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Microbiological Condition of Retail Beef Steaks: A Canadian Survey

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Abstract

The second national baseline microbiological survey of beef steaks offered for retail in Canada was conducted in 2015. A total of 621 steaks of four types (cross rib, CR; inside round, IR; striploin, SL; top sirloin, TS) collected from 135 retail stores in five cities across Canada were tested. Swab samples each from swabbing the entire upper surface of each steak were processed for enumeration of seven groups of indicator organisms: total aerobes (AER), psychrotrophs (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), *Brochothrix thermosphacta* (BRO), coliforms (COL) and *Escherichia coli* (ECO). The overall mean values (log CFU/100 cm^2) were 5.17 ±1.29, 4.92 ±1.36, 4.79 ±1.42, 3.26 ±1.49, 2.34 ±1.88, and 0.80 ±1.05 for AER, PSY, LAB, PSE, BRO, and COL, respectively. ECO were not recovered from 87.3% of the steaks and when there was recovery, the numbers were mostly ≤ 1 log CFU/100 cm^2. Strong correlation was found between the log numbers of AER and PSY, of AER and LAB, and of PSY and LAB, while the correlation between the log numbers of COL and ECO was weak. The numbers of COL and ECO from different groups of steak types or from different cities were not substantially different. Of the four types of steaks, IR had the lowest median values for AER, PSY, LAB, PSE and BRO, followed by CR. The microbiological condition of retail beef steaks in this survey was on par with that in the previous one, with very low numbers of generic *E. coli* being recovered from very few steaks and the indicators for microbial quality being at numbers much lower than the upper limit for shelf life of beef.

Keywords: beef steaks, retail, spoilage bacteria, *Escherichia coli*, coliforms

1. Introduction

National microbiological surveys of food products are useful for benchmarking the safety and/or microbial quality of food, depending on the types of microorganisms being examined. The data can be used by regulatory bodies for performance standard development, and by individual processors along the food chain to refine their process. On the global level, documented survey data from different countries can be used for best practice identification and may facilitate trade. Survey conducted in regular intervals using comparable techniques can provide long-term trends in product hygiene which can benefit both regulators and processors.

The present baseline study is the second organized as part of Canada's National Beef Quality Audit that included microbiological examination of retail beef steaks, though more national surveys in Canada on consumer acceptability and eating qualities have been conducted (Beef Cattle Research Council, 2017). The first nation-wide microbiological baseline was conducted in 2009 to establish a benchmark for the Canadian meat industry against which subsequent performance of the industry could be compared (Badoni, Rajagopal, Aalhus, Klassen, & Gill, 2012). In that survey, seven groups of bacteria were examined, including food safety indicators coliforms (COL) and *Escherichia coli* (ECO), and spoilage indicators total aerobes (AER), psychrotrophic organisms (PSY), lactic acid bacteria (LAB), pseudomonads (PSE), and *Brochothrix thermosphacta* (BRO). The key findings of the first survey were that *E. coli* was recovered from a very small fraction of steaks and at very low numbers when found, and the numbers of spoilage indicator organisms were much lower than the upper limit for spoilage. The Canadian beef industry is highly consolidated, with 98% of the processing capacity from four large beef plants (Canadian Food Inspection Agency, 2017). The current survey was conducted to determine the trend of the microbiological condition of retail steaks in Canada, with information on the same 7 groups of organisms.

2. Method

2.1 Collection of Steaks

During the period September-November 2015, steaks were collected from 135 retail stores in five cities across Canada (Calgary, Alberta; London, Ontario; Toronto, Ontario; Sherbrooke, Quebec; and St Eustache, Quebec). The types of steaks collected were inside round (IR), cross rib (CR), top sirloin (TS), and striploin (SL). On each occasion that steaks were collected, a portion of the steaks of each type from each store were assigned to microbiological testing, to obtain in total 161, 150, 156 and 154 of CR, IR, SL and TS steaks, respectively. Of the 621 steaks, 548 were from stores representing the majority of the market shares in Canada (Arbulu, 2017). Upon collection, the steaks were frozen at -20 ℃. 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

Bagged packages of steaks, previously stored at -20 ℃, were thawed at 4 ℃ overnight. The upper surface of each steak was sampled by swabbing with a sterile gauze swab (AMD-Ritmed; 5 cm by 5 cm) which had been moistened with 0.1% (w/v) peptone water supplemented with 15% glycerol. The total area swabbed of each steak was approximately 100 cm^2. The swabs were then placed in separate plastic stomacher bags. The stomacher bags were folded, put into Ziploc bags, sealed and stored at -80 ℃ until they were processed within 40 days of collection.

2.3 Enumeration of Bacteria

The bags containing the swabs were removed from the Ziploc bags. After thawing, 10 mL of 0.1% peptone water was added to the swab in each bag and pummeled in a stomacher for 2 minutes at 200 rpm. The resulting stomacher fluid was used to prepare ten-fold dilutions up to 10^{-5} in 0.1% peptone water. Subsequently, 0.1 mL of the undiluted fluid and each dilution were spread on two sets of tryptone soya agar (TSA; Oxoid, Mississauga, ON, Canada) as well as on one set each of de Man, Rugosa, Sharpe agar (MRS; Oxoid), cephaloridine fucidin cetrimide agar (CFC; Oxoid) and streptomycin thallous acetate acitidione agar (STAA; Oxoid). One set of the TSA plates, as well as the CFC and STAA plates, were incubated aerobically at 25 ℃ for 48 h. The second set of TSA plates were incubated aerobically at 4 ℃ for 10 days. The MRS plates were incubated anaerobically at 25 ℃ for 72 h. From the range of dilutions used for each type of agar plate, colonies were counted from the plate bearing between 20 and 200 colonies. The numbers of AER, PSY, LAB, PSE and BRO recovered from each swab sample were estimated from colony counts on TSA incubated at 25 ℃, TSA incubated at 4 ℃, MRS, CFC and STAA, respectively. All remaining undiluted stomacher fluid was each filtered through a hydrophobic grid membrane filter (HGMF; Neogen, Lansing, MI). These filters were placed onto plates of lactose monensin glucuronate agar (LMG; Oxoid) and incubated aerobically at 35 ℃ for 24 h. The number of squares containing blue colonies on each filter was enumerated. These counts were converted to MPN for the numbers COL using the formula MPN = N ln (N/N-X), where N is the total number of squares on a filter and X is the count of squares containing blue colonies. Afterward, the filters on the LMG plates were transferred to plates of buffered 4-methlyumbelliferyl-β-D-glucuronide agar (BMA; Oxoid) and incubated aerobically at 35 ℃ for 3 h. Following incubation, the filters were examined under long-wavelength ultraviolet light. The number of squares containing fluorescent blue-white colonies was enumerated. These counts were converted to MPN for the numbers of ECO recovered from each swab sample using above formula, where X equals the count of squares containing fluorescent blue-white colonies. The detection limit was 1 CFU/100 cm^2 for COL and ECO and 2 log CFU/100 cm^2 for the other five groups of bacteria.

2.4 Data Analysis

All bacteria counts were converted to log values with units of log CFU/100 cm^2, and the log values of each bacteria type were grouped into sets according to steak type (CR, IR, SL and TS) and store location (C, L, S, SE and T). The normal distribution of each of those sets was tested using a Shapiro-Wilk test (SAS, version 9.3). For groups of sets in which most of the sets were found to be not normally distributed, median values were separated by a Dunn's test using the NPAR1WAY procedure in SAS (SAS Institute, Cary, NC). A significance level of 0.05 was used for all statistical analysis.

3. Results

A total of 621 steaks from 135 retail stores across Canada were sampled for microbiological analysis. The median log numbers (log CFU/100 cm^2) of AER, PSY, LAB, PSE, BRO, and COL were 5.30, 5.03, 4.87, 3.45, 2.78, and 0.78, respectively. The corresponding mean log values (/100 cm^2) were 5.17 ±1.29, 4.92 ±1.36, 4.79 ±1.42, 3.26 ±1.49, 2.34 ±1.88, and 0.80 ±1.05. The fractions of steaks from which AER, PSY, LAB, PSE,

BRO and COL were not recovered were 1.1, 1.6, 2.1, 9.0, 26.4 and 24.5%, respectively (Table 1).

Table 1. The percent frequencies (%) at which bacteria[a] were not recovered (NR) or recovered at intervals of 1 log CFU /100 cm^2 from steaks collected from retail stores across Canada

Bacteria	Percent frequencies (%)									
	NR	0≤1	1≤2	2≤3	3≤4	4≤5	5≤6	6≤7	7≤8	8≤9
AER	1.1	-[b]	-	4.5	9.2	25.0	35.1	20.1	4.5	0.5
PSY	1.6	-	-	6.0	12.7	28.7	31.1	15.9	3.7	0.3
LAB	2.1	-	-	6.8	15.3	30.9	27.2	14.2	3.4	0.2
PSE	9.0	-	-	25.4	32.2	27.1	6.1	0.0	0.0	0.0
BRO	26.4	-	-	30.9	23.3	14.3	5.0	0.0	0.0	0.0
COL	24.5	35.1	27.1	10.6	2.7	0.0	0.0	0.0	0.0	0.0
ECO	87.3	11.8	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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

[b]-, Interval is below the detection limit of 2 log CFU /100 cm^2.

The criteria used for intervals are equal to or greater than and less than.

For the first three groups of organisms, most samples yielded numbers at 4-6 log CFU/100 cm^2. The numbers of PSE and BRO recovered from >50% of the steaks were ≤4 log CFU/100 cm^2. Over 90% of the steaks had COL at numbers below 2 log CFU/100 cm^2. ECO were not recovered from 87.3% of the steaks and when there was recovery, the numbers were mostly <1 log CFU/100 cm^2. Strong correlation was found between the log numbers of AER and PSY, of AER and LAB, and of PSY and LAB (Table 2). The correlation between the log numbers of COL and ECO was weak.

Table 2. Correlation matrix (R-values) for sets of log numbers (log CFU/100 cm^2) for various groups[a] of bacteria recovered from steaks from Canadian retail stores

	PSY	LAB	PSE	BRO	COL	ECO
AER	0.86	0.78	0.42	0.23	0.25	0.04
PSY	-	0.85	0.40	0.24	0.24	0.04
LAB	-	-	0.31	0.18	0.21	0.03
PSE	-	-	-	0.45	0.19	0.06
BRO	-	-	-	-	0.14	0.05
COL	-	-	-	-	-	0.08

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

Of the 63 data sets, 5 were normally distributed (Table 3). As such, median values, instead of mean values, were used to separate the groups. Of the four types of steaks, IR in general had the lowest median values for AER, PSY, LAB, PSE and BRO, followed by CR. The maximum difference, 0.9 log CFU/cm^2, was noted for LAB counts from IR and SL (Table 3). IR had fewer ($p<0.05$) COL than TS, though the difference was small, 0.35 log unit. The median values for ECO from different types of steaks were not significantly different ($p>0.05$). There was no significant difference ($p>0.05$) between median values for BRO, COL and ECO from different cities, with the exception that steaks from Toronto had a lower number of BRO than steaks from other cities. Steaks from London had higher ($p<0.05$) numbers of AER, PSY, and LAB than steaks from three other cities.

Table 3. Median values for sets of log numbers for various groups of bacteria[a] recovered from steaks from Canadian retail stores

Samples			Median values (log CFU/100 cm^2)						
Set type	Set	No.	AER	PSY	LAB	PSE	BRO	COL	ECO
Steak type[b]	CR	161	5.25BC	4.90*A	4.76A	3.36AB	2.48AB	0.70AB	-0.50A
	IR	150	4.98C	4.64A	4.45A	3.35A	2.48A	0.60B	-0.50A
	SL	156	5.62A	5.45B	5.38B	3.48AB	3.00B	0.87AB	-0.50A
	TS	154	5.31AB	5.23*B	5.01B	3.63B	3.11B	0.95A	-0.50A
Store location	C	71	4.91A	4.79A	4.60A	3.34AB	2.85A	0.90A	-0.50A
	L	89	5.85*B	5.45*C	5.28*C	4.09A	2.78A	1.11A	-0.50A
	S	141	5.29AC	5.01AB	4.76AB	3.52AB	3.04A	0.60A	-0.50A
	SE	124	5.58BC	5.18ABC	4.93ABC	3.84A	3.10A	0.95A	-0.50A
	T	196	5.03A	4.76AB	4.90AB	3.22B	2.48B	0.60A	-0.50A

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

[b]Steak types are: CR, cross rib; IR, inside round; SL, striploin; TS, top sirloin.

[c]Store locations are: C, Calgary, Alberta; L, London, Ontario; S, Sherbrooke, Quebec; SE, St. Eustache, Quebec; T, Toronto, Ontario.

*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 ($p > 0.05$).

4. Discussion

E. coli has long been regarded as an indicator organism for assessing meat as it sets upper limit for numbers of pathogenic *E. coli* which are of importance to human health (Brown et al., 2000). In this survey, *E. coli* was not recovered from 87.3% of the steaks and when there was recovery, the numbers were mostly <1 log CFU/100 cm^2. These findings are consistent with recent reports of drastically improved microbiological condition of carcasses produced in federally inspected Canadian beef packing plants (Liu, Youssef, & Yang, 2016; Yang, Badoni, Youssef, & Gill, 2012; Yang, Tran, Youssef, & Gill, 2015). In these studies, *E. coli* was not detectable on 96% of chilled carcasses entering the fabrication facilities, and on 56 to 93% of beef primal cuts at different production stages in different facilities. The lack of correlation between counts of COL and ECO indicates that most coliforms found on steaks were likely of environmental origin. Despite that the numbers of ECO and COL were both very small when there was recovery in both surveys, the fractions of steaks from which no ECO or COL were recovered were lower in this survey (Badoni et al., 2012). This may reflect the difference in sampling method used in the two surveys rather than the difference in the microbiological condition of steaks. Freezing of steaks and swab samples were unavoidable due to the large number of steaks sampled and the widely separated locations from which the steaks were collected in the surveys. In the previous survey, 25 steaks were sampled before and without freezing the swabs, and after freezing and with freezing swabs (Badoni et al., 2012). Although the freezing and thawing of steaks and swabs did not result in significant reduction in numbers of AER, PSY, LAB, PSE or BRO, it did reduce the number of COL by 0.44 log unit. In addition, the freezing reduced the total number of ECO and the number of steaks from which ECO was recovered from 0.90 log CFU/2,500 cm^2 and 7 to 0.60 log CFU/2,500 cm^2 and 3, respectively. To minimize the impact of freezing on small numbers of COL and ECO, peptone water that was used to moisten gauze swabs was supplemented with the cryoprotectant agent glycerol in this survey (Achá, Kühn, Mbazima, Colque-Navarro, & Möllby, 2005; Hollander & Nell, 1954). The lack of glycerol in the previous survey may have caused underestimation of both groups of organisms.

In Canada, vacuum packaged primal cuts are the primary form for distribution of beef from meat plants to warehouses and to retail stores at which they are prepared into steaks (Gill, Jones, LeBlanc, et al., 2002). The temperature at which primals are stored and transported is between 1 to 2 ℃ (Gill, Jones, Rahn, et al., 2002). The initially diverse microbial community on chilled vacuum packaged beef will be invariably replaced by a few species of LAB, which have growth advantage over other members of the meat microbiota at chiller temperatures under anaerobic condition provided by vacuum packaging (Holzapfel, 1998; Stanbridge & Davies, 1998; Youssef, Gill, Tran, & Yang, 2014; Youssef, Gill, & Yang, 2014). Spoilage usually occurs only some time after the microbiota has reached maximum numbers as LAB are generally of low spoilage potential, due to the

by-products of their metabolism being not highly offensive (Blixt & Borch, 2002; Castellano, González, Carduza, & Vignolo, 2010). The strong correlations between AER and PSY, AER and LAB, and PSY and LAB suggest that the primal cuts had been stored long enough to allow growth of LAB before they were fabricated into steaks. The number of total aerobes on all steaks was well below 8 log CFU/cm^2 at which spoilage can be expected to occur (Stanbridge & Davies, 1998).

Information on the microbiological condition of retail steaks from different countries is largely lacking. A few surveys have been conducted for beef primal cuts, the precursor material for steaks, at production. Ten types of primal cuts (n=1,022) produced at two processing plants in the USA were sampled for a period of 6 months (Stopforth, Lopes, Shultz, Miksch, & Samadpour, 2006). The numbers of aerobes, coliforms and *E. coli* ranged from 4.0 to 6.2, 1.1 to 1.8, and 0.8 to 1.0 log CFU/g, respectively. In Switzerland, beef primal cuts (n=200) at a European Union approved establishment had aerobes at 2.4 log CFU/cm^2 (Zweifel, Zychowska, & Stephan, 2005). An Australian survey of beef primal cuts fabricated at 29 establishments reported levels of aerobes at 1.3 and 1.5 log CFU/cm^2 and prevalence of *E. coli* at 11 and 25% for striploins (n=572) and outsides (n=572), respectively (Phillips, Bridger, Jenson, & Sumner, 2012). The prevalence of coliforms was about 20% higher than that of *E. coli* for both types of cuts. Two Canadian studies examined small numbers of beef primal cuts at production (n≤50) reported aerobes and *E. coli* being at ≤ 0.83 log CFU/cm^2, and ≤ 0 log CFU/100 cm^2, respectively (Yang et al., 2012; Yang et al., 2015).

In conclusion, the findings of this study show that the microbial safety of steaks offered for retail in Canada was similar across the country and the prevalence and number of coliforms and *E. coli* remained low. Even though the numbers of spoilage organisms varied with steak type or region, the maximum number observed was much lower than the upper limit at which spoilage is expected to occur.

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