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Contamination of refrigerator is a threat for infections

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ABSTRACT

Food that we eat may be perishable, semi-perishable and non-perishable. People generally use the refrigerator to store the perishable and semi-perishable food items. But during long time preservations without recalling may lead to the development of microbial contamination. Raw vegetables and fruits also play a major role in the spread of bacteria, fungi, and parasites. This may further lead to foodborne illness which is a threat to human infections. Cross contamination is also possible between the contaminated foods and fresh foods. Proper cleaning and maintenance reduce the bacteria as well as fungal load. Therefore, the monitoring of microbiota in the refrigerator is important for food safety.

Keywords— *Refrigerator, Contamination, Food Infections, Cleaning and Food Safety*

1. INTRODUCTION

Refrigeration is the most widely used method to prevent foods for some time. Mostly the perishable foods are kept for refrigeration to control the microbial contamination at 4-50C. (1, 2) Refrigeration not only minimizes the contamination of foods but also the chemical and enzymatic spoilage of food by retarding the growth of microbes (3). There are certain studies which proved that the perishable foods even preserved at refrigeration temperature also undergo spoilage (4). The food spoilage depends on the type of storage container or wrapping material. Several other factors related to the food stored also influence the rate of spoilage (5).

Most of the time inside the refrigerator (excluding the freezer) the temperature is about 7^0C which is called as sub-freezing temperature (6). This enables the maintenance of moisture and favours the growth of bacteria, molds and yeasts (7). The bacteria from uncleansed vegetables, fruits, milk packets, curd boxes, from wrapped foods directly purchased from the market such as cheese, jam, butter, ginger-garlic paste and so on grow spread to the other food materials inside the refrigerator. Long term preservation of these products leads to long term contamination (8, 9). The growth of fungi and bacteria rapidly increases leading to food and waterborne illness (10). Even the parasitic infestations caused by Cryptosporidium parvum and Giardia lamblia also are possible due to the improper maintenance of the refrigerator (11, 12).Bacteria such as Staphylococcus aureus, Listeria monocytogens, Escherichia coli, Salmonella spp, Campylobacter jejuni, Clostridium botulinum, Yersinia enterocolitica (13). Microbial contaminations of refrigerators have been studied because refrigerators are used to store food (14). Moisture and nutrients (food particles) in refrigerators provide favorable growth conditions for contaminating bacteria from unwashed raw foods, leaking packages, and hands. In particular, higher bacterial counts and temperatures in vegetable compartments could cause critical problems [15]. Recently, a German outbreak caused by Shiga-toxin producing Escherichia coli O104: H4 illustrated that unwashed vegetables could be a risk element [16]. Therefore, the study of bacterial contamination in the vegetable compartments of refrigerators is important for public health.

Most of the previously reported culture-dependent studies of kitchen and refrigerator microbes focused on pathogen detection [17]. The recent advent of next-generation sequencing techniques provides unprecedented data on the microbial composition, and the ecology of various environments, including indoor spaces [18].

2. MATERIALS AND METHODS

Samples were obtained from the refrigerator in the existing condition and after proper cleaning.

2.1 Samples obtained in the existing condition

2.1.1 The sampling procedure: Total of 200 samples were collected from 50 refrigerators (only at houses). Samples were obtained from all parts of the refrigerator (not the food samples) using sterile swabs dipped in saline solution. The swabs were dissolved first in sterile peptone water and then inoculated on different culture media.

2.2 Samples obtained after proper cleaning

After proper cleaning of refrigerator and wiping of the outer parts of all the containers, the samples were collected in the same manner as before.

2.2.1 Different culture media used to obtain the isolates: To obtain the bacteria isolated the samples were inoculated on CLED Agar, Mannitol Salt Agar, Salmonella-Shigella Agar, TCBS Agar and Blood Agar. To get the fungal isolates the samples were inoculated on Sabouraud Dextrose Agar (SDA). The samples were incubated and observed for the growth of colonies. The colonies were identified by the colony characters and Microscopy (Gram staining). The isolates were further identified by the biochemical tests such as IMViC tests, catalase test, Coagulase test, Oxidase test and fermentation tests.

3. RESULTS

3.1 Identification of bacteria and fungi from the samples in the existing condition

Total 10 bacteria and 4 fungi were identified from the samples collected from the refrigerator.

3.1.1 Bacteria identified from the samples

Table 1: Bacteria identified from the samples

Isolate	Organism identified	Gram staining	Lactose	Dextrose	Sucrose	H_2S production	Indole test	MR test	VP test	Citrate use	Urease test	Catalase test	Oxidase test	Starch hydrolysis
1	Escherichia coli	- Rod	AG	AG	A+	-	+	+	-	-	-	+	-	-
2	Staphylococcus aureus	+ Cocci	A	A	A	-	-	+	+	-	-	+	-	-
3	Shigella sp	- Rod	A	A	A	-	+	+	-	-	-	+	-	-
4	Alcaligens sp	Coccobacilli	-	-	-	-	-	-	-	+	-	+	+	-
5	Pseudomonas aeruginosa	- Rod	-	-	-	-	-	-	-	+	-	+	+	-
6	Klebsiella pneumoniae	- Rod	AG	AG	AG	-	-	-	+	+	+	+	-	-
7	Salmonella typhi	- Rod	AG	A	A	+	-	+	-	+	-	+	-	-
8	Proteus sp	- Rod	-	AG	AG	+	+	+	-	+	+	+	-	-
9	Micrococcus sp	+ Cocci	-	-	-	-	-	-	-	-	+	+	-	-
10	Bacillus cereus	+ Rod	-	A	A	-	-	-	+	-	-	+	-	-

3.1.2 Fungi observed in the samples

- **Aspergillus sp:** White colonies become black as culture matured. Single-celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the long conidiophores.
- **Alternaria sp:** Greyish-green colonies with aerial mycelium were observed. Pear-shaped multicelled conidia are observed and attached to single conidiophores arising from a septate mycelium.
- **Cladosporium sp:** Small, greenish-black, powdery colonies were observed. Conidia develop at the end of complex conidiophores.
- **Penicillium sp:** Greenish colonies were observed. Branched conidiophores were observed with the chains of conidia arising from the metula.

3.2 Counting the bacterial and fungal colonies after proper cleaning

A drastic reduction in the number of colonies of bacteria and fungi were observed indicating clearly that proper cleaning keeps the refrigeration safe.

4. DISCUSSION

There are so many factors which lead to refrigerator contamination of which cross-contamination of other stored foods and their persistence in the inner surfaces. According to Kumar and his associates, they isolated bacterial pathogens including Salmonella sp., Citrobacter sp., Shigella sp., and Proteus sp from the refrigerators of Vellore district in India. S. aureus, as a common inhabitant (up to 50%) of the human nose, throat, and skin is perhaps more likely to contaminate foods and refrigerators by direct or indirect human contact during domestic food handling and storage (19). Bacteria from indoor air contamination is also responsible for the inside contamination in the refrigerator as the door may be opened frequently for many purposes. [20]. Bacteroides vulgatus and Propionibacterium acnes were the most abundant on the surfaces of refrigerators (19).

Penicillium is isolated frequently from both air and surfaces in food processing areas and was the most frequent genus found in meat and dairy products (20, 21). In this study, Penicillium species was detected most commonly in refrigerators. Blue mould, caused by P. italicum is among the most economically important postharvest spoilage organisms, which affects fresh vegetables and other foods worldwide (22).

Aspergillus ochraceus, Aspergillus flavus, Aspergillus niger, Aspergillus terreus were the most common species from the Aspergillus genera found in mixed-food refrigerators. These moulds may cause invasive aspergillosis in immune compromised individuals (23). Mucor genera are associated with spoilage of cold-stored vegetables, cheese, and meat products (24). Many

species of Cladosporium are distributed worldwide and commonly found in air and indoor environments including food processing area (23, 25). Health effects caused by exposure to fungi and the role of fungal metabolites have been the focus of increased attention (26). Various species including Aspergillus and Penicillium genera are often associated with allergic symptoms of the respiratory system (27). Mycotoxin-producing fungi commonly found in the air of refrigerators include species of Aspergillus, Penicillium, and Alternaria (28, 29). Temperature control is important in restraining the growth of psychrotrophic bacteria and pathogenic microorganisms (Listeria monocytogenes, Salmonella sp.) in foods (30). Generally, microbial growth is related to increasing temperature (31). WHO (32) recommended the storage temperature of foods at a maximum of 5 °C in the refrigerator? Storing foods at an appropriate temperature is one of the best ways to slow the growth of dangerous bacteria. A refrigerator thermometer can make a big difference. Overpacking of food inside the refrigerator must be avoided in order to allow the cool air to circulate around all the food items. Cleaning the spills regularly minimizes the growth of Listeria bacteria. This practice even prevents cross contamination. Raw meat, poultry, seafoods, egg products are highly perishable. They should be kept quickly into the refrigerator, in order to stop the multiplication of bacteria. (33) It is advisable to throw out the recalled food. Removable parts inside the fridge must be cleaned with soap water and dried with a clean towel. The remaining body of the fridge also to be cleaned with soapy water and dried with a clean cloth. Then the cleaned parts should be arranged back for further use. (34)

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