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Research Note

Microbiological Status of Fresh Beef Cuts

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ABSTRACT

Fresh beef samples (n = 1,022) obtained from two processing plants in the Midwest (July to December 2003) were analyzed for levels of microbial populations (total aerobic plate count, total coliform count, and *Escherichia coli* count) and for the presence or absence of *E. coli* O157:H7 and *Salmonella.* A fresh beef cut sample was a 360-g composite of 6-g portions excised from the surface of 60 individual representative cuts in a production lot. Samples of fresh beef cuts yielded levels of 4.0 to 6.2, 1.1 to 1.8, and 0.8 to 1.0 log CFU/g for total aerobic plate count, total coliform count, and *E. coli* count, respectively. There did not appear to be substantial differences or obvious trends in bacterial populations on different cuts. These data may be useful in establishing a baseline or a benchmark of microbiological levels of contamination of beef cuts. Mean incidence rates of *E. coli* O157:H7 and *Salmonella* on raw beef cuts were 0.3 and 2.2%, respectively. Of the 1,022 samples analyzed, cuts testing positive for *E. coli* O157:H7 included top sirloin butt (0.9%) and butt, ball tip (2.1%) and for *Salmonella* included short loins (3.4%), strip loins (9.6%), rib eye roll (0.8%), shoulder clod (3.4%), and clod, top blade (1.8%). These data provide evidence of noticeable incidence of pathogens on whole muscle beef and raise the importance of such contamination on product that may be mechanically tenderized. Levels of total aerobic plate count, total coliform count, and *E. coli* count did not ($P \geq 0.05$) appear to be associated with the presence of *E. coli* O157:H7 and *Salmonella* on fresh beef cuts. *E.* O157:H7 was exclusively isolated from cuts derived from the sirloin area of the carcass. *Salmonella* was exclusively isolated from cuts derived from the chuck, rib, and loin areas of the carcass. Results of this study suggest that contamination of beef cuts may be influenced by the region of the carcass from which they are derived.

Bacterial foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* have been associated with recent outbreaks in the United States as a result of the consumption of undercooked or improperly handled beef products (*2–5*). This is particularly important for beef cuts intended for further processing, such as mechanical tenderization or injection, because surface contamination may be internalized during such processes. This internalization of pathogens in meat products may result in consequential temperature shielding by meat tissues imparting protection against cooking temperatures that traditionally have been sufficient for killing surface contamination (*1*) and especially against temperatures associated with undercooking of meat. Thus, it is important to establish baseline data relative to the microbiological status of fresh beef cuts that may be useful for determining public health risks and establishment of performance standards.

The objective of this study was to assess the levels of microbiological contamination, including prevalence of two pathogens, of fresh beef cuts. To achieve this objective, we determined the total bacterial load as well as that of indicator organisms (organisms commonly used as indicators of the sanitary quality of meat), such as coliforms and *E. coli*, and assessed safety by investigating incidence of *E. coli* O157:H7 and *Salmonella*.

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MATERIALS AND METHODS

Collection of fresh beef samples. Fresh beef cut samples were collected from two commercial meat processing facilities in the Midwest (July to December 2003) and transported on ice to the laboratory for analysis within 24 h. Samples included club ends (n = 60); strip loins (International Meat Purchase Specifications [IMPS] no. 175) (n = 52); top sirloin butt (IMPS no. 184) (n = 113); bottom sirloin butt (IMPS no. 185A) (n = 35); shoulder clod (IMPS no. 114) (n = 117); short loins (IMPS no. 174) (n = 238); clod, top blade (IMPS no. 114D) (n = 57); rib eye roll (IMPS no. 112A) (n = 133); butt, ball tip (IMPS no. 185B) (n = 94); and a collection of miscellaneous cuts (n = 123). Each fresh beef cut sample consisted of a 360-g composite sample comprised of 6-g portions excised aseptically (with blades sterilized in 82°C water for 20 s) from the surface of 60 individual representative cuts in a production lot. The 6-g portions were composited in sterile 18-oz (510.3-g) Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) for further analysis.

Microbial enumeration and multiplex PCR screening for pathogen incidence. Samples were enriched for PCR analysis by adding 180 ml of prewarmed (42°C) Trypticase soy broth (TSB; Becton, Dickinson and Company, Cockeysville, Md.) to each Whirl-Pak bag (Nasco) containing approximately 360 g of sample. All samples were pummeled in an IUL Masticator (IUL, S.A., Spain) for 60 s. A range of serial dilutions was made by initially transferring 1 ml of enrichment broth into 9 ml of sterile 0.1% buffered peptone water (Fisher Scientific, Houston, Tex.). A volume of 1 ml was aseptically removed and plated from appropriate dilutions onto Petrifilm Aerobic Count Plates (APC) and Petrifilm

TABLE 1. *Microbial profile of beef cuts*[a]

Type of cut	IMPS notation	No. of samples	Aerobic plate count	Total coliform count	*E. coli* count
Club ends		60	6.2 (1.1) A	1.3 (0.8) A	0.8 (0.3) A
Strip loins	175	52	5.9 (1.6) A	1.6 (1.2) A	0.8 (0.2) A
Top sirloin butt	184	113	5.9 (1.0) A	1.8 (1.3) A	0.9 (0.7) A
Bottom sirloin butt	185A	35	5.6 (1.3) A	1.1 (0.7) A	0.8 (0.2) A
Shoulder clod	114	117	5.0 (1.4) AB	1.4 (0.8) A	0.8 (0.3) A
Short loins	174	238	4.8 (1.5) B	1.2 (0.8) A	0.9 (0.5) A
Clod, top blade	114D	57	4.3 (1.4) B	1.2 (0.8) A	0.8 (0.4) A
Rib eye roll	112A	133	4.0 (1.6) B	1.3 (0.9) A	1.0 (0.7) A
Butt, butt, ball tip	185B	94	4.0 (1.4) B	1.5 (1.0) A	1.0 (0.6) A
Miscellaneous cuts		123	5.4 (1.7) AB	1.6 (1.1) A	1.0 (0.7) A
Total		1,022	5.0 (1.6)	1.4 (1.0)	0.9 (0.5)

[a] Values are mean log CFU per gram (standard deviation). Means within a column with different letters are statistically ($P < 0.05$) different. IMPS, International Meat Purchase Specifications.

E. coli/Coliform Count Plates (ECC and TCC) (3M, St. Paul, Minn.). Petrifilm plates were incubated at 37°C for up to 48 h and colonies enumerated according to the manufacturer's instructions. The TSB enrichments were incubated for 12 h at 37°C with agitation.

Following incubation, 1 μl of each TSB enrichment was transferred into two separate reaction tubes containing 50 μl of lysis reagent for *E. coli* O157:H7 (EC4 buffer, Molecular Epidemiology, Inc., Seattle, Wash.) and *Salmonella* (S3 buffer, Molecular Epidemiology, Inc.) analysis. Negative controls were performed by substituting 1μl of deionized water for sample enrichment in lysis reagent for both *E. coli* O157:H7 and *Salmonella* analysis. Positive controls were simultaneously analyzed by using 1 μl of control *E. coli* O157:H7 and *Salmonella* DNA to ensure appropriate conditions and visualization procedures. All lysis reactions were incubated for 20 min at 37°C, followed by 10 min at 95°C, according to manufacturer protocols (Molecular Epidemiology, Inc.). Lysate tubes were immediately placed at 4°C following incubation until commercially available multiplex PCR assays for *E. coli* O157:H7 (EC4, Molecular Epidemiology, Inc.) and *Salmonella* (S3, Molecular Epidemiology, Inc.) were used to test for pathogen incidence (sensitivity >99% and specificity >99% for both assays; from data provided by American Proficiency Institute, Traverse City, Mich.). The EC4 PCR assay for *E. coli* O157:H7 amplifies four specific genomic regions of the following sizes: 180, 255, 384, and 985 bp (Technical Specifications for EC4, Molecular Epidemiology, Inc.). The S3 PCR assay for *Salmonella* amplifies three specific genomic regions of the following sizes: 218, 382, and 803 bp (Technical Specifications for S3, Molecular Epidemiology, Inc.). Amplification was performed by cycling tubes in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany) for 33 cycles of a 95°C, 10-s denaturation followed by annealing temperature-time combination of 65°C for 30 s or 72°C for 20 s. Amplicons were resolved by 2% low electroendosmodis (EEO) agarose (Fisher Scientific) gel electrophoresis (Bio-Rad Laboratories sub-cell model 192, Hercules, Calif.) at 240 V for 35 min and stained with ethidium bromide (Fisher Scientific). Banding patterns were visualized with an Epi Chemi II Darkroom UV transilluminator (UVP, Inc., Upland, Calif.).

Isolates yielding presumptive positive electrophoresis banding patterns for *E. coli* O157:H7 were streaked onto sorbitol MacConkey agar (Becton, Dickinson and Company) with cefixime-tellurite (Dynal Inc., Lake Success, N.Y.) and incubated for 18 to 24 h at 37°C. Following incubation, suspect colonies were picked from sorbitol MacConkey agar with cefixime-tellurite, streaked onto TSA agar with 5% sheep blood (Hardy Diagnostics, Santa Maria, Calif.), and incubated for 18 to 24 h at 37°C. Colonies on 5% sheep blood agar were picked and tested for the presence of the O157 antigen with the Oxoid latex agglutination kit (Oxoid Limited, Wade Road, England, UK) following the manufacturer's guidelines. Samples generating positive latex agglutination were considered *E. coli* O157:H7–positive results. Isolates yielding presumptive positive electrophoresis banding patterns for *Salmonella* were streaked onto xylose lysine tergitol 4 agar (Becton, Dickinson and Company) and incubated at 37°C. Following incubation for 24 and 48 h, suspect black colonies were picked, streaked onto TSA agar with 5% sheep blood (Hardy Diagnostics), and incubated for 18 to 24 h at 37°C. Colonies were then picked and tested with the S3 PCR assay for *Salmonella* (Molecular Epidemiology, Inc.) according to the same PCR protocols outlined above. Samples producing positive banding patterns following secondary screening with the S3 PCR assay for *Salmonella* were considered positive for *Salmonella*.

Experimental design and statistical analysis. The experiments were conducted using a completely randomized design to test the population differences between types of cuts. Bacterial populations were expressed as mean log CFU per gram with associated standard deviations. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. Viable population data were separated by APC, TCC, and ECC and evaluated for differences between least squares means of types of cuts by one-way analysis of variance procedures of SAS version 8.2 (SAS Institute, Cary, N.C.). Pathogen prevalence data were separated by *E. coli* O157:H7 and *Salmonella* and evaluated for differences using chi-square test for equal proportions. Pearson's correlations were performed to evaluate if any significant correlation between microbial loads and pathogen incidence on fresh beef cuts exists. All differences were reported at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

APC ranged from 4.0 log CFU/g on butt, ball tips and rib eye rolls to 6.2 log CFU/g on club ends (Table 1). APC from various cuts were statistically different ($P < 0.05$); however, mean population counts were within one standard deviation of the overall mean counts (5.0 ± 1.6 log CFU/

TABLE 2. *Incidence of* E. coli *O157:H7 and* Salmonella *on beef cuts*[a]

Type of cut	IMPS notation	No. of samples	*E. coli* O157:H7		*Salmonella*	
			No. of positives	% positive	No. of positives	% positive
Club ends		60	0	0 A	0	0 B
Strip loins	175	52	0	0 A	5	9.6 A
Top sirloin butt	184	113	1	0.9 A	1	0.9 B
Bottom sirloin butt	185A	35	0	0 A	0	0 B
Shoulder clod	114	117	0	0 A	4	3.4 B
Short loins	174	238	0	0 A	8	3.4 B
Clod, top blade	114D	57	0	0 A	1	1.8 B
Rib eye roll	112A	133	0	0 A	1	0.8 B
Butt, butt, ball tip	185B	94	2	2.1 A	0	0 B
Miscellaneous cuts		123	0	0 A	2	2.4 B
Total		1,022	3	0.3	22	2.2

[a] Means within a column with different letters are statistically ($P < 0.05$) different. IMPS, International Meat Purchase Specifications.

g) for all cuts and, thus, were not substantially different. Furthermore, there were no obvious trends in difference of APC between individual cuts (Table 1). TCC and ECC on beef cuts ranged from 1.1 to 1.8 log CFU/g and 0.8 to 1.0 log CFU/g, respectively, and were not different ($P > 0.05$) (Table 1).

The beef industry currently is required by the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) to enumerate *E. coli* as a means of ''verifying that the slaughter process is under control'' *(6).* There are, however, no microbiological guidelines for fresh beef cuts and, as such, no industry-wide benchmark for producers. Results of the current study may be used in the establishment of a microbiological baseline for fresh beef cuts. Ideally, each establishment producing fresh beef cuts would develop its own performance criteria for process control based on in-house data and/or industry-developed benchmarks. Because no such baseline data exists, there may be a need for future surveys, in addition to the current study, on microbiological quality (APC, TCC, and ECC) of fresh beef cuts and other products (such as trimmings, variety meats, etc.) from plants in different geographic regions over different seasons. Such a baseline will assist individual establishments producing beef cuts and other products in (i) assessing performance of their process control relative to an industry benchmark, (ii) meeting performance standards for exporting product, and (iii) meeting microbiological specifications of customers.

Of the 1,022 samples analyzed, cuts testing positive for *E. coli* O157:H7 included top sirloin butt (1 of 113) and butt, ball tip (2 of 94) (Table 2). Overall, samples testing positive for *E. coli* O157:H7 yielded an incidence rate of 0.3% (3 of 1,022) (Table 2). This result was similar to an earlier study reporting prevalence of 0.2% *E. coli* O157:H7 on beef cuts *(10).* Of the 1,022 samples analyzed, cuts testing positive for *Salmonella* included short loins (8 of 238), strip loins (5 of 52), rib eye roll (1 of 133), shoulder clod (4 of 117), and clod, top blade (1 of 57) (Table 2). Overall, samples testing positive for *Salmonella* yielded an incidence rate of 2.2% (22 of 1,022) (Table 2).

Although there is no established industry-wide baseline for the incidence of *E. coli* O157:H7 on cuts, the national incidence rate in ground beef was estimated at 0.17% (14 of 8,010) in 2004 and 0.18% (13 of 7,104) in 2005 *(9),* which indicates that *E. coli* O157:H7 has a similar incidence rate on whole muscle beef cuts as it does in ground beef. The USDA-FSIS considers intact products destined for further processing into nonintact products (i.e., mechanical tenderization) and raw ground beef to be adulterated if contaminated with *E. coli* O157:H7, unless they are further processed to destroy the pathogen *(7).* Nonintact beef, including steaks injected with tenderizing and flavor-enhancing compounds, has been implicated as the source of *E. coli* O157:H7–related illness in recent foodborne outbreaks *(8).* Mechanical tenderization of beef cuts involves the penetration of meat by sets of needles or double-edged blades to improve tenderness by disrupting the muscle structure and is usually combined with the infusion or injection of marinade mixtures or processing ingredients to assist in flavor enhancement and product tenderization. The concern with mechanical tenderization techniques is that the procedures may translocate pathogens from the surface to the interior of intact beef cuts and cause potential shielding of the dislocated pathogens to subsequent lethal effects of heating during cooking. This problem is exacerbated by the possibility that consumers are not always aware that (i) beef cuts purchased have been subjected to mechanical tenderization, and (ii) mechanically tenderized beef cuts may increase risk of illness if cooked to the same internal temperatures as intact cuts. Similar incidence rates of *E. coli* O157:H7 on ground beef *(9)* and fresh beef cuts as determined in the current study are important because cuts obtained from commercial meat processors for this study were intended for injection tenderization. Until now, there has been intense regulatory focus and industry effort to alleviate the problem of *E. coli* O157:H7 in ground beef; however, results of this study suggest that incidence of this pathogen on fresh beef cuts intended for mechanical tenderization is similar to that in ground beef. The public health importance of fresh beef cuts intended for mechanical tenderization found to be contaminated with pathogens such as *E. coli* O157:H7 and *Salmonella* may thus be underes-

timated. It should be noted that caution should be used when using data about cuts in the present study for direct comparative purposes with those of ground beef in the USDA-FSIS study *(8)* because (i) the products are not the same, (ii) the studies do not use the same method of sampling, and (iii) the studies do not use the same microbiological techniques for pathogen detection.

APC, TCC, and ECC on fresh beef cuts were 5.0 ± 1.6, 1.4 ± 1.0, and 0.9 ± 0.5 log CFU/g, respectively. These counts did not appear to influence the presence of *E. coli* O157:H7 and *Salmonella* on fresh beef cuts. Results of this study did, however, indicate that *E. coli* O157:H7 was exclusively isolated from cuts derived from the sirloin area, whereas *Salmonella* was exclusively isolated from cuts derived from the chuck, rib, and loin areas. Thus, contamination of beef cuts may have been influenced by the region of the carcass they were derived from. The results suggest that pathogens such as *E. coli* O157:H7 and *Salmonella* are invariably present on fresh beef surfaces and particularly cuts that have already been exposed to primary interventions intended to reduce, control, and/or eliminate such pathogens. It should be noted that sampling of fresh beef cuts occurred at a stage prior to decontamination of the cuts with acidified sodium chlorite and, as such, these results do not necessarily represent the microbiological status of fresh beef cuts entering the food supply but, more so, fresh beef cuts that may not be subjected to a further antimicrobial intervention. Future investigations should consider surveying fresh beef cuts at a stage following retail packaging because this would most accurately represent the microbiological status of fresh beef cuts that are intended for consumption.

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