Journal of Food Protection, Vol. 71, No. 3, 2008, Pages 608–612

Research Note

Occurrence of *Listeria* and *Enterobacteriaceae* in Domestic Refrigerators

AGNES KILONZO-NTHENGE,[1]* FUR-CHI CHEN,[1] AND SANDRIA L. GODWIN[1,2]

[1]*Institute of Agricultural and Environmental Research, and* [2]*Department of Family and Consumer Sciences, Tennessee State University, Nashville, Tennessee 37209-1561, USA*

MS 07-172: Received 28 March 2007/Accepted 26 October 2007

ABSTRACT

Consumers' refrigeration practices have a significant impact on the safety and quality of foods. To determine the prevalence and the identity of microorganisms in domestic refrigerators, swab samples were taken from various locations in the refrigerators from 137 households in middle Tennessee. The swabs were inoculated into different media, and standard procedures were used to characterize the isolates. API 20E and API *Listeria* were used for identification of *Enterobacteriaceae* and *Listeria* spp., respectively. The Kirby-Bauer technique was used to test resistance of the isolates. Actual counts for aerobic and *Enterobacteriaceae* ranged from not detected to 8.53 and 8.39 log CFU per sample, respectively. *Klebsiella pneumoniae* (23.4%), *Klebsiella oxytoca* (6.8%), *Klebsiella terrigena* (4.0%), *Enterobacter sakazakii* (2.2%), and *Yersinia enterocolitica* (0.7%) were some of the bacteria of concern that were isolated from domestic refrigerators. Resistance to antibiotics was most common in erythromycin (39.9%), followed by ampicillin (33.8%), cefoxitin (12.8%), tetracycline (5%), streptomycin (4.0%), nalidixic acid (2.1%), kanamycin (1.4%), and colistin (0.7%). None of the isolates tested was resistant to ciprofloxacin or gentamycin. *Listeria* spp. were also detected in six refrigerators. These findings underline the need for greater consumer education regarding proper refrigerator cleaning and safe food handling practices.

Although several efforts have been made to improve food safety and quality, foodborne diseases still present a serious threat to the health of millions of individuals *(33).* Approximately 76 million cases of human illness occur each year in the United States due to food-related disease *(20).* Foodborne illness is initiated in private homes three times more frequently than in commercial operations *(19, 27, 29).* Many of these are attributable to improper food storage, lack of safe food handling procedures, poor cleanliness, and refrigerator management *(23, 26, 30).*

Refrigerators form an important link in the wider chain of cross-contamination, and a significant factor in 28% of outbreaks of domestic foodborne disease *(26).* Interior surfaces of domestic refrigerators can be contaminated with foodborne pathogens, which can be transferred to prepared foods and other parts of kitchen. Unwashed raw foods, leaking packages, and hands can introduce bacteria to domestic refrigerators and other foods *(22).* Survival and growth of microorganisms in refrigerators present a potential hazard in refrigerated foods, particularly in products that are consumed without further cooking. Various studies have indicated the potential for ready-to-eat products to be cross-contaminated through surfaces of the refrigerator *(2, 28).* There is also a concern about antibiotic resistance development in foodborne pathogens and subsequent transfer to humans through contaminated food *(32). Klebsiella pneumoniae* strains resistant to antibiotics have been isolated from farms and ground turkey, retail beef, and chicken products from retail stores *(18).* It stands to reason that antibiotic-resistant bacteria might be transferred from contaminated meat packages to interior surfaces of domestic refrigerator (meat drawers).

Currently, there is a paucity of data quantifying microbial contamination on interior surfaces of domestic refrigerators. In addition, little is known about antimicrobial resistance of microorganisms in domestic refrigerators. Therefore, the objectives of this study were (i) to assess the overall microbial contamination on interior surfaces of domestic refrigerators and (ii) to determine whether the domestic refrigerators are a potential source of antibiotic-resistant bacteria. The emphasis of this study was on *Listeria* spp. and *Enterobacteriaceae,* because some of these microorganisms are of great concern in the public food safety.

MATERIALS AND METHODS

Collection of samples from home refrigerators. As part of a wider study, 137 households in middle Tennessee volunteered to participate in this study. A list of participants was obtained from church groups and social service personnel. Three swabs were taken from the interior (shelves, meat and vegetable drawers, or middle drawer) of each refrigerator. Approximately 400 cm^2 were swabbed using sterile and moistened hydra sponges (Biotrace International, Muncie, Ind.). The sponges were transported back to the laboratory in a cooler and examined within 2 h. Butterfields phosphate buffer (25 ml) was added to each sponge sam-

* Author for correspondence. Tel: 615-963-5437; Fax: 615-963-1557; E-mail: akilonzontheng@tnstate.edu.

TABLE 1. *Identity of bacteria isolates from different locations of domestic refrigerators*

Identity	% of homes[a]	Location(s)[b]
Listeria spp.	4.4	Mpd, Vb, Bs
Klebsiella pneumoniae	23.4	Mpd, Bs, Ms, Vb, Ts
K. oxytoca	6.8	Mpd, Bs, Ms, Vb
K. terrigena	4.0	Mpd, Bs, Ms, Vb
Enterobacter sakazakii	2.2	Vb
E. cloacae	20.5	Mpd, Bs, Ms, Vd, Ts
E. aerogenes	2.9	Mpd, Ms, Vb
E. cancergenus	0.7	Mpd
E. amnigenus	4.4	Mpd, Bs, Vb
Pantoea spp.	13.9	Mpd, Bs, Ms, Vb, Ts
Escherichia hermannii	0.7	Mpd
E. vulneris	0.7	Bs
Yersinia enterocolitica	0.7	Vb
Citrobacter freundii	0.7	Vb
Rahnella aquatilis	2.9	Mpd, Bs, Vs
Ewingella americana	1.5	Mpd, Bs
Enterobacter asburiae	1.5	Bs, Vb
Serratia liquefaciens	2.2	Bs, Vb, Ts
Hafnia alvei	0.7	Ms
Unidentified	5.3	

[a] Percentage of homes with specific microorganisms from 137 homes.

[b] Mpd, meat/poultry drawer; Bs, bottom shelf; Ms, middle shelf; Vb, vegetable bin; Ts, top shelf.

ple and pummeled in a Stomacher 400 Circulator (Seward Limited, London, UK) at 230 rpm for 2 min. The homogenate was then used for further analysis. All samples were analyzed for *Listeria* spp., aerobic plate count, and *Enterobacteriaceae* count.

Isolation and identification of *Listeria* ssp. A volume of 10 ml of the homogenate from each sponge sample was transferred to 10 ml of University of Vermont broth and incubated for 24 h at 30°C. After primary enrichment, 1 ml of University of Vermont broth was transferred to 10 ml of secondary enrichment Fraser broth and incubated for 48 h at 35°C. Fraser broth culture tubes showing blackening were streaked to *Listeria* selective agar (SR020E, Oxoid, Basingstoke, Hampshire, UK) plates with subsequent incubation for 48 h at 35°C. Following incubation, five typical colonies per plate were transferred onto tryptic soy agar with 6% yeast and incubated for 24 h at 35°C. Presumptive *Listeria* spp. colonies were confirmed by Latex agglutination (Remel, Lenexa, Kans.), Gram stain, oxidase test, and catalyst test and identified by biochemical test strips (API *Listeria,* bioMérieux, Hazelwood, Mo.).

Enumeration of *Enterobacteriaceae* and aerobic counts. Homogenized samples were serially diluted from 10^{-1} to 10^{-5} for subsequent plating on plate count agar and Petrifilm *Enterobacteriaceae.* Plate count agar and Petrifilm plates were incubated for 48 and 24 h at 35°C, respectively. Typical colonies on Petrifilm were transferred to tryptic soy agar and incubated for 24 h at 35°C. After incubation, there were many colonies, but only three (presumptive *Enterobacteriaceae*) were isolated to make bacterial suspension to inoculate the API 20E strips. Oxidase tests and biochemical strips (API 20E, bioMérieux) were used to identify these isolates to the species or genus level.

Antimicrobial susceptibility testing. The Kirby-Bauer technique was used to determine sensitivity to the following antimicrobial agents: tetracycline (30 μg), streptomycin (10 μg), ampicillin (10 μg), erythromycin (15 μg), ciprofloxacin (10 μg), cefoxitin (30 μg), colistin (10 μg), gentamycin (10 μg), kanamycin (30 μg), and nalidixic acid (30 μg). *Escherichia coli* 25922 was used as the quality control organism. Test isolates were grown with shaking in 5 ml of Luria-Bertani broth at 37°C for 24 h. Each overnight culture was spread evenly onto Mueller-Hinton agar plate with cotton swab. Antibiotic disks were placed onto Mueller-Hinton plates and incubated at 37°C for 24 h. The diameter of the zone around the disk was measured and interpreted according to the standard procedures outlined in the Clinical and Laboratory Standards Institute guidelines *(4).*

TABLE 2. *Sample distribution of* Enterobacteriaceae *counts from domestic refrigerators*[a]

Location	No. of samples at log CFU/sample of: 0–1.9	2.0–3.9	4.0–5.9	6.0–7.9	8.0–9.9	Total samples
Top shelf	15	5	2	0	0	22
Middle shelf	31	24	15	0	1	71
Meat/poultry drawer	43	24	5	2	0	74
Vegetable bin	43	41	42	10	0	136
Bottom shelf	45	33	25	5	0	108
Total	177	127	89	17	1	411

[a] Range of *Enterobacteriaceae* counts (log CFU per sample) from domestic refrigerators. Samples were collected from 137 homes; three samples were collected from each domestic refrigerator.

Statistical analysis. Readings of aerobic colony counts (ACC) and *Enterobacteriaceae* counts (ETC) were converted to log CFU per sample before statistical analyses were performed. The mean values were compared by the GLM procedure with SPSS 12.0 for windows (SPSS Inc., Chicago, Ill.). Differences were considered significant at $P < 0.05$.

RESULTS

***Listeria* spp.** *Listeria monocytogenes* was not isolated in any of domestic refrigerators sampled. However, *Listeria innocua* (4.4%) was detected in meat drawers, vegetable bins, and on the bottom shelves in some refrigerators.

***Enterobacteriaceae* and aerobic colony counts.** Bacteria isolates from different locations of domestic refrigerators are shown in Table 1. *Enterobacter sakazakii* (2.2%) and *Yersinia enterocolitica* (0.7%) were isolated from the vegetable bins. Additional species that were isolated include *K. pneumoniae* (23.4%), *Klebsiella oxytoca* (6.8%), *Klebsiella terrigena* (4.0%), *Enterobacter cloacae* (20.5%), and *Pantoea* spp. (13.9%). Some isolates from a number of homes could not be identified (5.3%) with the biochemical strips (API 20E, bioMérieux).

Sample distribution of ACC and ETC isolated from domestic refrigerators are listed in Tables 2 and 3. The ETC ranged from 1.0 to 8.39 log CFU per sample, and the ACC ranged from 1.0 to 8.53 log CFU per sample. The highest ETC (8.39 log CFU per sample) and ACC (8.53 log CFU per sample) recorded were found in the vegetable bins. For ETC, the highest mean log CFU per sample count was in the vegetable bins (3.00 ± 0.18), followed by bottom shelves (2.38 ± 0.21), middle shelves (2.19 ± 0.5), meat

TABLE 3. *Sample distribution of aerobic counts from domestic refrigerators*[a]

Location	No. of samples at log CFU/sample of: 0–1.9	2.0–3.9	4.0–5.9	6.0–7.9	8.0–9.9	Total samples
Top shelf	2	9	10	1	0	22
Middle shelf	0	28	38	4	1	71
Meat/poultry drawer	2	23	40	9	0	74
Vegetable bin	1	23	61	50	1	136
Bottom shelf	3	27	48	28	2	108
Total	8	110	197	92	4	411

[a] Range of aerobic counts (log CFU per sample) from domestic refrigerators. Samples were collected from 137 homes; three samples were collected from each refrigerator.

drawers (1.53 ± 0.22), and top shelves (1.09 ± 0.37). ETC mean count recovered from vegetable bins was significantly higher ($P < 0.05$) than mean counts recovered from meat drawers and top shelves. *Enterobacteriaceae* counts on top shelves were significantly ($P < 0.05$) lower than in the vegetable bins. The mean ACC log CFU per sample was highest in vegetable bins (5.38 ± 0.12), followed by bottom shelves (5.01 ± 0.15), middle shelves (4.48 ± 0.13), meat drawers (4.42 ± 0.15), and top shelves (3.74 ± 0.33). The mean log CFU per sample for ACC recovered from the vegetable bins was significantly higher ($P < 0.05$) than the mean count recovered from the bottom, middle, and top shelves, and meat drawers. Means of ACC from the bottom, middle, and top shelves, and in meat drawers were not significantly different ($P \geq 0.05$) from each other.

***Enterobacteriaceae* antimicrobial resistance.** Only the results of resistant isolates are presented in Figure 1. Some ETC isolates were intermediate and susceptible to the selected antibiotics (data not shown). Percentage of resistant isolates was highest to erythromycin (39.9%), followed by ampicillin (33.8%), cefoxitin (12.8%), tetracycline (5%), streptomycin (4.1%), nalidixic acid (2.0%), kanamycin (1.4%), and colistin (0.7%). None of the tested isolates were resistant to ciprofloxacin or gentamycin (Fig. 1).

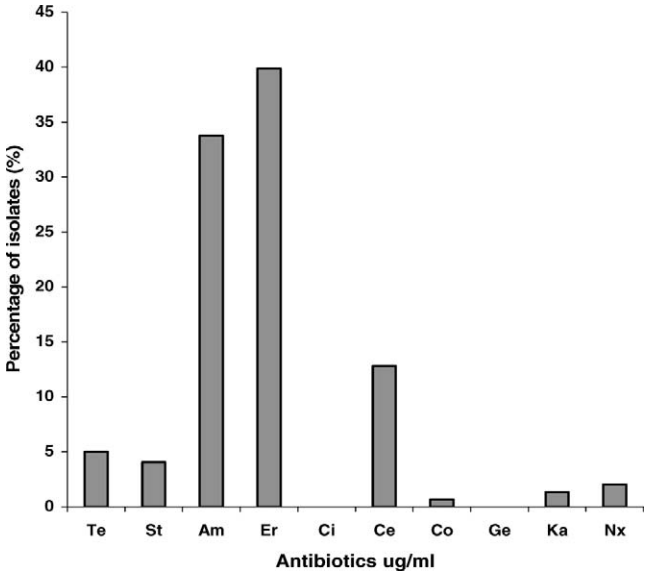

FIGURE 1. *Percentage of* Enterobacteriaceae *isolates resistant to selected antibiotics. Te, tetracycline; St, streptomycin; Am, ampicillin; Er, erythromycin; Ci, ciprofloxacin; Ce, cefoxitin; Co, colistin; Ge, gentamycin; Ka, kanamycin, Nx, nalidixic acid.*

DISCUSSION

L. monocytogenes was not found in any of the domestic refrigerators. However, *L. innocua* was present in 4.4% of the homes. Our results are consistent with a study conducted in Portugal that reported low incidence of *Listeria* spp. (3.5%) in refrigerators *(2)*. The prevalence of *L. monocytogenes* in refrigerators was reported between 0 and 2.9% *(12, 13, 28)*. One possible source of *L. innocua* in the domestic refrigerators might be contamination from stored food products. *L. monocytogenes* has been found in turkey and chicken products *(8),* dairy products *(31),* and a variety of vegetables including bean sprouts, cabbage, lettuce, tomatoes, and mushrooms *(3)*. Foods, especially raw materials, that are stored in refrigerators frequently contain pathogenic microorganisms including *L. monocytogenes (21)*. The presence of *L. innocua* in meat drawers, vegetable bins, and on bottom shelves suggest favorable conditions for *L. monocytogenes* to grow and persist in domestic refrigerators. *L. monocytogenes* grows under refrigeration conditions, often causes listeriosis in elderly people, people with underlying diseases, and neonates *(25)*. Foods implicated in listeriosis are typically refrigerated ready-to-eat products that have a long shelf life, allowing *L. monocytogenes* to grow to high numbers *(6, 10)*. *L. monocytogenes* persistence in ready-to-eat foods and its ability to grow at refrigeration temperatures indicates a public health concern. Poor food handling practices may introduce *L. monocytogenes* in domestic refrigerators and, if allowed to persist, pose a significant risk to the consumer.

Commonly recognized foodborne pathogens were not detected on interior surfaces of domestic refrigerators sampled in this study. Although there were no isolates of *Salmonella, E. coli, Shigella,* and *Proteus,* there was isolation of *E. sakazakii* (2.2%) and *Y. enterocolitica* (0.7%) from the domestic refrigerators. *E. sakazakii* is an emerging foodborne pathogen that has caused illnesses and deaths in infants, elderly, and the immunocompromised *(17)*. *E. sakazakii* has been isolated in variety of foods and food sources, indicating that it is widespread *(24, 30)*. Detection of *Y. enterocolitica* in our study is in compliance with another study, which found *Y. enterocolitica* in 2% of Irish domestic refrigerators *(16)*. *Y. enterocolitica* is a psychrotroph and grows at temperatures near 0°C. This is of great concern because refrigeration is used to control the growth of spoilage and pathogenic microorganisms in foods *(1)*. Approximately 85,000 cases of *Y. enterocolitica* occur annually in the United States *(20)*. Although pork is considered to be the main source of *Y. enterocolitica,* other foods such as beef, milk, lamb, seafood, and vegetables have also been implicated *(14)*. Because of the presence of *E. sakazakii* and *Y. enterocolitica* (Table 1) isolates in the vegetable bins, it is strongly recommended that fresh produce be thor-

oughly washed before consumption. Refrigeration supports the growth of *Y. enterocolitica* and, therefore, can survive in foods stored for extended periods in refrigerators *(7). K. pneumoniae* and *E. cloacae* occurred frequently on the interior surfaces of domestic refrigerators. These microorganisms are present in the environment and gastrointestinal tracts of humans and animals, and thus are an indication of poor cleanliness and refrigerator management. *Pantoea* spp., *Enterobacter aerogenes, E. cloacae, Citrobacter freundii, Serratia liquefaciens,* and *Hafnia alvei,* which are widely distributed in soils and plants, were also isolated from the refrigerators. Most of the bacteria (Table 1) identified in the study are not usually associated with foodborne pathogens and are considered nonpathogenic to healthy adults. However, their presence highlights the diversity of microorganisms that can survive on domestic refrigerator surfaces.

Multidrug-resistant *Klebsiella* spp. have been isolated in farm environments, retail poultry, and beef products *(18),* and dishcloths and sponges *(15).* In this study, multidrug resistance was found in *Klebsiella* spp., but not in *Y. enterocolitica* or *E. sakazakii.* These findings suggest that antibiotic-resistant *Klebsiella* is likely to be transmitted from contaminated poultry, beef products, and fresh produce in domestic refrigerators to other parts of domestic kitchens. *Y. enterocolitica* and *E. sakazakii* isolates from this study were resistant to ampicillin, erythromycin, and cefoxitin. It stands to reason that antibiotic-resistant bacteria on raw poultry and beef products can be transferred to the interiors of domestic refrigerators. The public health significance of this is unknown, but there is growing concern that antibiotic resistance among foodborne pathogens associated with uncooked plant and animal products can represent a potential hazard to humans.

Consumers can cause their own illnesses due to poor hegemony practices, lack of hand washing, poor cleanliness of refrigerators, and lack of knowledge of proper refrigeration *(9, 16, 30).* A consumer study found that only 6% of those surveyed cleaned their refrigerators weekly, whereas more than 80% cleaned only monthly or less frequently *(2).* Raw meat, poultry, and fish should be stored separately from other ready-to-eat foods *(5)* to avoid cross-contamination. Regular cleaning and disinfection of refrigerators eliminate pathogen contamination *(11).* Bicarbonate solutions restrict the growth of moulds and bacteria, and therefore can be used to clean the interiors of domestic refrigerators *(2).* Educational programs should emphasize the importance of regular cleaning of domestic refrigerators.

In conclusion, findings from this study are informative and clearly indicate the need for greater consumer education regarding proper domestic refrigerator cleaning and safe food handling practices in domestic kitchens. Effective management and cleaning of domestic refrigerators makes the refrigerator less likely to act as a significant niche for persistence and dissemination of foodborne pathogens.

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