Microbiological Quality of Household Refrigerators in Three Cities South-West of Nigeria

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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 examine the consumers’ knowledge and hygiene status of household refrigerators in Ibadan, Lagos and Abeokuta. One hundred and eighty households were randomly selected. Questionnaires covering social demographic characteristics, refrigerator quality and maintenance practices, householders’ knowledge about food borne diseases, and frequency of electricity supply were administered. The interior of each refrigerator was swabbed using a sterile swab stick that had been pre-soaked in pentone diluent. Each sample collected was plated out on four different culture media viz. Nutrient agar, MacConkey agar, Mannitol Salt agar and Potato Dextrose agar. Results showed that majority of the householders had good knowledge of food borne disease, powered their refrigerators with generators and have some levels of education. Some refrigerators were completely free from contamination (neither bacteria nor fungi was detected) while others were found to be contaminated. Total viable count of bacteria ranged between 0 and 14.1 × 10^6 cfu/ml while fungi count ranged from 0 to 6.8 × 10^4 cfu/ml. Bacteria and fungi isolated from the refrigerators were Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Enterobacter spp., Klebsiella spp. and Shigella spp., Aspergillus flavus, A. niger, A. fumigatus, Saccharomyces cerevisiae and Rhizopus spp. Findings show the presence of microorganisms which can cause food spoilage and food borne diseases. Educational programmes are needed to improve the refrigeration practices and the issue of regular supply of electricity is paramount in the hygiene of refrigerators.

Keywords: Household refrigerators; Knowledge about food safety; Demographics; Electricity; Microbial quality; Food spoilage; Food borne illness; Education; Nigeria

Introduction
Consumers store various kinds of food in refrigerator at an inappropriate temperature which could give rise to food borne diseases or illness [1]. Food borne pathogens are the leading cause of illness and death in less developed countries. In the developed countries food borne pathogens are responsible for millions of cases of infections such as gastrointestinal diseases each year [2]. 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 [3].

Refrigerators are one of the most important kitchen appliances found in homes 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 [4]. Lower temperature in a confined volume lowers the reproduction rate of bacteria, 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. The device is thus used to reduce the rate of spoilage of foodstuffs [5].

Foods are stored in the refrigerators for preservation so as to be available for future use. Refrigeration is employed to control the rate of certain chemical and enzymatic reactions as well as rate of growth of food microorganisms [4]. 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).

A study has 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 stored in and duration of storage are also important factors that influence the type of microbial growth, toxicity and spoilage of food during refrigerated storage. Although low temperature retards spoilage but even a sub-freezing temperature of about -7°C does not prevent multiplication of all microorganisms. Refrigerated foods are therefore subjected to spoilage by moulds, yeasts and bacteria [7].

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 [8]. Inadequate food handling at processing sites is frequently implicated in outbreaks [9,10]. Consumers associate Food Borne Illness (FBI) with the consumption of foods outside of home; however, it is well known that foods prepared and consumed at home also represent a risk for FBI outbreaks [11-13]. Indeed, FBI originates in private homes three times more frequently than in food service operations [14]. Research indicates that perhaps as many as 50% of household FBI can be attributed to an inappropriate food storage including ineffective chill storage and refrigerator management [15]. Bacteria 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 [16].

It was reported that foodborne illnesses in the African region recorded a dramatic rise in 2008; anthrax in Zimbabwe; typhoid fever in Uganda, chemical poisoning due to consumption of seed beans and maize in Nigeria and Kenya; cholera from several countries e.g., Mozambique, Nigeria, Congo, Zambia, DRC, Kenya, Tanzania, South Africa, Zimbabwe; pesticide poisoning from cabbage and other vegetables in Senegal; fish mouse in Mauritius; mushroom poisoning in Algeria; Botulism and Hepatitis A in Uganda; Gala Night Dinner Meal, Nigeria [17].

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. The aims of this study are to examine the food safety knowledge of householders, determine the general hygiene status of different household refrigerators and the temperature status of domestic refrigerators in three cities south west of Nigeria.

Materials and Methods

Study protocol (questionnaire distribution)

In this study, the three cities selected are Ibadan, Sango-Ota and Lagos. In these selected cities, we focused on high density areas which were mainly inhabited by the middle and low income individuals. In each city, sixty households that were willing to participate in the study were randomly selected. The evaluation of householders’ knowledge in relation to the use of refrigerator was done by giving questionnaires consisting of questions covering social demographics, refrigeration practices, refrigerator maintenance practices, incidence of food borne diseases etc. to each household (owner of refrigerator) of the selected houses with plea to be sincere with their responses.

Refrigeration temperature determination

A thermometer was inserted into the refrigerator and removed after few minutes. The temperature was read and noted.

Microbiological analysis of samples

A total of 180 household refrigerators were sampled in Ibadan, Lagos and Abeokuta. With the consent of the householders the interior of each refrigerator was swabbed using a sterile swab stick that had been soaked in peptone 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:

| Media                           | Test                              |
|---------------------------------|-----------------------------------|
| Nutrient Agar                   | for total mesophilic count         |
| MacConkey Agar for Enterobacteriaceae count |                      |
| Mannitol Salt Agar for Staphylococcus aureus count |                      |
| Potato Dextrose Agar for fungal count |                      |
| The bacteria plates were incubated at 37°C for 48 hours, and the fungi plates were incubated at room temperature (28 ± 2°C) for five days. |

Characterization and identification of bacterial isolates

This was done according to standard methods described by Olutitiola et al., [18]. The methods involved Gram staining, morphological, and cultural and biochemical characteristics

(Catalase, coagulase, oxidase, methyl red, Voges Proskauer, citrate reduction, indole and sugar fermentations test).

Characterization and identification of fungal isolates

Two drops of lactophenol blue was put on a clean glass slide containing mycelia growth. The mould isolates were examined macroscopically and microscopically using the schemes of Klich [19].

Results

Table 1 shows the social demographic, refrigerator maintenance practices and food safety knowledge of the householders that participated in the study. Educational statuses of respondents show that they all have some levels of education ranging from National Certificate of Education to Doctor of Philosophy.

Between 15% and 25% of households had 3 hours of light in the cities reported while 40 - 70% had electricity for 6-9 hours and 5 - 20% had electricity for 10-12 hours. Sango-Ota had no erratic electricity supply. No respondent reported all round electricity supply. It was seen that the different locations in the three cities had different electricity distribution patterns. Sango Ota had regular electricity supply than Ibadan and Lagos. This led to the issue of using generators to power their refrigerators. The time each respondent empowered his refrigerator with generator varies from individual to individual.

Another factor that could have affected the microbial quality of refrigerators was the cleaning regimes. Most respondents reported varying degrees of cleaning regimes. On weekly basis, 25 - 80% of respondents reported cleaning their refrigerators as shown in Table 1. Between 20% and 75% reported cleaning their refrigerators on monthly basis and no respondent reported yearly cleaning.

A key factor in determining the microbiological quality of refrigerators is the temperature regimes of the sampled refrigerators. Results show that 53 households had refrigerators whose temperatures were less than 5°C, while 127 refrigerators had greater than 5°C (Table 2). The microbiological analysis of the refrigerators revealed various degrees of microbiological contamination. Out of the 180 refrigerators sampled microbiologically, 14 refrigerators had total viable bacterial counts of less than or equal to 10⁶ cfu/ml while 166 refrigerators had greater than or equal to 10⁶ cfu/ml. S. aureus counts showed that 42 refrigerators had counts less than or equal to 10⁶ cfu/ml when compared with 138 refrigerators that had counts greater than or equal to 10⁶ cfu/ml. Thirty refrigerators had Enterobacteriaceae counts of less than or equal to 10⁶ cfu/ml while 150 refrigerators had greater than or equal to 10⁶ cfu/ml. Fungal counts of 20 refrigerators were less than or equal to 10⁶ propagules while 160 refrigerators had above 10⁶ propagules /ml (Table 3). Bacteria and fungi isolated include: Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter sp., Penicillium sp., Saccharomyces cerevisae, Muor, Aspergillus niger, A. fumigatus, Klebsiella sp.

Discussion

Consumers associate food borne illness with the consumption of foods outside of home; however, it is well known that foods prepared and consumed at home also represent a risk for Food Borne Illness (FBI) outbreaks [11-13]. Indeed, FBI originates in private homes three times more frequently than in food service operations [14]. Research indicates that perhaps as many as 50% of household FBI can be attributed to an inappropriate food storage including ineffective chill
storage and refrigerator management [15]. Bacteria 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 [16].

In this study it was seen in the questionnaires that most householders do not have steady electricity supply and this affected the temperature regimes of refrigerators (Table 2). Out of the 180 refrigerators sampled only 53 (29.4%) had temperature of ≤ 4°C. 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 (70.6%) which will allow growth of mesophilic microorganisms which can be pathogenic and therefore increases the risk of food borne disease [20,21]. 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 [22]. This may not be the case for some householders that get alternative source to power their refrigerators in some selected rich communities and some industrial areas especially are specially favored in the electricity distribution pattern like SangoOta. The total viable count contamination levels observed in this study show that 14 out of 180 refrigerators had bacteria count below a million colonies (Table 3). The bacteria counts for S. aureus and coliforms shown in Table 3 were high. 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 domestic refrigerators are likely to be influenced by a range of factors including the nature and levels of initial contamination introduced on 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 [16].

The presence of microorganisms in the refrigerator affects the microbial quality of foods kept in it. During the course of this study and with the aid of the results from the questionnaires given for knowledge survey it was observed that irregular power supply, level of cleanliness and general knowledge of food safety greatly accounted for the presence of spoilage and pathogenic microorganisms inside the refrigerator.

This study identified the presence of undesirable food related pathogens such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas spp., Aspergillus fumigatus, A. niger, Penicillium spp. which were isolated from 170 refrigerators sampled. S. aureus and E. coli were the most frequently isolated pathogen in this study and were recovered from 75% of the 170 refrigerators examined. Unlike the other microbial pathogens, which principally

| Variable                             | Frequency (%) |
|--------------------------------------|---------------|
|                                       | Sango-Ota     | Ibadan        | Lagos         |
| Sex                                   |               |               |               |
| Male                                  | 48 (80)       | 6 (10)        | 18 (30)       |
| Female                                | 12 (20)       | 54 (90)       | 42 (70)       |
| Age                                   |               |               |               |
| 20-30                                 | 36 (60)       | 24 (40)       | 12 (20)       |
| 31-40                                 | 18 (30)       | 24 (40)       | 30 (50)       |
| 41-50                                 | 6 (10)        | 9 (15)        | 18 (30)       |
| >50                                   | -             | 3 (5)         | -             |
| Educational Qualification             |               |               |               |
| ND/NCE                                | 9 (15)        | 9 (15)        | 18 (30)       |
| HND/BSc                               | 39 (65)       | 27 (45)       | 36 (60)       |
| MSc                                   | 9 (15)        | 12 (20)       | 6 (10)        |
| PhD                                   | 1 (5)         | 12 (20)       | -             |
| How many Refrigerators do you have?   |               |               |               |
| One                                   | 45 (75)       | 30 (50)       | 45 (75)       |
| Two                                   | 15 (25)       | 27 (45)       | 15 (25)       |
| More than two                         | -             | 3 (5)         | -             |
| How old is your Refrigerator?         |               |               |               |
| <1 yr                                 | 9 (15)        | 15 (25)       | 6 (10)        |
| 1-5 years                             | 45 (75)       | 27 (45)       | 30 (50)       |
| >5 years                              | 6 (10)        | 18 (30)       | 24 (40)       |
| How often do you have electricity in a day? |           |               |               |
| At least 3 hours on the average       | 9 (15)        | 15 (25)       | 15 (25)       |
| 6-9 hours on the average              | 42 (70)       | 24 (40)       | 33 (55)       |
| 10-12 hours on the average            | 9 (15)        | 12 (20)       | 3 (5)         |
| Round the clock                       | -             | -             | -             |
| Electricity supply is very erratic     | -             | 9 (15)        | 9 (15)        |
| Do you power your refrigerator when there is power outage? |           |               |               |
| Occasionally                          | 12 (20)       | 24 (40)       | 36 (60)       |
| Regularly                             | 42 (70)       | 24 (40)       | 15 (25)       |
| Rarely                                | 6 (10)        | 12 (20)       | 9 (15)        |
| How often do you clean your refrigerator? |             |               |               |
| Daily                                 | -             | -             | 9 (15)        |
| Weekly                                | 15 (25)       | 48 (80)       | 30 (50)       |
| Monthly                               | 45 (75)       | 12 (20)       | 21 (35)       |
| Yearly                                | -             | -             | -             |
| What do you use to clean your refrigerator? |         |               |               |
| Water only                            | 3 (5)         | 6 (10)        | 18 (30)       |
| Water and Soap                        | 48 (80)       | 48 (80)       | 27 (45)       |
| Disinfectant                          | 9 (15)        | 6 (10)        | 3 (5)         |
| Dry wipes                             | -             | -             | 12 (20)       |
| Is your refrigerator door always tightly closed? |       |               |               |
| Yes                                   | 60 (100)      | 60 (100)      | 60 (100)      |
| No                                    | -             | -             | -             |
| Have you heard about food safety?     |               |               |               |
| Yes                                   | 60 (100)      | 60 (100)      | 48 (80)       |
| No                                    | -             | -             | 12 (20)       |
| Do you know anything about food borne disease/food poisoning? | | | |
| Yes                                   | 51 (85)       | 60 (100)      | 51 (85)       |
| No                                    | -             | -             | -             |
| If yes, how did you get informed?     |               |               |               |
| Through the media                     | 45 (75)       | 3 (5)         | 27 (45)       |
| Through friends                       | -             | 42 (70)       | 12 (20)       |
| A relative has been affected          | -             | 3 (5)         | 9 (15)        |
| Others                                | 15 (25)       | 12 (20)       | 12 (20)       |
| Does your food item stay long in the refrigerator? | | | |

Table 1: Social demographic, refrigerator maintenance practices and food safety knowledge of householders in three South-Western Nigerian cities.
enter domestic kitchens from previously contaminated raw foods, *S. aureus* as a common inhabitant (up to 50%) of the human nose, throat, and skin [24] is perhaps more likely to contaminate foods and refrigerators by direct or indirect human contact during domestic food handling and storage. As a gram-positive organism, it is relatively resistant to drying and is, therefore more likely to become dominant than more desiccation-sensitive organisms, especially in the low water activity conditions which prevail in domestic refrigerators.

*E. coli* is widely accepted indicator of fecal contamination suggesting that the refrigerator internal surfaces are frequently contaminated by import of contaminated raw foods or by poor personal hygiene. It is impossible to completely exclude food pathogens from the kitchen; 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. *E. coli*, *Klebsiella sp.*, *Enterobacter spp.* and *Shigella spp.* are enteric organisms that can cause gastroenteritis, Hemolytic Uremic Syndrome and shigellosis [25,26].

The three *Aspergillus spp.* are agents isolated from household refrigerators that can cause food intoxication as they possess toxins which are harmful to the body when contaminated foods are consumed. Majorly *A. flavus* isolates produce mycotoxins such as aflatoxins. Aflatoxin B1 is known to cause toxic carcinogenic, mutagenic and teratogenic effect in patient. The International Agency for Research on Cancer (IARC) has designated aflatoxin as a human liver carcinogen.

This study has shown that food pathogens can survive on refrigerator surfaces and could therefore, pose a cross contamination risk. The need to have constant power supply and making sure the refrigerator is working at the normal chilling temperature is highly important to reduce the growth of these microorganisms. The potential risk of these organisms is heightened by their ability to multiply at refrigeration or mild abused temperatures. The recommended time in which, chilled foodstuffs are stored at required temperatures in case of power failure vary e.g. from 2 to 6 hours. The pathogens found in domestic refrigerators could contaminate food directly or indirectly and pose a major health risk to consumers in terms of food poisoning.

Thus, food safety knowledge with a focus on kitchen hygiene and cleaning and disinfection of food contact site. It is impossible to completely exclude food pathogens from the refrigerator internal surfaces are frequently contaminated by import of contaminated raw foods or by poor personal hygiene. Majorly *A. flavus* isolates produce mycotoxins such as aflatoxins. Aflatoxin B1 is known to cause toxic carcinogenic, mutagenic and teratogenic effect in patient. The International Agency for Research on Cancer (IARC) has designated aflatoxin as a human liver carcinogen.

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Thus, food safety knowledge with a focus on kitchen hygiene and preventing of cross contamination is necessary if the scale and effect of domestic food borne illness is to be reduced. Storage (refrigeration), at appropriate temperature should be practiced.

**Table 2: Temperature regimes of 180 household refrigerators**.

| Temperature | ≤ 4°C | ≥ 4°C |
|-------------|-------|-------|
| Number of refrigerators | 53 | 127 |

**Table 3: Microbial counts obtained from swabbed surfaces of 180 household refrigerators.**

| Microorganisms | Frequency ≤ 10^9 | Frequency ≥ 10^9 |
|----------------|------------------|------------------|
| Total Viable Count | 14 | 166 |
| *S. aureus* Count | 42 | 138 |
| Enterobacteriaceae Count | 30 | 150 |
| Fungi Count | 20 | 160 |

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