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Bacterial Contamination of Some Domestic and Laboratory Refrigerators in Port Harcourt Metropolis.

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ABSTRACT

Background: A refrigerator is a potential source of pathogen contamination leading to food spoilage and food borne diseases. It forms an essential connection in broad chain of cross contamination which might possibly lead to the outbreaks of food borne diseases.

Purpose: The aim of the study was to evaluate bacterial Contamination of Some Domestic and Laboratory Refrigerators in Port Harcourt Metropolis.

Methodology: This study was done in Port Harcourt metropolis in Rivers state, Nigeria. Sterile swab sticks moistened with peptone water were used to swab the handles, bases, sides and shelves of domestic (90) and laboratory (60) refrigerators. One hundred and fifty (150) samples obtained in total were inoculated and identified using standard conventional methods of bacteria identification.

Results: The results showed 100% bacterial contamination. With prevalence rates of *Staphylococcus aureus*(30.6%), *Klebsiella pneumonia* (5.3%), *Vibro cholera* (4.7%), *Escherichia coli* (4%), *Salmonella typhi* (3.3%), *Pseudomonas aeruginosa* (2%), *Bacillus cereus* (1.3%), *Clostridium botulinium* (1.3%), *Shigellaspecies* (1.3%), *Listeria monocytogenes* (1.3%), *Proteus mirabilis* (1.3%), *Vibroparahaemolyticus* (1.3%), *Yersinia enterocolitica* (1.3%) and *Clostridium perfringens*(0.7%). Chi square confirms that relationships exist between bacteria isolate with the batch sampled (chi square =139.239; df=54; p=0.00), Gram reaction (chi square =150.000; df=18; p=0.00) and the refrigerator type (chi square =35.325; df=18; p=0.00). However, no statistically significant association exists between isolates and positions on Refrigerator (chi square = 68.080; df=72; p=0.61).

Conclusion: The outcome of this study confirms the presence of mesophilics and psychrotrophic bacteria in refrigerators, including potential foodborne pathogens which can cause serious health challenges. Hence, refrigerators are potential pathogen contamination sources. Therefore, proper hand hygiene, awareness and sensitization on maintenance of appropriate, good storage and refrigerator management is recommended for all.

Keywords: *Bacteria, Contamination, Domestic, Laboratory, Refrigerators, Food borne diseases.*

1.0 INTRODUCTION

Foodborne diseases are the result of ingestion of contaminated food materials. This contamination can either be caused by microorganisms or chemicals. The contamination of food may occur at any stage in the process from food production to consumption otherwise referred to as "farm to fork". This can result from environmental contamination, including pollution of water, soil or air. It is an issue of public health concern particularly with its varying epidemiology. Food borne pathogens cause millions of cases of sporadic illnesses and chronic complications, as well as huge and demanding outbreaks over many states and nations [1]. Emergence and re-emergence of these pathogens including the worldwide spread have worsened the challenge. Several microorganisms, including Salmonella, Escherichia *coli* O157:H7, *Campylobacter,* and *Yersinia enterocolitica*, have reservoirs even in healthy food animals, from which they spread to a rising variety of foods. In 2016, disease rates increased in the United States for many foodborne pathogens, including Shiga toxin-producing *E. coli* according to a study by Eskin in 2017 [2].

The menace of foodborne disease is not limited to developing countries rather developed countries are faced with similar challenge. In 2017, Eskin in a report stated that United States has continued to make minimal improvements driving down the rates of infection by bacteria commonly transmitted through food. This was based on FoodNet 2016 outcome. Besides, preliminary FoodNet 2016 results show disturbing trends. Illness rates for *Campylobacter, Listeria, Salmonella, Shigella,* Shiga toxin-producing *E. coli O157*and *Yersinia* did not change compared with 2006-2008 despite the fact that rates for *Vibrio* and *Cryptosporidium* showed a statistically significant increase [2]. Illnesses associated with food to a large extent are preventable, but it can lead to long-term health challenges due to complications and even death, particularly for more vulnerable groups like the immune compromised, paediatrics and geriatrics.

Foodborne diseases cover a wide continuum of illnesses and are a rising public health problem globally. There are various signs and symptoms of foodborne diseases but the most common clinical presentation takes the form of gastrointestinal symptoms; nonetheless, such diseases can also have neurological, gynaecological, immunological including other symptoms. Multi-organ failure and even cancer may result from the ingestion of contaminated foods, consequently representing a huge burden of disability as well as mortality [3].

The process of storage of food can promote the growth of microorganisms if present in the food. Refrigeration is the commonly used method of preservation and storage especially for foods that cannot be consumed immediately. Domestic refrigerators are of immense aid for domestic use for food items and other materials. Domestic refrigerators are built in such a way that temperatures are regulated even without a visible thermometer. Food preservation is usually guaranteed with its use. On the other hand, lack of periodic maintenance, improper handling and other issues have contributed to malfunctioning of these refrigerators including the manufacturing process and materials used. The resultant effect is the loss of preserving capacity of these refrigerators. Hence, food stored in the domestic refrigerator is prone to spoilage due to bacterial contamination either from internal or external sources.

Domestic refrigerator is a low temperature appliance used in homes for the preservation and storage of food products [4]. Refrigerator follows the principle of refrigeration whereby microbial growth is controlled by inhibition at a low temperature to achieve optimum preservation. It is one of the most widely practiced methods of controlling microbial growth on perishable products of which temperature specification of four to five degree Celsius (4-5°C) is considered desirable. The underlying mechanism is that it controls the rate of certain chemical and enzymatic reactions as well as the rate of growth of microorganisms; spoilage slows down as molecular motion slows, which retards growth of bacteria that causes spoilage [5].

Nonetheless, some studies have revealed that perishable materials like food will deteriorate even at refrigerator temperature as a result of spoilage consequent to some factors like presence of microorganisms, enzymes and oxidation [6]. Furthermore, there are other determinants of spoilage such as material or container used in storing as well as the duration of storage. These factors which determine spoilage are important because they influence the type of bacteria that can grow, the toxins produced as well as its toxicity consequent to spoilage during refrigerated storage [7].

Remarkably, low temperature retards spoilage, but even a sub-freezing temperature of about 7°C does not prevent multiplication of all microorganisms. Refrigeration of foods and other materials are for that reason subjected to spoilage by microorganisms like bacteria and fungi that might possibly survive the refrigeration temperature [8]. For domestic refrigerator, Bacteria from unwashed raw foods, leaking packages, unclean container surfaces introduced into refrigerators may perhaps cause direct contamination of other stored materials and persist on the interior surfaces. This in turn creates the risk of indirect long term contamination. The internal surface of refrigerator generally creates a bad environment for a lot of pathogenic bacteria apart from the fact that, most of them possess the capacity of growing and surviving adverse condition like reduced temperature. However, inadequate maintenance may make the refrigerator a breeding place for such bacteria [9].

Basically, the role of the refrigerators as a domestic or a laboratory preservation device cannot be underestimated. A refrigerator is a great asset to any domestic, medical facility, laboratory or supply room. This is based on the nature of activities done there as per food preservation and maintenance of the quality of laboratory materials without deterioration.

For laboratory refrigerator, this main point of storage and preservation is not without a challenge as there is always the risk that the interior or exterior of the refrigerator will get dirty. However, this is not limited to laboratory refrigerator but affects domestic refrigerator as well. Along with the dirt that is to be expected with daily use for storing specimens and liquids there is also the risk of contamination and bacterial growth within the laboratory refrigerator itself. This type of contamination can have very serious consequences so it is important to have an effective routine cleaning program in place.

Unlike a standard domestic refrigerator, laboratory refrigerators are constructed with easy cleaning and decontamination in mind. The high quality laboratory refrigerators will be made with molds and will have no internal seams. This means that the unit is one complete surface inside, without seams where bacteria or contaminants can become lodged or grow and thrive. Without any seams in the interior it is also very easy to wipe down and clean. Shelves and

bins are typically removable for easy cleaning plus the shelves may be wired to prevent liquids that spill from pooling and collecting on the shelf. The interior of most refrigerators is white, allowing for easy determination of spills which have occurred in the interior. Spot cleaning is certainly possible with a simple wipe down with a cloth. Knowing what material was spilled in the interior will also determine if a full cleaning and decontamination with cleanser and disinfectant is necessary.

Cleaning on a specific routine or schedule is important both to remove containers and materials that are outdated or forgotten on the shelves but also to routinely clean and disinfect the entire interior surface. Routine cleaning and disinfecting of the interior and exterior of laboratory refrigerators then becomes a standard practice and requires very little time when completed on a standard schedule. The specific schedule that is best suited for laboratory or medical facility's needs; will depend on the use, types of samples and materials, and the overall condition of the unit on a regular basis. Besides, cleaning with warm water and commercial disinfectants that is approved for use you the facility should provide all the cleaning required. It is important to avoid using any type of scraping device, including steel wool or cleansers that may scratch or damage the surface. Scratches in the interior surface will lead to an increased risk of contamination within the laboratory refrigerators. If necessary a cloth with warm water and cleanser that is left on the material will help to soften and lift gummy material or stains.

Microorganisms are ubiquitous in nature, its presence in a particular location and condition suitable for it multiplication dictate it pathogenicity or not. The presence of these microorganisms, including potential foodborne pathogens and others, in domestic and laboratory refrigerators portends serious health implications due to the disease causing capacities of these microorganisms. Contamination of refrigerators has been linked to poor hygienic conditions as microorganisms tend to thrive under such conditions. Microorganisms exist mainly in abundance in the presence of nutrients, moisture and temperature suitable for growth and multiplication [10].

The ubiquitous nature of microorganisms make it possible for organisms to exist almost everywhere including air, food, body surfaces and other close environments. Its presence in a location has varying effects which could either be beneficial or harmful. Based on this, microorganisms are also found in the refrigerator and some are known to survive extreme refrigeration (psychrophiles) and cause spoilage. Interestingly, non psychrophiles have also been isolated from refrigerators [9]. These have posed lots of threats to users in recent time therefore; this present study was carried out to consider some of these public health implications that are caused bacterial contamination and inadequate refrigeration.

2.0. Materials and Methods

2.1. Study Area

The study area was Port Harcourt metropolis with Coordinates 4.75°N 7°E. Port Harcourt lies along the Bonny Stream and is arranged in the Niger Delta. Beginning at 2016, the Port Harcourt urban domain has a population of 1,865,000 inhabitants, up from 1,382,592 beginning at 2006 [11].

Figure 1. Map of Rivers State showing Port Harcourt (Study Area)

2.2. Study Design

The study was an observational study of Bacterial Contamination of some Domestic and Laboratory Refrigerators in Port Harcourt Metropolis.

2.3. Selection Criteria

All households and laboratories that agreed to participate in the study and Refrigerators that were actively working with materials in-stock were included into the study**.** All households and laboratories that denied approval and those outside the metropolitan city of Port Harcourt.

2.4. Sample Size Determination

The formula was used to estimate the sample size:

$N = Z^2 p (1-p)/e^2$ (Cochran, 1977)

Where N = Minimum sample size

Z = Standard normal deviant curve corresponding to 95% = 1.96

p = Proportion/prevalence of Prevalence of Bacteria (*Aeromonas hydrophilla*) in Refrigerator shelves = 9.7% [12].

e = Precision or error margin = 0.05

Hence, **$N = Z^2 p (1-p)/e^2$**

$N = (1.96)^2\ 0.097\ (1-0.097)/0.05^2$

N -=133

10% Non Response to make up for losses = 10% of minimum sample size = 10/100 x 133 = 13.3. Therefore, 133 + 13 = 146. A sample size of 146 was made up to 150 and used for the study.

2.5. Sampling Technique

In order to reduce bias, simple random sampling techniques was used to give every subject an equal opportunity of been part of the study. Also, unannounced visits were made to the various households and laboratories used for this study. Households were randomly selected within the identified sample area of Port Harcourt metropolis using a random walk technique until the required sample size was achieved [13].

2.7. Sample Collection

With the consent of the house owner and the laboratory heads the interior (base, shelves and sides) of each refrigerator was swabbed. Sterile swab sticks moistened with peptone water were used to swab the refrigerator parts of interest. The swabs were aseptically transferred to appropriate culture media and the cultures incubated at 37°C for 24 hours. The base, shelves and inner sides of selected domestic and laboratory refrigerators

A hundred and fifty (150) samples were obtained from domestic (90) and laboratory (60) refrigerators accessed with consent of the owners. Samples were taken from the base, shelves and sides of the refrigerator using sterile swab sticks moistened in sterile peptone water and transported back to the Laboratory for analysis within one hour of collection.

2.8. Sample Analysis

The samples taken with sterile swab sticks were moistened with sterile peptone water and cultured on different culture media such asThioglycollate bile salt (TCBS), Salmonella shigella Agar (SSA), Cystein lactose electrolyte deficient (CLED), Chocolate, Blood and MacConkey media. After 24 hrs incubation at 37^{o}C isolates on the culture plates were identified following standard microbiological procedures.

2.9. Statistical Analysis

SPSS version 21 and Winpepi statistical software were used for the analysis. Frequency, percentage and prevalence were calculated. Chi square was also used for test of significance at $p<0.05$.

3. RESULTS

The results obtained were represented in the tables.Table 4.1 represents overall frequency distribution/percentage occurrence of isolates from the study, *Staphylococcus aureus*had the highest 67(44.7%) while *Bacillus anthracis, Clostridium tetani, Streptococcus pneumonia* and *Streptococcus pyogenes* had the lowest 1 (0.7%). This shows multiple lowest occurrences. Details of other the frequency/distribution of microorganisms isolated are also presented on table 4.1.

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Table 4.1: Overall Frequency Distribution/Percentage Occurrence of Isolates from the Study

Isolates	Frequency	Percentage (%)	Remark
Bacillus anthracis	1	0.7*	Lowest
Bacillus cerus	5	3.3	
Clostidium perfringens	3	2.0	
Clostridium botulinium	6	4.0	
Clostridium perfringens	6	4.0	
Clostridium tetani	1	0.7*	Lowest
Escherichia coli	11	7.3	
Klebsiella pneumonia	13	8.7	
Listeria monocytogenes	4	2.7	
Proteus mirabilis	2	1.3	
Pseudomonas aeruginosa	10	6.7	
Salmonella typhi	6	4.0	
Shigella species	2	1.3	
Staphylococcus aureus	67	44.7	Highest
Streptococcus pneumonia	1	0.7*	Lowest
Streptococcus pyogenes	1	0.7*	Lowest
Vibro cholera	7	4.7	
Vibro parahaemolyticus	2	1.3	
Yersinia enterocolitica	2	1.3	

Note: Superscript ***** indicates similar percentage occurrence

Table 4.2 represents the Percentage Occurrence of Bacteria Isolated by Refrigerator types (Domestic and Laboratory). The study revealed that *Staphylococcus aureus*had the highest percentage occurrence of 51.1% and 35.0% for domestic and laboratory refrigerators respectively as well as an overall percentage occurrence of 44.7% for combination of domestic and laboratory refrigerators. Multiple lowest percentage occurrences of 0.7% was observed in *Bacillus anthracis, Clostridium tetani, Streptococcus pneumonia and Streptococcus pyogenes.* However, the study showed varying percentage occurrences for the both domestic and laboratory refrigerators studied. Domestic refrigerators had *Clostridium perfringens*(1.1%) whereas, laboratory refrigerators had *Bacillus anthracis , Clostridium tetani , Salmonella typhi , Streptococcus pneumonia* and *Streptococcus pyogenes* which occurred at similar rate with 1.7% been the lowest.

Also, there is dissimilarity in the occurrence of some bacteria isolated from the different refrigerators like domestic refrigerators harboured *Proteus mirabilis, Shigella species, Vibro cholera, Vibroparahaemolyticus*and *Yersiniaenterocolitica*while these were not detected in the laboratory refrigerators. On the other hand, laboratory refrigerator had *Bacillus anthracis, Clostidiumperfringens, Clostridium tetani, Streptococcus pneumonia* and *Streptococcus*

*pyogenes*while the domestic refrigerators did not habour these bacteria as reported in this study.

Conversely, *Bacillus cerus, Salmonella typhi, Listeria monocytogenes*and others as observed were reported in both domestic and laboratory refrigerators although at varying percentages ass seen in table 4.2.

Table 4.2: Percentage Occurrence of Bacteria Isolated by Refrigerator Type (Domestic and Laboratory)

Isolate	Refrigerator		Total
	Domestic(N=90)	Laboratory(N=60)	(N=150)
Bacillus anthracis	0.0%	1.7%	0.7%
Bacillus cerus	2.2%	5.0%	3.3%
Clostidium perfringens	0.0%	5.0%	2.0%
Clostridium botulinium	2.2%	6.7%	4.0%
Clostridium perfringens	1.1%	8.3%	4.0%
Clostridium tetani	0.0%	1.7%	0.7%
Escherichia coli	6.7%	8.3%	7.3%
Klebsiella pneumoniae	8.9%	8.3%	8.7%
Listeria monocytogenes	2.2%	3.3%	2.7%
Proteus mirabilis	2.2%	0.0%	1.3%
Pseudomonas aeruginosa	3.3%	11.7%	6.7%
Salmonella typhi	5.6%	1.7%	4.0%
Shigella species	2.2%	0.0%	1.3%
Staphylococcus aureus	**51.1%**	**35.0%**	**44.7%**
Streptococcus pneumonia	0.0%	1.7%	0.7%
Streptococcus pyogenes	0.0%	1.7%	0.7%
Vibro cholera	7.8%	0.0%	4.7%
Vibro parahaemolyticus	2.2%	0.0%	1.3%
Yersinia enterocolitica	2.2%	0.0%	1.3%

Equally, prevalence rate of Bacteria was estimated in this study. *Staphylococcus aureus* was the most prevalent bacteria with an overall prevalence rate of 44.7%. Specific prevalence rates revealed highest prevalence rate of 30.6% for *Staphylococcus aureus* for domestic refrigerator.

Comparably, specific prevalence for laboratory refrigerator showed a lower rate of about 14% for *Staphylococcus aureus.* Nonetheless, *Staphylococcus aureus* appeared as the most prevalent bacteria as well in the laboratory refrigerator notwithstanding the lower rate when weighed against the domestic refrigerator.

A least prevalence rate of 0.7% was recorded in this study for general and specific groups (domestic and laboratory refrigerators). However, the bacteria differ in some as others shared similarities as seen in table 4.3.

Table 4.3: Prevalence of Bacteria Isolated by Refrigerator Types (Domestic and Laboratory)

Isolate	Domestic	Laboratory	Total
Bacillus anthracis	0.0%	0.7%	0.7%
Bacillus cerus	1.3%	2%	3.3%
Clostidium perfringens	0.0%	2%	2%
Clostridium botulinium	1.3%	2.7%	4%
Clostridium perfringens	0.7%	3.3%	4%
Clostridium tetani	0.0%	0.7%	0.7%
Escherichia coli	4%	3.3%	7.3%
Klebsiella pneumonia	5.3%	3.3%	8.7%
Listeria monocytogenes	1.3%	1.3%	2.7%
Proteus mirabilis	1.3%	0.0%	1.3%
Pseudomonas aeruginosa	2%	4.7%	6.7%
Salmonella typhi	3.3%	0.7%	4%
Shigella species	1.3%	0.0%	1.3%
Staphylococcus aureus	30.6%	14.0%	44.7%
Streptococcus pneumonia	0.0%	0.7%	0.7%
Streptococcus pyogenes	0.0%	0.7%	0.7%
Vibro cholera	4.7%	0.0%	4.7%
Vibro parahaemolyticus	1.3%	0.0%	1.3%
Yersinia enterocolitica	1.3%	0.0%	1.3%
Total	60%	40%	

Table 4.4 represents Chi square distributions of isolates with batch sampled, position on refrigerator, Gram reaction and refrigerator was done to measure the relationship between the isolates and the other variables. The study demonstrates that relationships exist between bacteria isolate with the batch sampled (chi square =139.239; df=54; p=0.00), Gram reaction (chi square =150.000; df=18; p=0.00) and the refrigerator type (chi square =35.325; df=18; p=0.00). However, no statistically significant association exist between isolates and Position on the Refrigerator (chi square = 68.080; df=72; p=0.61). See table 4.4 for detail.

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Table 4.4: Chi Square Distribution of Isolates with Batch, Position On Refrigerator, Gram Reaction and Refrigerator

Variable	Classification	Frequency (%)	Chi Square	Df	p-value	Remark
Batch	First	22 (14.7)	139.239	54	0.00	Sig
	Second	49 (32.7)				
	Third	39 (26.0)				
	Fourth	40 (26.7)				
Position On Refrigerator	Base	34 (22.7)	68.080	72	0.61	N/S
	Handle	32 (21.3)				
	Shelf	29 (19.3)				
	Side	28 (18.7)				
	Top	27 (18)				
Gram Reaction	Positive	95 (63.3)	150.000	18	0.00	Sig
	Negative	55 (36.7%)				
Refrigerator	Domestic	90 (60)	35.325	18	0.00	Sig
	Laboratory	60 (40)				

Sig=Significant=p<0.05; N/S=Not Significant=p>0.05. df=degree of freedom. %=Percent

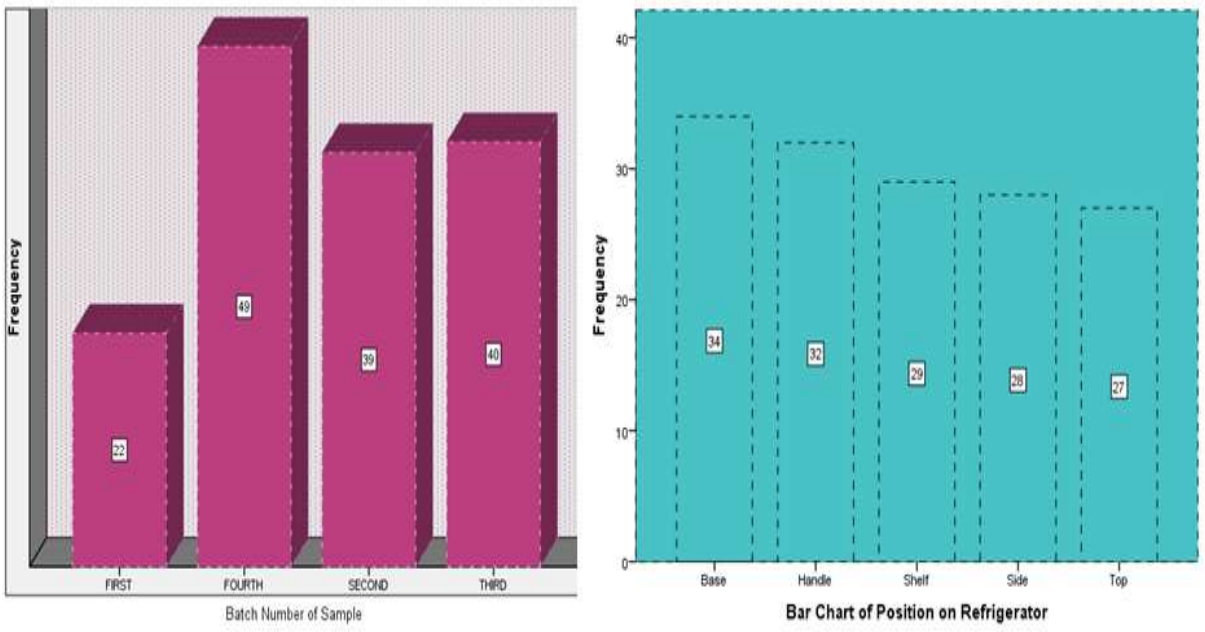

Figure 4.1: Bar Chart of Batch Number of Samples and position on Refrigerators

Figure 4.1 represents number of Samples and position on Refrigerators were samples were taken. With 22, 49, 39 and 40 samples taken in the first to fourth batches of sample collection. On the other hand, 34 samples were collected from the base of the refrigerators, 32(handle), 29 (shelf), 28(side) and 27 (top) respectively. Figure 4.2 on the other hand represents isolates from both the domestic and laboratory Refrigerators while. Figure 4.3 represents bacteria isolated from different batches and positions on the Refrigerators.

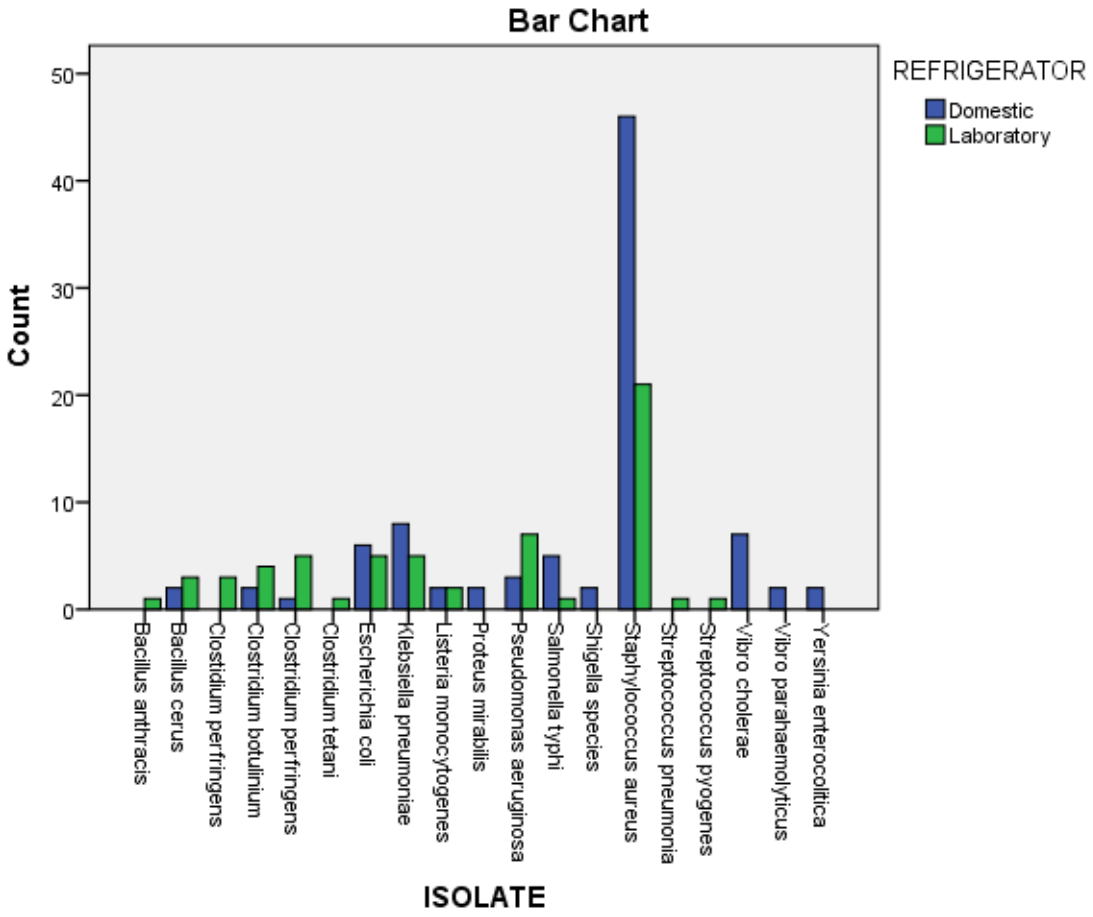

Figure 4.2: Bar Chart of Isolates from Refrigerators

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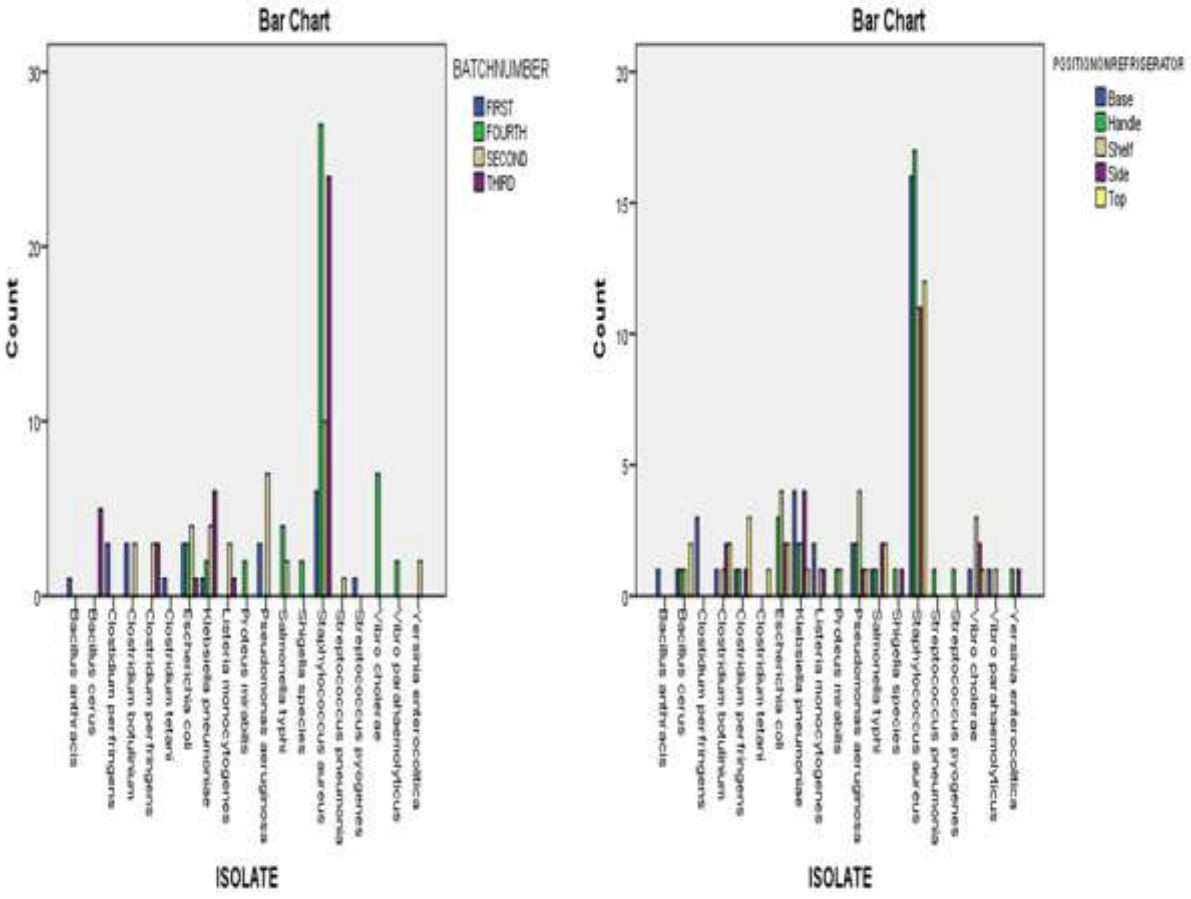

Figure 4.3: Bar Chart of Bacteria Isolated from different Batches and positions on Refrigerator.

4. Discussion

These results indicate the presence of different types of bacteria in refrigerators. Domestic refrigerators could be considered as one of the major potential sources of food borne diseases. Laboratory refrigerator is not free from contamination as well hence, the probability of cross infection could occur in the refrigerators. The refrigerators sampled were seen to be contaminated with bacteria with *Staphylococcus auerus* being the most prevalent bacteria in both refrigerator groups.

This study revealed higher prevalence rate (30.6%) in domestic refrigerator than reported in previous studies like: 20% [14] and 27.4% [15]. Also historically, an earlier study reported a lower rate 5% [16].

The result of this present study is similar to the work of Otu-Bassey and colleagues [12] which reported bacterial contamination in all the refrigerators sampled. However, the

studies differ in the percentage occurrence and infection rate characterization of the various strains isolated. This study demonstrated prevalence rate in descending order as; *Staphylococcus aureus* (30.6%), *Klebsiella pneumonia* (5.3%), *Vibro cholera* (4.7%), *Escherichia coli* (4%), *Salmonella typhi*(3.3%), *Pseudomonas aeruginosa*(2%), *Bacillus cerus*(1.3%), *Clostridium botulinium* (1.3%), *Shigella species*(1.3%), *Listeria monocytogenes* (1.3%), *Proteus mirabilis* (1.3%), *Vibro parahaemolyticus* (1.3%), *Yersinia enterocolitica* (1.3%) and *Clostridium perfringens*(0.7%) whereas, the Calabar based study by Otu-Bassey and colleagues showed a contradictory rate of bacteria genera in descending order of frequency; *Staphylococcus aureus* (27.3%), *Escherichia coli* (20.2%), *Shigella* spp (13.0%), *Pseudomonas aeruginosa* (11.9%), *Aeromonas hydrophilia* (8.3%), *Salmonella typhi (*5.9%), *Klebsiella pneumonia* (5%), *Streptococcus pyogenes* (4.7%) and *Proteus mirabilis* (2.3%) [12].Although, both studies are comparable in terms of the most prevalent bacteria been *Staphylococcus aureus* they differing in percentages as this study had higher rate compared to that of Otu-Bassey and colleagues[12]study.

A preventative approach to promote food safety: Bacterial contamination of domestic refrigerators was a headline in British Food Journal accounted for presence of some pathogens in domestic refrigerator [17] similar to some of the bacteria isolated in this present study. The study equally isolated *L. monocytogenes, S. aureus, E. coliand Salmonella spp*as observed in this study. Nonetheless, the rate of occurrence differed significantly from thispresent study. This difference can be attributed to methodological approach used. This present study utilized the conventional microbiological method of culture and biochemical identification whereas; Esfarjani and colleagues [17] screened the domestic refrigerators using Polymerase Chain Reaction method.

Notably, Keping and his co-researchers [18] have a contrast view as regards the highest percentage occurrence with this study; this is evident as the outcome of their study showed that *Bacillus* and *Acinetobacter* were the predominant bacteria in domestic refrigerators. *Acinetobacter was* present in 87.5% of the refrigerators but was absent in the refrigerators sampled in this study. Therefore, Keping *et al*. [18] disagreed with percentage occurrence obtained in this study for *Bacillus spp*. The report of the study showed a marked detection of *Bacillus spp* (100%) compared to the result of this present study.

In addition, Catellani et al. [19] found about 5.6% of *Bacillus cereus* while the record of this study (1.3% and 2% for domestic and laboratory refrigerators respectively) did not approximate to that account including the overall percentage. *Bacillus anthracis* was not isolated in domestic fridge but in laboratory refrigerator about 0.7% which gave an overall percentage of 2.7% of *Bacilllus spp* isolated in this study. This frequency is in opposition to studies by Hasan (2018) which reported a higher occurrence of *Bacillus spp.* (5.4%). Outstandingly, four classes of *Clostidium spp such Clostidium perfringens, Clostridium botulinium, Clostridium perfringens and Clostridium tetani* which were isolated in this study were not seen in earlier studies [12, 20].

Furthermore, 7.3% of *Escherichia coli* was isolated in the refrigerators analysed in this study with about 4% from domestic refrigerator. This is in opposition with an earlier study by Otu-Bassey et al. [12] which reported a higher prevalence in domestic refrigerator. Hasan [20] also isolated *E.coli* at a rate (13.5%) higher than this study. Furthermore, the assessment of Kumar & Osborne [21] is consistent with this study as the investigations proved the presence

of bacteria isolate *Escherichia coli*. It is worthy of note that *Escherichia coli* has been recognized as generally uncommon with frequency of the low infective pathogens in the human food chain and its capacity to form nonviable non-culturable forms [16, 22]. It is widely accepted indicator of faecal contamination signifying that the internal surfaces of refrigerators are frequently contaminated by introduction of contaminated materials such as raw foods or by poor personal hygiene practices. For domestic refrigerator and kitchen hygiene, it is impossible to completely exclude food pathogens from the kitchens. However, bacteria spread; growth and survival can be controlled with correct food storage, preparation of practices and regular cleaning in addition to disinfection of food contact site. Also, the laboratory is masked with lots of infectious activities hence zero contamination may not be seen. The microbiology laboratory stores many items from urine, faecal, blood samples to reagent and media for later use. These and more could introduce bacteria to the refrigerator which may likely multiply rendering the refrigerator contaminated; hence, this account seen in this study.

With respect to *Klebsiella* spp, this study showed an overall prevalent rate of 8.7%; 5.3% for domestic and 3.3% for laboratory refrigerators and only a specific strain of the species which is *Klebsiella pneumonia* was isolated. On the contrary, Hasan [20] in a study on determination of prevalence and antibiotic susceptibility pattern of the bacterial isolates collected from different parts of domestic refrigerators revealed *Klebsiella spp* to be among the frequently isolated bacteria (21.62%) even though the strains were not identified as *Klebsiella pneumonia.* But Otu-Bassey et al. [12] in Calabar, Nigeria reported *Klebsiella pneumonia* 5.9%.

Isolation of psychrotroph bacteria, this study is in contrary to [23] which found no *Listeria monocytogenes* which was isolated in both domestic and laboratory refrigerators. Furthermore, *L. monocytogenes* had a rate of 1.3% each for domestic and laboratory refrigerators with a total prevalence of 2.7% in this study. This did not corresponds with the Otu-Bassey et al., [12] that isolated at a varied frequency of 1.6% in domestic refrigerators. Earlier studies are in agreement with this study as the studies had isolated this psychrotroph at a rate approximately 3% and less indicating its presences in refrigerators [15, 23, 24, 25 ,26].

As a psychrotrophic organism, *L. monocytogenes* is capable of growth at cold temperatures. By implication, the presence of a small number can replicate into huge amount capable of causing infection. If this is found in edible item stored in the refrigerator, this contaminating effect might result to foodborne infections. The nature of *Listeria monocytogenes posses strict adherence to certain surfaces and* has been proven to adhere to numerous of surfaces, including stainless steel, glass and rubber [27]. The attachment of *Listeria monocytogenes* to surfaces has moreover been correlated to an increase in resistance to antiseptic agents and other antimicrobial agents, highlighting the call for thorough cleaning prior to disinfection of surfaces [28, 29].

Remarkably, 1.3% *Proteus mirabilis* been only isolated in domestic refrigerator proved low in occurrence as compared to2.3%*Proteus mirabilis* isolated in other studies [12, 30]. Moreover, the studies performed by Kumar & Osborne [21] is consistent with this study based on the report that the investigation confirmed the occurrence of bacteria isolates *Proteus sp*.

Pseudomonas spp were also detected in some refrigerators in this study likewise the position of Keping *et al*. [18] and Hasan [20] who isolated 13.5%*Pseudomonas spp*. Similarly, 11.9% was also isolated by Otu-Bassey et al., [12] from domestic refrigerator. This quantity contradicts this report which isolated 6.7% overall with domestic refrigerator having 2% and laboratory refrigerator 4.7%.

Salmonella spp. was found in 1.7% according to the report of one study done in Italy on the levels of microbial contamination of domestic refrigerators by Catellani *et al*., [19]. This is lower than what was found in this study which had 3.3% of *Salmonella typhi* isolated from domestic refrigerator. But a less figure was seen with a prevalence of 0.7% for laboratory refrigerator in this study. On the contrary Hasan [20] reported higher rate *Salmonella spp* (13.5%). Nonetheless, *Salmonella typhi*5.9 % was observed by Otu-Bassey et al., [12]. History has it that, *Salmonella sp.* was seen as effectively spread all through the local condition although some posited inability to identify *Salmonella sp.* in refrigerator yet in a wide scope of destinations analyzed in local kitchens [16]. Furthermore, the observation of Kumar & Osborne [21] is consistent with this study in the detection of *Salmonella sp.*

In addition, *Salmonella sp*. is a continuous contaminant of numerous prepared foods hence a general challenge to health of the populace especially as it relates to cross contamination. *Salmonella sp*. is effectively spread through the household condition where they can persist for as long as four days. Surface related *Salmonella sp*. still on the grounds that a noteworthy cross contamination hazard implies this pathogen can multiply under state of mild temperature misuse in cross polluted substances [31].

However, a lesser occurrence of prevalence of *Shigella spp* was noticed in this study compared to the work of Otu-Bassey et al., [12] which is thirteen times the result of this study.

From the report obtained in this study *Staphylococcus spp* was the most frequently isolated bacteria. A high discrepant prevalence exists between this study and Catellani *et al*., [19] which found *staphylococci* in 4% much lower than what was reported in this study. This is dissimilar with previous work of Ojima *et al*., [14]. Similarly, the study of Spiers *et al*., [15] contradicts this current study. The report of this study showed higher percentage occurrence cum prevalence compared to the two studies mentioned. Hasan [20] reported *Staphylococcus auerus* as the most prevalent bacteria in refrigerator, this report is in consonance with this present study. *S aureus* was the most frequently isolated pathogen in some previous studies [32]. Furthermore, previous studies have isolated *S aureus* from refrigerator sample and associated it with contamination of food at differing rate of occurrence [14, 15, 16].

About 50% of *Staphylococcus auerus* is a common inhabitant of the human body parts such as nose, throat, and skin [33]. *Staphylococcus auerus* is perhaps more likely to contaminate foods and refrigerators by direct or indirect human contact during domestic food handling and storage. With regards to the morphology of *Staphylococcus auerus* been a gram-positive organism, it is relatively resistant to drying and is, consequently more likely to become prevailing than more desiccation-sensitive organisms, especially in the low temperature conditions which exist in refrigerators. This distinguished quality is unlike other bacteria which mainly enter domestic kitchens, on previously ' contaminated raw foods.

More to the point, another species of Gram positive bacteria- *Streptococcus spp* were present although not frequent. *Streptococcus spp* seen in this study appeared distinct from other studies which had a higher rate for *Steptococcus pyogenes (*4.7%) [12]. Two different species were recorded namely; *Streptococcus pneumonia* and *Streptococcus pyogenes* at equal incidence; all in laboratory refrigerators.

Additionally,*Vibrio spp* was not left out. A total of 6% *Vibrio spp* isolated in this study classified as *Vibro cholera* 4.7% and *Vibroparahaemolyticus*1.3% , both been isolated from domestic refrigerator. This prevalence rate is not equivalent to the percentage isolated by some prior studies isolated 10.8% *Vibrio spp*[20].In addition,Otu-Bassey *et al.*, [12]did not isolate *Vibrio spp*.

Also, Yersinia enterocolitica detection in this study differs from others. Catellani *et al*., [19] did not isolate *Yersinia enterocolitica, this contradicts the findings of this study which isolated Yersinia enterocolitica* although at a low frequency was only detected in domestic refrigerator.

Variation in the occurrence of these organisms in refrigerator by different studies could be as a result of geographic location, methods used for analysis, hygienic practices of refrigerator users as well as awareness. Also, the intensity of bacterial contamination observed in domestic refrigerators are probably to be influenced by some factors including the scenery and extent of introduction of preliminary contamination on food materials stored, the existence and lack of effective packaging, the hygienic practices of handlers when the food is been placed into the refrigerator and the efficiency as well as frequency of refrigerator preventive or periodic maintenance along with cleaning.

The challenge of contamination of refrigerator is a global issue and of public health concern. The rate of bacterial contamination in this study mainly foodborne pathogens suggest the huge burden of food borne illnesses. These findings indicate very poor standards of domestic refrigerator management and hygiene posing risks to households' health. Besides, the refrigeration temperature might possibly be compromised since this study was a cross sectional study with one time sample collection and no track record of the refrigerator temperature over a period as a result, temperature which is a strong factor for refrigerator could not be ascertained.. Thus, the findings of this study highlight the importance of adequate temperature control and thorough, regular cleaning of refrigerators to ensure prompt food safety. This highlight has the support of Bharathirajan et al., [32].

Findings of this study revealed presence of bacteria which can cause food spoilage and food borne diseases and this is in parallel with other studies [34] likewise, the work of Macías-Rodríguez and colleagues[35].

5. Conclusion

Mesophilic and psychrotrophic bacteria are predominant in refrigerator notwithstanding the initial presumption that mesophilics bacteria may not survive cold temperature. *Staphylococcus auerus* is most prevalent in the refrigerators. Bacteria responsible for diarrhea and gastroentritisis are harboured in refrigerators too; hence food stored in refrigerators contaminated with these bacteria may cause foodborne infections. The presence of bacterial contamination can make the refrigerator lose its preservative capacity. A minor foodborne

pathogen that is rare (***Listeria monocytogenes)*** was detected and refrigerators could be a source of cross contamination hence a potential reservoir of bacteria

Based on empirical evidence from this study, all refrigerators (domestic and laboratory) sampled showed varying degrees of bacterial contamination although domestic refrigerators had more bacteria presence as compared to laboratory refrigerators however, this might possibly be due to the fact that more domestic refrigerators were sampled.

Remarkably, *Staphylococcus auerus* had the highest percentage occurrence as well most prevalent bacteria in this current study. Other foodborne pathogens and rare food borne pathogens including psychotrophs were also evident in this study.

Recommendation

Consequently, refrigeration been a means of storage exclusively for preservation is not completely free from contamination. Hence, it is crucial to alert the households, laboratory even the general public about refrigeration best practices. This includes but not limited to importance of temperature control and regular efficient hygienic practices, maintenance of appropriate good storage and refrigerator management including preventive maintenance. Proper hand hygiene is highly suggested because individuals taking care of items to be put into the refrigerator but careless about their hands (potential vehicle for transmission) may perhaps introduce bacteria into the refrigerator.

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