 17.55% of the refrigerators examined.*Listeria monocytogenes*is the species of pathogenic bacteriathat cause thelisteriosisinfection.It can grow and reproduce inside the host's cells and is one of the most virulent foodborne pathogens, with 20 to 30% of food borne listeriosis infections in high-risk individuals may be fatal[12].*Pseudomonas spp.*are found widely in the environment, such as in soil, water, and plants. They usually do not cause infections in healthy people. Infectious species include *P. aeruginosa*, *P. oryzihabitans*, and *P. plecoglossicida*. *P. aeruginosa* flourishes in hospital environments. It is the second-most common infection in hospitalized patients (nosocomial infections).

E. coli was isolated from 10.63% of refrigerators in this study widely. It is accepted indicator of fecal contamination suggesting that the refrigerator internal surfaces are frequently contaminated by the import of contaminated raw foods or by poor personal hygiene.

Salmonella sp.was recovered from 14.03% refrigerators. It is a frequent contaminant of many retail foods posses public health challenges in terms of potential cross contamination to food and food preparation surfaces during routine food preparation. *Salmonella* sp. are equally easily spread through the domestic environment where they can persist for up to four days[13].Surface associated *Salmonella* sp. still because a significant cross contamination risk means this pathogen can multiply under condition of mild temperature abuse in cross contaminated foods[14]. *Salmonella sp.* was found to be easily spread throughout the domestic environment. *E. coli* was identified in this study, which is relatively rare occurrence of the low infectious dose pathogens in the human food chain and its ability to form viable non-culturable forms[15].

From 37 refrigerators (45.8%) of them were from urban areas showed no bacterial growth compared with those from rural areas (38.5%). Householders from urban areas may be more interested in cleaning and maintenance of refrigerators and preparing healthy food according to their knowledge about foodborne diseases. The results showed that 67.6% of respondents did not clean vegetables and fruits before keeping in refrigerators may because of the common belief that store them without washing makes them resistant to rot for longer time. That action leads to spread of bacteria in refrigerators. The use of chromagarmedia give rapid detection to differentiate between the bacterial colonies by color and morphology characteristics[16].

It is impossible to completely exclude food pathogens from the kitchens; however their spread, growth and survival can be controlled with correct food storage and preparation of practices and regular cleaning and disinfection of food contact site.This study concludes that most of the refrigerators under study were contaminated with some bacterial species and some of these are pathogenic and lead to diseases such as *Listeriaand Salmonella*. Continuous cleaning and using disinfectant agents reduces bacterial contamination.

Ethical Clearance: Approval to conduct the study was obtained from the dean of college of Nursing, University of Basrah.

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Conflict of Interest Statement: Nil.

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