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Research Paper

Prevalence of Multidrug-Resistant Bacteria from U.S.-Grown and Imported Fresh Produce Retailed in Chain Supermarkets and Ethnic Stores of Davidson County, Tennessee

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ABSTRACT

The aim of this study was to determine whether U.S.-grown and imported fresh produce retailed in ethnic stores and chain supermarkets was a reservoir of antibiotic-resistant bacteria. A total of 360 (129 imported and 231 U.S.-grown) samples of fresh produce were purchased from retail stores and analyzed for *Enterobacteriaceae,* including three pathogenic bacteria (*Escherichia coli* O157:H7, *Shigella,* and *Salmonella*), using standard methods. Presumptive pathogenic isolates were confirmed using PCR. The mean *Enterobacteriaceae* counts for imported produce were 6.87 ± 0.15 log CFU/g and 7.16 ± 0.11 log CFU/g in ethnic stores and chain supermarkets, respectively. For U.S.-grown produce, the contamination levels were at 8.35 ± 0.17 log CFU/g and 7.52 ± 0.13 log CFU/g in ethnic stores and chain supermarkets, respectively. *Salmonella* (0 and 0.3%), *Shigella* (1.7 and 0.6%), *E. coli* (3.1 and 1.4%), *Enterobacter* (9.4 and 8.6%), *Klebsiella* (6.7 and 0.6%), and *Serratia* (5.8 and 1.4%) were detected in produce from ethnic stores and chain supermarkets, respectively. None of the samples were positive for *E. coli* O157:H7. Regarding distribution by produce type, leafy vegetables had a significantly ($P < 0.05$) higher prevalence of *Enterobacteriaceae* (19.2%) than the other types, followed by root vegetables (6.4%), tomatoes (5.6%), and fruits (3.9%). Antibiotic-resistant *Salmonella, Shigella, E. coli, Enterobacter, Klebsiella,* and *Erwinia* bacteria were also isolated from fresh produce. The frequencies of vancomycin resistance (98.1 and 100%) were significantly higher ($P < 0.05$) than the frequencies of ampicillin resistance (42.3 and 72.9%) for imported and U.S.-grown produce, respectively. Despite the increased attention to the role of imported produce as a source of antimicrobial resistance, this study indicates that U.S.-grown produce is also contaminated with antibiotic-resistant bacteria. Good agricultural practices on the farms and washing of fresh produce before consumption are greatly recommended to avoid possible public health hazards.

Key words: Antibiotic resistance; *Enterobacteriaceae*; Ethnic; Fresh produce; Imported

The demand for fresh produce in the United States is rising, owing in part to its nutritional value and to consumer health awareness *(35)*. U.S. imports of fruits and vegetables also continue to grow due to the increased purchasing power, shifting consumer perceptions and habits towards better health, and a growing U.S. population of immigrants accustomed to fresh produce diets *(28)*. The shifting demographic makeup of the U.S. population has greatly raised the demand for fresh produce *(14)*. The United States has to import fresh produce to fill the gaps where domestic production is too small or off-season. Even though U.S. fruit and vegetable exports exceeded $7 billion in 2011, U.S. imports of fruits and vegetables exceeded $18 billion, resulting in a gap between imports and exports of $11.2 billion *(21)*. The increasing number of international trade treaties and the demand for fresh produce have led to a significant growth in U.S. produce imports. Although fresh produce is often promoted as a healthy food, it is also a vehicle of foodborne illnesses *(5)*. Pathogenic *Escherichia coli* strains, such as *E. coli* O157:H7, are considered to be one of the greatest concerns regarding foodborne disease associated with leafy greens *(19)*. Imports allow a continuing and abundant supply of fresh produce in the United States; however, foodborne pathogens may diffuse into the country as a result of contaminated produce from other countries. In previous reports, tests for *Salmonella* (3.48 and 0.58%), *Shigella* (0.89 and 0.48%), and *E. coli* O157:H7 (0 and 0%) were positive for imported and U.S.-grown produce, respectively *(42, 43)*.

Bacteria from fresh produce include a number of opportunistic human pathogens which may be resistant to several antibiotics; their resistances may be disseminated to other gut commensal or pathogenic bacteria in food products *(16)*. Antibiotic resistance has been recognized as a global health problem and as one of the uppermost health challenges facing us in the 21st century *(41)*. Approximately 2 million illnesses and 23,000 deaths are caused by antibiotic-resistant bacteria in the United States *(12)*. The emergence and spread of antimicrobial resistance is, among

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other reasons, due to certain human actions, such as unfitting use of antimicrobial remedies in animal production *(7)*. Antibiotic-resistant bacteria have been identified in animal waste, wastewater, river sediments, and farmland soil *(27)*. Antimicrobial-resistant bacteria may have the potential to make their way over to fresh produce through contaminated irrigation water and manure applied to agricultural fields. Fresh produce is a vehicle for bacteria while passing from farm to table and may be a reservoir and path for antimicrobial resistance. There is a concern that antibiotic-resistant bacteria may have the potential to spread globally through the exports and imports of fresh produce. Since fresh produce is usually consumed raw and has been identified as a source of foodborne pathogens, this study examined the prevalence of antibiotic-resistant bacteria in U.S.-grown and imported fresh produce vended in ethnic stores and chain supermarkets.

MATERIALS AND METHODS

Sample collection. In this study, fresh fruits and vegetables were purchased from chain supermarkets and ethnic stores in Davidson County, Tennessee, during the summer and fall of 2014. Prior to conducting the study, a survey was conducted to determine the locations of the stores. Retail stores were assigned identification numbers which were randomly selected using a random number generator in SAS 9.1. *(34)*. To keep their confidentiality, the stores were identified as DA, DB, and DC for chain supermarkets, and EA, EB, and EC for ethnic stores. The chain supermarkets were large mainstream stores with shared central organization, and the ethnic retail stores were small stores that retailed foods of Asian types. In total, 360 fresh produce samples (129 imported and 231 U.S. grown) were analyzed for microbial contamination. The types of produce tested were spinach, mushrooms, broccoli, bean sprouts, tomatoes, cilantro, onions, carrots, bananas, kiwi, berries, avocados, apples, cantaloupe, and mangoes. Fresh produce was purchased in bunches or in the original packaging material (plastic boxes, bags, or wraps) before its best-before date. All produce samples were labeled with the grocery store identification letters, origin, and date of collection. Microbial analyses were initiated within 2 h after sample collection. Samples were analyzed for the presence of *Enterobacteriaceae,* including *E. coli, E. coli* O157:H7*, Salmonella,* and *Shigella*.

Enumeration of *Enterobacteriaceae*. Twenty-five-gram subsamples were weighed and diluted 1:10 in 0.1% buffered peptone water (BPW; BD, Franklin Lakes, NJ) and thereafter homogenized for 2 min at 230 rpm in a Stomacher 400 (Seward, London, UK). Next, 10-fold serial dilutions were prepared in 0.1% BPW for microbial analysis. Briefly, 0.1-ml aliquots from serial dilutions were plated on violet red bile agar (Oxoid, Basingstoke, Hants, England) plates and incubated at 37°C for 24 h. Red to dark-purple colonies surrounded by red-purple halos were identified as *Enterobacteriaceae*. Presumptive *Enterobacteriaceae* colonies were transferred to tryptic soy agar and incubated for 24 h at 35°C. After incubation, colonies were biochemically identified by using oxidase and the API 20E (bioMérieux, Hazelwood, MO) test methods. Strips were inoculated with samples following the manufacturer's instructions, and the isolates were identified by using the apiweb software (bioMérieux). *Enterobacteriaceae* identified at and above the 90% confidence level were recorded in this study. Identified isolates were stored at −80°C in 20% glycerol for antimicrobial susceptibility testing and further confirmation by PCR.

Detection of pathogens. Composite samples, each made up of three subsamples, were prepared for pathogen detection. For *Salmonella* detection, 25 g of produce was homogenized in 225 ml of BPW, followed by incubation at 37°C for 24 h. One-milliliter and 0.1-ml amounts of the BPW preenrichment were transferred to 10 ml of tetrathionate enrichment broth (BD, Sparks, MD) and 10 ml of Rappaport-Vassiliadis broth (Oxoid), respectively. The tetrathionate and Rappaport-Vassiliadis enrichment cultures were incubated at 37°C and 42°C for 24 h, respectively. After enrichment, 10 μl of each sample was streaked onto xylose lysine Tergitol 4 (Oxoid) and *Salmonella* chromogenic agar (Oxoid) plates. After incubation at 37°C for 24 h, colonies which were red to yellow with black centers on xylose lysine Tergitol 4 agar or mauve (rose to purple) on *Salmonella* chromogenic agar were identified as presumptive *Salmonella* spp. *(3)*. For further characterization, presumptive *Salmonella* colonies were inoculated onto nutrient agar (BD) at 37°C for 22 h and subsequently subjected to the oxidase test, the *Salmonella* agglutination test (FT0203; Oxoid), and testing with API Rapid 20E strips (bioMérieux).

For *E. coli* O157:H7 detection, 25-g produce samples were first enriched in 225 ml of enterohemorrhagic *E. coli* enrichment broth (20 μg/ml novobiocin) at 37°C for 24 h. After the incubation, 0.1 ml of the enrichment was plated on sorbitol MacConkey agar plates supplemented with potassium tellurite and cefixime (50 ng/ml) and potassium tellurite (25 mg/ml) (CT-SMAC) *(22)*. After incubation at 37°C for 24 h, three colonies were screened for the presence of the O157 antigen using the commercial *E. coli* O157 latex test kit (DR0620M, Oxoid). For *Shigella* detection, 25-g produce samples were incubated in *Shigella* broth supplemented with 0.5 μg/ml novobiocin (BD) at 42°C for 20 h. After incubation, a loop (10 μl) of the enrichment was streaked on MacConkey agar (BD) and incubated at 37°C for 20 h. Colonies that were slightly pink and translucent, with or without rough edges, on MacConkey agar plates were identified as presumptively *Shigella (22)*. The Wellcolex Colour *Shigella* Test kit (R30858401; Oxoid) was used for a *Shigella* agglutination test following the manufacturer's instructions.

Confirmation of *Salmonella, E. coli* O157:H7, and *Shigella* using PCR. *Salmonella* isolates were confirmed by PCR. Biochemically identified *Salmonella* isolates were cultivated overnight in modified tryptic soy broth (TSB; Difco BD). DNA was extracted from the overnight cultures ($> 5 \times 10^6$ cells) using the PureLink Genomic DNA Mini Kit (Life Technologies, Grand Island, NY). DNA concentrations were determined using a NanoDrop 2000 (Thermo Scientific, Pittsburgh, PA), and DNA integrity was confirmed using agarose gel electrophoresis. The final working concentration of template DNA was 25 ng/μl. A PCR CORE Kit (Sigma, St. Louis, MO) was used in this study. Each reaction mixture (25 μl) contained 125 ng of DNA template, 0.5 μM each forward and reverse primers, 400 μM deoxynucleoside triphosphates, 3 mM $MgCl_2$, 2.5 μl of 10× PCR buffer, and 2.5 U *Taq* DNA polymerase. The primer pair sequences and expected PCR products are shown in Table 1. PCR was performed using a GeneAmp PCR system 2700 thermal cycler (Applied Biosystems, Foster City, CA). After an initial denaturation at 95°C for 2 min, 30 cycles of 1 min at 95°C, 1 min at 57°C, and 2 min at 72°C were performed to amplify the *ompC* gene specific to *Salmonella* spp., followed by a final extension at 72°C for 5 min *(2)*. The target PCR amplicon size was 204 bp. For reference strains, *Salmonella*

TABLE 1. *Primer sequences and PCR product sizes for selected pathogens*

Bacterial identification	Sequence	Target gene	T_m (°C)[a]	Size (bp)	Reference
Salmonella spp.	5′-ATCGCTGACTTATGCAATCG-3′	*ompC*	58.4	204	*2*
	5′-CGGGTTGCGTTATAGGTCTG-3′		62.4		
Shigella spp.	5′-TGCCCAGTTTCTTCATACGC-3′	*invC*	60.4	875	*29*
	5′-GAAAGTAGCTCCCGAAATGC-3′		60.4		
Escherichia coli O157:H7	5′-GTGAAGGTGGAATGGTTGTC-3′	*rfbE*	60.4	314	*1*
	5′-TCTTTCCTCTGCGGTCCTA-3′		60.2		

[a] T_m, melting temperature.

Typhimurium ATCC 13311 was used as the positive control and *E. coli* ATCC 25922 as the negative control.

In addition, PCR was applied for the confirmation of *E. coli* O157:H7. A specific primer pair was used for the detection of the *rfbE* regions of *E. coli* serotype O157:H7, generating PCR products of 314 bp (Table 1). Cycles of 15 s at 94°C, 30 s at 55°C, and 40 s at 72°C were applied, followed by a final extension at 72°C for 5 min *(1). E. coli* O157:H7 ATCC 35150 and water were used as the positive and negative control, respectively. *Shigella* isolates were detected by using the PCR procedures. The primers for *Shigella* are shown in Table 1. Cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C and an extension at 72°C for 5 min *(29)* were performed to amplify the *invC* gene specific for *Shigella sonnei*. The target PCR amplicon size was 875 bp. Twenty-five-microliter aliquots of the reaction mixtures were electrophoresed through 2.5% agarose gels, and fragments were revealed by staining with ethidium bromide. The separated PCR products were visualized and photographed under UV light. *S. sonnei* (Levine) Weldin ATCC 25931 and water were used as the positive and negative control, respectively.

Antibiotic susceptibility testing. The antimicrobial susceptibilities of identified isolates were determined using the Bauer and Kirby disk diffusion technique *(24)*. Briefly, overnight TSB cultures were adjusted to 0.5 McFarland standard and spread evenly on Mueller-Hinton agar plates (Difco, BD). After 10 min at ambient temperatures, antibiotic susceptibility disks (BBL, BD) were applied on Mueller-Hinton plates with sterile forceps. The plates were observed for inhibition zones after an incubation period of 24 h at 37°C. The following antibiotic susceptibility disks (BD), with the disk strength in parentheses, were used: amikacin (AMK; 30 μg), ampicillin (AMP; 10 μg), cefotaxime (CTX; 30 μg), chloramphenicol (CHL; 30 μg), ciprofloxacin (CIP; 5 μg), erythromycin (ERY; 15 μg), gentamicin (GEN; 120 μg), kanamycin (KAN; 30 μg), streptomycin (STR; 10 μg), and vancomycin (VAN; 30 μg). The interpretation of the results was based on the Clinical and Laboratory Standards Institute guidelines *(15)* for human medicine as resistant, intermediate, or susceptible. *E. coli* ATCC 25922 was used as the reference strain.

Statistical analysis. All plate count data were converted to log CFU per gram values prior to statistical analysis**.** The antibiotic resistance values are expressed as percentages. Differences in microbiological counts and prevalence among treatment means were determined with one-way analysis of variance using SPSS software for Windows, version 12 (Chicago, IL), and the chi-square test, respectively. *P* values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Table 2 displays mean counts for *Enterobacteriaceae* isolated from U.S. and imported produce retailed in chain supermarkets and ethnic stores. The bacterial mean count (6.87 ± 0.15 log CFU/g) for imported produce at ethnic stores was significantly lower ($P < 0.05$) than that of imported produce sold in chain supermarkets (7.16 ± 0.11 log CFU/g). However, the U.S.-grown produce sold in ethnic stores had significantly higher ($P < 0.05$) counts of *Enterobacteriaceae* (8.35 ± 0.17 log CFU/g) than that retailed in chain supermarkets (7.52 ± 0.13 log CFU/g). The low levels of *Enterobacteriaceae* from imported produce in ethnic retail stores might be because of a high demand for imported produce by the increasing population of immigrants leading to short display periods and less bacterial growth. U.S.-grown produce had lower contamination levels in chain supermarkets than in ethnic stores. Due to demand, U.S.-grown produce also had short display times. This might be explained by the fact that when it comes to food safety, most consumers trust U.S.-grown produce more than imported produce retailed in chain supermarkets. According to Flessa et al. *(17),* the length of storage time for the produce plays a vital role in the growth of bacteria. Overall, the incidence of *Enterobacteriaceae* (30.3%) isolated from fresh produce from ethnic stores was significantly higher ($P < 0.05$) than the incidence for produce retailed in chain supermarkets (15.0%). The long chain of distribution (transport) of the imported fresh produce may also have

TABLE 2. *Viable* Enterobacteriaceae *counts from fresh produce in ethnic stores and chain supermarkets*

Type of store	No. of fresh produce samples	Mean *Enterobacteriaceae* count ± SD (log CFU/g) in[a]: Imported produce	U.S.-grown produce
Ethnic stores	180 (57 imported; 123 U.S. grown)	6.87 ± 0.15 B	8.35 ± 0.17 A
Chain supermarkets	180 (72 imported; 108 U.S. grown)	7.16 ± 0.11 A	7.52 ± 0.13 B
P value		0.002	0.002

[a] Mean values within columns with no common letter differ significantly ($P < 0.05$).

TABLE 3. *Occurrence of* Enterobacteriaceae *in fresh produce*

Genus or species	No. (%) of produce samples positive for indicated microbe from[a]: Ethnic stores	Chain supermarkets
Acinetobacter	1 (0.3) A	0 (0.0) A
Citrobacter spp.	7 (2.0) A	2 (0.6) B
Escherichia coli	11 (3.1) A	5 (1.4) B
E. coli O157:H7	0 (0.0) A	0 (0.0) A
Enterobacter spp.	34 (9.4) A	31 (8.6) A
Erwinia spp.	4 (1.1) A	3 (0.9) A
Hafnia spp.	0 (0.0) A	3 (0.9) A
Klebsiella spp.	24 (6.7) A	2 (0.6) B
Proteus spp.	1 (0.3) A	0 (0.0) A
Salmonella spp.	0 (0.0) A	1 (0.3) A
Serratia spp.	21 (5.8) A	5 (1.4) B
Shigella spp.	6 (1.7) A	2 (0.6) B
Total	109 (30.3) A	54 (15.0) B

[a] Occurrence of *Enterobacteriaceae* (percentage of samples) was determined as follows: (number of fresh produce samples with *Enterobacteriaceae* isolates/total number of fresh produce samples) ($n = 360$). Mean percentages within rows and columns with no letter in common differ significantly ($P < 0.05$).

contributed to the high levels of contamination, due to spoilage *(44)*.

The prevalence rates of bacterial species isolated from fresh produce are provided in Tables 3 and 4; some of these species reside in the intestines of humans and animals *(26)*. *Salmonella* (0 and 0.3%), *Shigella* (1.7 and 0.6%), *E. coli* (3.1 and 1.4%), *Enterobacter* (9.4 and 8.6%), *Klebsiella* (6.7 and 0.6%), *Serratia* (5.8 and 1.4%), and *Citrobacter* (2.0 and 0.6%) were isolated from produce from ethnic stores and chain supermarkets, respectively. The *Enterobacteriaceae* species isolated in the current study are in agreement with the findings of Falomir et al *(16)*; they reported the presence of these species in a variety of fresh produce. The percentages of *Shigella, E. coli, Klebsiella, Serratia,* and *Citrobacter* spp. were significantly higher ($P < 0.05$) in produce obtained from ethnic stores than in produce from chain supermarkets. *E. coli,* a commensal bacterium, is considered the indicator for fecal contamination *(25)* and can be detected in produce if it was watered with tainted water or fertilized with contaminated manure *(23)*. Commensal bacteria may be a risk factor for infection, and thus, consumption of raw produce should be considered an impending food safety concern for immunocompromised individuals *(20)*.

Leafy vegetables (Table 4) had the highest ($P < 0.05$) prevalence of *Enterobacteriaceae* (19.2%), followed by root vegetables (6.4%), tomatoes (5.6%), fruits (3.9%), green peppers (3.3%), and then mushrooms (3.1%). Broccoli and bean sprouts had the lowest prevalence of *Enterobacteriaceae* (0.8%). Although this prevalence was not statistically different from those of beans and cucumber, it was significantly lower ($P < 0.05$) than those of leafy vegetables, fruits, mushrooms, green peppers, tomatoes, and root vegetables. Leafy greens are grown in exposed fields and are at risk of contamination from manure, soil, and

TABLE 4. *Numbers and percentages of* Enterobacteriaceae *species isolated from different types of fresh produce*

Type of produce ($n = 360$)[a]	No. (%) of samples positive for[b]: ACI	CIT	ESC	ENT	ERW	HAF	KLE	PRO	SAM	SER	SHI	Total no. (%)[c]
Broccoli			2 (0.6)	1 (0.3)					0 (0.0)			3 (0.8) C
Beans				2 (0.6)			1 (0.3)			1 (0.3)		4 (1.1) C
Cucumbers		2 (0.6)								2 (0.6)		4 (1.1) C
Fruit		1 (0.3)	2 (0.6)	8 (2.2)			1 (0.3)			1 (0.3)	1 (0.3)	14 (3.9) B
Leafy vegetables	1 (0.3)	3 (0.8)	9 (2.5)	21 (5.8)	3 (0.8)	1 (0.3)	13 (3.6)	1 (0.3)	1 (0.3)	12 (3.3)	4 (1.1)	69 (19.2) A
Mushrooms					2 (0.6)		4 (1.1)			3 (0.8)	2 (0.6)	11 (3.1) B
Green peppers		1 (0.3)	1 (0.3)	5 (1.4)			5 (1.4)		0 (0.0)			12 (3.3) B
Bean sprouts				2 (0.6)	1 (0.3)							3 (0.8) C
Tomatoes		1 (0.3)	1 (0.3)	16 (4.4)	1 (0.3)		1 (0.3)		0 (0.0)			20 (5.6) B
Root vegetables		1 (0.3)	1 (0.3)	10 (2.8)		2 (0.6)	1 (0.3)			7 (1.9)	1 (0.3)	23 (6.4) B
Total	1 (0.3) Z	9 (2.5) Y	16 (4.4) XY	65 (18.1) W	7 (1.9) Y	3 (0.8) Z	26 (7.2) X	1 (0.3)	1 (0.3) Z	26 (7.2) X	8 (2.2) Y	163 (45.3)

[a] The numbers of samples of fresh produce tested were as follows: broccoli, 9; beans, 15; cucumbers, 12; fruit, 51; leafy vegetables, 147; mushrooms, 12; green peppers, 21; bean sprouts, 15; tomatoes, 33; and root vegetables, 45. Fruit included apples, avocado, banana, berries, cantaloupe, mangoes, kiwi, etc. Leafy vegetables included spinach, lettuce, bok choy, celery, Chinese nappa, cilantro, gai choy, green onion, etc. Root vegetables included bulb onion, carrot, chayote, ginger root, radish, etc.

[b] ACI, *Acinetobacter* spp.; CIT, *Citrobacter* spp.; ESC, *Escherichia coli*; ENT, *Enterobacter* spp.; ERW, *Erwinia* spp.; HAF, *Hafnia* spp.; KLE, *Klebsiella* spp.; PRO, *Proteus* spp.; SAM, *Salmonella* spp.; SER, *Serratia* spp.; SHI, *Shigella* spp. Mean values within the last row with no letters (W, X, Y, Z) in common differ significantly ($P < 0.05$).

[c] Mean percentages within the column with no letter (A, B, C) in common differ significantly ($P < 0.05$).

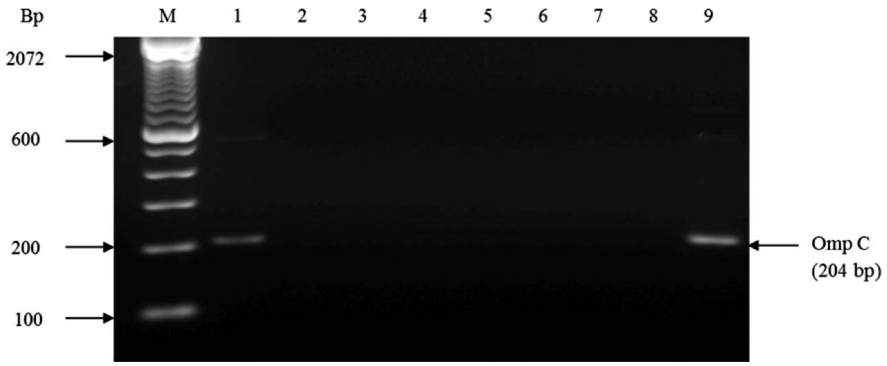

FIGURE 1. *PCR amplification products generated by* Salmonella*-specific primers from isolates recovered from fresh produce. Lane M, 100-bp DNA marker; lane 1, positive control (*Salmonella *Typhimurium ATCC 13311); lane 2, negative control (*Escherichia coli *ATCC 25922); lanes 3 through 9, DNA samples isolated from fresh produce (lane 9,* Salmonella *positive). BP, base pairs. Arrows point to the 204-bp band and corresponding weights of the DNA marker.*

irrigation water; they have been recognized as the fresh produce commodity group of utmost concern from a microbiological safety perspective *(6, 39)*. The elevated levels of *Enterobacteriaceae* contamination in leafy greens may also be due to the large surface areas for microbial attachment *(4)*. Overall, none of the sprouts tested were found to be contaminated with *Salmonella, Shigella,* or *E. coli* O157:H7. Sprouts are one of the most common vehicles for produce-associated bacterial foodborne illnesses *(36)*. Producers are advised to use potable water for irrigation and test spent irrigation water for improved sprout safety *(40)*. In 2009, raw alfalfa sprouts grown in the United States were implicated in an outbreak *(11)*.

Amplification of the *ompC* (Fig. 1) and *invC* (Fig. 2) genes confirmed the presence of *Salmonella* and *Shigella,* respectively, in the fresh produce surveyed. Among the 360 samples of produce tested during the study, only 0.8 and 0% (Table 5) of imported and U.S.-grown produce samples were positive for *Salmonella,* respectively. S*almonella* at low levels was detected in iceberg lettuce originating from Chile and purchased from a chain supermarket. Even at low occurrence, the incidence of *Salmonella* in produce has an expansive impact on public health due to its wide distribution and the fact that it is often consumed raw *(30)*. *Salmonella* Typhimurium has been implicated in tomatoes originating from Ohio *(9)*. *Salmonella* Reading and *Salmonella* Abony were also indicated in alfalfa sprouts supplied by Sprouts Extraordinaire of Denver, CO *(13)*. In the current study, *Shigella* was isolated from both imported (3.1%) and U.S.-grown (1.7%) produce. In 2008, a *Salmonella* Saintpaul outbreak in the United States was associated with jalapeño peppers, serrano peppers, and tomatoes imported from Mexico *(10)*. *Shigella* was detected in mushrooms from China and Korea, red lettuce, cilantro, spinach, and bok choy from the United States, chayote squash from Mexico, and bananas from Honduras (Table 5). From 1996 to 2006, the percentages of outbreaks attributed to leafy vegetables were reported to be 75 and 64% in Brazil and Australia, respectively. Through globalization of the food trade, foodborne pathogens may be reintroduced or transported between countries as a result of contaminated produce. *E. coli* O157:H7 was not detected in any of the imported or U.S.-grown produce examined. *Salmonella, Shigella, E. coli* O157:H7, and various commensal bacteria are major risk factors, particularly for immunocompromised individuals *(22)*.

Antimicrobial resistance test. A total of 137 *Enterobacteriaceae* isolates (52 imported fresh produce and 85 U.S. grown) were tested against 10 types of antibiotics frequently used in clinical and agricultural settings**.** Our results recorded the presence of antibiotic-resistant bacteria in retail U.S.-grown and imported fresh produce (Table 6). The emergence of resistance to antimicrobials is a major public health problem and justifies the monitoring of foodborne pathogens in foods. According to Teuber et al. *(38),* the food chain has become known as one of the principal routes for the diffusion of antibiotic resistance between animal and human populations. Antibiotic resistance among the *Enterobacteriaceae* isolates from imported produce was highest to vancomycin (98.1%), followed by erythromycin (92.3%) and ampicillin (42.3%). The differences in the percentages of resistance to vancomycin and erythromycin were not statistically significant ($P > 0.05$), but they were significantly higher than the percentage of resistance to ampicillin ($P < 0.05$). The percentages of resistance to cefotaxime (1.9%) and kanamycin (7.7%) were

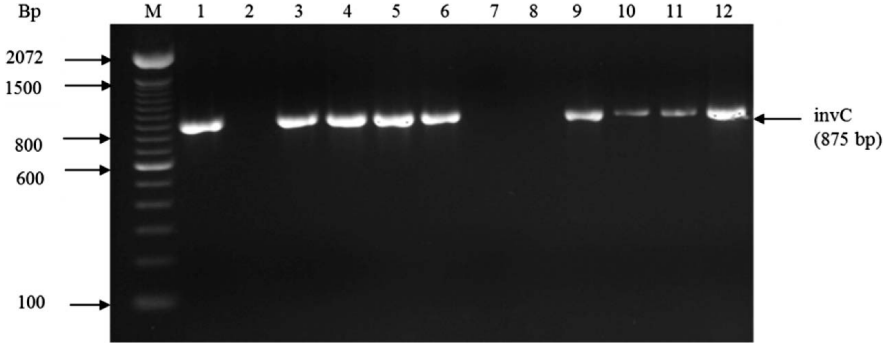

FIGURE 2. *PCR amplification products generated by* Shigella*-specific primers from* Shigella *isolates recovered from fresh produce. Lane M, 100-bp DNA marker; lane 1, positive control [*Shigella sonnei *(Levine) Weldin ATCC 25931]; lane 2, negative control (*Escherichia coli *ATCC 25922); lanes 3 through 12, DNA samples isolated from fresh produce (lanes 3 through 6 and 9 through 12,* Shigella *positive). BP, base pairs. Arrows point to the 875-bp band and corresponding weights of the DNA marker.*

TABLE 5. *Prevalence of pathogenic bacteria isolated from U.S.-grown and imported fresh produce*

Type of produce	No. of samples	No. of samples (country[ies] of origin or %) positive for[a]: *Salmonella* Imported ($n = 129$)	*Salmonella* U.S. grown ($n = 231$)	*Shigella* Imported ($n = 129$)	*Shigella* U.S. grown ($n = 231$)
Bananas	33	0	NA	1 (Honduras)	NA
Chayote	18	0	NA	1 (Mexico)	NA
Cilantro	30	NA	0	NA	1
Bok choy	12	NA	0	NA	1
Iceberg lettuce	30	1 (Chile)	0	0	0
Mushrooms	12	0	0	2 (China, South Korea)	0
Red lettuce	24	NA	0	NA	1
Spinach	33	NA	0	NA	1
Other produce	168	0	0	0	0
Total	360	1 (0.8) B	0 (0.0) B	4 (3.1) A	4 (1.7) A

[a] NA, sample was not available. Mean percentages within the last row with no letter in common are significantly different ($P < 0.05$).

low compared with the results for other antibiotics. None of the isolates from imported produce were resistant to ciprofloxacin, amikacin, or gentamicin.

For U.S.-grown produce, the highest resistance was to vancomycin (100.0%), followed by erythromycin (90.6%) and ampicillin (72.9%). The percentages of *Enterobacteriaceae* isolates resistant to vancomycin and erythromycin were not different ($P > 0.05$), but they were significantly higher ($P < 0.05$) than the percentage of isolates resistant to ampicillin. Resistance to cefotaxime (1.2%) and chloramphenicol (3.5%) was noted to be low among the isolates. None of the isolates from U.S.-grown produce were resistant to amikacin, ciprofloxacin, or gentamycin. The prevalence of antibiotic-resistant bacteria is reported to be higher in some countries, including China and Spain, than in the United States, owing to more liberal use of antibiotics in food animals *(45)*. Antibiotic-resistant bacteria can enter the produce chain through contaminated soils and spraying and irrigating produce with contaminated water containing resistant bacteria. The use of contaminated water for irrigation water and the application of pesticides is thought to be a primary source for the diffusion of resistant bacteria into agricultural lands *(8)*.

The findings for highly multidrug-resistant bacteria are shown in Table 7. An isolate was considered to be multidrug resistant when it displayed resistance to three or more antibiotics. In total, 16 different resistance profiles were noted, among which were AMP-CTX-CHL-ERY-VAN (0 and 11.5%), AMP-ERY-VAN (9.4 and 11.5%), and ERY-VAN (1.2 and 30.7%) for *Enterobacter* isolates from U.S.-grown and imported produce, respectively. *E. coli* isolates exhibited eight phenotype patterns; among them were AMP-CTX-CHL-ERY-KAN-VAN (8.2 and 0.0%), AMP-CTX-CHL-ERY-STR-VAN (2.4 and 0%), and AMP-CTX-CHL-ERY-KAN-STR-VAN (1.2 and 0.0%) for U.S. and imported produce, respectively. *Klebsiella,* a commensal and an opportunist pathogen, showed AMP-ERY-STR-VAN (10.6 and 9.6%) and AMP-ERY-VAN (9.4 and 1.9 %) patterns for isolates from U.S. and imported produce, respectively. An AMP-ERY-KAN-STR-VAN (1.9%) pattern for *Salmonella* isolates was detected in iceberg lettuce imported from Chile. The most common multidrug resistance phenotype of

TABLE 6. *Antibiotic susceptibilities of* Enterobacteriaceae *isolates from fresh produce*

Antibiotic[a]	% of isolates with antibiotic susceptibility in[b]: Imported fresh produce Susceptible	Imported fresh produce Intermediate	Imported fresh produce Resistant	U.S.-grown fresh produce Susceptible	U.S.-grown fresh produce Intermediate	U.S.-grown fresh produce Resistant
Amikacin	98.1 A	1.9 D	0.0 E	100.0 A	0.0 D	0.0 E
Ampicillin	32.7 D	25.0 A	42.3 B	20.0 D	7.1 D	72.9 B
Cefotaxime	84.6 B	13.5 B	1.9 E	74.1 B	24.7 B	1.2 E
Chloramphenicol	84.6 B	0.0 D	15.4 C	77.6 B	18.8 C	3.5 E
Ciprofloxacin	100.0 A	0.0 D	0.0 E	98.8 A	1.2 D	0.0 E
Erythromycin	0.0 E	7.7 C	92.3 A	0.0 E	9.4 D	90.6 A
Gentamicin	100.0 A	0.0 D	0.0 E	100.0 A	0.0 E	0.0 D
Kanamycin	92.3 AB	0.0 D	7.7 D	77.6 B	9.4 D	12.9 D
Streptomycin	65.4 C	19.2 B	15.4 C	38.8 C	34.1 A	27.1 C
Vancomycin	1.9 E	0.0 D	98.1 A	0.0 E	0.0 E	100.0 A

[a] Antibiotics selected for susceptibility testing.
[b] Mean values within columns with no letter (A, B, C, D, E) in common differ significantly ($P < 0.05$).

TABLE 7. *Antibiotic resistance profiles of bacteria isolated from fresh produce*

Bacterial species	Antibiotic resistance phenotype[a]	No. (%) of isolates with phenotype in[b]: U.S.-grown produce ($n = 85$)	Imported produce ($n = 52$)
Acinetobacter spp.	AMP-VAN	1 (1.2) B	0 (0.0) C
Citrobacter spp.	AMP-CHL-ERY-STR-VAN	0 (0.0) B	1 (1.9) C
	AMP-ERY-VAN	1 (1.2) B	0 (0.0) C
	ERY-STR-VAN	3 (3.5) AB	1 (1.9) C
	ERY-VAN	3 (3.5) AB	0 (0.0) C
E .coli spp.	AMP-CTX-CHL-ERY-KAN-STR-VAN	1 (1.2) B	0 (0.0) C
	AMP-CTX-CHL-ERY-STR-VAN	2 (2.4) B	0 (0.0) C
	AMP-CTX-CHL-ERY-KAN-VAN	7 (8.2) A	0 (0.0) C
	AMP-ERY-KAN-STR-VAN	1 (1.2) B	1 (1.9) C
	CTX-CHL-ERY- KAN-VAN	0 (0.0) B	1 (1.9) C
	AMP-CTX-ERY-VAN	1 (1.2) B	0 (0.0) C
	ERY-KAN-VAN	0 (0.0) B	1 (1.9) C
	ERY-VAN	1 (1.2) B	0 (0.0) C
Enterobacter spp.	AMP-CTX-CHL-ERY-KAN-VAN	1 (1.2) B	0 (0.0) C
	AMP-CTX-CHL-ERY-VAN	0 (0.0) B	6 (11.5) B
	AMP-ERY-KAN-STR-VAN	1 (1.2) B	1 (1.9) C
	AMP-ERY-STR-VAN	6 (7.1) AB	1 (1.9) C
	AMP-ERY-VAN	8 (9.4) A	6 (11.5) B
	AMP-CTX-ERY	0 (0.0) A	2 (3.8) C
	ERY-VAN	1 (1.2) B	16 (30.7) A
Erwinia spp.	AMP-ERY-VAN	2 (2.4) B	1 (1.9) C
	ERY-VAN	2 (2.4) B	2 (3.8) C
Hafnia spp.	AMP-ERY-VAN	1 (1.2) B	0 (0.0) C
	ERY-VAN	2 (2.4) B	0 (0.0) C
Klebsiella spp.	AMP-CHL-ERY-STR-VAN	1 (1.2) B	0 (0.0) C
	AMP-ERY-STR-VAN	9 (10.6) A	5 (9.6) B
	AMP-ERY-VAN	8 (9.4) A	1 (1.9) C
	ERY-STR-VAN	1 (1.2) B	1 (1.9) C
Proteus spp.	AMP-ERY-KAN-VAN	1 (1.2) B	0 (0.0) C
Salmonella spp.	AMP-ERY-KAN-STR-VAN	0 (0.0) B	1(1.9) C
Serratia spp.	AMP-ERY-STR-VAN	9 (10.6) A	0 (0.0) C
	AMP-ERY-VAN	4 (4.7) AB	0 (0.0) C
	ERY-KAN-VAN	1 (1.2) B	0 (0.0) C
	ERY-STR-VAN	2 (2.4) B	0 (0.0) C
Shigella spp.	AMP-ERY-VAN	2 (2.4) B	0 (0.0) C
	AMP-VAN	2 (2.4)B	0 (0.0) C
	ERY-VAN	0 (0.0) B	4 (7.7) BC

[a] AMP, ampicillin; VAN, vancomycin; CHL, chloramphenicol; ERY, erythromycin; STR, streptomycin; CTX, cefotaxime; CHL, chloramphenicol; KAN, kanamycin.

[b] Values in the same row with no letter in common are significantly different ($P < 0.05$).

Salmonella is reported to confer resistance to ampicillin and streptomycin *(18)*. According to Rusul et al. *(32),* multidrug-resistant *Salmonella* strains found in vegetables are a major concern for food safety. Multidrug-resistant *Shigella* was also identified in the current study, including patterns of AMP-ERY-VAN (2.4 and 0%) in isolates from red lettuce, AMP-VAN (2.4 and 0%) in isolates from spinach, and ERY-VAN (0 and 7.7%) in isolates from mushrooms from U.S. and imported produce, respectively. The ERY-VAN resistance pattern was observed in *Shigella* isolates from imported produce from Honduras, Mexico, China, and South Korea. Among isolates from U.S. fresh produce, the antibiotic resistance profile AMP-ERY-STR-VAN was more prominent ($P < 0.05$) than other profiles in *Klebsiella* and *Serratia* isolates. On the other hand, among isolates from imported produce, the antibiotic profile ERY-VAN was more prominent ($P < 0.05$) than other profiles in *Enterobacteriaceae*. Other resistance patterns for commensal bacteria in the current study were found in *Citrobacter, Erwinia, Hafnia,* and *Proteus* isolates. Our results are in agreement with the study of Schwaiger et al. *(35),* which detected antibiotic-resistant bacteria on vegetable products at the retail level. According to Salyers et al. *(33),* commensal bacteria may be a reservoir of antibiotic resistance determinants that can be transferred to human pathogens through the food chain.

The practice of using antibiotics in animal production is reflected as a major factor contributing to the emergence of antibiotic-resistant bacteria *(31)*. In many parts of the world, animal manure is applied to the soil, and potentially resistant

bacteria can end up on fresh produce in the field. The fresh produce trade has the potential to disseminate antibiotic-resistant bacteria between countries; a good example is the 2005 nationwide outbreak of multidrug-resistant *Salmonella* Typhimurium DT104B in Finland, which was due to contaminated lettuce imported from Spain *(37)*. Our results and previous investigations *(35)* demonstrate the occurrence of multiple-antibiotic-resistance profiles among bacterial isolates on imported and U.S.-grown produce. Imported produce and U.S.-grown produce are indicated to be reservoirs of antibiotic-resistant bacteria, and therefore, there is need for international efforts to combat antimicrobial resistance in agroecosystems. Consumers are recommended to follow hygienic practices and thoroughly wash fresh produce, as it might have an important role as a source of multiple-antibiotic-resistant bacteria. Even though our data are limited to one U.S. county, the sample size was adequate and the results can be used for further studies to scale the microbiological quality and the prevalence of antimicrobial-resistant bacteria on both imported and U.S.-grown produce.

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