Microbiological Evaluation of Imported Produce and Domestically Produced Sprouts Purchased at Retail Outlets Across Canada

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Background:
Over the past decade, produce has increasingly been linked to recalls and, more seriously, outbreaks of foodborne disease.[1,2,3] Improved surveillance and detection capabilities play a role in this, but do not solely account for the increase. Other contributing factors involve changing consumer preferences, increased consumption of fresh, minimally processed produce, globalization of our food supply and consumer expectations of year-round produce availability.[3,4] Unlike most foods, produce is consumed fresh, with no heat intervention prior to consumption. As such, the presence of foodborne pathogens, such as *Escherichia coli* O157:H7 and *Salmonella*, are a serious concern to the public.

Project Goal:
Examine the microbiological quality of domestically produced sprouts and imported produce commodities available at retail outlets in Canada.

Materials and Methods:
Samples of produce with expiry dates beyond the expected testing dates were purchased from five Canadian cities (Halifax, Regina, Toronto, Winnipeg and Vancouver) and shipped in pre-cooled coolers by overnight courier. Overall, 48 leafy green (salads and spinach), 58 herb (various) and 44 sprout-based products were analyzed for total microbial and coliform counts, enterococci, *E. coli* O157:H7 and *Salmonella* using standard methods. Briefly, 25 g of product (for herbs, composite samples from two or three packages were used) was added to 225 ml of buffered peptone water and homogenized by stomaching (260 rpm for 150 sec). From this, enterococci, generic *E. coli* and *Salmonella* were screened using previously published methods.[5] In addition, coliform and total microbial counts were estimated by serial dilution plating on violet red bile agar and tryptic soya agar, respectively. A separate 25 g sample was mixed with 225 ml of modified tryptic soy broth with novobiocin (mTSB-n) and used to screen for *E. coli* O157:H7 according to MFLP-80 using the Reveal 20 hr detection system.[6]

Results:
Escherichia coli O157:H7 and *Salmonella* were not detected in any samples. Overall, coliforms and enterococci were detected in 45 and 66% of samples, respectively (Table 1). When analysed by produce type, coliform detection ranged from 36% in herbs to 60% in leafy greens. Levels of total coliform contamination did not correlate with generic *E. coli* detection, with 3.3% (n=5) samples testing positive. Of these, four were imported herbs originating from Columbia (bay leaves, mint), Mexico (oregano) and an unknown origin (cilantro); the remaining positive was associated with a sprout sample. Significant variation was observed in enterococci contamination; only 13% of leafy greens were contaminated whilst 93% of sprout products were positive for enterococci. From these samples, 34% of recovered enterococci were identified as *Enterococcus faecium*, 15% as *Enterococcus faecalis* and the remainder *Enterococcus* spp. Total microbial loads were highest in sprouts, with 78% of the samples being too numerous to count (*i.e.* $>2x10^8$ CFU/g) whereas no spinach samples and ≤13% of herb and leafy greens exceeded this limit. The lowest microbial loads were observed on spinach where 60% of samples possessed microbial counts $<10^7$ CFU/g.

Conclusions:
Overall, levels of microbial loads were generally acceptable in herbs, salads and spinach, with counts frequently at or below $\leq 10^7$ CFU/g; exceptions to this, however, could be observed for most brands examined. Due to the nature of sprout production, high microbial loads were both expected and observed. Over 78% of sprouts had levels of microorganisms too numerous to count. In addition, one sample was contaminated with generic *E. coli* and nearly all samples had enterococci detected, including *E. faecalis* and *E. faecium*. Although high levels of microorganisms in sprouts were expected, the extensive detection of enterococci and potential significance are not well documented in scientific literature. *Enterococcus* spp. are inherently resistant to some antibiotics, and are known for their ability to acquire and subsequently disseminate antibiotic resistance genes to other bacteria. As such, the observed high levels

of contamination in sprouts (93%), as well as herbs (79%) and spinach (50%), warrants further investigation, and may present an issue in the dissemination of antimicrobial resistance through foodborne means. Regarding herbs, four generic *E. coli* positive samples (7%) were observed and is similar to other reports in Canada,[7,8] but may suggest issues with in-field or processing-based fecal contamination. Lastly, while the lack of pathogen detection is reassuring, it is important to interpret these results with caution. Low pathogen concentrations and infrequent contamination make detection of pathogens in produce an ongoing challenge for food industry and public health officials.

Table 1. Summary of microbiological data evaluating the quality of retail produce products (n=150) in Canada.

Category	**% Samples TNTC[a]**	**% <10^7 CFU/g**	**Coliforms % Positive**	**% *E. coli* Positive**	**% Enterococci Positive**	**% *Salmonella* Positive**	**% *E. coli* O157:H7 Positive**
Herbs	13	54	36	7	79	0	0
Leafy Greens	4	15	60	0	13	0	0
Spinach	0	60	50	0	50	0	0
Sprouts	78	0	50	2	93	0	0

[a]-Too numerous to count (>2x10^8 CFU/g).

References:

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