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Microbiological quality of fresh produce obtained from retail stores on the Eastern Shore of Maryland, United States of America

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ABSTRACT

The aim of this study was to investigate the microbiological quality of six types of fresh produce obtained from three retail stores located on the Eastern Shore of Maryland, USA. A total of 414 samples representing basil, cilantro, lettuce, scallion, spinach, and parsley were analyzed for total aerobic bacteria (APC), total coliforms, *Escherichia coli*, and three pathogenic bacteria (*E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*), using standard methods. Presumptive pathogenic isolates were confirmed using BAX Polymerase Chain Reaction. Total aerobic populations varied widely between samples, while 38.41% were positive for total coliforms and only 10.15% for *E. coli*. Median abundance (log CFU/g) of total coliforms and *E. coli* were less than the limit of detection and that of APC ranged from 5.78 to 6.61 over the six produce types. There was a statistically significant difference in prevalence of total coliforms among the retail stores, but not for abundance of APC or prevalence of *E. coli*. *E. coli* O157:H7 and *L. monocytogenes* were detected in one spinach sample each, while one parsley and one cilantro sample were positive for *Salmonella*. There were no statistically significant differences in microbiological quality among produce types. Although the results of this study provided some indices of sanitary and/or spoilage level, no relationship was observed among the total aerobic bacteria, total coliforms, *E. coli*, and the presence of pathogenic bacteria in the samples tested.

1. Introduction

Fresh produce is an important part of a healthy diet and has been reported to be consumed in larger quantities in the United States than ever before (Bowen et al., 2006). The incidence of human pathogens on fresh produce is a serious concern in most industrialized countries. *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* are among the most common pathogens associated with fresh produce illness outbreaks (Lapidot et al., 2006). Rajkowski and Fan (2008) reported findings of leafy greens sold in the markets contaminated with the pathogens. Their study revealed that microbiological quality of fresh produce is a concern for both food safety and product shelf-life. It is estimated that 30% of produce is lost due to microbial spoilage between the time of harvest and consumption. Few studies on the microbiological quality of leafy vegetables have been documented. Pingulkar et al. (2001) examined microbiological quality of salad in India and found a prevalence of 73% for *Listeria* but did not detect the presence of *Salmonella*. Also, they found higher bacterial counts in salads compared to freshly-washed vegetable ingredients. Furthermore, Johnston et al. (2005) reported a mean of 4.5–6.6 log CFU/g for total aerobic bacteria in leafy vegetables. Some studies and reports on fresh produce linked *Listeria* to cases of illnesses and deaths in the 1980s (Mead et al., 1999). The occurrence of these outbreaks led the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to establish "a Zero Tolerance" policy under which ready-to-eat food contaminated with *Listeria-monocytogenes* at a detectable level are deemed adulterated (Harris, 2002; Suslow and Harris, 2000).

Historically, foodborne outbreaks had been associated with the consumption of products with animal origin. However, recently outbreak cases have been increasingly linked to raw and minimally processed fruits and vegetables. Outbreaks associated with *Salmonella* have been traced back to contaminated irrigation water, wild birds, animals, wash water during processing, handling by

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workers, and contact with contaminated surfaces (Beuchat, 1997; Chisholm et al., 2006; Tauxe et al., 1997). The Centers for Disease Control and Prevention (CDC) estimate up to 2500 cases of *Listeriosis* resulting in 500 deaths yearly in the United States (Scallan et al., 2011). Statistics have shown that foodborne outbreaks linked to contaminated leafy greens rose significantly from the 1970s through the 1990s. During this period the number of illnesses due to outbreaks rose from 0.7% to 6.0% of all the foodborne related cases (Scallan et al., 2011; Aparecida et al., 2010; Mead et al., 1999; Buck et al., 2003; Sivapalasingam et al., 2004).

Foodborne illnesses linked to *E. coli* O157:H7 have been estimated at 73,000 in the US each year. One hundred eighty-three outbreaks of foodborne illness associated with E. *coli* O157:H7 have been reported in the US since 1982. Of those outbreaks, 21% were linked to produce sources including lettuce, apple juice, salad, coleslaw, melons, sprouts, and grapes (Rangel et al., 2005; Cooley et al., 2007). Microbiological quality of imported fresh produce obtained from retail stores and fresh produce from local farms which were analyzed for APC and coliform counts, *E. coli*, *E. coli* O157:H7, and *Salmonella* spp. showed a consistency of APCs across commodities regardless of country of origin, ranging from mean $\log_{10}$ CFU/g of 6.1–7.4 with no significant differences observed. These studies reported widely varied concentration of coliforms, ranging from undetectable to too numerous to count (Allen et al., 2013; Wood et al., 2015).

Surveys of prevalence of contamination of fresh produce by indicator and pathogenic bacteria have shown evidence of the presence of these microorganisms on leafy produce. Both conventional and organic fresh produce have been reported to have been contaminated with fecal coliforms and *E. coli*. Since vegetables such as basil and cilantro require less process or treatment before consumption, there is a possibility that the pathogens present in these produce can be easily transferred to the consumers (Pan et al., 2014; Maistro et al., 2012). Though microbiological quality of some of fresh produce generally consumed raw in some parts of the world have been reported, information is limited on microbial quality of produce in Maryland, USA. Therefore, the overall goal of this study was to determine the microbiological quality of fresh produce obtained from retail stores on the Eastern Shore of Maryland. The specific objectives were: 1) To evaluate the prevalence of total aerobic bacteria, total coliforms, and *E. coli* on fresh produce collected from food stores on the Eastern Shore of Maryland, and 2) To detect the presence of three pathogenic bacteria: *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* on fresh produce.

2. Materials and methods

2.1. Leafy greens samples

Six fresh produce samples were collected bi-weekly for a period of one year from three different retail chain stores (A, B, C) on the Eastern Shore of Maryland, United States of America, but they were in different locations. The six types of produce selected are common and popular in this area. Samples were collected only once in December due to inclement weather. At each sampling time, samples were obtained from the same three stores. All samples were domestic origin and sold under the same brand. These samples were: basil, lettuce, cilantro, scallion, spinach, and parsley. Basil, scallion and spinach were packed in breathable film. Cilantro and parsley were in small bunches tied up with rubber bands. Lettuce was covered with plastic wrap.

3. Microbiological analyses

All samples were analyzed for total aerobic bacteria, total coliforms, *E. coli*, and three pathogens (*E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*).

3.1. Enumeration of total aerobic bacteria, total coliforms and E. coli

A 25 g sample from each produce was stomached using Stomacher®-400 (Seward, Norfolk, UK) for 2 min at 230 rpm in 225 ml of 0.1% peptone water. The pH (6–7) of the sample was recorded, and then 10-fold serial dilutions were prepared by adding 1 ml of sample in 9 ml 0.1% peptone water. Samples were subsequently processed for enumeration of total aerobic bacteria, total coliforms and *E. coli* using Aerobic Count Plate Petrifilm™ and Coliform/*E. coli* Petrifilm™ (3M Microbiology, St Paul, MN), respectively.

3.2. Detection of pathogens

3.2.1. Escherichia coli O157:H7

For isolation of *E. coli* O157:H7, a 25 g sample of each type of produce was stomached in a Stomacher®-400 (Seward) with sterile filter bags for 2 min at 230 rpm in 225 ml *E. coli*.- Broth (Ec. broth) with Novobiocin (20 mg/L) at 37 °C for 24 h (Oxoid, Lenexa, KS). After incubation, a 0.1 ml sample was inoculated onto sorbitol-MacConkey Agar (Oxoid) and plates were incubated at 37 °C for another 24 h. After incubation, three to five presumptive *E. coli* O157:H7 colonies were picked for confirmation (Feng and Weagant, 2002; Norton et al., 2001; Johnston et al., 2005).

3.2.2. L. monocytogenes

For isolation of *L. monocytogenes*, a 25 g sample from each type of produce was stomached in a Stomacher® 400 (Seward) 225 ml of *Listeria* Enrichment Broth (LEB) (Fisher Sci.) at 230 rpm for 2 min. Stomached samples were then incubated at 30 °C for 4 h followed by the addition of 0.45 ml Acriflavin (0.5%), 1.8 ml Nalidixic acid (0.5%), and 1.125 ml Cycloheximide (1%). After 24 h, a 0.1 ml of samples were inoculated onto an Oxford Agar base (MOX) (BD Diagnostics, Sparks, MD) with modified *Listeria* supplement and incubated at 30 °C for 24 h. Three to five presumptive colonies were picked for confirmation (Hitchins, 1998; Norton et al., 2001; Johnston et al., 2005; Pagadala et al., 2012).

3.2.3. Salmonella

For isolation of *Salmonella*, a 25 g sample from each type of produce was stomached in a Stomacher® 400 in 225 ml of Lactose Broth (BD Diagnostics) at 230 rpm for 2 min. Stomached samples were incubated at 37 °C for 24 h. After 24 h, 0.1 ml of suspension was inoculated into 10-ml Rappaport-Vassiliadis (RV) Enrichment Broth medium (BD Diagnostics), incubated at 37 °C for 24 h. A 0.1 ml of RV-medium with sample was streaked onto XLT4 agar (Xylose lysine agar (XL) (BD Diagnostics) supplemented with Tergitol 4 (T4) (BD Diagnostics). Then XLT4 plates were incubated at 37 °C for 24 h. Three to five blackish colonies were picked for confirmation (Andrews et al., 1998; Norton et al., 2001; Johnston et al., 2005).

3.3. Confirmation of pathogens

All bacterial isolates were confirmed using BAX® Polymerase Chain Reaction (PCR)-based system according to the manufacturer instruction (DuPont Qualicon Inc., Wilmington, DE).

3.4. Statistical analysis

Analysis of Variance (ANOVA) and logistic regression were conducted to determine and evaluate the significance of differences in abundance of total aerobic bacteria and the prevalence of