

Original Research Article

Microbial management of household cold storage exploratory study in Jeddah, Saudi Arabia

¹R.Bharathirajan, ²R.Gopinathan and ²M.Prakash*

¹Department of Microbiology, Saudi Modern food factory, Jeddah - 21421, Kingdom of Saudi Arabia

²Department of Microbiology, Kanchi Shri Krishna College of arts and Science, Kilambi, Kancheepuram - 631 551, Tamilnadu, India

*Corresponding author: mprakashmicro@gmail.com

ABSTRACT

KEYWORDS

Pathogenic bacteria;
Refrigerator;
households;
bacteriological
quality;
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The people of Saudi Arabia are often suffering from food poisoning and other health diseases. The isolation of pathogens from domestic refrigerators was performed to determine the prevalence of pathogenic microorganisms. Samples (n = 150) were obtained from domestic refrigerators of various parts of Jeddah, Saudi Arabia. In these samples, *Staphylococcus aureus* was isolated about 9.55%, *Salmonella* sp 3.8%, *E. coli* 2.2 %, and *Listeria monocytogenes* from 1.6% of examined refrigerators. But, *Campylobacter* spp. were not obtained from any refrigerators. The study estimated total viable count (TVCs) ranging from 3.8 log₁₀ cfu/cm² to 9.7 log₁₀ cfu/cm² and total coliform count (TCCs) ranging from 0.060 log₁₀ cfu/cm² to 7.65 log₁₀ cfu/cm². These findings indicate very poor standards of consumer refrigerator management and hygiene, and posing risks to consumer health. The findings of this study highlight the importance of adequate temperature control and thorough, regular cleaning of their refrigerators to ensure prompt food safety.

Introduction

The incidence of pathogens found in domestic refrigerator in Jeddah (Saudi Arabia) is unacceptably high, even though it is also significant underestimation of the true magnitude of the problem (Sheard, 1986). Food-borne diseases encompass a wide spectrum of illnesses and are a growing public health problem worldwide. They are the result of ingesting contaminated foodstuffs, and range from diseases caused by a multitude of microorganisms (WHO, 2008). Food-borne diseases are widespread and a growing public health problem, both in developed and developing countries. 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 (WHO, 2008). It has been suggested that the food-borne illness is initiated in domestic refrigerator three times more frequently than in commercial

refrigerator (Borneff, *et al.*, 1988). Many cases are attributable to inappropriate food storage including ineffective chill storage and refrigerator management (Ryan, *et al.*, 1996). Failure to follow correct practices in the adjustment, maintenance, use or cleaning of domestic refrigerators poses a number of risks to consumers.

Refrigerators form an important link in the wide chain of cross-contamination which leads to the outbreak of domestic food-borne disease (Ryan *et al.*, 1996). Many domestic refrigerators are incorrectly adjusted and are not capable of supporting sub-optimum but significant growth of mesophilic organisms such as *Staphylococcus aureus*, *E. coli* and *Salmonella* sp. (Flynn, *et al.*, 1996). Even when correctly adjusted, refrigerators can support the growth of psychrotrophic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica*, which can, therefore, grow to clinically significant numbers in foods stored for

extended periods in these domestic refrigerators (Flynn *et al.*, 1992 and Johnson *et al.*, 1998). Bacterial contamination caused by unwashed raw foods, hands, leaking packages, utensil surface, etc are introduced to domestic refrigerator would directly contaminate other stored foods (Michaels *et al.*, 2001). Bacteria can colonize a wide range of substrates like food preparation surface, utensils, domestic dish clothes, sponges and others cleaning materials from which they can be transfers into food and further could cause harmful effects on human (Scott *et al.*, 1982; Rusin *et al.*, 2002; Rusin *et al.*, 1998). There is, however, little information on their spread to, and persistence on, the interior surfaces of domestic refrigerators, making it difficult to quantify the true burden of such pathogens in these environments, or to estimate the risks they pose to consumers. Therefore, this study was carried out and examined the incidence of the pathogen on the surface of domestic refrigerators in selected houses in Jeddah (Saudi Arabia) to provide insights and the risk posed by these pathogens in domestic refrigeration system.

Materials and Methods

Location/household distribution

Sampling locations were chosen from different part of Jeddah (Saudi Arabia) for the isolation of pathogens from domestic refrigerators.

Household selection

Unannounced visits were made to 150 homes in Jeddah. Houses were randomly selected within the identified sample locations using a random walk method. Briefly in each selected cluster, the interview team started at a central point selected a random direction from that point, and choice a dwelling at random among those along the line from the centre to the edge of the area (Milligan *et al.*, 2004).

Sampling site and method of sampling

With the consent of the householder the interior (base, shelves and sides) of each refrigerator (approximately 100 cm²) were swabbed using a 10 cm². Sterile sponge moistened with 5 ml Buffered Peptone Water (BPW). The sponge was transported back to the laboratory under chilled conditions (4°C ± 1.0) and examined within 6 h.

Microbiological analysis

Samples were examined as follows; sponges were

stomached in 250 ml BPW (sample stock solution). All bacteriological media were prepared according to manufacturers. Aliquots from the sample stock solution were allowed to recover for 4 h, these serially diluted in Maximum Recovery Diluent (MRD), and plated on Plate Count Agar (PCA), incubated for 48 h at 35°C and examined to for TVCs. Violet Red Bile Glucose Agar (VRBGA), incubated for 48 h at 35°C, and examined for TCCs. Mannitol salt agar (MSA), incubated for 48 h at 35°C, and examined for the presence of presumptive *S. aureus*. Macconkey agar (MAC), incubated for 72 h at 35°C, and examined for the presence of *E.coli*.

Morphological Characterization

The cultures were morphologically characterized by Gram staining, methylene blue and Hanging drop were performed for examination of culture (Cogan, *et al.*, 1999).

Biochemical Characterization

All presumptive isolates were characterized by the various biochemical (IMVIC Test) i.e. Indole, Methyl red, Voges Proskauer, Citrate, TSI, Coagulase, Catalase (Gilbert, *et al.*, 1993). From the biochemical analysis the pathogenic microbes was identified by comparing the results with Bergey's manual (Griffin, *et al.*, 1991) (Table 1).

Statistical analysis: All counts were converted to log₁₀ CFU/cm² for analysis. The differences for total viable counts were evaluated by using analysis of variance for one way classification according to (Snedecor, *et al.*, 1968).

Results and Discussion

Isolation of specific food pathogens

Campylobacter sp were not detected in any of the refrigerators examined. *S aureus* was the most frequently isolated pathogen in this study, being recovered from 9.55 % of refrigerators. *Salmonella* sp was recovered from 3.8 % of refrigerator surfaces, *E. coli* was recovered from 2.2 % of refrigerator surfaces, *Listeria monocytogenes* was recovered from 1.6 % of refrigerator surfaces (Fig 1). Additional species also morphological and biochemical characterization identified, (Table -1) it can be understand that different types of strains are shown with different type of activity. But not quantified, respective media included *Shigella* sp.

Table 1: Biochemical tests for the isolates of *L. monocytogenes*

Strain	Gram staining	Coagulase	Catalase	Indole	M.R	V.P	Citrate	TSI	Motility	Organism
1	+	+	+	-	-	-	-	-	-	<i>S. aureus</i>
2	-	-	+	-	+	-	+	+	+	<i>Salmonella</i> sp.
3	-	-	+	+	+	-	-	+	+	<i>E. coli</i>
4	+	-	+	-	+	-	+	+	+	<i>L. monocytogenes</i>
5	-	-	+	-	+	-	-	-	-	<i>Shigella</i> sp

Where MR= Methyl red test, V.P= Voges proskauer test, TSI= Triple sugar iron test

General hygiene status of refrigerators

The TVCs obtained, ranged from 3.8 log₁₀ cfu/cm² to 9.7log₁₀ cfu/cm² with a mean of 7.4 log₁₀ cfu/cm². TCCs were obtained from 57% of refrigerators with an average incidence level of 4.3 log₁₀ cfu/cm² and a range of 0.06-7.65 log₁₀ cfu/cm². *E. coli* was isolated from 2.2 % refrigerator surfaces (Fig. 2).

Campylobacter sp. can survive for extended periods on damp surfaces, especially at low temperature (Tholozan *et al.*, 1999), but rapidly becomes undetectable in conditions of low water activity (Aw) (Fernandez *et al.*, 1985, Humphrey *et al.*, 1995 and Kusumaningrum *et al.*, 2003). However, the true extent, and public health significance, of *Campylobacter* in domestic refrigerators remain uncertain until it have a better understanding of the survival and pathogenic significance of the VNC state in this pathogen.

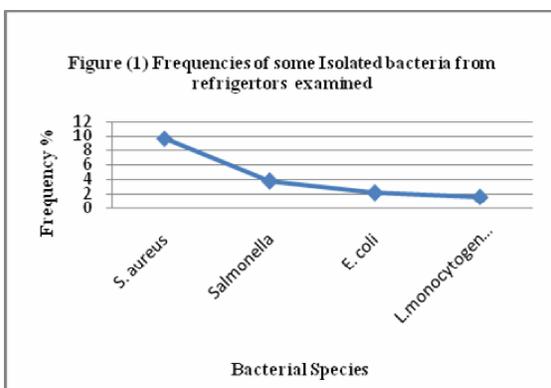
S. aureus was the most frequently isolated pathogen in this study and was recovered from 9.5% of the 150 refrigerators examined. This result is higher than some previous reported detections, e.g. 5% (Scott *et al.*, 1982), but lower than the 20% reported by (Ojima *et al.*, 2002), or the 27.4% reported by (Spiers *et al.*, 1995). Unlike the previously considered pathogens, which principally enter domestic kitchens, on previously 'contaminated raw foods, *S. aureus*, as a common inhabitant (up to 50%) of the human nose, throat, and skin (Arbuthnott, 1990) 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 Aw conditions which prevail in domestic refrigerators.

Salmonella sp. was isolated in 3.8% of refrigerators analysed in this study. (Spiers *et al.*, 1995) reported the failure to detect these pathogens, not only in refrigerator, but in a wide range of sites examined in domestic kitchens. However, *Salmonella* sp. was found to be easily spread throughout the domestic environment (Scott *et al.*, 1982). *E. coli* was identified in this study, which is relatively rare occurrence of the low infective dose pathogens in the human food chain and its ability to form viable non-culturable forms (Dillon *et al.*, 1992 and Scott *et al.*, 1982).

Salmonella sp. is a frequent contaminant of many retail foods poses' 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. 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 (De Boer *et al.*, 1990).

E.coli strain was isolated in 2.2% of refrigerators analysed in this study were isolated from almost every refrigerator surface. These result supported the report of Scott *et al* (1982) which is much unexpected pathogen which founds in mainly meat products and raw food contact surfaces. 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 contaminated foods, the presence and absence of effective packaging, the hygiene of those preparing and placing foods into the refrigerators and the efficiency and frequency of refrigerator maintenance and cleaning.

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 kitchens; however their spread, growth in survival can be controlled with correct food storage and preparation of practices and regular cleaning and disinfection of food contact site. As this work was rely more and more on refrigeration as a means of food preservation it is crucial to aware the public about the refrigeration better handlings. The importance of temperature control and regular efficient cleaning should be communicated to the public.

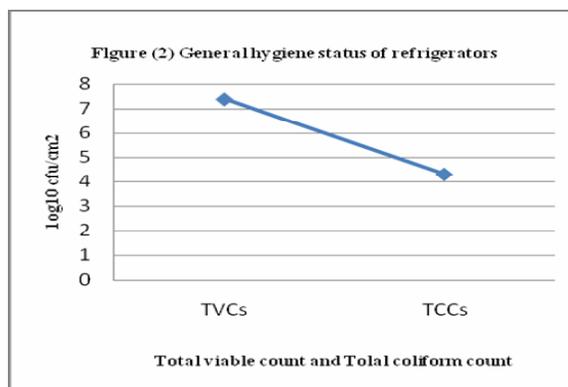


L. monocytogenes was isolated in 1.6% of refrigerators analysed in this study. This is in agreement with previous reports of *L. monocytogenes* in between 0% and 2.9% refrigerators (Beumer *et al.*, 1996; Cox *et al.*, 1989; Jackson *et al.*, 1993; Sergelidis *et al.*, 1997 and Spiers *et al.*, 1995). Being a psychrotrophic organism, *L. monocytogenes* is capable of growth at refrigeration temperatures, which means that low numbers of initially contaminating cells may proliferate and become hazardous if present on or transferred to ready-to-eat foods. It has been shown to adhere to many kinds of surfaces, including stainless steel, glass and rubber (Mafu *et al.*, 1990). Its ability to attach to surfaces has also been linked to an increase in resistance to sanitizers and other antimicrobial agents, highlighting the need for thorough cleaning prior to disinfection of surfaces (Frank and Koffi, 1990; Shin *et al.*, 1991 and Somers *et al.*, 1994).

The TVC contamination levels observed in this study extend across a wide range of values, ranging from 3.8 log₁₀ cfu/cm² to 9.7 log₁₀ cfu/cm² with a mean of 7.4 log₁₀ cfu/cm². 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 contaminated foods, the presence and absence of effective packaging, the hygiene of those preparing and placing foods into the refrigerators, and the efficiency and frequency of refrigerator maintenance and cleaning. Similarly, progress in reducing the significant extent of temperature abuse which allows undesirably rapid growth of both mesophilic and psychrophilic bacteria wider domestic refrigeration conditions (Flynn *et al.*, 1992; Johnson *et al.*, 1998; Kennedy *et al.*, 2005; Kennedy *et al.*, 2005)

It concludes that major factor contributing to food-borne illness, especially in the home, is the mishandling of food in the final preparation steps. This study has shown that food pathogens can survive on refrigerator surfaces and could, therefore, pose a cross contamination risk. Thus a number of undesirable foods related pathogens, i.e. *L. monocytogenes*, *E. coli* and *S. aureus* were isolated from a small but significant percentage of refrigerators. The risk potential of these organisms is heightened by their ability to multiply at refrigeration or mild abuse temperatures.



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