

A study of the microbial content of the domestic refrigerators in Khartoum area (Khartoum North)

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Summary

A study was conducted to determine the prevalence of some pathogenic bacteria and the general hygienic status on the interior surfaces of some domestic refrigerators ($n = 150$). *Campylobacter* spp., and *Salmonella* spp. were not recovered from any refrigerators, but *Staphylococcus aureus* was recovered from 9.54%, *Listeria monocytogenes* 3.8%, *E. coli* from 2.1 % and *Yersinia enterocolitica* from 1.6% of examined refrigerators. The study estimated total viable count (TVCs) ranging from $3.8 \log_{10} \text{ cfu/cm}^2$ to $9.7 \log_{10} \text{ cfu/cm}^2$ and total coliform count (TCCs) ranging from $0.060 \log_{10} \text{ cfu/cm}^2$ to $7.65 \log_{10} \text{ cfu/cm}^2$ indicating 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 domestic refrigerators to ensure prompt food safety.

Introduction

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 a widespread and 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). The reported incidence of food-borne illness in Europe is high, even though there is also a significant underestimation of the true magnitude of the problem (Anonymous, 2003a; Anonymous, 2003b; Anonymous, 2003c and Wheeler *et al.*, 1999).

Although restaurants, hotels and take-aways are the most frequently cited sites of outbreaks of food-borne disease (Bonner *et al.*, 2001), it has been suggested that food-borne illness is initiated in private homes three times more frequently than in commercial operations (Borneff *et al.*, 1988; Scott, 1996; Scuderi *et al.*, 1996 and Sheard, 1986). Many of these cases, perhaps up to 50%, are attributable to inappropriate food storage including ineffective chill storage and refrigerator management (Ryan *et al.*, 1996).

Bacteria contaminating unwashed raw foods, leaking packages, hands, and surfaces, introduced to domestic refrigerators may directly contaminate other stored foods, or attach to and persist on the internal surface of the refrigerator posing risks of indirect longer term contamination during subsequent food preparation activities (Michaels *et al.*, 2001). Many domestic refrigerators are incorrectly adjusted, operating above the recommended temperature and are therefore capable of supporting sub-optimum but significant growth of mesophilic organisms such as *Staphylococcus aureus* and *Salmonella* spp. (Flynn *et al.*, 1992; Johnson *et al.*, 1998). Even when correctly adjusted, refrigerators can support the growth of psychotropic 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; Johnson *et al.*, 1998).

Bacteria can colonize a range of food preparation surfaces, utensils, domestic dishcloths, sponges and other cleaning materials (Rusin *et al.*, 1998; Scott *et al.*, 1982, Sharp and Walker, 2003 and Spiers *et al.*, 1995), from which they can be transferred into food (Rusin *et al.*, 2002). 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, the objective of this study was to examine the presence of *Campylobacter* spp., *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *E. coli*, and *Y. enterocolitica* on the surfaces of domestic refrigerators in selected houses in Khartoum north.

Materials and Methods

Household selection : Unannounced visits were made to 150 homes in Khartoum North over a one month period from October to November 2007. 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, Oxoid). 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, Oxoid), and plated on:

(1) Plate Count Agar (PCA, Oxoid), incubated for 48 h at 25 °C and examined to provide TVCs.

(2) Chromocult Agar (Merck), incubated for 48 h at 35 °C, and examined to provide TCCs.

(3) Baird Parker Agar (BP, Oxoid), incubated for 48 h at 37 °C, and examined for the presence of presumptive *S. aureus* (grey/black shiny colonies with/without lipase activity).

(4) Yersinia Selective Agar (CIN, Oxoid), incubated for 24–48 h at 37 °C, and examined for the presence of presumptive *Y. enterocolitica* (red “bullseye” colonies).

Other aliquots from the sample stock solution were:

(1) Enriched in Listeria Enrichment Medium (LEM, Oxoid) for 24 h at 30 °C, plated on Listeria Selective Agar—Oxford formulation (LSA, Oxoid) for 24–48 h at 30 °C and examined for presumptive colonies of *L. monocytogenes* (cream/yellow colonies with a sunken centre surrounded by a black zone).

(2) Enriched in Preston Selective Enrichment Broth (PSEB, Oxoid) for 24 h at 42 °C (aerobically), plated on Preston Campylobacter Selective Agar (PCSA, Oxoid) for up to 48 h at 42 °C (anaerobically), and examined after 24 and 48 hours for presumptive colonies of *Campylobacter* spp. (grey/pink mucoid colonies which tend to swarm).

(3) Enriched in BPW for 24 h at 37 °C and then further enriched in Rappaport-Vassiliadis broth (RV, Oxoid) for 24 h at 42 °C. The resultant cultures were plated on Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB, Oxoid) and Brilliant Green Agar (BGA, Oxoid) for 24 h at 37 °C and examined for presumptive colonies of *Salmonella* spp. (red colonies on BGA and purple/black colonies on MLCB).

All presumptive isolates were confirmed using appropriate biochemical tests. (Barrow and Feltham, 1993).

Statistical analysis: All counts were converted to \log_{10} CFU cm^{-2} for analysis. The differences for total viable counts were evaluated by using analysis of variance for one-way classification according to Snedecor and Cochran (1968).

Results

Isolation of specific food pathogens

Campylobacter spp. were not detected in any of the refrigerators examined. *S. aureus* was the most frequently isolated pathogen in this study, being recovered from 9.54 % of refrigerators. *L. monocytogenes* was recovered from 3.8 % of refrigerator surfaces and *Y. enterocolitica* from 1.6 %.

Additional species identified, but not quantified, from Chromocult agar included *Proteus mirabilis*, *Klebsiella* spp., *Enterobacter cloacae*, *Enterobacter agglomerans* and *Pseudomonas* spp. (Fig. 1).

General hygiene status of refrigerators

The TVCs obtained ranged from 3.81 log₁₀ cfu/cm² to 9.7 log₁₀ 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 2.1% of refrigerator surfaces. (Fig. 2).

Discussion

Campylobacter spp. 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 (A_w) (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 we have a better understanding of the survival and pathogenic significance of the VNC state in this pathogen.

Similar concerns apply to *Salmonella* spp., which although less frequent contaminants of domestic kitchens, are equally easily spread throughout the domestic environment (de Boer and Hahné, 1990), where they can persist for up to 4 days, even under conditions of low A_w (Kusumaningrum *et al.*, 2003). Thus, surface associated *Salmonella* spp. still pose a significant cross-contamination risk, while their abilities to grow at low temperatures, i.e. as low as 5 °C (Jay, 2000), means that this pathogen can multiply under conditions of mild temperature abuse in cross-contaminated foods.

S. aureus was the most frequently isolated pathogen in this study and was recovered from 6.4% 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 A_w conditions which prevail in domestic refrigerators.

L. monocytogenes was isolated in 1.2% 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).

Y. enterocolitica was isolated from only 0.6% of refrigerators examined. A previous similar study (Spiers *et al.*, 1995) did not detect this pathogen in domestic refrigerators, but did recover it from 4.3% of domestic sinks. Alternatively, the low rates of detection of *Y. enterocolitica* in this study may be related to the relatively limited sensitivity of currently available culture methods which continue to pose problems in clinical, environmental and other food related investigations of this pathogen (Fredriksson *et al.*, 2003).

The TVC contamination levels observed in this study extend across a wide range of values, ranging from $3.81 \log_{10} \text{ cfu/cm}^2$ to $9.7 \log_{10} \text{ cfu/cm}^2$ with a mean of $7.4 \log_{10} \text{ cfu/cm}^2$. 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 psychophilic bacteria under domestic refrigeration conditions (Flynn *et al.*, 1992; Johnson *et al.*, 1998; Kennedy *et al.*, 2005; Kennedy *et al.*, 2005)

We conclude that major factor contributing to food-borne illness, especially in the home, is the mis-handling 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 food related pathogens, i.e. *L. monocytogenes*, *Y. enterocolitica* 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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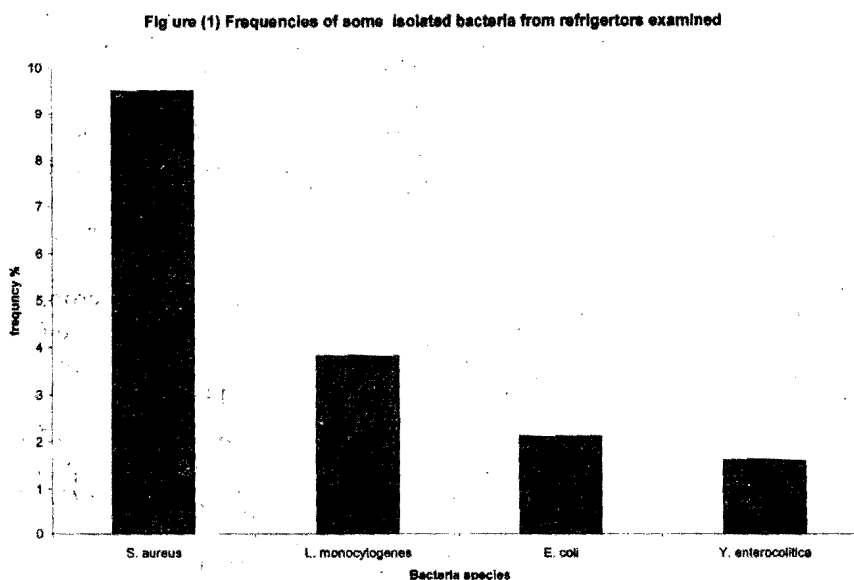


Figure (2) General hygiene status of refrigerators

