

# The Microbiological Condition of Canadian Beef Steaks Offered for Retail Sale in Canada

M. Badoni<sup>1</sup>, S. Rajagopal<sup>1</sup>, J. L. Aalhus<sup>1</sup>, M. D. Klassen<sup>2</sup> & C. O. Gill<sup>1</sup>

<sup>1</sup> Agriculture and Agri-Food Canada Lacombe Research Centre, Lacombe, Canada

<sup>2</sup> Canadian Cattlemen's Association, Calgary, Canada

Correspondence: M. Badoni, Agriculture and Agri-Food Canada Lacombe Research Centre 6000 C & E Trail, Lacombe, Alberta T4L1W1, Canada. Tel: 1-403-782-8100. E-mail: madhu.badoni@agr.gc.ca

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## Abstract

Steaks of 4 types collected from 113 retail stores in 4 Canadian cities were frozen for storage. Swab samples collected from approximately 100 cm<sup>2</sup> of each of 598 thawed steaks were processed for enumeration of bacteria. The fraction of steaks from which total aerobic counts (AER), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE) and *Brochothrix thermosphacta* (BRO) were not recovered at  $\geq 2$  log cfu/100 cm<sup>2</sup> were 3, 12, 8, 25 and 51%, respectively. The fractions of steaks from which coliforms (COL) and *Escherichia coli* (ECO) were not recovered at  $\geq 0$  log cfu/100 cm<sup>2</sup> were 56 and 92%, respectively. The log number per 100 cm<sup>2</sup> recovered from  $\geq 90\%$  of steaks were  $< 6$  for AER, PSY and LAB,  $< 5$  for PSE,  $< 4$  for BRO, and  $< 2$  for COL. The microbiological conditions of groups of steaks of different types, from different cities or from different groups of stores were not substantially different.

**Keywords:** beef steaks, retail sale, aerobic counts, spoilage bacteria, *Escherichia coli*

## 1. Introduction

Surveys of the microbiological conditions of fresh meats offered for retail sale have usually been concerned with the detection of one or more pathogens, such as verotoxigenic *Escherichia coli* (VTEC), *Salmonella*, *Campylobacter*, etc (Arslan & Evi, 2010; Etcheverria et al., 2010; Sammarco, Ripabelli, Fanelli, Grasso, & Tamburro, 2010). In some surveys, total aerobic counts, Enterobacteriaceae or coliforms and, sometimes, *E. coli* have been enumerated; and in surveys that included examination of beef, samples commonly were obtained from ground beef rather than cuts (Bohaychuk et al., 2006; Pao & Ettinger, 2009; Phillips, Jordan, Morris, Jensen, & Sumner, 2008). Thus, information on the numbers and compositions of the spoilage microflora that are present on premium beef cuts offered for retail sale in developed markets is largely lacking.

The beef packing industry in North America has undergone extensive consolidation during the past 30 years (MacDonald, 2003). Consequently, most premium beef of North American origin now comes from relatively few packing plants that each slaughter some 2000 or more animals per day. Over 80% of Canadian premium beef comes from only four such plants (Alberta Beef Producers, 2011; Cargill Canada, 2011). In addition, in recent years, large beef packing plants in North America have implemented various carcass decontaminating treatments and have undertaken other actions aimed at enhancing the microbiological safety of their products (Byelashov & Sofos, 2009). Enhanced control over hazardous microbiological contamination might well result in some reduction in product contamination with spoilage organisms. Although generally desirable, the consequent improvement of product storage stability might, in some circumstances, increase risks from product that still is contaminated with hazardous organisms (Koutsoumanis, 2009). However, information of the effects of current North American practices on the spoilage microflora of retail beef is largely lacking.

In both the USA and Canada, beef industry organizations have undertaken national surveys of the quality of beef from their own plants that have included investigation of the acceptability to consumers and eating qualities of beef steaks from retail stores (Canadian Cattlemen's Association, 2009; George, Tatum, Belk, & Smith, 1999). When arranging for such a survey in 2009, the Canadian Cattlemen's Association considered that information on the microbiological conditions of beef that could be related to product storage stability and safety would be desirable; not only for knowledge of current conditions but also to provide benchmark data by which to assess

improvement or otherwise of control over microbiological contamination from data that will be obtained in future surveys. Therefore, the work reported here was undertaken to determine the numbers and compositions of the spoilage flora, and the numbers of *E. coli* present on Canadian steaks offered for retail sale in Canada.

As in previous surveys, steaks were collected from stores in three cities in the east and one in the west of Canada. Three of the four large packing plants that provide prime Canadian beef are located in or close to the western city, Calgary, which is 3000 to 3700 km from each of the eastern cities. Data were analyzed with respect to steak type, city and store group for indication of whether there were differences in the microbiological conditions of steaks that might arise from regional or company differences in processes for acquiring, fabricating and distributing beef for retail sale.

## 2. Materials and Methods

### 2.1 Collection of Steaks

During the period August-September 2009, steaks for consumer, organoleptic, and physical, chemical and microbiological testing were obtained from 113 retail stores in three cities (Montreal, Quebec; Toronto, Ontario; London, Ontario) in eastern Canada and one city (Calgary, Alberta) in Western Canada (Juarez, Klassen, Larsen, & Aalhus, 2012).

The types of steak collected were cut from inside round (IR), cross rib (CR), top sirloin (TS), and striploin (SL) primal cuts of Canadian origin. On each occasion that steaks were collected, between 20 and 25% of the steaks of each type from each store were assigned to microbiological testing, to obtain in total 150 of each of IR, TS and SL steaks, and 148 CR steaks.

When each steak was collected, the type of steak, the store from which it was collected, the location of the store and the date of collection were recorded and the steak was assigned a code number. Each steak was then removed from its retail packaging and placed with a tag bearing the code number in a 17 x 21 cm zip sealable plastic pouch (Ziploc; S.C. Johnson, Brantford, Ontario, Canada) designed for storage of frozen foods. Air was squeezed from each pouch to apply the film closely to all meat surfaces before the pouch was zipped closed. Groups of 5 steaks in sealed pouches were each placed in a plastic bag from which air was squeezed before it was tied shut, and placed in a domestic freezer operating at -20°C. When all steaks collected in each city had been frozen they were transported to the laboratory as a single consignment in a refrigerated road trailer.

### 2.2 Microbiological Sampling of Steaks

Steaks were stored at the laboratory at -20°C. At times between 5 and 8 months after steaks were collected, batches of 10 steaks were removed from frozen storage. Each frozen steak was taken from its plastic bag, placed on a drip pad in a polystyrene tray, and overwrapped with a plastic film of high oxygen permeability. The overwrapped steaks were placed in a single layer on a rack in a refrigerator operating at  $4 \pm 1$  °C and allowed to thaw overnight (Juarez et al., 2012).

After removal of the wrapping film, the whole of the upper surface of each steak, i.e. about 100 cm<sup>2</sup>, was swabbed with a sterile gauze swab (Curity gauze sponge; Kendall Canada, Peterborough, Ontario, Canada) that had been moistened with 0.1% (w/v) peptone water (Difco, Becton Dickinson, Sparks, MD, USA), and the swab was then placed in a plastic bag (Gill, Badoni, & Jones, 2001). When all the steaks in a batch of 10 had been sampled, within 20 min, the swabs used on the steaks were placed in a single layer in a freezer operating at -80°C. The frozen swabs were held at -80°C until they were processed.

### 2.3 Enumeration of Bacteria

The *swab* samples were processed for enumeration of bacteria between 3 and 9 months after they were frozen. Swabs were thawed by immersing the bottoms of the bags in which they were contained in water at a temperature of 30°C for 15 min. Then, 10 ml of peptone water was added to each bag and the swab and diluent were pummeled for 2 min in a stomacher operated at high speed. A 1 ml portion of the stomacher fluid was used to prepare ten-fold dilutions of the fluid to 10<sup>-6</sup>. Then, 0.1 ml portions of the undiluted fluid and each dilution were spread on duplication plates of tryptose soy agar (TSA; Difco) and on single plates of de Man, Rogosa, Sharpe agar (MRS; Difco), cephaloridine fucidin cetrinide agar (CFC; Difco), and streptomycin thallos acetate acitidione agar (STAA; Difco). One set of plates of TSA inoculated with the undiluted stomacher fluid and each dilution, and plates of CFC and STAA were incubated aerobically at 25°C for 48 h. The other set of TSA plates was incubated at 4°C for 10 days. Plates of MRS were incubated anaerobically; at 25°C for 72 h. Colonies were counted on plates bearing between 20 and 200 colonies. The numbers of total aerobic counts (AER), presumptive psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE) and *Brochothrix*

*thermosphacta* (BRO) recovered from each steak were estimated from counts on TSA incubated at 25°C, TSA incubated at 4°C, MRS, CFC and STAA, respectively (Correy, 2007).

The swab within each bag was squeezed to expel most of the stomacher fluid and all the available fluid was removed from the bag by means of a 10 ml, graduated pipette. The volume of fluid that was obtained was recorded. The fluid was mixed with 1 ml of a papain solution (EZ-Enzyme; Oxoid, Nepean, Ontario, Canada) and the mixture was incubated at 25°C for 20 min before it was filtered through a hydrophobic grid membrane filter (HGMPF; Oxoid). The filter was placed on a plate of lactose monensin glucuronate agar (LMG; Oxoid) and incubated at 35°C for 24 h. Squares on the filter that contained blue colonies were counted. That count was converted to a most probable number (MPN) for the number of coliforms recovered from the stomacher fluid by application of the formula  $MPN = N \log_n (N/N-X)$ , where N is the total number of squares on a filter and X is the count of squares containing blue colonies. The MPN was corrected for the volume of stomacher fluid obtained from the swab to estimate the number of coliforms recovered per 100 cm<sup>2</sup>.

The filter was transferred to a plate of buffered 4-methylumbelliferyl-β-D-glucuronide agar (BMA; Oxoid) and incubated at 35°C for 2 h. Then, the filter was examined under long wave length UV light, and squares containing blue-white, fluorescent colonies were counted. A MPN for the number of *E. coli* recovered per 100 cm<sup>2</sup> was calculated from that count and the volume of stomacher fluid recovered from the swab as was the number of coliforms per 100 cm<sup>2</sup> (Entis Boleszczuk, 1990).

#### 2.4 Effects of Freezing Steaks and Swabs on Recovering of Bacteria

Before any frozen swab samples were processed, two or three striploin steaks were purchased from each of seven retail stores, to obtain a total of 25 steaks. The steaks were placed in a plastic bag and tumbled together for 5 min, to homogenize the microflora on the steaks. Each steak was then placed in a plastic bag, frozen at -20°C and, after 14 days, thawed as were the steaks collected for the survey. Each thawed steak was sampled by swabbing and the swab samples were frozen at -80°C, as described above.

After 5 days, the swab samples were processed, and dilutions of the stomacher fluids were prepared, as described before. Plates of TSA and the selective agars were spread with undiluted and dilutions of stomacher fluids, as described above. In addition, for each stomacher fluid, undiluted fluid and dilutions were spread on a third set of TSA plates and sets of each of the selective agars that were overlaid with 10 ml of TSA and inoculated within 30 min of setting of the TSA (Wu & Fung, 2001). The additional plates of TSA were incubated at 25°C for 4 h before they were incubated at 4°C for 10 days. The overlaid plates were incubated as were the corresponding plates without overlaid TSA.

A further twenty-five steaks purchased from seven retail stores were tumbled together, as before. One surface of each steak was sampled by swabbing, and then the steak was placed on a polystyrene tray with the swabbed surface down. The swab was pummeled with diluent, and the stomacher fluid was used for enumeration of bacteria as were the stomacher fluids obtained from swabs used with steaks collected in the survey.

Each swabbed steak was sealed in a plastic pouch, and groups of 5 packaged steaks in plastic bags were frozen as were the steaks collected in the survey. After frozen storage for two weeks, the steaks were thawed and the upper surface of each was sampled by swabbing, with thawing and sampling being as for the steaks collected in the survey. The swabs were frozen, thawed and used for enumeration of bacteria as were the swabs used with steaks collected in the survey, with the frozen swabs being stored for two weeks before they were thawed.

#### 2.5 Analysis of Data

All counts were transformed to log values and tabulated as log cfu/100 cm<sup>2</sup> on the assumption that the surface area sampled on each steak was 100 cm<sup>2</sup>. For calculations, when bacteria of any group were not recovered a log value of 1.5 or -0.5 log cfu/100 cm<sup>2</sup> was assigned when the detection limit for the organisms was 2 or 0 log cfu/sample, respectively. The sets of counts of each type obtained for all steaks collected in the survey were categorized by intervals of 1 log cfu/100 cm<sup>2</sup>, and a correlation coefficient was calculated for each pair of those sets. The counts of each type were arranged as sets by reference to steak type, store location and store affiliations. Each of those sets was tested for normal distribution by a Shapiro-Wilk test, with  $P < 0.05$ . Correlation coefficients were calculated, and Shapiro-Wilk tests were performed using Minitab, releases 12 (Minitab Inc., State College, PA, USA). Median values for pairs of sets of the same type of count for steak type, store location or store affiliation were separated by a Kruskal-Wallis test, using the NPARIWAY procedure in SAS, version 9.2 (32) (SAS 2012.) with  $P < 0.05$ .

The log mean values for sets of the same type of count for steak type, store location or store affiliation were calculated by summing the counts in each set, calculating the mean, and obtaining the log of the mean.

Correlations between log mean values and numbers of samples from which colonies were not recovered were calculated using Minitab.

The mean ( $\bar{x}$ ) and standard deviation (s) were calculated for all sets of log counts for bacteria recovered from the groups of 25 steaks in which assigned values for numbers below the detection limit were  $> 20$  of the 25 counts. When  $\bar{x}$  and s were calculated for a set, a value for the log mean (log A) was calculated also using the formula  $\log A = \bar{x} + \log_{10} s^2/2$ . Each set of those counts was subject to the Shapiro-Wilk test. The mean values for sets of counts of the same type recovered from the same steaks were separated using the t-test for paired comparisons of the PROC MEANS procedure in SAS.

### 3. Results and Discussion

Because steaks were collected in widely separated locations and utilized for several purposes, freezing of steaks and swab samples was unavoidable. Bacteria can be injured or inactivated by freezing and thawing, and frozen storage; but the effects of freezing vary widely with species and strains of bacteria, the composition of menstria and rates of freezing and thawing (Gill, 2012). However, bacteria in raw meats and swab samples are generally little affected by freezing and frozen storage (Black, Hirneisen, Hoover, & Kniel, 2009; Lianou & Koutsoumanis, 2009; Musser & Gonzalez, 2011), particularly if freezing and thawing is rapid (Lund, 2000). It was then expected that the freezing of steaks and swabs would not greatly affect the numbers of bacteria recovered in the survey. Even so, some testing of this expectation seemed to be required for assurance that the assumption of minimal effects of the freezing and thawing was warranted.

When PSY, LAB, PSE and BRO were recovered from a group of 25 steaks with or without resuscitation at a non-selective temperature or on a non-selective agar overlay, both sets of BRO log counts included two assigned values while all other sets of log counts included none. All the sets of counts were normally distributed ( $P > 0.05$ ). Differences between the mean log values for the sets of PSY, LAB, PSE and BRO log counts recovered with or without resuscitation were (with - without) -0.13, 0.22, -0.19 and 0.06 log unit, respectively; and differences between the log mean values were -0.03, 0.45, -0.23 and 0.05 log unit, respectively. The mean log values in each pair of sets were not significantly different ( $P > 0.05$ ).

When bacteria were recovered from a group of 25 steaks before and after freezing, and without or with freezing of swabs, bacteria of each group except ECO were recovered from all samples. All sets of log counts except that for AER from not frozen steaks were normally distributed ( $P > 0.05$ ). Differences between the mean log values for sets of AER, PSY, LAB, PSE, BRO and COL log counts from steaks before and without freezing of swabs or after freezing and with freezing swabs (not frozen - frozen) were -0.01, -0.10, -0.31, -0.21, 0.04 and 0.44/log unit respectively. The mean log values for the pairs of LAB log counts and the pair of ECO log counts were significantly different ( $P < 0.05$ ). ECO were recovered from 7 steaks that had not been frozen at total numbers of 0.90 log cfu/2500 cm<sup>2</sup> and from 3 frozen steaks at total numbers of 0.60 log cfu/2500 cm<sup>2</sup>.

LMG allows resuscitation of injured coliforms and *E. coli* (Entis & Bolezszcuk, 1990). The similar recovery of bacteria of each of the other groups of bacteria on a selective agar, or at a selective temperature, whether or not the plates were overlaid with a non-selective agar or incubated initially at a non-selective temperature to allow resuscitation of injured cells, indicates that there was little injury of cells in any of those groups as a result of the freezing treatments. The numbers of most groups of bacteria recovered from steaks were not reduced by freezing of the steaks and the swabs with which they were sampled. Freezing may have reduced the relatively small numbers of coliforms and very small numbers of *E. coli* on steaks, but any such reductions were small. Therefore, it appears that, as was expected, the flora recovered from the steaks likely did not differ greatly from the flora present on steaks at the times of their collection.

AER, PSY, LAB and PSE were recovered at numbers  $> 2$  log cfu/100 cm<sup>2</sup> from majorities of steak that ranged from 97 to 75% but BRO were recovered at that level from only 49% of the steaks (Table 1). AER, PSY, LAB and PSE were recovered from  $\geq 50\%$  of steaks at numbers  $\geq 3$  log cfu/100 cm<sup>2</sup>. Only AER and PSY were recovered at numbers  $\geq 7$  log cfu/100 cm<sup>2</sup>, from only 1% of the steaks. COL and ECO were recovered at numbers  $\geq 0$  log cfu/100 cm<sup>2</sup> from 44 and 8% of the steaks respectively. COL were recovered at numbers  $\geq 2$  log cfu/100 cm<sup>2</sup> from only 6% of the steaks, while ECO were not recovered at that level from any steak.

Table 1. The percent frequencies (%) at which total aerobic counts (AER), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), *Brochothrix thermosphacta* (BRO), coliforms (COL) and *Escherichia coli* (ECO) were not recovered (NR) or were recovered, at intervals of 1 log cfu/100 cm<sup>2</sup>, from steaks collected from retail stores across Canada

Bacteria		Interval							
		0 < 1	1 < 2	2 < 3	3 < 4	4 < 5	5 < 6	6 < 7	7 < 8
AER	3	- <sup>a</sup>	-	5	17	34	31	9	1
PSY	12	-	-	12	21	26	20	8	1
LAB	8	-	-	8	27	34	20	3	0
PSE	25	-	-	25	31	15	3	1	0
BRO	51	-	-	26	18	5	1	0	0
COL	56	19	18	6	1	0	0	0	0
ECO	92	7	1	0	0	0	0	0	0

<sup>a</sup> -, interval is below the level of detection.

Correlation coefficients (R) for pairs of the sets of log counts for AER, PSY, LAB, PSE and BRO were mostly  $\geq 0.4 < 0.6$  (Table 2). Only the coefficient for the pair of sets for AER and LAB was  $> 0.6$ . Correlation coefficients for the set of log counts for COL and each of the other sets of log counts were all  $< 0.4$ . Correlation coefficients for the set of log ECO counts and all sets of log counts other than the log COL counts were about 0.1.

Table 2. Correlation matrix (R values) for sets of log numbers (log cfu/100cm<sup>2</sup>) for various groups of bacteria<sup>a</sup> recovered from steaks from Canadian retail stores

	PSY	LAB	PSE	BRO	COL	ECO
AER	0.49	0.65	0.52	0.41	0.31	0.12
PSY	-	0.46	0.51	0.42	0.20	0.08
LAB	-	-	0.47	0.40	0.36	0.12
PSE	-	-	-	0.56	0.39	0.13
BRO	-	-	-	-	0.27	0.07
COL	-	-	-	-	-	0.30

<sup>a</sup>Groups of bacteria are: AER, total aerobic counts; PSY, psychrotrophs; LAB, lactic acid bacteria; PSE, pseudomonads; BRO, *Brochothrix thermosphacta*; COL, coliforms; ECO, *Escherichia coli*.

All beef from which steaks of the types examined in this study are prepared is dispatched from packing plants as vacuum packaged primal cuts. Most of that meat is maintained at temperatures of  $1 \pm 1^\circ\text{C}$  throughout distribution and is more than 10 days old before it is prepared for retail sale (Gill et al., 2002a). Temperatures of the displayed beef are mostly  $4 \pm 2^\circ\text{C}$ , and most retail packs of beef remain in display cases for less than 2 days (Gill et al., 2002b). The limited available data indicate that the numbers of aerobes on beef primal cuts at the time of vacuum packing at large North American plants can now be about 2 log cfu/cm<sup>2</sup> (Hamling, Jenschke, & Calkins, 2008 ; Yang, Badoni, Youssef, & Gill, 2012). The low initial numbers and good control of products temperatures would account for the numbers of bacteria on all steaks being well below the 8 log cfu/cm<sup>2</sup> (i.e. 10 log cfu/100 cm<sup>2</sup>) at which spoilage can be expected to occur (Stanbridge & Davies, 1998). The usually substantial periods of storage of the meat in vacuum pack would account for the relatively large fractions of LAB in most flora. However, the correlation between log numbers of AER and LAB is not strong and the correlations between log numbers of most groups of bacteria are weak. This probably reflects the considerable variation in the time cuts are in vacuum pack before they are used for steak preparation and, possibly, variation in the numbers and types of bacteria added to the meat during steak fabrication. The findings for Canadian steaks are very different than those reported for pork cuts stored aerobically and ground at the time of sale (Andritsos,

Matargas, Mavrou, Stamatiou, & Drosinos, 2012). In that study, as could be expected, pseudomonads (PSE) and *B. thermosphacta* (BRO) were found to be the major components of the often numerous flora, and their log numbers strongly correlated with the log total viable counts (AER).

Table 3. Median values for sets of log numbers (log cfu/100cm<sup>2</sup>) for various groups of bacteria<sup>a</sup> recovered from steaks from Canadian retail stores

Set type	Set	No. of samples	Median values (log cfu/100 cm <sup>2</sup> )						
			AER	PSY	LAB	PSE	BRO	COL	ECO
Steak type <sup>b</sup>	IR	150	4.92 B	5.00 D	4.11 A	3.15 B	2.00 A	-0.50 A	-0.50 A
	CR	148	4.52 *A	4.65 *C	3.93 A	3.11 B	2.30 A	-0.50 B	-0.50 A
	TS	150	4.74 *A	4.28 B	4.14 A	2.87 AB	2.30 A	-0.50 B	-0.50 A
	SL	150	4.76 A	2.98 A	4.14 A	2.78 A	2.00 A	0.49 B	-0.50 A
Store location <sup>c</sup>	M	232	4.85 A	3.95 A	3.84 A	2.81 A	2.30 B	-0.50 A	-0.50 A
	T	193	4.87 B	4.68 B	4.40 B	3.18 B	2.00 A	-0.50 AB	-0.50 AB
	L	94	4.93 AB	4.48 B	4.53 A	3.18 B	2.65 B	-0.50 AB	-0.50 B
	C	79	4.60 *A	4.28 *AB	3.96 * A	3.18 B	2.30 B	0.27 B	-0.50 A
Store group <sup>d</sup>	I	188	4.57 A	3.71 A	3.80 A	2.60 A	2.00 A	-0.50 A	-0.50 A
	II	156	4.30 A	3.75 A	3.69 A	2.78 A	2.26 A	-0.50 A	-0.50 A
	III	113	4.90 A	4.18 A	4.38 A	2.90 A	2.00 A	-0.50 A	-0.50 A
	IV	141	5.11 B	5.03 B	4.68 *B	3.71 *B	2.60 B	0.77 B	-0.50 B

<sup>a</sup>Groups of bacteria are: AER, total aerobic counts; PSY, psychrotrophs; LAB, lactic acid bacteria; PSE, pseudomonads; BRO, *Brochothrix thermosphacta*; COL, coliforms; ECO, *Escherichia coli*.

<sup>b</sup>Steak types are: IR, inside round; CR, cross rib; TS, top sirloin; SL, striploin.

<sup>c</sup>Store locations are: M, Montreal, Quebec; T, Toronto, Ontario; L, London, Ontario; C, Calgary, Alberta.

<sup>d</sup>Store groups: Stores in each of groups I, III, and IV are operated by a single company; stores in group II are variously operated by 10 companies.

\*The data in the set are normally distributed ( $P > 0.05$ ).

Median values for the same type of set and group of bacteria with the same letter are not significantly different ( $P > 0.05$ ).

Level of detection for AER, PSY, LAB, PSE and BRO was 2 log cfu/100 cm<sup>2</sup>; a log value of 1.5 log cfu/100 cm<sup>2</sup> was assigned as the log count for any group of bacteria not recovered from a sample.

Level of detection for COL and ECO was 0 log cfu/100 cm<sup>2</sup>; a log value of -0.5 log cfu/100 cm<sup>2</sup> was assigned as the log count when coliforms or *E. coli* were not recovered from a sample.

In various surveys of North American raw beef products, *E. coli* was recovered from most samples from ground beef and beef roasts and steaks offered for retail sale (Kegode, Doetkott, Khairisa, & Wesley, 2008; Zhao, Blickenstaff, Bodeis-Jones, Gaines, Tong & McDermott, 2012); and from all primal cuts at numbers  $> 1$  cfu/cm<sup>2</sup>, i.e.  $> 2$  log cfu/ 100 cm<sup>2</sup> (Stopforth, Lopes, Schulz, Miksch, & Samadpur, 2006). These findings obviously contrast with the finding in this study of *E. coli* being present on only few steaks in small numbers. A recent study at a large Canadian beef packing plant found that control over microbiological contamination of product had greatly improved in recent years, with the numbers of *E. coli* on primal cuts being about 0 log cfu/100 cm<sup>2</sup> (Yang et al., 2012). The numbers of *E. coli* recovered from steaks in this study are compatible with that finding.

Thus, the findings for *E. coli* suggest that all the large Canadian beef packing plants are now producing primal cuts that carry historically low numbers of *E. coli*; and that few *E. coli* are added to product during the fabrication of steaks from primal cuts.

Stores in Calgary are close to the packing plants that supply their beef while all other cities in which steaks were obtained are distant from their main suppliers of beef. The cities also differ with respect to the proportions of supermarket chain stores and independent stores that sell meat, with the fractions of chain stores being 76%, 60% and 36% in the Calgary, London and Toronto, and Montreal regions, respectively (Osec, 2011). Moreover, the predominant supermarket chains differ between the cities (Canada FAQ, 2012). In surveys of microbiological conditions of ground beef from different stores in North American cities, significant differences were found in the microbiological conditions of the meat from different groups of stores within the same city (Koro, Anandan, & Quinlan, 2010; Zhao et al., 2001). Some differences between sets of microbiological data for steaks from different cities or different groups of stores, and perhaps between steaks of different types could then be expected.

Table 4. Log mean numbers for sets of numbers (cfu/100cm<sup>2</sup>) for various groups of bacteria<sup>a</sup> recovered from steaks from Canadian retail stores and the percent fraction of zero counts in each set

Set type	Set	No. of samples	Log mean number (log cfu/100 cm <sup>2</sup> /zero counts (%))						
			AER	PSY	LAB	PSE	BRO	COL	ECO
Steak Type <sup>b</sup>	IR	150	6.03/3	6.17/4	5.49/6	4.20/14	3.18/39	1.15/72	-0.98/95
	CR	148	5.79/1	5.84/1	4.77/4	4.11/14	3.58/37	1.85/52	-0.09/91
	TS	150	5.85/1	5.66/9	4.96/8	4.29/22	3.79/37	1.21/58	-0.01/89
	SL	150	5.36/5	4.75/24	5.12/3	4.27/20	3.58/39	2.47/46	-0.29/92
Store Location <sup>c</sup>	M	232	5.68/3	5.60/12	4.93/9	3.88/24	3.71/35	1.70/62	-0.74/94
	T	193	5.85/1	5.72/9	5.14/2	4.11/14	2.90/44	2.16/54	0.04/90
	L	94	6.07/8	6.18/9	5.57/5	4.45/17	3.90/38	1.56/54	-0.05/85
	C	79	5.56/3	5.68/8	5.09/3	4.85/14	3.38/35	1.85/47	-0.72/95
Store Group <sup>d</sup>	I	198	5.74/4	5.82/13	4.93/8	3.75/27	3.40/50	1.66/63	-0.29/92
	II	156	5.75/5	5.67/8	5.14/8	4.56/20	3.24/42	1.21/65	-0.94/95
	III	113	5.82/1	5.82/11	5.17/1	3.95/18	3.47/34	1.73/52	0.01/91
	IV	141	5.96/1	5.84/6	5.42/1	4.51/4	3.88/24	2.46/38	0.02/87

<sup>a</sup>Groups of bacteria are: AER, total aerobic counts; PSY, psychrotrophs; LAB, lactic acid bacteria; PSE, pseudomonads; BRO, *Brochothrix thermosphacta*; COL, coliforms; ECO, *Escherichia coli*.

<sup>b</sup>Steak types are: IR, inside round; CR, cross rib; TS, top sirloin; SL, striploin.

<sup>c</sup>Store locations are: M, Montreal, Quebec; T, Toronto, Ontario; L, London, Ontario; C, Calgary, Alberta.

<sup>d</sup>Store groups: Stores in each of groups I, III, and IV are operated by a single company; stores in group II are variously operated by 10 companies.

Level of detection for AER, PSY, LAB, PSE and BRO was 2 log cfu/100 cm<sup>2</sup>.

Level of detection for COL and ECO was 0 log cfu/100 cm<sup>2</sup>.

When the log numbers for each group of bacteria were arranged by steak type, store location and store group, only 7 of the 36 sets of log numbers for AER, PSY and LAB were normally distributed ( $P > 0.05$ ). Of the other 48 sets of log numbers only one set, of the numbers of PSE recovered from steaks from store group IV, were normally distributed ( $P > 0.05$ ). There were significant differences ( $P < 0.05$ ) between median values among sets of log counts of the same type from different types of steak. However, none of the steak types consistently gave

sets of log counts with median values that were significantly higher or lower ( $P < 0.05$ ) than the corresponding median values for the log counts from any other type of steak. Similarly, there was no consistent pattern of differences between median values for corresponding sets of log counts for steaks from stores in different cities, but median values for sets of log counts for steaks from store group IV were all significantly higher ( $P < 0.05$ ) than the corresponding median values for sets of log counts for steaks from other groups or stores. The significant differences ( $P < 0.05$ ) in median values for sets of log counts of the same type for different types of steak, store locations or store groups indicates real differences between corresponding sets. However, within groups of counts of the same type for steak type, store location or store group, log mean values mostly differed by  $< 0.5$  log unit (Table 4). Correlation coefficients for log mean values and numbers of samples from which colonies were not recovered were  $< -0.1$  and  $-0.2$  for sets of counts of AER and LAB, respectively. For all other types of count, the corresponding correlation coefficients were  $\geq 0.6$ .

Differences in bacterial counts of  $< 0.5$  log units are microbiologically inconsequential and, for most sets, correlation between counts and numbers of samples that yielded no colonies were good. Thus, there is little indication of consistent differences of possible practical importance between corresponding sets. Therefore, the findings indicate that the microbiological condition of Canadian steaks on retail sale in Canada is broadly similar for all types of steaks in retail stores of all groups across Canada. This somewhat unexpected finding probably reflects the continuing consolidation of Canadian beef packing and distribution systems; and the widespread adoption in recent years of effective measures for controlling hazardous microbiological contamination of raw meats.

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