Enterobacteriaceae Associated with Meats and Meat Handling

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The source of Enterobacteriaceae on meats was shown to be associated with the meat-handling work surfaces in the two packing plants studied. A total of 2,343 Enterobacteriaceae were isolated and identified from meat samples and work surfaces at the packing plants and at the retail facilities. Escherichia coli biotype I and Serratia liquefaciens were detected at all stages of meat handling, indicating that they may be present in meats throughout the meat-handling system. Enterobacter agglomerans and S. liquefaciens were the predominant Enterobacteriaceae at the retail level, but they had limited indicator potential for sanitation and hygiene. Klebsiella pneumoniae was a frequent isolate among Enterobacteriaceae from meats and meat-handling surfaces in the packing plants but not at the retail level, indicating that this organism might signal unhygienic handling of meats at the retail level.

Escherichia coli biotype I is one of the predominant Enterobacteriaceae in ground beef (11, 28). With good manufacturing practices, E. coli I contamination on meat is generally considered to come from the skin or hide of animals during processing (1, 14, 30) and possibly represents both fecal and nonfecal contamination (27). Furthermore, E. coli I in the stationary growth phase survives well in frozen and nonfrozen meat (18, 32) and grows in meats at improper storage temperatures. This fact led many workers to criticize the use of E. coli as an indicator of sanitation and hygiene in raw meats (13, 15, 20). In contrast, Mossel and co-workers (5, 21, 24, 25) criticized the use of E. coli as an indicator of food safety for dehydrated, frozen, and refrigerated foods, because they found that E. coli does not survive well under such conditions. As a result, they recommended the use of Enterobacteriaceae as indicators of food safety (22, 23, 26). Greater concern for non-E. coli coliforms has also been proposed because of their increasing involvement in diarrheal diseases (34).

E. coli biotype I (IMViC [indole, methyl red, Voges-Proskauer, citrate] ++-- and gas positive in EC broth at 45°C) is generally recognized as an indicator of direct or indirect fecal contamination of foods, and E. coli or coliform bacteria in pasteurized milk and cooked foods generally indicate postprocessing contamination (16). Klebsiella pneumoniae is also a frequent but not exclusive inhabitant of the intestines of animals and humans (4, 5, 9, 19), has been implicated in human infections (3, 12), and has a similar fecal origin, and hence public health significance, as typical E. coli (4, 17). Other Enterobacteriaceae are of less specific origin; for example, Citrobacter freundii is found in feces but also in soil (4, 29), and Enterobacter spp. are reported to occur only rarely in the human intestine (5).

In an earlier study (28), the principal Enterobacteriaceae in ground beef were found to be E. coli type I, Enterobacter agglomerans, and Serratia liquefaciens. Other Enterobacteriaceae were also identified on selective media, including C. freundii, Enterobacter aerogenes, E. cloacae, E. hafniae, and K. pneumoniae. The significance of these organisms in retail meats could not be determined. Hence, our object was to study Enterobacteriaceae at different stages of the meat-processing chain to determine whether any associations could be made between bacterial isolates and stage in the meat-handling process.

MATERIALS AND METHODS

Sample collection. Samples were collected at packing plants and retail points of meat handling for subsequent isolation and identification of Enterobacteriaceae. In the packing plant study, samples were collected on 18 occasions (7 times at plant A and 11 times at plant B) from two federally inspected Canadian packing plants, each operating beef and hog kill floors and meat-processing departments. Samples were taken to include (i) meat and nonmeat contact surfaces and (ii) the beef, hog, and processed-meat areas of the plants. For sampling, we used Rodac
contact plates and violet red bile agar (VRBA). At the retail level, three stores that received supplies directly or indirectly from these packing plants were used to obtain samples that included: (i) vacuum-packaged beef primal cuts before they were opened at the retail store; (ii) vacuum-packaged beef trimmings for use in ground beef manufacture; (iii) packaged pork loins (not vacuum packaged) before they were opened at the retail store; (iv) frozen and thawed pork sausage packaged by manufacturers; and (v) meats handled at the retail level, including beef steaks, pork chops, and ground beef.

**Sample preparation.** Samples of integral meats were collected by a spray gun technique (7) with a 100-ml sterile 0.1% peptone water wash. Samples were collected aseptically in a graduated Erlenmeyer flask (approximately 20 ml per sampling point). Commuted meat samples were refrigared and taken to the laboratory for immediate analysis. Commuted meats were sampled by blending 11 g of meat with 99 ml of sterile 0.1% peptone water for 5 min in a Colwirth stomacher (model 400).

**Bacteriology.** Appropriate dilutions were plated onto VRBA by the pour plate technique. All VRBA plates were overlaid with 5 ml of VRBA and incubated at 35°C for 18 to 24 h. After incubation, representative numbers of bile-precipitating and non-bile-precipitating colony types were randomly picked from the VRBA plates and purified by streaking first on MacConkey agar and then on nutrient agar. Biochemical tests were done on an isolated colony from the nutrient agar plates. Enterobacteriaceae were screened by Gram stain and oxidase test. The biochemical tests were an expanded Minitest (BBL, Benton-Dickinson and Co., Mississauga, Canada) identification system, described previously (31), involving: arabinose, citrate, dulcitol, H2S-indole, inositol, lysine, malonate, o-nitrophenyl-β-D-galactopyranoside, ornithine, phenylalanine, raffinose, rhamnose, and urea disks. Isolates were also inoculated on phenol red glucose and lactose (1%) broths, nitrate-motility agar, triple sugar iron agar, and methyl red-Voges-Proskauer medium. Supplementary tests were carried out as required.

**RESULTS**

Table 1 shows the incidence of *Enterobacteriaceae* on various packing-plant surfaces. The raw meat-handling surfaces, when in use, were the areas most heavily contaminated with *Enterobacteriaceae*. Rodac plates with >1,000 to too numerous to count *Enterobacteriaceae* per plate frequently involved the meat saw tables and stainless-steel work surfaces. In addition, the carcass-splitting saw and beef carcass shrouds were implicated on two occasions each. Cooked-meat contact surfaces were less contaminated with *Enterobacteriaceae*, and surfaces with no meat contact (walls, doors, etc.) were not apparent reservoirs of *Enterobacteriaceae*.

A total of 2,343 *Enterobacteriaceae* isolates were identified, 1,372 from packing-plant samples and 971 from meats sampled at the retail level. The distribution of *Enterobacteriaceae* types isolated at the two packing plants (Table 2) indicated that *K. pneumoniae*, *E. coli*, and *S. liquefaciens* were the principal *Enterobacte-

riaceae* isolated, each accounting for 10 to greater than 20% of the isolates. *C. freundii*, *Enterobacter agglomerans*, and *E. cloacae* were of lesser importance, accounting for 5 to 12% of the isolates. Each of the other types accounted for less than 5% of the isolates. Some differences were observed in the order of the frequency with which different *Enterobacteriaceae* were isolated at each packing plant. *E. coli* I was the most common isolate in plant A, whereas *K. pneumoniae* was the most common at plant B.

In general, the frequency of isolating *Enterobacteriaceae* at plant A was lower than that at plant B. A total of 86% of the plates with confluent growth were obtained at plant B, compared with only 14% at plant A. The sanitation practices in both plants virtually eliminated all *Enterobacteriaceae* from inanimate meat-handling surfaces (stainless steel, polyethylene cutting boards, and PVK or Neoprene rubber food belting). Occasional survivors included: *E. aerogenes*, *K. ozaenae*, *K. pneumoniae*, and *S. liquefaciens*. No *E. coli* I were isolated on equipment after cleanup.

The data could also be examined on the basis of the *Enterobacteriaceae* associated with different areas and surfaces within the packing plants (Table 3). The number of isolates for each surface type was proportional to the number of organisms growing on the Rodac sampling plates. This finding confirmed the earlier indication that the raw-meat-handling surfaces were the most heavily contaminated with *Enterobacteriaceae*, cooked-meat contact surfaces were less contaminated, and surfaces with no meat contact were not apparent reservoirs of *Enterobacteriaceae* in the plants. On raw-meat contact surfaces, *E. coli I*, *K. pneumoniae*, and *S. lique-

faciens* predominated, whereas *E. agglomerans* and *K. pneumoniae* were the main types identified on cooked-meat contact surfaces.

Similar frequency distributions (Table 4) were calculated on the basis of the *Enterobacteriaceae* associated with the different product types (beef, pork, and cooked meat). *K. pneumoniae*, *E. coli*, and *C. freundii* were the principal isolates from beef work surfaces; *E. coli I*, *S. liquefaciens*, and *K. pneumoniae* (in that order) were the principal isolates from pork; and *S. liquefaciens*, *E. cloacae*, *K. pneumoniae*, and *E. agglomerans* were the principal isolates from cooked-meat-handling surfaces. On cooked-meat work surfaces, *E. coli I* and *C. freundii* were relatively minor isolates (<10%).

The incidence of *E. coli* I on equipment and
meat-handling surfaces in use was determined for each meat type. In the beef-cutting areas of both plants, *E. coli* I was detected on meat-handling surfaces in 14 of 40 samples. *E. coli* I was also detected on a hand wash sink on the beef kill floor of plant B in four of nine samples. In contrast, *E. coli* I was found on only 1 of 14 tests on walls in the beef kill area. In the beef-cutting area for vacuum-packaged primal cuts, *E. coli* I was detected on meat-handling surfaces in 26 of 102 samplings.

Similar tests for *E. coli* I in the pork preparation areas resulted in fewer *E. coli* I isolates on the floor kill equipment (5 positive of 34 tests) than on the beef kill floor. However, there was a marked increase in the frequency of *E. coli* I detected in the pork-cutting areas (51 positive of 130 tests). For example, stainless-steel chutes for pork cuts had 8 of 18 samplings that were *E. coli* I positive; plastic cutting boards, 7 of 20; rubber belting, 7 of 19; and stainless-steel work tables, 13 of 22.

The emulsion preparation area for cooked-
meat products gave many E. coli I-positive samplings (18 of 86 tests). The incidence of E. coli I in this area of plant B was higher than at plant A. However, in the cooked-product handling areas of the two plants, the incidence of E. coli I was markedly reduced (only 4 positive of 123 samplings), and all of these positive samples were from the wiener-handling equipment at plant A (4 of 28 tests).

Throughout the two plants, surfaces with no meat contact (walls, doors, etc.) were tested on 102 occasions. Only one swab gave a positive E. coli I test. The incidence of Enterobacteriaceae in general on these surfaces was low (Table 1).

The distribution of different types of Enterobacteriaceae (Table 5) on vacuum-packaged beef revealed S. liquefaciens as the predominant isolate (29.7%) and E. coli I as the next most important isolate. In contrast, for ground beef prepared from supplies that had been centrally prepared but not vacuum packaged, the predominating Enterobacteriaceae were E. agglomerans, S. liquefaciens, and E. coli I. Frozen pork sausage, packaged by the manufacturer, revealed that S. liquefaciens was the predominant Enterobacteriaceae; E. agglomerans and E. coli I were also major isolates. In contrast to the packing-plant samples, K. pneumoniae was an isolate of minor importance in samples collected at the retail level.

Enterobacteriaceae were also isolated from retail store trim. Of 115 isolates, the principal organisms were E. agglomerans (43.5%), E. coli I (20.9%), and S. liquefaciens (13.9%). Samples from beef steaks and pork chops contained far fewer Enterobacteriaceae, and as a result only 56 and 45 isolates were identified from these samples, respectively. E. coli I was not detected among the beef steak isolates and represented only 2.2% of the isolates from pork chops. The predominant Enterobacteriaceae on both meat types were S. liquefaciens, which accounted for 32.1% of beef steak and 46.7% of pork chop isolates; E. agglomerans accounted for 30.4 and 20.0%, respectively. The only other isolate of any significance was E. hafniae, which accounted for 16.1% of the isolates from beef steak and 8.9% of those from pork chops.

Whereas E. coli isolates were principally bio-type I, with IMViC reaction +++++, the K. pneumoniae isolates had heterogeneous IMViC bio-types. The 284 K. pneumoniae of packing-plant origin included 59 (20.8%) IMViC type -+++ , 112 (39.4%) ++++, and 93 (13.7%) ++++. In comparison, the 118 K. pneumoniae isolates from meats at the retail level included 43 (36.4%) IMViC type -+++ , 47 (39.8%) ++++, and 10 (8.5%) +++++. This represents a high percentage of indole-positive strains.

**DISCUSSION**

The distribution of Enterobacteriaceae in the packing plants was primarily on meat-handling surfaces. Surfaces that did not normally come in contact with meats were not implicated as reservoirs of Enterobacteriaceae, and any buildup of Enterobacteriaceae during the day’s operations appeared to be controlled by sanitation practices. Nonetheless, some work surfaces developed excessively high contaminating loads of Enterobacteriaceae during the work day, which would act as continual sources of contamination for products coming in contact with them. From these results, we conclude that the Enterobac-
**ENTEROBACTERIACEAE IN MEAT HANDLING**

**TABLE 5. Distribution of Enterobacteriaceae isolated from VRBA plates from different meats sampled before handling at the retail level**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Vacuum-packaged beef trim and primal cuts</th>
<th>Ground beef from packaged supplies</th>
<th>Packaged pork sausage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High fat</td>
<td>Low fat</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> I</td>
<td>22 (15.2)*</td>
<td>48 (20.9)</td>
<td>28 (14.8)</td>
</tr>
<tr>
<td>Other <em>E. coli</em></td>
<td>3 (2.1)</td>
<td>2 (0.9)</td>
<td>4 (2.1)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>9 (6.2)</td>
<td>15 (6.5)</td>
<td>10 (5.3)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>8 (5.5)</td>
<td>10 (4.3)</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td><em>E. agglomerans</em></td>
<td>14 (9.6)</td>
<td>59 (25.6)</td>
<td>59 (31.2)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>9 (6.2)</td>
<td>19 (4.3)</td>
<td>15 (7.9)</td>
</tr>
<tr>
<td><em>E. hafniae</em></td>
<td>17 (11.7)</td>
<td>12 (5.2)</td>
<td>8 (4.2)</td>
</tr>
<tr>
<td><em>K. ozoaena</em></td>
<td>5 (3.5)</td>
<td>3 (1.3)</td>
<td>8 (4.2)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>1 (0.7)</td>
<td>6 (2.6)</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td><em>S. liquefaciens</em></td>
<td>43 (29.7)</td>
<td>53 (23.0)</td>
<td>37 (19.6)</td>
</tr>
<tr>
<td><em>S. rubidaea</em></td>
<td>6 (5.1)</td>
<td>3 (1.3)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>5 (3.5)</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other <em>Enterobacteriaceae</em></td>
<td>3 (2.1)</td>
<td>8 (3.5)</td>
<td>5 (2.6)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are percentages.*

Enterobacteriaceae on the meat contact surfaces are a buildup from meats and their ongoing handling. As such, the stage at which the surfaces can be considered unsanitary is difficult to decide, especially since buildup can be attributed, in part, to growth of the bacteria on these surfaces.

*E. coli* I, *K. pneumoniae*, and *S. liquefaciens* were generally the dominant Enterobacteriaceae in the raw-meat-handling areas of the packing plants. In contrast, the incidence of Enterobacteriaceae on cooked (processed)-meat work surfaces and products was dramatically reduced, as would be expected with the heat treatment. However, *E. cloacae*, *S. liquefaciens*, *K. pneumoniae*, and *E. coli* I were the predominant isolates from surfaces before processed products were cooked, whereas *E. agglomerans*, *K. pneumoniae*, and *S. liquefaciens* were the most common isolates from cooked-meat work surfaces used after the products had been cooked. These data indicated that *K. pneumoniae* and *E. coli*, both of which could be of fecal origin (4, 9), were predominant Enterobacteriaceae on meat-handling surfaces in the packing plants.

At the retail level, even on meats before handling by the butchers, the predominating Enterobacteriaceae had changed to *S. liquefaciens*, *E. agglomerans*, and *E. coli* I. *K. pneumoniae* had become a relatively infrequent and minor isolate. This suggested a change in the Enterobacteriaceae surviving on meats, growth of psychrotrophic Enterobacteriaceae (5) on meats during the marketing process, or both. *E. coli* I was a frequent Enterobacteriaceae isolate on all samples except processed meats sampled after cooking. As a result, *E. coli* I in a product such as raw ground beef could have no meaning for sanitation at the retail level. *S. liquefaciens* was also present as a major Enterobacteriaceae isolate throughout the meat-handling process. However, *E. agglomerans* was a minor isolate at the packing plants, but it frequently became a major isolate, with *S. liquefaciens*, in retail samples.

The Enterobacteriaceae with greatest indicator potential in these samples was *K. pneumoniae*. This organism was frequently isolated as a dominant Enterobacteriaceae in meats and on meat-handling surfaces in the packing plant, but it was only infrequently found on meats at the retail level. This observation was confirmed by data of Cox and Mercuri (11) in their study of Enterobacteriaceae on retail meats, whereas Newton et al. (27) found that 21.5% of the Enterobacteriaceae from hides and meat at packing plants were *K. pneumoniae*. These results suggest that this organism has a short survival time in meats, so that its detection at the retail level could indicate recent contamination, which in turn could indicate unsanitary and unhygienic handling.

Studies on the distribution of *Klebsiella* spp. in a hospital kitchen indicated that 6 of 13 raw meats contained *Klebsiella*, but that heaviest contamination (>10^5/g) was observed on salads (8). Evidence of contamination in the kitchen was presented. The use of *E. coli* I as an indicator organism has always been predicated on its restricted occurrence in nature compared with the ubiquitous nature of *E. aerogenes* (5). Differentiation of *K. pneumoniae* and *E. aerogenes* poses even greater difficulties because of the similarity in their biochemical characteristics (2, 10, 11, 22). *K. pneumoniae* can be selectively enumerated, based on morphology on methyl violet agar medium (6); if contaminants on vegetables can be related to fecal origin (3), this organism might indeed be a more meaning-
ful Enterobacteriaceae to use as an indicator for sanitation and hygiene of meats at the retail level than E. coli I.

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LITERATURE CITED

16. International Commission of Microbiological Speci-