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Microbial Safety and Quality of Fresh Herbs from Los Angeles, Orange County, and Seattle Farmers’ Markets

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BACKGROUND: Farmers’ markets have been growing in popularity in the United States, but the microbial quality and safety of the food sold at these markets is currently unknown. The purpose of this study was to assess the microbial safety and quality of fresh basil, parsley, and cilantro sold at farmers’ markets in the Los Angeles, Orange County, and greater Seattle areas.

RESULTS: A total of 133 samples (52 basil, 41 cilantro, and 40 parsley) were collected from 13 different farmers’ markets and tested for Salmonella and generic Escherichia coli. One sample (parsley) was confirmed positive for Salmonella and 24.1% of the samples were positive for generic E. coli, with a range of 0.70-3.15 log CFU/g. Among the herbs tested, basil showed the highest percentage of samples with generic E. coli (26.9%), followed by cilantro (24.4%), and then parsley (20.0%). For 12% of samples, the levels of generic E. coli exceeded guidelines established by the Public Health Laboratory Service for microbiological quality of ready-to-eat foods.

CONCLUSION: Overall, this study indicates the presence of Salmonella and generic E. coli in fresh herbs sold at farmers’ markets; however, additional studies are needed to determine the sources and extent of contamination.
Introduction

Farmers’ markets have become an important source of produce for many consumers in the United States.¹ These markets are generally held in the summer months and allow for consumers to purchase locally grown fruits and vegetables directly from the producer or farmer. According to the United States Department of Agriculture (USDA) Economic Research Service, farmers’ markets have been increasing since 2009 near urban areas, particularly along the East and West coasts.¹ In August 2013 there were over 8,000 farmers’ markets listed in the USDA’s National Farmers’ Market directory, a 3.6% increase from 2012.² While farmers’ markets can become certified to ensure that each farmer is actually growing the commodities being sold, food safety is not addressed as part of the certification process. Some potential areas of concern with regard to food safety at these markets are the storage conditions of the produce throughout the day, the farming practices, and the farmer’s pre- and post- harvest handling techniques.

Certain herbs, such as parsley, basil, and cilantro have been implicated in many food outbreaks over the past two decades.³⁻⁶ In 1999, there were 41 restaurant-associated illnesses and 35 sporadic cases involving Salmonella enterica serotype Thompson in fresh, room temperature cilantro that was suspected to originate from Mexico.⁵ In 2006, about 200 teachers and students in Denmark were infected with Salmonella and enterotoxigenic Escherichia coli (ETEC) from the consumption of a pasta salad with pesto.³ A retrospective cohort study determined that fresh basil used in the preparation of the pesto was most likely the source of illness due to contamination with ETEC and S. enterica serotype Anatum.³ Furthermore, in 2007, Salmonella was found in 18 out of 3,760 ready-to-eat fresh herb samples collected from different retail stores and tested by 30 laboratories in the UK.⁶ Eight of the 18 contaminated samples consisted of fresh basil obtained from a single grower in Israel.⁴,⁶ Increasing concern
over foodborne outbreaks in fresh produce has also led to testing for generic E. coli as an
indicator of fecal contamination and potential pathogen presence. Fecal contamination in fresh
herbs and other types of fresh produce is problematic, as these items are commonly consumed
raw, with no intervention step to inactivate potential pathogens.

There is currently limited information on food safety at farmers’ markets and some
studies conducted thus far have reported concerning results. For example, a study in
Pennsylvania, USA, reported the presence of Salmonella and Campylobacter in raw chicken
sold at farmers’ markets at detection frequencies of 28% and 90%, respectively. In
comparison, raw chicken samples from conventional supermarkets showed detection
frequencies of 8-20% for Salmonella and 28-52% for Campylobacter. Teng et al. investigated
the food handling practices of cheese vendors at farmers’ markets located in Ontario, Canada. It
was found that 47% of the vendors had problems with refrigeration and a majority of the
vendors did not wash their hands prior to handling the cheese. Furthermore, a study surveying
supermarkets and farmers’ markets in Ontario, Canada, reported the presence of thermotolerant
Campylobacter spp. in a number of fresh produce items sold at the farmers’ markets, including
parsley, and no detections in fresh produce items sold at the supermarkets. Despite the
potential for foodborne illness from fresh herbs and other fresh produce sold at farmers’ markets
in the United States, there is currently a lack of knowledge regarding the microbial safety and
quality of these items.

Due to the prevalence of farmers’ markets along the U.S. West Coast and the association
of fresh herbs with outbreaks of foodborne illness, the overall objective of this study was to
conduct a survey of the microbial safety and quality of fresh basil, parsley, and cilantro sold at
farmers’ markets in the Los Angeles, Orange County and greater Seattle areas. Specifically, the fresh herbs were tested for *Salmonella*, *E. coli*, and total coliforms.

**Materials and Methods**

**Media and bacterial strains.** Unless otherwise stated, all media were obtained from Hardy Diagnostics (Santa Maria, CA, USA). For the Los Angeles County, CA, and Orange County, CA, portion of the study, *S. enterica* serotype Abaetetuba ATCC 35640 and generic *E. coli* ATCC 51813 were used as positive control strains. For the greater Seattle area, WA, portion of the study an environmental *S. enterica* isolate, *S. enterica* LT2 (courtesy of the laboratory of Dr. Sobsey at the University of North Carolina, Chapel Hill, USA), and generic *E. coli* ATCC 11303 were used as positive controls.

**Sample collection.** Thirteen different farmers’ markets were visited in the Los Angeles, Orange County and greater Seattle areas (Table 1), and a total of 133 samples of basil, parsley, and cilantro were aseptically collected from the display tables using plastic sampling bags. Farmers’ markets were selected on the basis of geographical proximity to the research laboratories to allow for samples to be analyzed on the same day that they were collected. The number of samples collected was determined based on budgetary constraints as well as the availability of samples at farmers’ markets. Sample collection took place between 8 and 10 am in Orange and Los Angeles Counties and between 10:30 am and 2:30 pm in the greater Seattle area. Samples were collected between July and October 2013 and each farmers’ market was visited between 1 and 3 times depending on sample availability (Table 1). Each sample unit collected was equivalent to at least 454 grams (1 pound). Following sample collection, herbs were transported on ice in a cooler to the laboratory at Chapman University (Orange, CA, USA).
Salmonella testing of fresh herbs. Samples were prepared for Salmonella testing according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM). Twenty-five grams of each herb sample were aseptically weighed into 24 oz. Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA). Lactose broth (225 ml) was added and mixed by vigorously swirling the bag 25 times clockwise and then counterclockwise. The samples were incubated for 24 ± 2 h at 35 ± 2 °C. Then, 0.1 ml of each sample was transferred to a test tube containing 10 ml of Rappaport Vassiliadis (RV) broth and 1.0 ml of each sample was transferred to a test tube containing 10 ml tetrathionate (TT) broth. The inoculated RV and TT tubes were incubated for 24 ± 2 h at 42 ± 1 °C. Next, a sterile inoculating loop was used to streak samples from the RV and TT tubes onto individual plates of xylose lysine deoxycholate (XLD), bismuth sulfite (BS), and hektoen enteric (HE) agar for isolation, resulting in six plates per sample. The plates were inverted and incubated for 24 ± 2 h at 35 ± 2 °C.

After incubation, typical Salmonella colonies were selected from XLD, BS, and HE agar plates and confirmed, as described below. Typical colonies on XLD agar appear pink with or without black centers. Typical colonies on BS agar appear brown, gray or black with an occasional metallic sheen, and typical colonies on HE agar appear blue to blue-green with or without black centers. In the absence of typical colonies on HE and XLD after 24 ± 2 h incubation, one atypical Salmonella colony was selected per sample for confirmation testing. If typical or suspicious colonies were not present on BS agar after 24 ± 2 h, the plates were re-incubated for an additional 24 ± 2 h. If typical or suspicious colonies were not present after 48 ± 2 h incubation, then one atypical colony was selected per sample for confirmation testing.
The colonies were transferred to triple sugar iron (TSI) agar and lysine iron agar (LIA) slants and incubated at 35 ± 2°C for 24 ± 2 h. Samples showing typical TSI/LIA slants were then confirmed with API 20E test kits (bioMérieux, Durham, NC, USA).

**Generic Escherichia coli and total coliform testing of fresh herbs.** Herbs were tested for generic *E. coli* and total coliforms according to the Association of Official Analytical Chemists Method 991.14.13. Samples (50 g each) were aseptically weighed into Whirl-Pak bags and 450 ml of Butterfield’s phosphate buffer was added. Samples were then mixed at 230 rpm for 30 s in a Stomacher 400 Circulator (Seward, Norfolk, UK). Each sample was plated in duplicate by pipetting 1 ml of the sample homogenate onto an *E. coli*/Coliform Petrifilm plate (3M, Saint Paul, MN, USA). The Petrifilm plates were incubated at 35 ± 2°C for 48 ± 2 h in stacks of 20 or less and then enumerated for *E. coli* and total coliforms. The average *E. coli* and total coliform counts were determined for each sample. In cases where the number of colonies was outside of the countable range of 15-150, an estimated plate count was obtained.

**Statistical analyses.** The levels of *E. coli* and total coliforms were statistically compared across herb types using a one-way analysis of variance (ANOVA), with a predetermined significance level of *p* < 0.05. The percentages of samples that were positive for *E. coli* and total coliforms were compared across herb types with a Pearson’s chi-square test, with a pre-determined 2-sided significance value of *p* < 0.05. All statistical analyses were carried out using IBM SPSS Statistics 21 (IBM SPSS Inc., Armonk, NY, USA).

**Results and Discussion**

**Sample collection.** Overall, 133 samples of fresh herbs were collected for testing from 13 different farmers’ markets (Table 1). Samples were collected from 49 different vendors at these markets, with an average of 3 samples collected per vendor. Among the samples collected,
basil represented the highest percentage (39%), followed by parsley (30%), and then cilantro (31%). Figure 1 provides a breakdown of the number of each type of herb collected within the three major geographic sampling regions of Orange County, Los Angeles, and the greater Seattle areas. The greatest number of samples was collected in Orange County, CA (n = 68), followed by the greater Seattle area, WA (n = 41), and Los Angeles County, CA (n = 24).

**Salmonella results.** Of the 133 samples collected, 15 samples had typical or suspicious growth on HE, XLD, and/or BS agar. However, only one sample confirmed positive for *Salmonella* on TSI/LIA and the API 20E test strip. This was a sample of parsley collected from a Los Angeles County farmers’ market (LA1) that showed typical growth on both HE and XLD agars. According to the biochemical reactions, the profile given on the API 20E test strip was 6704752 with 99.9% identification of *Salmonella* spp. The remaining 118 samples either showed no growth or atypical colonies on HE, XLD, and BS agars. These samples were ruled out as negative with the TSI/LIA slants and, when necessary, an API 20E test strip.

The overall prevalence of *Salmonella* in parsley was 2.5%. The prevalence of *Salmonella* in fresh herbs found in this study was similar to percentages reported previously for *Salmonella* in FDA field investigation studies. These studies reported *Salmonella* prevalence rates of 0-2.5% in imported and domestic parsley samples and 1.2-9% in imported and domestic cilantro samples. The FDA studies each collected 84-90 samples of parsley and 85-177 samples of cilantro, compared to 40 parsley samples and 41 cilantro samples collected in the current study. Further testing of these herbs from farmers’ markets will be useful in verifying *Salmonella* prevalence. Although it is not known whether the *Salmonella* detected was present at infectious levels, contamination of fresh herbs with *Salmonella* is concerning considering that these herbs are commonly consumed raw. Salmonellosis symptoms include:
diarrhea, abdominal cramps, and fever about 12 to 72 hours after consumption that lasts about four to seven days. In severe cases, the diarrhea may be so detrimental that the patients must be hospitalized because the infection can spread from the intestines to the bloodstream and other sites in the body. The severe illness generally occurs in the elderly, infants and those with compromised immune systems. Overall, the results of the current study illustrate the possibility of *Salmonella* contamination in fresh herbs sold at farmers’ markets and demonstrate a need for more extensive investigation into this topic.

**Generic *Escherichia coli* and total coliform results.** Among the 133 fresh herb samples tested in this study, 24.1% were positive for generic *E. coli* (Fig. 1) and 84.2% were positive for total coliforms, with a range of 0.70-3.15 and 0.70-4.15 log CFU/g, respectively (Table 2). Interestingly, the parsley sample found to be positive for *Salmonella* was positive for total coliform growth (0.70 log CFU/g) but not for *E. coli*. The average generic *E. coli* count for all positive samples combined was 1.81 log CFU/g and the average total coliform count was 2.45 log CFU/g. There were no significant differences in levels of *E. coli* or total coliforms when compared across the three types of herbs tested, according to a one-way ANOVA, with significance set at $p < 0.05$. A total of 16 samples had average *E. coli* counts considered to be unsatisfactory (≥ 2 log CFU/g) according to guidelines established by the Public Health Laboratory Service for microbiological quality of ready-to-eat foods. The herbs in this category included seven basil samples, five cilantro samples, and four parsley samples. These samples were collected from two farmers’ markets in Orange County, CA (OC1 and OC2), two farmers’ markets in the greater Seattle area (SC1 and KC3), and one farmers’ market in Los Angeles County, CA (LA2). Among the herbs tested, basil showed the highest percentage of samples with growth for generic *E. coli* (26.9%), followed by cilantro (24.4%) and then parsley.
(20.0%). On the other hand, cilantro showed the greatest percentage of samples positive for total coliforms (87.8%), followed by basil (82.7%), and parsley (82.5%). There were no significant differences in the percentage of samples positive for *E. coli* or total coliforms across herb types, according to a Pearson’s chi-square test with significance set at *p* < 0.05. As shown in Fig. 1, Orange County farmers’ markets had the highest percentage of samples with *E. coli* growth, at 26.5%, followed by farmers’ markets in the greater Seattle area (24.4%), and Los Angeles County farmers’ markets (16.7%). The percentages of positive samples were not statistically compared across locations due to differences in sample sizes.

Although generic *E. coli* are generally more useful than total coliforms as indicators of fecal contamination in fresh produce, total coliform levels were also recorded in this study in order to enable comparison with existing research on microbiological quality of fresh herbs. In general, the levels and detection frequencies of generic *E. coli* and total coliforms in the current study were similar to or higher than those found in previous studies examining the microbiological quality of fresh herbs. For example, in a series of two studies, Johnston *et al.*\(^1\)\(^7\)\(^,\)\(^8\) reported average levels of generic *E. coli* to be 0.70-1.31 log CFU/g and total coliform levels to be 1.3-3.4 log CFU/g for commercially grown parsley (n = 141) and cilantro samples (n = 187) collected during multiple steps in the production and packaging process. In comparison, average levels of 1.82 log CFU/g (generic *E. coli*) and 2.36 log CFU/g (total coliforms) were found in the current study for these two herbs combined (excluding basil).

Furthermore, Allen *et al.*\(^1\)\(^9\) tested a variety of herb samples (n = 61), including basil and cilantro, sold at retail stores in five Canadian cities and found that only 6.6% of the samples showed *E. coli* growth and 37.7% of the samples showed growth of total coliforms, compared to 24.1% and 84.2%, respectively, in the current study. However, the average total coliform
counts of 1.3 to 2.6 log CFU/g reported by Allen et al.\textsuperscript{19} were similar to those observed in the current study for all herbs combined (2.45 log CFU/g). Finally, a study by Arthur et al.\textsuperscript{20} also found lower detection frequencies of generic \textit{E. coli} in fresh herbs sold at retail distribution centers and farmers’ markets in Ontario, Canada, with growth in 13.4\% of parsley samples (n = 127) and 4.9\% of cilantro samples (n = 61). Interestingly, the authors reported \textit{E. coli} to be present at higher maximum levels in these herbs as compared to the current study, with up to 4.2 log CFU/g found in parsley and up to 3.9 log CFU/g found in cilantro, compared to up to 3.15 log CFU/g in parsley and up to 2.66 log CFU/g in cilantro found in the current study (Table 2).

Overall, the majority of fresh herb samples tested in the present study were compliant with microbiological criteria established by the Public Health Laboratory Service for microbiological quality of ready-to-eat foods; however, 12\% of samples showed levels of generic \textit{E. coli} determined to be unsatisfactory by these guidelines. Further research is needed to determine the source(s) of contamination and whether contamination is greater at farmers’ markets compared to other retail sources of fresh produce.

\textbf{Conclusions}

With the growing popularity of farmers’ markets, the lack of food safety regulations at these markets, and the association of fresh produce with foodborne illness, it has become increasingly important to monitor the microbiological safety and quality of these items. Overall, a relatively high level of microbiological contamination was found in the herbs collected in this study as compared to previous studies. However, additional studies are needed to verify this trend. While a direct comparison between fresh herbs from farmers’ markets and conventional supermarkets was not carried out in this study, storing herbs at ambient
temperatures in the open environment during warm summer days could impact the microbiological safety and quality of these items. At conventional supermarkets, fresh herbs and other perishable produce items are held under controlled temperature and humidity conditions and they are required to be handled according to the Good Manufacturing Practices. In order to assess the importance of these factors, additional research is needed comparing the microbial quality and safety of herbs held in controlled environments, such as those in a conventional supermarket, to those held at ambient temperatures in outdoor environments, such as at a farmers’ market. Since farmers’ markets are generally held in the summer months, another important area of research will be to monitor microbial changes that occur in fresh herbs and other perishables throughout the day as the temperature increases from the morning to the afternoon. The current study, along with future research in this area, will be important in heightening our understanding of the safety of perishable foods sold at farmers’ markets.

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References


**Figure Captions**

**Figure 1.** Number of samples collected from farmers’ markets categorized by geographic region and by herb type. The number of samples that tested positive for *Escherichia coli* growth is displayed within the total sample number for each category. The greater Seattle area includes data from both King and Snohomish Counties.
### Tables

**Table 1.** Details on farmers’ markets sampled in this study

<table>
<thead>
<tr>
<th>Location</th>
<th>Farmer’s market ID</th>
<th>No. of vendors collected from</th>
<th>No. of times visited</th>
<th>No. of samples collected</th>
<th>Months visited</th>
</tr>
</thead>
<tbody>
<tr>
<td>King County, WA</td>
<td>KC1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>August</td>
</tr>
<tr>
<td>King County, WA</td>
<td>KC2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>August</td>
</tr>
<tr>
<td>King County, WA</td>
<td>KC3</td>
<td>7</td>
<td>3</td>
<td>18</td>
<td>September, October</td>
</tr>
<tr>
<td>King County, WA</td>
<td>KC4</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>August, September</td>
</tr>
<tr>
<td>Snohomish County, WA</td>
<td>SC1</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>August, September</td>
</tr>
<tr>
<td>Los Angeles County, CA</td>
<td>LA1</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>August</td>
</tr>
<tr>
<td>Los Angeles County, CA</td>
<td>LA2</td>
<td>7</td>
<td>1</td>
<td>16</td>
<td>August</td>
</tr>
<tr>
<td>Los Angeles County, CA</td>
<td>LA3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>August</td>
</tr>
<tr>
<td>Orange County, CA</td>
<td>OC1</td>
<td>13</td>
<td>3</td>
<td>53</td>
<td>July, August</td>
</tr>
<tr>
<td>Orange County, CA</td>
<td>OC2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>July, August</td>
</tr>
<tr>
<td>Orange County, CA</td>
<td>OC3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>August</td>
</tr>
<tr>
<td>Orange County, CA</td>
<td>OC4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>August</td>
</tr>
<tr>
<td>Orange County, CA</td>
<td>OC5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>August</td>
</tr>
</tbody>
</table>
Table 2. Generic *E. coli* and total coliform levels in positive samples of basil, parsley and cilantro

<table>
<thead>
<tr>
<th>Herb type</th>
<th>Positive samples (n)</th>
<th>Generic <em>E. coli</em></th>
<th>Total coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average (log CFU/g)</td>
<td>Range (log CFU/g)</td>
</tr>
<tr>
<td>Basil</td>
<td>14</td>
<td>1.79</td>
<td>0.70-2.95</td>
</tr>
<tr>
<td>Cilantro</td>
<td>10</td>
<td>1.71</td>
<td>0.70-2.66</td>
</tr>
<tr>
<td>Parsley</td>
<td>8</td>
<td>1.96</td>
<td>1.00-3.15</td>
</tr>
<tr>
<td>Overall</td>
<td>32</td>
<td>1.81</td>
<td>0.70-3.15</td>
</tr>
</tbody>
</table>
Negative for E. coli growth
Positive for E. coli growth

Greater Seattle Area, WA
Los Angeles County, CA
Orange County, CA