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Pilot Study: Microbiological Survey of Select Fresh Produce Acquired from Small Independent Retailers and Large Chain Supermarkets in Food Desert Areas of Central Virginia, USA

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Abstract

Low profit margins drive exodus of large chain supermarkets (LCM) in food deserts. Food desert residents lacking transportation shop for fresh foods at nearby small independently owned markets (SIM). Studies demonstrate SIM incur higher food safety code violations compared to LCM. The study conducted, assessed microbiological quality differences of select fresh produce sold at SIM and LCM within identified Virginia food desert areas of Petersburg and Colonial Heights. Evaluation of 122 fresh produce samples from 10 SIM and nine LCM between September 2018 and April 2019 took place. Higher counts of aerobic mesophile were present in all SIM samples, as compared to LCM. Regardless of SIM or LCM, *Campylobacter*, *E. coli* and *Listeria* were detected in 10.7%, 4.9% and 3.3% of samples, respectively. The SIM accounted for majority of isolated *Campylobacter* (76.9%). Evaluation of 28 bacterial isolates consisting of *Campylobacter*, *E. coli*, and *Listeria* for susceptibility to 12 antimicrobials occurred. Ampicillin resistance showed highest frequency among *Campylobacter* (84.6%) while nalidixic acid resistance was highest in *Listeria* isolates (100%). Approximately 85% *Campylobacter* and 27% *E. coli* isolates exhibited multidrug resistance (MDR). Study findings document unique food safety risks associated with food desert SIM. Additional research efforts are needed to conduct a larger-scale sample size of SIM fresh foods. Validation of observed presence of opportunistic foodborne pathogens and antimicrobial resistance associated with SIM fresh foods is a critical research element. Increased knowledge may improve existing SIM food safety best practices in support of increased availability of safe fresh food products to food desert residents.

Keywords: *Food Desert; Large Chain Supermarkets; Small Independently Owned Markets; Fresh Produce; Bacteria; Antimicrobial Resistance; Multidrug Resistance*

Introduction

The USDA designates a food desert as an area where over one-third of low-income residents must drive 1 - 10 miles to shop at a supermarket [1]. An estimated 38M people live in a USDA designated food desert [2]. Each day, over 9M adults go hungry in US food deserts, daily skipping meals because they cannot afford to eat [3]. The Virginia Department of Social Services [4] estimated the COVID-19 pandemic

increased food insecurity by 22.5%. The designated study areas of Petersburg, VA and Colonial Heights, VA includes 28,560 low income individuals with low access to supermarkets [5]. Within the study area, 2,346 households have no car to access distant supermarkets [5].

In food deserts, low-income households without convenient transportation shop most often at small independently owned corner markets and convenience stores. Lack of nearby supermarkets results in overreliance on neighborhood food outlets (small independently owned corner markets, convenience stores, roadside stands, farmers markets, home gardens, etc.) to acquire fresh foods (i.e. produce, meats, dairy, and eggs). Alwitt and Donley [6] and Beaulac., *et al.* [7] indicated fewer chain stores and a prevalence of small, independently owned food outlets in low-income areas.

Products handled by small independently owned markets (SIM) on a relatively small scale may be quite different from those handled by their large-scale counterparts in that they are generally unregulated and may come with self-prescribed handling and sanitation practices procedures that do not correlate with governmental and industry regulatory guidance. Additionally, local food outlets located in food deserts may not practice or be aware of food safety activities in the form of Good Handling Practices (GHP). Thus, small-scale retailer practices may increase the risk of product contamination and human health hazard, while lack of effective GHP at the available food desert fresh food access markets opens food safety vulnerabilities with the eventual foodborne illness outbreak within low-income communities.

Research examining the quality of food available at small, independent retail food outlets indicates high potential for reduced product quality and safety of perishable foods sold [7-10]. Hendrickson., *et al.* [11] also reported that available foods in food desert stores were fair to poor quality in comparison to the food available in non-food desert retailers. Darcey and Quinlan [12] reported that small independent markets might have more critical and non-critical code violations in food safety. Analyses of reported cases have found increased rates of some foodborne illnesses among minority racial and ethnic populations associated with food insecurity region [13].

In addition, researchers indicated the prevalence of opportunistic foodborne pathogens and their antimicrobial resistance associated with fresh produce procured farmers markets in the US. For example, Kim., *et al.* [14] reported the prevalence of *Campylobacter*, *E. coli*, and *Listeria* in 8.7%, 9.4%, and 8.0% of the total 138 selected fresh produce samples procured from farmers' markets in Central Virginia. Pan., *et al*. [15] and Scheinberg., *et al*. [16] detected *E. coli* in 20% and 25%, respectively, of fresh produce obtained from selected farmers' markets in Northern California and Pennsylvania. Roth., *et al*. [17] and Scheinberg., *et al*. [16] detected *L. monocytogenes* in 2.6% of leafy greens and 0% of lettuce, respectively, obtained from selected farmers' markets in Florida and Pennsylvania. The study conducted by Kim., *et al*. [14] also indicated ampicillin resistance among *Campylobacter* (100%) and *E. coli* (47.8%) isolates and nalidixic acid resistance among *Listeria* isolates (72.7%). At least 17% of each *Campylobacter*, *E. coli*, and *Listeria* isolates obtained from their study exhibited multidrug resistance (MDR).

However, food safety risks associated with local food retailers particularly in food deserts appear to be inadequately addressed in research practice or literature. It also appears from a review of existing local foods and food safety published literature that no assessment has been done on the microbiological safety of fresh produce sold at food retailers in food deserts of Virginia. Furthermore, due to the potential differences in handling and manufacturing practices, any differences in antibiotic resistance in bacterial isolates from SIM and large chain supermarket (LCM) operations are of interest. There is a valid research need in this area since more than 1M Virginia residents live in food insecurity [18].

Therefore, this study was designed to assess the differences in the prevalence of opportunistic foodborne pathogens on fresh produce, which contributes to the majority of documented illnesses in the US [19], sold at small independent retailers and comparable products found at large chain supermarkets in and surrounding food desert areas of Petersburg and Colonial Heights, VA.

Materials and Methods

Fresh produce samples used: Visual inspections for the market compliance to the simplified food establishment checklist adopted from the Virginia Beach Department of Public Health [20], the McHenry County Department of Health [21], and the South Dakota De-

partment of Health [22] were performed by three trained researchers during sample acquisition visits at markets and the list is shown in table 1. The market conditions and employee hygiene practices for nine operational compliance elements associated with food safety were assessed. The nine elements were categorized into four with respect to risk factors, including (1) food dry storage, (2) food storage practices, (3) food cold storage, and (4) employee practices. Food storage practices were assessed via visual observation of mold and the extent of blemish on the produce, the freshness of the produce, storage room cleanness, and most of all, consumer acceptance. Employee practices were assessed observing the instances of wearing hair net, gloves, and uniform or at least no street clothing.

Food Safety Checklist	Food outlets[a]/Compliance (%)	
	LCM (n = 9)	SIM (n = 10)
Food Dry Storage		
All produce is stored 6-8 inches off the floor	100	100
No evidence of pests is present	100	100
Food Storage Practices		
Produce is wholesome and in good condition	100	100
Produce storage rooms are clean and organized	100	88.9
Food Cold Storage		
Produce is stored or displayed at 41°F (5°C) or below	77.8	50.0
Raw meat & poultry stored separate or below produce	88.9	90.0
Refrigerators maintained clean (shelving, etc.)	100	80.0
Employee Practices		
Employees use good hygiene practices while handling food	77.8	70.0
Employees do not consume food in produce storage areas	100	90.0

Table 1: *Checklist of food safety inspections conducted and market compliance*.*

**The list was excerpted from Virginia Beach Department of Public Health [20], Illinois McHenry County Department of Health [21], and South Dakota Department of Health [22] educational materials, and adapted for this study.*

[a]LCM: Large Chain Supermarkets; SIM: Small Independently Owned Markets; n: Number of Food Outlets Tested.

A total of 122 fresh produce items were randomly procured from registered ten small independently owned markets (SIM) and nine large chain supermarkets (LCM) located in food desert areas of Petersburg and Colonial Heights in Virginia. The fresh produce obtained from SIM is locally sourced, while the fresh produce obtained from LCM is nationally sourced. Products procured represented seven different types of fresh produce and are shown in table 2. Figure 1 indicates the food desert areas of Petersburg and Colonial Heights where selected food products for this study were obtained. All purchases were made in duplicate between September 2018 and April 2019. The produce purchased came directly from market displays in the form of baskets, cardboard boxes, or plastic bags at the point of purchase. Purchased samples were transported to our laboratory in insulated containers packed with ice. All products were kept in the refrigerator (4 ± 2°C) and used for microbial testing within two days of arrival.

Microbial testing: A FDA approved method [23] for microbial analysis was used with modification using peptone water. For blendable products (i.e. cilantro, collard green, and kale), approximately 25g of each sample portion (taken from multiple locations in a sample) was homogenized with 225 ml of sterile 0.1% peptone water (PW) in a laboratory blender (Model 400 Circulator, Seward Ltd., West Sussex, UK) at 260 rpm for 2 minutes. For other non-blendable products (i.e. bell pepper, jalapeno pepper, tomato, and turnip), each whole sample

Name of sample	Market source (store number)			
	LCM (n = 9)		SIM (n = 10)	
	No. of samples	Price ($/lb or bunch)[b]	No. of samples	Price ($/lb or bunch)
Bell pepper	12	1.33 ± 0.68 a	14	2.33 ± 1.23 b
Cilantro	18	1.31 ± 0.70 a	4	1.16 ± 0.41 a
Collard green	6	2.10 ± 1.51 a	6	2.04 ± 0.29 a
Jalapeno pepper	4	1.62 ± 0.64 a	6	1.99 ± 0.65 a
Kale	4	5.02 ± 0.65 a	4	2.29 ± 0.33 b
Tomato	18	1.87 ± 0.45 a	18	1.96 ± 0.54 a
Turnip	6	1.36 ± 0.44 a	2	0.69 ± 0.00 b

Table 2: *Market source of fresh produce procured from 2018 to 2019[a].*

[a]Samples were purchased from large chain supermarkets (LCM) and small independently owned markets (SIM) from September 2018 to April 2019; a total of 122 samples consisted of 68 and 54 samples from LCM and SIM, respectively, were procured; in the same row within each sample, means preceded by the same lower letter are not significantly different (P > 0.05).

[b]Mean ± standard error of sample price (excluding tax).

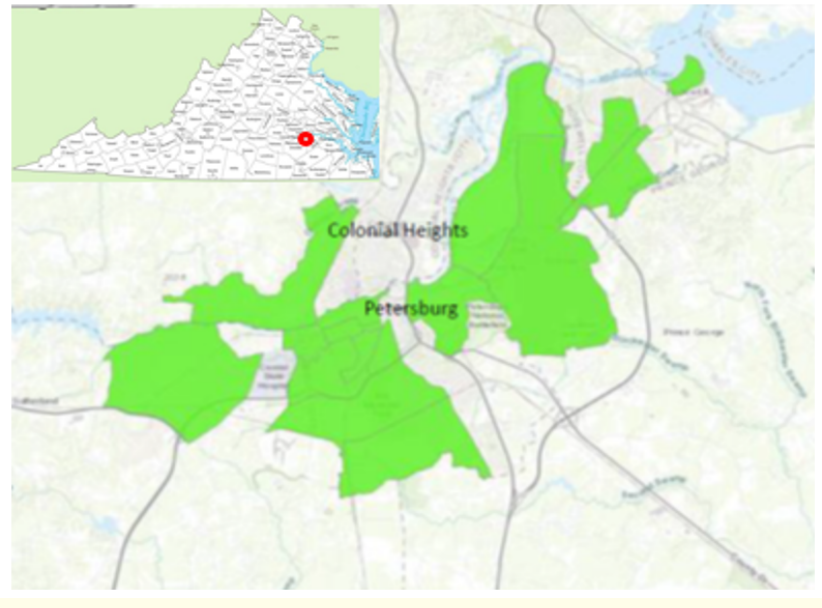

Figure 1: *Map of Virginia (insert, http://ontheworldmap.com/usa/state/virginia/virginia-county-map.html) showing Petersburg and Colonial Heights (red-circled area). Study area (green shaded) of low income and low access areas (food desert) where residents must travel 1-10 miles to access supermarket is indicated. Map was generated using a food access research atlas [5].*

was aseptically transferred into a stomacher bag filled with equal weight of sterile PW. Each sample was then agitated and vigorously rubbed by gloved hand for 2 minutes to detach microorganisms. Appropriate dilutions of the homogenate were surface plated with a de-

tection limit of 100 cells per g for blendable products and 20 cells per g for non-blendable products, respectively, using standard method agar (SMA; unless otherwise stated, all media were from Bacto, BD, Sparks, MD) for total aerobic mesophile counts after incubation at 36°C for 48h [24].

The level of total coliform and *E. coli* were determined using the three-tube most-probable-number (MPN) evaluation with a detection limit of three cells per g [23]. After incubation at 36°C for 48h, a loopful of culture from each lauryl sulfate tryptose broth tube that produced gas was transferred to brilliant green bile broth (BGBB) and EC broth containing 4-methylumbelliferyl-b-D-glucuronide (EC-mug), respectively. After incubation for 48h, BGBB tubes with growth and gas production at 36°C confirmed the presence of coliforms, and EC-mug tubes with growth at 45.5°C and fluorescence under long-wave UV light at 365 nm indicated the presence of *E. coli* [23]. All positive EC tubes were streaked on eosin-methylene blue agar; purple colonies (with or without a green metallic sheen) were evaluated by API 20E test strips (bioMe´rieux, Hazelwood, MO) for *E. coli* confirmation. One randomly selected and confirmed isolate from each positive EC tube and API 20E test strip was used for further study.

Salmonella, *Listeria*, and *Campylobacter* were identified using performance tested methods [14,25]. For *Salmonella*, each sample homogenized with sterile PW was pre-enriched in buffered peptone water (225 ml) at 36°C for 24h, enriched in Rappaport-Vassiliadis broth at 42°C for 18h, and post-enriched in M broth at 36°C for 8h. M broth cultures were surface-streaked onto xylose lysine deoxycholate (XLD) agar for isolation. The colonies of assumptive *Salmonella* on XLD agar were confirmed with the API 20E test. For *Listeria*, each sample homogenized with sterile PW was enriched in the University of Vermont Medium (UVM) *Listeria* enrichment broth at 30°C for 48h before one loopful of the enrichment broth was surface-streaked onto Oxford *Listeria* (OL) agar for isolation. The colonies of assumptive *Listeria* on OL agar were identified to species with the API *Listeria* kit. For *Campylobacter*, each sample homogenized with sterile PW was enriched in modified Bolton broth (OXCM0983, Oxoid Ltd., Basingstoke, UK) supplemented with 5% laked horse blood (R54072, Thermo Fisher/Remel™, Lenexa, KS) and Bolton broth selective supplement (OXSR0183E, Oxoid Ltd.) at 42°C for 48h. Enrichment broth cultures were surface-streaked onto a modified *Campylobacter* blood-free selective agar (CM0739) with cefoperazone and amphotericin B (Antibiotic Supplements SR0155E, Oxoid Ltd.) and incubated microaerobically using the AnaeroPack System with Pack-MicroAero (Mitsubishi Gas Chemical, New York, NY) at 42°C for 48h. Colonies with *Campylobacter*-like morphology on the plates and gram-negative seagull-like cell morphology under a light microscope were presumed to be *Campylobacter* [24].

For the confirmation of *Campylobacter* spp., *Campylobacter* DNA was extracted from presumptive *Campylobacter* isolates in Bolton broth using a boiling method. Briefly, 2 ml of broth was centrifuged at 10,000 rpm for 4 minutes and the supernatant was discarded. One ml of molecular-grade water was added to the bacterial pellets and re-suspended into the solution by vortexing. The suspension was centrifuged again for 4 minutes at 10,000 rpm, and the supernatant was discarded. In the final step, 300 µl of molecular-grade water were added to the pellet and re-suspended by vortexing. This was followed by heating the bacterial suspension at 100°C for 20 minutes. The sample was centrifuged at full speed (14,600 rpm) for 4 minutes and the supernatant containing the DNA was transferred into new tubes. The DNA concentration was measured using a NanoDrop 2000C spectrometer (Thermofisher, MA) and subsequently stored at -80°C until PCR was performed.

A conventional PCR using primers targeting the *Campylobacter* spp. conserved 23sRNA gene was used to confirm isolates. Isolates were confirmed using a *C. jejuni*- and *C. fetus*-specific SYBR green-based real-time PCR assay (Catalog No. A25741, SYBR® Green PCR Master Mix-Life Technologies, MA). The forward and reverse primer sequence used for *C. jejuni* was TATACCGGTAAGGAGTGCTGGAG and ATCAATTAACCTTCGAGCACCG (a 650bp region of conserved 23sRNA gene), respectively. The forward and reverse primer sequence used for *C. fetus* was GCAAATATAAATGTAAGCGGAGAG and TGCAGCGGCCCCACCTAT, respectively. The conventional PCR protocol utilized the Amplitaq 360 gold master mix kit with recommended conditions at an annealing temperature of 60°C. Positive and negative controls were included for all reactions. Samples were run on a 1.5% agarose gel and visualized under UV light using the E-Gel Imager (Thermofisher).

All confirmed *Campylobacter*, *E. coli*, and *Listeria* isolates obtained above were suspended in brucella broth containing 20% glycerol and stored at -80°C until used for further evaluation of antimicrobial resistance (AMR).

Antimicrobial resistance: Following the procedure described by Kim., *et al*. [14], antimicrobial susceptibility tests were performed on Mueller Hinton Agar (MHA) using the Kirby-Bauer disk diffusion method [26]. In brief, the confirmed *Campylobacter*, *E. coli*, and *Listeria* isolates were tested for susceptibility to 12 antimicrobial agents, approved by the US Food and Drug Administration for clinical use, and their categories are shown in table 3. The following 12 antimicrobial agents acquired from Oxoid, Ltd. were tested: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), meropenem (MEM), amikacin (AMK), gentamycin (GEN), streptomycin (STR), tobramycin (TOB), tetracycline (TCY), ciprofloxacin (CIP), nalidixic acid (NAL), chloramphenicol (CHL), and trimethoprim-sulfamethoxazole (SXT). Antimicrobial susceptibility, classified as "susceptible", "intermediate", and "resistant", was interpreted in accordance with criteria established by the National Committee of Clinical Laboratory Standards [26]. In addition, bacteria classified as either resistant or intermediate were defined as "non-susceptible", and those exhibiting resistance to at least one antimicrobial agent in three or more antimicrobial categories were defined as multi-drug resistant (MDR) [27,28]. *E. coli* ATCC 25922 was used as a control strain for the performance of antimicrobials used in this study.

Antimicrobial category	Antimicrobial agent and its abbreviation	Concentration (µg/disk)	Zone diameter (mm)		
			S	I	R
Penicillins	Ampicillin (AMP)	10	>17	14-16	<13
β-lactamase inhibitor combinations	Amoxicillin-clavulanic acid (AMC)	30	>18	14-17	<13
Carbapenems	Meropenem (MEM)	10	>23	20-22	<19
Aminoglycosides	Amikacin (AMK)	30	>17	15-16	<14
	Gentamicin (GEN)	10	>15	13-14	<12
	Streptomycin (STR)	10	>15	12-14	<11
	Tobramycin (TOB)	10	>15	13-14	<12
Tetracyclines	Tetracycline (TCY)	30	>15	12-14	<11
Fluoroquinolones	Ciprofloxacin (CIP)	5	>21	16-20	<15
Quinolones	Nalidixic acid (NAL)	30	>19	14-18	<13
Phenicols	Chloramphenicol (CHL)	30	>18	13-17	<12
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (SXT)	25	>16	11-15	<10

Table 3: *A list of antimicrobials and interpretive criteria used in this study (CLSI 2015)*.*

**Interpretive criteria: S, Susceptible; I, Intermediate; and R, Resistant to antimicrobial agents tested.*

***Campylobacter*:** One loop of confirmed *Campylobacter* was transferred into 5 ml of Modified Bolton Broth (MBB) and incubated at 42°C for 24h. Then, 0.1 ml of each *Campylobacter* in MBB was transferred into a new 10 ml MBB and incubated at 42° for 24h. One-tenth ml of each MBB, adjusted to approximately 8 log CFU/ml, was transferred to blood agar and spread uniformly. Before applying the antimicrobial discs, the plates were left for 10 minutes to allow any excess surface moisture to be absorbed. Then, antimicrobial discs were transferred by using a 12-capacity disc dispenser. Plates were incubated for 24h at 42°C with a Pack-MicroAero and the inhibition diameter zones were measured in millimeters for each plate with a caliper and recorded for each sample. *E. coli* ATCC 25922 cultured and sub-cultured in Mueller Hinton Broth (MHB) as described under *E. coli* and *Listeria* section below was used as a control.

***E. coli* and *Listeria*:** One loop of each confirmed *E. coli* and *Listeria* isolate was transferred to 10 ml of MHB and incubated at 36°C for 24h. The isolates were again sub-cultured in MHB broth to ensure that they were all viable and fresh before antimicrobial resistance testing. *E. coli* ATCC 25922 was also cultured and sub-cultured similarly in MHB. One-tenth ml of each MHB, adjusted to approximately 8 log CFU/

ml, was transferred onto MHA plates and spread uniformly. Before applying the antimicrobial discs, the plates were left for 10 minutes to allow any excess surface moisture to be absorbed. Then, antimicrobial discs were transferred onto the plates by using a 12-capacity disc dispenser. Plates were incubated for 24h at 36°C and the inhibition diameter zones were measured in millimeters for plate with a caliper and recorded for each sample.

Data analysis: Log-transformed microbial (aerobic mesophile, coliform, and *E. coli*) populations obtained from duplicates of each sample were averaged and subjected to an analysis of variance and Duncan's multiple range test (SAS Institute, Cary, NC) to determine the significance of the differences ($P < 0.05$) in mean populations of microorganisms. SAS correlation analysis (SAS Institute, Cary, NC) was implemented to evaluate prevalence relationship among *Campylobacter*, *E. coli* and *Listeria* investigated (0 = absent; 1 = present). Associations were considered significant when $P < 0.05$.

Results and Discussion

Using the food safety inspection checklist (Table 1) established by the VBDH, MCDH, and SDDH, 12 (63%) out of 19 markets assessed did not comply with at least one element in the checklist (Data not shown). In specific, approximately 56% (5/9) LCM and 70% (7/10) SIM outlets were out of compliance for at least one element. All produce at both LCM and SIM was stored 6-8 inches off the floor and there was no evidence (i.e., droppings, gnaw marks) of pests in both types of markets. In Food Storage Practices, 100% LCM and 100% SIM sell produce in wholesome and good condition whereas 11.1% of SIM did not comply with the guideline of health department for food storage rooms that should be clean and organized. As for the Cold Storage of Food, only 77.8% of LCM and 50% of SIM stored produce at 41°F (5°C) or below, which was observed from the thermometer installed in the display case. It was noted that high percentage (50%) of violations at SIM was associated with the elements of missing or broken thermometers or refrigerator temperatures set above recommended range in the category of Food Cold Storage, which directly reflects the economic viability in the area. In addition, a rusty pipe was found with fresh produce in the refrigerator at one SIM. Approximately 22% (2/9) of LCM also had violations of either missing thermometers or temperatures set above the recommended range. Approximately 11% LCM and 10% SIM were observed with inappropriate separation of raw meat and poultry from produce in a cold storage section. Shelving in refrigerators were maintained clean in only 80% of SIM. In Employee Practices, approximately 22% of LCM and 30% of SIM employees did not follow health department guidelines of using good hygiene practices while handling food. The majority (60%) of violations at LCM were associated with employee hygiene element of not wearing hair restraints or gloves in the food preparation area whereas 40% (4/10) only of SIM outlets were out of compliance in the category. In addition, employees in 10% of SIM were observed to consume food in produce storage areas. The rate of non-compliance for LCM and SIM varied considerably, ranging from 0% to 22.2% and from 0% to 50%, respectively. Overall, average compliance rates of LCM and SIM were 93.8% and 85.4%, respectively.

Both LCM and SIM, in general, sold fresh produce at about the same price. However, the average prices of kale ($2.29 ± 0.33/lb) and turnip ($0.69 ± 0.00/lb) at SIM were about 2 times lower than the similar products at LCM, whereas bell pepper was about 1.8 times higher at SIM ($2.33 ± 1.23/lb) compared to LCM (Table 2). The higher price of a bell pepper at SIM may have to do with the product available in a limited quantity at SIM. Additionally, some of SIM studied in the area obtain the product from wholesalers or LCM rather than from local small-scale producers, and market the product to consumers (personal communications).

The observed difference between LCM and SIM include: 1) a relatively limited variety of fresh produce was available at SIM, 2) the majority of fresh produce sold at SIM had unknown origins, 3) the sale price at both LCM and SIM was about the same with the exception of few items, 4) the turnover rate for ownerships in SIM was higher than those in LCM, and 5) the majority of owners of SIM consisted of diverse ethnicity.

Microbial evaluation: Results of the levels of aerobic mesophile, coliform, and *E. coli* counts in the 122 samples analyzed are shown in table 4. Overall, there was high variability in the aerobic mesophile counts, depending on the types of fresh produce sold at LCM and SIM ranging from 3.48 ± 1.75 to 7.80 ± 0.37 log CFU/g and 5.80 ± 1.75 to 8.48 ± 0.23 log CFU/g, respectively. The mean aerobic mesophile

counts were highest in turnips (7.80 ± 0.37 log CFU/g) and lowest in tomatoes (3.48 ± 1.75 log CFU/g) for LCM and highest in cilantro (8.48 ± 0.23 log CFU/g) and lowest in tomatoes (5.80 ± 1.75 log CFU/g) for SIM. Although there was a limited availability of same commodities at different markets in the studied food desert area, aerobic mesophile counts in cilantro, collard green, and tomato obtained from SIM were significantly higher ($P < 0.05$) than those obtained from LCM. It is also noteworthy that despite having high bacterial counts (> 8.0 log CFU/g), samples such as cilantro and turnip showed no sensory signs of defect or spoilage.

Sample type	Number of samples	Market source	Microorganisms (log CFU/g or log MPN/g)[a]			
			Aerobic mesophiles	ICMSF (%)[b]	Coliforms	*E. coli*
Bell pepper	12	LCM	AB 6.45 ± 1.12 a	0.0, 58.3, 41.7	AB 2.78 ± 1.78 a	0.78 ± 0.00 a
	14	SIM	AB 6.91 ± 1.14 a	14.3, 35.7, 50.0	A 1.81 ± 1.49 a	0.78 ± 0.00 a
Cilantro	18	LCM	AB 6.98 ± 1.04 b	11.1, 16.7, 72.2	AB 2.71 ± 1.28 a	0.78 ± 0.00 a
	4	SIM	A 8.48 ± 0.23 a	0.0, 0.0, 100.0	A 3.18 ± 1.68 a	0.78 ± 0.00 a
Collard green	6	LCM	B 6.09 ± 0.89 b	16.7, 66.7, 16.7	BC 1.47 ± 0.87 a	0.78 ± 0.00 a
	6	SIM	AB 7.33 ± 0.55 a	0.0, 33.3, 66.7	A 2.56 ± 1.43 a	0.78 ± 0.00 a
Jalapeno pepper	4	LCM	B 5.85 ± 0.40 a	0.0, 100.0, 0.0	BC 1.96 ± 1.47 a	0.78 ± 0.00 a
	6	SIM	AB 7.28 ± 1.25 a	0.0, 33.3, 66.7	A 3.15 ± 1.26 a	1.80 ± 1.34 a
Kale	4	LCM	AB 6.85 ± 0.33 a	0.0, 75.0, 25.0	AB 2.59 ± 0.94 a	0.78 ± 0.00 a
	4	SIM	AB 7.27 ± 0.46 a	0.0, 25.0, 75.0	A 2.93 ± 0.88 a	0.78 ± 0.00 a
Tomato	18	LCM	C 3.48 ± 1.75 b	77.8, 22.2, 0.0	C 0.98 ± 0.52 b	0.78 ± 0.00 a
	18	SIM	B 5.80 ± 1.75 a	27.8, 44.4, 27.8	A 2.41 ± 1.52 a	0.81 ± 0.11 a
Turnip	6	LCM	A 7.80 ± 0.37 a	0.0, 0.0, 100.0	A 3.48 ± 0.69 a	0.78 ± 0.00 a
	2	SIM	A 8.31 ± 0.28 a	0.0, 0.0, 100.0	A 3.78 ± 1.27 a	0.78 ± 0.00 a

Table 4: *Level of microorganisms in the samples procured from large chain super markets (LCM) and small independently owned markets (SIM).*

[a]In the same column within the same market source and each microorganism, means preceded by the same uppercase letter are not significantly different (P > 0.05). In the same column within the same sample type, means followed by the same lowercase letter are not significantly different (P > 0.05). [b]Values are the percentages of samples with aerobic mesophile counts within the recommended range for good quality (≤ 5 X 10^5 CFU/g), marginally acceptable (5 X 10^5 to 5 X 10^7 CFU/g), and unacceptable (≥ 5 X 10^7 CFU/g), respectively, according to the limits established by the International Commission on Microbiological Specifications for Foods [29].

The International Commission on Microbiological Specifications for Foods [29] does not specify the limit of aerobic mesophile counts for raw vegetables and fruits for which production and processing history is not known. However, to better understand the microbiological quality of samples acquired for this study, the aerobic mesophile counts within the range of ≤ 10^5 CFU/g, 10^5 to 10^7 CFU/g, and ≥ 10^7 CFU/g were presented in table 4. Again, the majority (70% - 100%) of cilantro and turnip regardless of market source and kale obtained from SIM had aerobic mesophile counts > 7 log CFU/g. These results may indicate that commodities grown close to ground (soil) are prone to be contaminated with environmental microbiota resulting in higher microbial counts on the samples (i.e. cilantro, turnip) while those of peppers and tomato were somewhat apart from ground, resulting in lower microbial counts. However, approximately 28% of tomatoes procured from SIM had an aerobic mesophile count even greater than 7.0 log CFU/g. It is concerning given the consideration that this type of product may be consumed as ready-to-eat foods.

Furthermore, among all seven commodities, turnip obtained from both LCM (3.48 ± 0.69 log MPN/g) and SIM (3.78 ± 1.27 log MPN/g) had the highest coliform counts, respectively. The level of coliforms in tomatoes (2.41 ± 1.52 log MPN/g) acquired from SIM was signifi-

cantly ($P < 0.05$) higher than LCM-acquired tomatoes (0.98 ± 0.52 log MPN/g). In addition, although the level of *E. coli* detected in jalapeno peppers acquired from SIM was the highest (1.80 ± 1.34 log MPN/g), no significant difference among all samples, regardless market source, was observed.

Regarding the higher microbial levels observed in SIM samples than LCM-acquired ones in the present study, we speculate that the fresh produce in SIM may have been exposed to variations in temperature during display or in the store refrigerator, whereas LCM products on average were well-maintained at recommended temperatures (41°F or below) or handled better. This result is also consistent with the previous findings (Table 1) that SIM had a lower compliance rate (average of 85.4%) in the Food Storage and Employee Practices than LCM (average of 93.8%).

Bacterial prevalence: Prevalence of bacteria in the fresh produce samples analyzed in this study is shown in table 5. *Campylobacter* and *E. coli* were detected in 13 (10.7%) and 6 (4.9%), respectively, out of 122 samples collected during the study period. Using the conventional PCR method, 13 out of 28 assumptive *Campylobacter* isolates from enrichment broth were confirmed positive based on the presence of a 650-bp band on the gel (Figure 2). Approximately, 11% of cilantro and 25% of kale obtained from LCM tested positive for *Campylobacter*. The majority (76.9%, 10/13) of *Campylobacter* detected from the samples were associated with SIM. It is also noteworthy that regardless of the market source, *Campylobacter* commonly linked to outbreaks associated with poultry, dairy products, and seafood [30] was detected from at least one of all fresh produce sample types tested in our study.

Out of six samples with the presence of *E. coli* (Table 5), eleven isolates were obtained from the highest population of the EC-mug three-tube MPN evaluation and confirmed as *E. coli* by the API 20E test kit. Eight different API profiles (prevalence in % per 11 isolates) obtained among 11 isolates are as follows: 1044572 (9.1%), 5044172 (9.1%), 5044512 (9.1%), 5044572 (18.2%), 5144542 (9.1%), 5144572 (18.2%), 7144172 (9.1%), and 7144572 (18.2%). The detection of *E. coli* in tomatoes procured from LCM and SIM in this study is concern-

Sample name	Number of samples	Market source	Number (%) of positive samples		
			Campylobacter	*E. coli*	*Listeria* spp.
Bell pepper	12	LCM	0 (0.0)[a]	1 (8.3)	0 (0.0)
	14	SIM	2 (14.3)	0 (0.0)	1 (7.1)
Cilantro	18	LCM	2 (11.1)	1 (5.6)	0 (0.0)
	4	SIM	0 (0.0)	1 (25.0)	0 (0.0)
Collard green	6	LCM	0 (0.0)	0 (0.0)	0 (0.0)
	6	SIM	2 (33.3)	0 (0.0)	0 (0.0)
Jalapeno pepper	4	LCM	0 (0.0)	0 (0.0)	0 (0.0)
	6	SIM	2 (33.3)	1 (16.7)	0 (0.0)
Kale	4	LCM	1 (25.0)	0 (0.0)	1 (25.0)
	4	SIM	2 (50.0)	0 (0.0)	1 (25.0)
Tomato	18	LCM	0 (0.0)	1 (5.6)	0 (0.0)
	18	SIM	1 (5.6)	1 (5.6)	0 (0.0)
Turnip	6	LCM	0 (0.0)	0 (0.0)	1 (16.7)
	2	SIM	1 (50.0)	0 (0.0)	0 (0.0)

Table 5: *Prevalence of bacteria (*Campylobacter, E. coli, and Listeria*) in the samples procured from large chain super markets (LCM) and small independently owned markets (SIM).*

[a]*Not detected.*

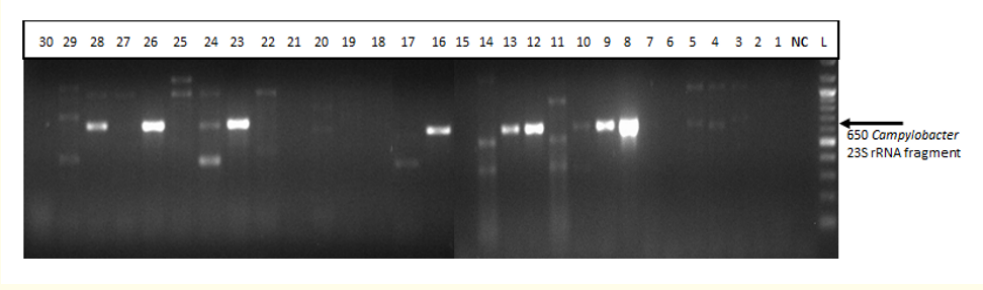

Figure 2: *A representative amplification fragments of multiplex PCR detection and identification of* Campylobacter *spp. isolated from samples. Lane L: 100-bp ladder, NC: PCR-negative control, Lanes 8 and 28: positive controls (*C. jejuni *ATCC 29428), Lanes 3, 4, 5, 9, 10, 12, 13, 16, 20, 22, 23, 24, and 26:* Campylobacter *positives.*

ing. Because the presence of *E. coli* in fresh produce indicates possible fecal contamination during harvesting, handling, and/or processing, these findings should be taken seriously given that pathogenic *E. coli* O157:H7 often can come from the same contamination sources [31,32]. In addition, as addressed earlier, it is particularly concerning given the consideration that this type of product may be consumed as ready-to-eat foods without any further processing of "kill step", which may eradicate any opportunistic pathogens from the product.

Listeria spp. was detected in four (3.3%) out of the 122 samples tested. *Listeria* spp. detected from kale and turnip obtained from LCM were *L. seeligeri/ivanovii* whereas *Listeria* spp. detected from bell pepper and kale obtained from SIM were *L. welshimeri*. Although samples tested revealed the presence of *Campylobacter*, *E. coli*, and *Listeria*, overall no correlation ($r < 0.09$, $P > 0.3059$) among the prevalence of the bacteria was observed. In specific, Pearson correlation coefficients for the prevalence of *Campylobacter*, *E. coli*, and *Listeria* in fresh produce procured from LCM and SIM were $r < 0.25$ with $P > 0.0393$ and $r < 0.28$ with $P > 0.044$, respectively. The very low correlation observed in the current study affirms our previous findings [14] that commodities with the presence of *E. coli*, the best bacterial indication of fecal contamination [33], does not warrant a presence of harmful, disease-causing microorganisms or vice versa.

In the meantime, none of the samples analyzed were detected with the presence of *Salmonella*. The prevalence of bacteria obtained in the current study was comparable to the prevalence found in our previous study [14] that surveyed 138 fresh produce samples from farmers' markets in the same region of VA. In the previous study, we detected *Campylobacter*, *E. coli*, and *Listeria* in 8.7%, 9.4%, and 8.0% of samples, respectively. The present overall prevalence of *Campylobacter* (10.7%) detected in the fresh produce obtained from the food outlets in food desert areas was considerably higher than farmers' market-acquired fresh produce (8.7%). However, the overall prevalence of *E. coli* (4.9%), and *Listeria* (3.3%) obtained from the present study was considerably lower than the previous study (9.4% and 8.0% for *E. coli* and *Listeria*, respectively). Differences among sample commodities, mode of display (i.e., refrigeration and open air) and transportation associated with farmers' market products and our present study could have led to the disparity in results. Market sanitary conditions during production, processing and retail, could also have contributed to the differences. Therefore, more information on contamination at different points in the production and supply chain associated with LCM, SIM, and farmers' markets is needed to interpret these differences.

Antimicrobial resistance: Prevalence of antimicrobial resistance in *Campylobacter*, *E. coli*, and *Listeria* isolates to the 12 antimicrobials tested are summarized in table 6. The susceptible, intermediate, and resistant patterns of *Campylobacter* isolates obtained from LCM and SIM in relation to the antimicrobials tested are presented in figure 3A and 3B, respectively. The majority (84.6%) of the *Campylobacter* isolates obtained from our study showed MDR (Table 6). All *Campylobacter* isolates obtained were resistant to at least one antimicrobial

agent. Of the total 13 *Campylobacter* isolates obtained, resistance to AMP was the most common in 84.6%, followed by CHL (69.2%), SXT (69.2%), AMC (53.8%), STR (53.8%), TOB (30.8%), and NAL (23.1%) (Figure 3A and 3B).

Bacteria	Nature of susceptibility[a]	Market source (n)[b]	Prevalence (%) of resistance or non-susceptibility to each quantity of antimicrobial agents[c]								
			1	2	3	4	5	6	7	8	MDR(≥3)[d]
Campylobacter spp.	R	LCM (3)	0.0	0.0	66.7	0.0	33.3	0.0	0.0	0.0	100
		SIM (10)	10.0	10.0	10.0	40.0	10.0	20.0	0.0	0.0	80.0
		Total (13)	7.7	7.7	23.1	30.8	15.4	15.4	0.0	0.0	84.6
	R+I	LCM (3)	0.0	0.0	0.0	33.3	0.0	33.3	33.3	0.0	NA[e]
		SIM (10)	0.0	10.0	10.0	0.0	20.0	30.0	30.0	0.0	NA
		Total (13)	0.0	7.7	7.7	7.7	15.4	30.8	30.8	0.0	NA
E. coli	R	LCM (5)	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	20.0
		SIM (6)	0.0	16.7	0.0	0.0	0.0	16.7	16.7	0.0	33.3
		Total (11)	0.0	9.1	0.0	0.0	0.0	9.1	18.2	0.0	27.3
	R+I	LCM (5)	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	NA
		SIM (6)	33.3	16.7	0.0	0.0	0.0	0.0	16.7	16.7	NA
		Total (11)	18.2	9.1	0.0	0.0	0.0	0.0	18.2	9.1	NA
Listeria spp.	R	LCM (2)	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		SIM (2)	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Total (4)	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R+I	LCM (2)	0.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	NA
		SIM (2)	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0	NA
		Total (4)	0.0	75.0	25.0	0.0	0.0	0.0	0.0	0.0	NA

Table 6: *Antimicrobial resistance prevalence of 13* Campylobacter, 11 E. coli, *and* 4 Listeria *isolates in fresh produce samples procured from large chain super markets (LCM) and small independently owned markets (SIM) between September of 2018 and April of 2019*. *Susceptibility categorization was carried out in accordance with interpretive criteria provided by the National Committee of Clinical Laboratory Standards recommendations [26]; [a]R: Resistant; I: Intermediate; R+I: Non-Susceptible to antimicrobial agents tested; [b]number of isolates tested; [c]prevalence (%) was presented in resistance and non-susceptibility of isolates to the total number of antimicrobial agents tested [i.e., an isolate exhibiting resistant and intermediate, respectively, to two and four antimicrobial agents was presented under 2 of Resistance and 6 of Non-susceptibility (R+I).]; [d]multidrug resistance; [e]not applicable.*

All three *Campylobacter* isolates detected in cilantro and kale obtained from LCM showed MDR (Table 5 and 6). These isolates were resistant to AMP and SXT in 100%, to STR and CHL in 66.7%, and to AMC in 33.3% (Figure 3A). In contrast, the isolates were susceptible to MEM, AMK, GEN, TCY, and CIP. One isolate displayed resistance (33.3%) and non-susceptibility (33.3%) to five and seven antimicrobials, respectively (Table 6). Among the 10 *Campylobacter* isolates obtained from SIM, resistance to AMP was also the most common in eight (80%) isolates, followed by CHL (70%), AMC (60%), SXT (60%), STR (50%), TOB (40%), and NAL (30%) (Figure 3B). These isolates were susceptible to MEM, AMK, GEN, and TCY in 100%. All types of samples except cilantro carried MDR *Campylobacter* isolate. In addition, two isolates showing the highest level of resistance were obtained from collard green and tomato, and displayed resistance to six antimicrobials, while three isolates showing the highest level of non-susceptibility were obtained from kale and turnip, and displayed non-susceptibility to seven antimicrobials.

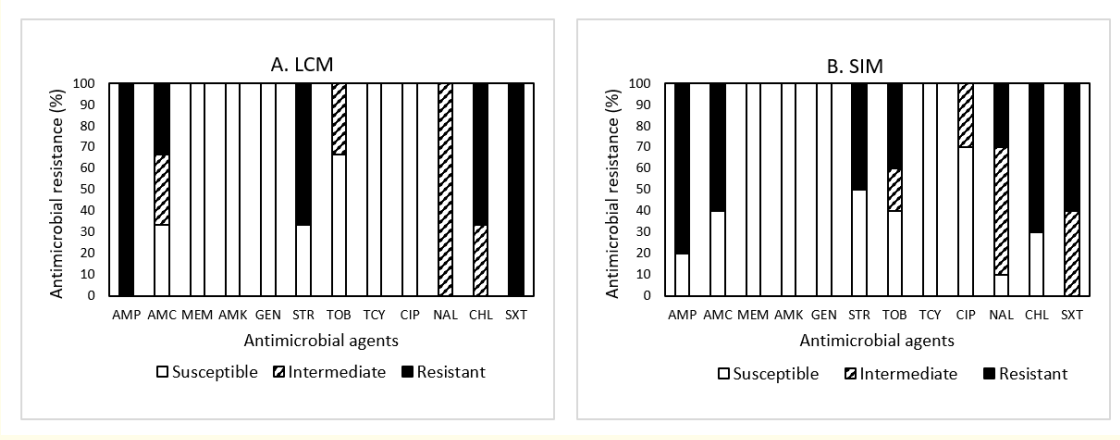

Figure 3A and 3B: *Prevalence of resistance to 12 antimicrobial agents in 3 and 10* Campylobacter *isolates in fresh produce procured from large chain super markets (LCM) and small independently owned markets (SIM), respectively.*

Our previous studies [14,33] on the prevalence of AMR in *Campylobacter* isolates obtained from farmers' markets, farm animals, wild-life, and food samples in the eastern United States found similar pattern of non-susceptibility to all the antimicrobials tested. In detail, Kim., *et al*. [34] reported that 97.4% of *Campylobacter* isolates obtained from farms were non-susceptible to at least one antimicrobial. Another study [14] also found that all *Campylobacter* isolates in fresh produce procured from farmers' markets in Central Virginia were non-susceptible to at least one antimicrobial agent and 91.7% of the isolates showed MDR. Their resistance to AMP was the most common in 100%, followed by AMC (91.7%), NAL (83.3%), CHL (83.3%), and SXT (75.0%). In addition, 66.7% of the isolates were resistant to all 12 antimicrobials tested. These isolates were obtained from green onion, rutabaga, leek, beet, and parsley.

Overall, although the prevalence of AMR in *Campylobacter* isolates obtained from SIM in our study demonstrated similar patterns of non-susceptibility to the LCM-obtained isolates, the prevalence of MDR in the isolates obtained from SIM (80%) was much lower than the prevalence from LCM (100%). The isolates obtained from SIM also revealed lower resistance to AMP and SXT compared to LCM-obtained isolates. Besides, 30% and 33.3% of *Campylobacter* isolates obtained from SIM and LCM, respectively, displayed non-susceptibility to seven antimicrobials. Lower prevalence of AMR, MDR, and non-susceptibility in *Campylobacter* isolates detected in fresh produce samples obtained from SIM (Table 6) may have to do with the economic viability of the small-scale production system mainly relying on organic processing. In the meantime, due to the management differences, *Campylobacter* isolates in samples obtained from LCM may have likely been exposed to the practice of antimicrobial usage during large-scale agricultural production systems. Several scientists reported that the practice of antimicrobial usage in agricultural production influences the prevalence of AMR in bacteria [35,36]. More importantly, although isolates obtained from both LCM and SIM were susceptible to MEM, AMK, GEN, TCY GEN and TOB, none of the isolates obtained from both LCM and SIM were susceptible to all antimicrobials tested in this study.

Among the 11 *E. coli* isolates, resistance to AMP, NAL, CHL, and SXT was the most common in 3 (27.3%) isolates, respectively, followed by AMC (18.2%), MEM (18.2%), STR (18.2%), TCY (18.2%), AMK (9.1%), and CIP (9.1%) (Figure 4A and 4B). Overall, 27.3% of *E. coli* isolates showed MDR (Table 6). Five (45.5%) isolates were susceptible to all tested antimicrobials, indicating that 54.5% of *E. coli* isolates were non-susceptible to at least one antimicrobial (Table 6). Prevalence of AMR in *E. coli* obtained in our study was comparable to the prevalence found in another study [14] on the prevalence and AMR in *E. coli* isolates obtained from fresh produce in farmers' markets of Central Virginia, which located in the proximity to the present study area. In the study, they found that 65.1% and 87% of *E. coli* isolates

were resistant and non-susceptible, respectively, to at least one antimicrobial. In addition, 17.4% of the isolates showing MDR were obtained from bell pepper and sweet potato. In their study, the resistance of *E. coli* isolates to AMP was the most common in 47.8%, followed by STR (34.8%), AMC (26.1%), TCY (8.1%), SXT (8.7%), and NAL (4.3%).

The present study revealed that the majority (80%) of *E. coli* isolates obtained from LCM were susceptible to all antimicrobials tested whereas one isolate obtained from tomato showed resistant to seven antimicrobials (Table 6). This isolate was resistant to AMP, AMK, TCY, CIP, NAL, CHL, and SXT (Figure 4A). In contrast, the isolate was susceptible to AMC, MEM, GEN, STR, and TOB. Among the six *E. coli* isolates obtained from SIM, resistance to AMP, AMC, MEM, STR, NAL, CHL, and SXT was the most common in two (33.3%) isolates each, followed by TCY (16.7%) (Figure 4B). These isolates were susceptible to AMK, GEN, TOB, and CIP in 100%. Five (83.3%) isolates were non-susceptible to at least one antimicrobial whereas one isolate only showed susceptibility to all antimicrobials. In addition, two isolates showing the highest level of resistance and non-susceptibility were obtained from tomato and cilantro, and displayed resistance and non-susceptibility to six and seven antimicrobials, respectively.

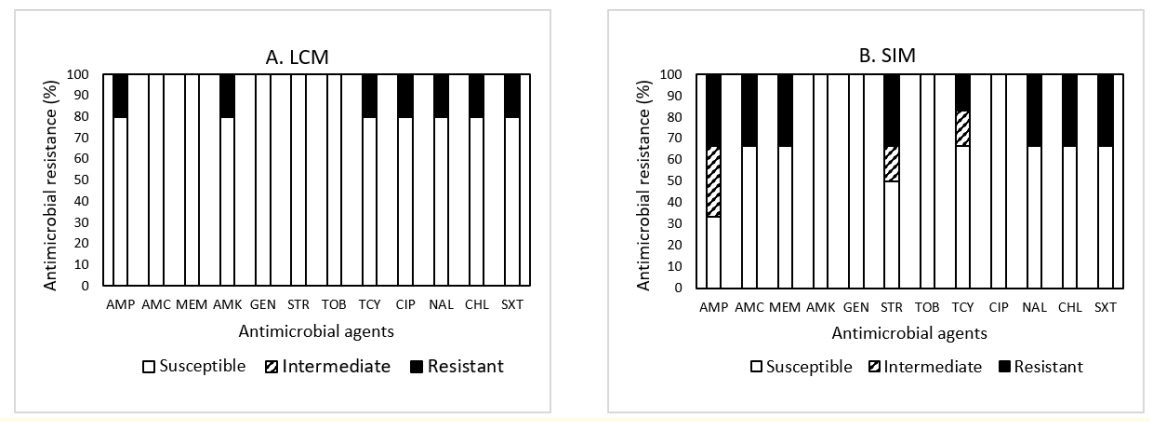

Figure 4A and 4B: *Prevalence of resistance to 12 antimicrobial agents in 5 and 6* E. coli *isolates in fresh produce procured from large chain super markets (LCM) and small independently owned markets (SIM), respectively.*

Overall, although the prevalence of AMR in *E. coli* isolates obtained from SIM in our study demonstrated similar patterns of resistance to the LCM-obtained isolates, the prevalence of MDR in the isolates obtained from SIM (33.3%) was higher than the prevalence from LCM (20%). The isolates obtained from SIM revealed higher resistance to the majority of antimicrobials except AMK, TCY, and CIP compared to LCM-obtained isolates. Besides, 33.3% and 20% of *E. coli* isolates obtained from SIM and LCM, respectively, displayed non-susceptibility to at least seven antimicrobials. Higher prevalence of AMR, MDR, and non-susceptibility in *E. coli* isolated from fresh produce samples obtained from SIM (Table 6) was shown compared to LCM-obtained isolates. All isolates obtained from both SIM and LCM were completely (100%) susceptible to GEN and TOB only of antimicrobials tested in this study. Since the presence of *E. coli* is mostly associated with good handling practices (i.e., hygiene practices) rather than agricultural practices (i.e. application of antimicrobials), contradictory results of higher prevalence of AMR, MDR, and non-susceptibility in *E. coli* isolates obtained from LCM samples may not necessarily represent the exposure of the bacteria to antimicrobials during the production and processing practices.

The prevalence of resistance to antimicrobials in 4 *Listeria* isolates obtained in this study revealed that NAL resistance was most common (100%), followed by resistance to AMP (50.0%) (Figure 5A and 5B). In addition, 75% of the *Listeria* obtained showed intermediate to STR. The isolates were susceptible to the majority of antimicrobials tested in this study. None of the isolates showed MDR. However, all

of the *Listeria* isolates tested were resistant to at least one antimicrobial agent (Table 6). The prevalence of AMR in *Listeria* obtained in our study was comparable to the prevalence found in another study [14]. In the study, they found that the resistance of *Listeria* isolates to NAL was the most common in 72.7%, followed by AMP (63.6%). In addition, 54.5% of the isolates showing MDR were obtained from asparagus, bok choy, spinach, crimini mushroom, and green onion. The most effective antimicrobial tested in their study was CIP showing 100% susceptibility.

The present study revealed that all two *Listeria* isolates detected in turnip and kale obtained from LCM showed resistance to two antimicrobials (AMP and NAL) (Table 6 and figure 5A). One of the isolates obtained from kale additionally showed intermediate to STR. In contrast, two *Listeria* isolates obtained from bell pepper and kale of SIM showed resistant and intermediate to NAL only and STR, respectively (Figure 5B).

Overall, although the prevalence of AMR in *Listeria* isolates obtained from LCM in our study demonstrated similar patterns of non-susceptibility to the SIM-obtained isolates, the isolates obtained from LCM only revealed additional resistance to AMP (Figure 5A and 5B). In other words, the isolates obtained from LCM revealed higher resistance to the antimicrobials compared to SIM-obtained isolates. Besides, 100% of *Listeria* isolates obtained from both LCM and SIM displayed resistance to NAL.

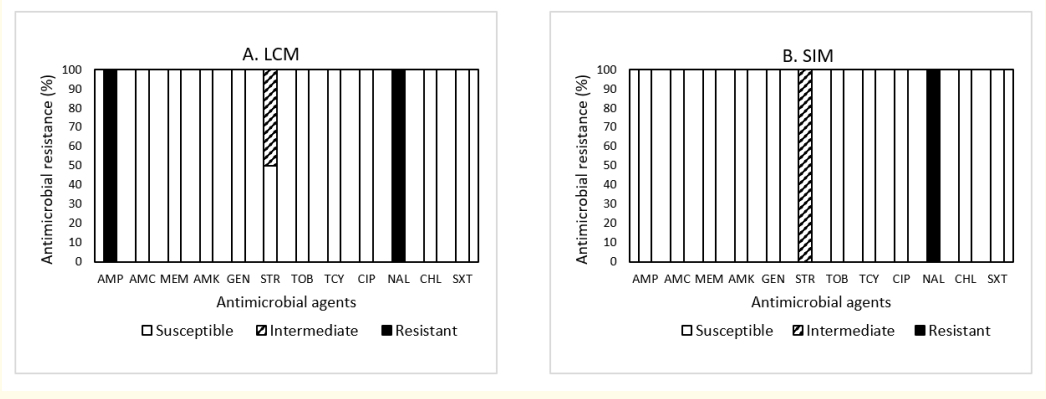

Figure 5A and 5B: *Prevalence of resistance to 12 antimicrobial agents in each 2* Listeria *isolates in fresh produce procured from large chain super markets (LCM) and small independently owned markets (SIM).*

The present survey revealed that the prevalence of MDR to 12 antimicrobials tested in the current study was the highest in *Campylobacter* (84.6%), followed by *E. coli* (27.3%), and *Listeria* spp. (0.0%) (Table 6). Among all the tested antimicrobials, AMP and NAL showed the highest frequency of resistance among *Campylobacter* (84.6%) and *Listeria* (100%), respectively. *Campylobacter* was most resistant to AMP, CHL and SXT. *E. coli* was most resistant to AMP, NAL, and CHL while *Listeria* were most resistant to NAL only demonstrating different resistance patterns among the bacteria in this study (Figure 3-5). The most effective antimicrobials tested in this study were MEM, AMK, GEN, and TCY for *Campylobacter*, and GEN and TOB for *E. coli*. Overall, the most effective antimicrobial was GEN showing 100% susceptibility to all of *Campylobacter*, *E. coli*, and *Listeria*. Findings from the present study revealed diverse AMR profiles and specificity in regard to fresh produce type, market source, and bacterial species in the study area.

Conclusion

This study demonstrated a potential for bacteriological health hazards associated with fresh produce obtained from LCM and SIM in the studied food desert area. Additionally, it emphasizes the importance of and need for good handling practices regardless of the market

source. The food safety compliance rate of LCM following the health department guidelines was higher compared to that of SIM, as demonstrated by the lower levels of bacterial counts and lower prevalence of *Campylobacter* observed in the respective samples. However, lower prevalence of AMR, MDR, and non-susceptibility in *Campylobacter* isolates and lower prevalence of AMR and non-susceptibility in *Listeria* isolates from SIM samples may have to do with the practices of small-scale producers. Small-scale, limited-resource producers likely may be less reliant on antimicrobials, and they market primarily to SIM due to a limited scale of production. In contrast, LCM purchase produce from large-scale producers, and samples of these items may have been exposed, either intentionally or incidentally, to antimicrobials. We suggest that the management differences in the production and processing practices of large-scale and small-scale producers may have influenced the prevalence of AMR in bacteria in fresh produce obtained from LCM and SIM. It is also noteworthy that many of the small-scale retailers we worked with spoke English as a second language and may have reading and comprehension challenges in their attempt to understand and apply complex food safety regulations. Additionally, SIM may have frequent turnover of ownership and management. Nevertheless, this study supports the hypothesis that economic viability in food desert areas may increase food safety risk for low-income residents heavily reliant on SIM to acquire fresh foods. The current research also shows that risks differ depending on circumstance; there are benefits and risks associated with microbiological quality of fresh produce at both LCM and SIM. Whereas the risk associated with overall microbiological contamination appears to be lower at LCM, the risk for exposure to AMR bacteria is higher. How to best address this disparity is a particularly vexing problem given the current litigious nature of society, but one approach might involve enhanced education both for consumers and producers at all scales of production and processing. Educational materials and approach may be developed to enhance understanding by translating information into retailer's preferred language. Regardless, continued research efforts on a larger-scale sample size with a greater diversity of products are warranted to determine the cause(s) of the observed differences in the prevalence of the pathogens and AMR profiles, and appropriate intervention and mitigation strategies to address and limit the potential foodborne illness in the area. This information will contribute to developing and disseminating future food safety training and educational programs for stakeholders associated with food establishment venues in food desert areas.

Acknowledgments

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Significance

This study is the first systematic approach documenting the unique food safety risks associated with economic viability at food outlets in food desert areas of Virginia. Continued research efforts on a larger-scale sample size with a greater diversity of products are warranted to determine the cause(s) of the observed differences in the prevalence of the pathogens and AMR profiles, and appropriate intervention and mitigation strategies to address and limit the potential foodborne illness in the area. Therefore, these findings will contribute to developing and disseminating future food safety training and educational programs for stakeholders associated with food establishment venues in food desert areas.

Disclaimer

This study simply indicates the occurrence and AMR of major opportunistic foodborne pathogens on randomly selected fresh produce commodities available at SIM and LCM food outlets in food desert areas of Virginia. Due to the limited availability of the same commodities at different food outlets between SIM and LCM, each item acquired in duplicate may not represent all fresh produce and fruits in the study area. However, the findings are noteworthy to understand the characteristics of opportunistic foodborne pathogens on those commodities sold in the studied food desert area in the broad spectrum. Furthermore, the mention of trade names or commercial products in this publication is solely for the purpose of providing specific information. It does not imply a recommendation or endorsement by Virginia State University. The authors want to state that this work does not, nor was it intended to, suggest that commodities available at SIM are

neither safer nor healthier than LCM acquired ones or vice versa. The authors would like to declare that this study was carried out, mainly for academic research purpose, without any conflict of interest.

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