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Evaluation of Antimicrobial Resistance in Enterobacteriaceae and Coliforms Isolated on Farm, Packaged and Loose Vegetables in Kentucky

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Abstract

Fresh produce normally carry epiphytic microorganisms; however, it can be contaminated with pathogenic bacteria. Categorized as 'ready-to-eat', most vegetables are consumed raw, thus, may present a food safety risk. Over the last three decades, global consumption of fresh vegetables has increased considerably, and the market has expanded by more than 20%. Concomitantly, the number of outbreaks involving fresh vegetables has increased significantly. Enterobacteriaceae members are involved in most of the bacterial outbreaks linked to fresh produce. There is a worldwide concern about the increased use of antimicrobials in agriculture. Antimicrobial resistant bacteria enter the food chain from the farm, often due to the use of animal manure. In the current study, the presence of entero-coliform bacteria and their antimicrobial susceptibilities in fresh vegetables sampled from small farms to retail was evaluated. Samples of vegetables were (i) collected directly from small farms from central Kentucky (n=59) and (ii) from four supermarkets in Frankfort, KY including loose and pre-packaged produce (n=72), analyzed for isolation of entero-coliform species. Members of the Enterobacteriaceae were detected on 25% of farm and 40% of retail produce, respectively. Approximately, 61% of the packaged produce and 19.4% of the loose produce had entero-bacterial presence, respectively. Their resistance to fourteen common antimicrobials was tested using Kirby-Bauer method. Approximately, 63% of isolates from farm and 70% of isolates from retail produce displayed resistance to at least three antimicrobial agents, while 18% of the isolates from farm and 41% from retail samples displayed resistance to at least ten antimicrobial agents. We conclude that 'ready-to-eat' fresh vegetables can be a source of exposure to pathogens with multiple drug resistance (MDR), defined as resistance to at-least three antimicrobial agents, leading to greater risks in immunocompromised individuals, and may serve as reservoirs for resistance gene transfers in human colon.

Keywords: Enterobacteriaceae; Produce; Farm; Retail; Packaged; Loose; Antimicrobial resistance; Multiple drug resistance

Introduction

Over the last three decades the global consumption of fresh vegetables has increased significantly, thus the market segment for fresh produce has expanded by more than 20% [1]. A survey conducted on American consumers' choice of supermarkets revealed that freshness of the produce and its availability across the year was the single most deciding factor. In an effort to understand consumers' attitudes toward produce packaging, the Produce Marketing Association, Yerecic Label and the Perishables Group conducted a three-part study. Almost 90% of the participants responded that the most important feature of packaged produce is its ability to preserve freshness and taste [2]. All this has led to a greater availability of "four range" produce, a term that refers to packaged, cleaned, possibly chopped and sometimes-mixed produce ready for consumption. Consumers also mentioned that they are attracted to packaged produce because of labels that contain information about the origin of the produce, recipes and cooking ideas, nutritional information, and sell-by-date [3].

Fresh fruits and vegetables available at retail supermarkets, unlike produce at the farm have gone through the entire process of the food supply chain. These products are vulnerable for potential contamination at multiple avenues as they go through farm production, processing, distribution, and retail. Some of these products have also gone through packaging, which can create an additional chance for contamination to occur. In the United States, more than 1500 different types of packagings are in use including items such as bags, crates, hampers, baskets, cartons, bulk bins, and pallets [4].

Classified as 'ready-to-eat' food, most fresh fruits and vegetables can be and are consumed raw, without needing further processing, such as cooking, thereby can pose a food safety problem. Most fresh vegetables normally carry non-pathogenic epiphytic microorganisms, however, contamination at the farming sites can arise because of various types of soil treatments such as organic fertilizers, which include sewage sludge and manure, use of contaminated irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables [3,5]. The number of reported outbreaks involving fresh vegetables have increased significantly. The top five most concerning foodborne pathogens associated with recent outbreaks involving fresh fruits and vegetables were *Listeria monocytogenes*, *Salmonella* (non-typhoidal serotypes only), *Escherichia coli* (E.coli) O157:H7, *E. coli* non- O157 STEC, and *Campylobacter* [6]. On average, *Listeria monocytogenes* causes 1600 illnesses each year in the U.S. Of these illnesses, 1400 result in hospitalizations, Resulting in 250 deaths [7]. Since March 2016, the Centers for Disease Control and Prevention (CDC) has been collaborating with public health officials in several states and the US Food and Drug Administration (FDA) to investigate a multistate outbreak of *Listeria monocytogenes* infections [8]. In March 2016, a specific brand of pistachios were implicated in a *Salmonella* outbreak

[9]. There was a multistate outbreak of Shiga toxin-producing *Escherichia coli* O157 infections linked to Alfalfa sprouts produced by Jack & The Green Sprouts [10].

Enterobacteriaceae family of bacteria have a natural habitat in the digestive tract of warm-blooded animals. They are commonly found in soils, plants, and in water [11] and can survive in soil depending on the soil type, temperature and moisture content [12,13]. Many of these bacteria found in fresh produce carry resistance factors to multiple antimicrobials, and thereby pose additional safety concerns for consumers [14,15].

There is a worldwide concern about the increased prevalence of antimicrobial resistance in bacteria. In 2011, 13,569,037 kg of antibiotics were used in production of food animals in the US. Of these antibiotics, the FDA deemed only 8,255,697 kg as "medically important" [16]. In the conventional livestock industry, animals are usually maintained indoors in small and unsanitary living conditions, fed antibiotics mixed in the feed, sometimes daily, as a "medically important" substitute to their living conditions [17]. The remaining amount of these antibiotics found a use mostly for promoting feed efficiency, weight gain, and faster production rates. Thus, commercial animal husbandry, especially involving pig and cattle are the largest users of antibiotics as growth promoters [18]. Antimicrobial resistant bacteria enter the food chain from the farm environment, often through water runoff, animal manure, and spread to agricultural plants [19]. The genes for antibacterial resistance have the potential for horizontal transfer to other related and non-related species, including the gastro-intestinal tracks of livestock, to the manure and can survive even in composted animal waste.

The nonselective and widespread use of antimicrobial agents in food animal production systems remains to be the main cause for increased resistance in pathogenic bacteria [20,21]. Most antimicrobial agents used in the food production system are the same antibiotics used in treating humans [22]. Since 1995, there has been a significant increase in the use of fluoroquinolones in treating and preventing *E. coli* infections in chicken and turkeys prior to slaughter [23]. Over the past decade, there has been a 25% increase in fluoroquinolone resistance for treating human *E. coli* infections [24]. Other top antibiotics used in the pig and cattle industry include β-lactams, such as various penicillins, and other classes of antibiotics such as macrolides, and tetracyclines.

Bacteria with antimicrobial resistance are found in soil, and water even in produce farms that do not use manure for fertilization. The likely source of the antimicrobial resistant bacteria in such farms include water runoff, often from neighboring food animal farms. Many growers create buffer zones of unfarmed land to try to alleviate the risk of water runoff from neighboring farms. However, information regarding the effective location and size of the buffer zone required to minimize the risk of water runoff from such farms is yet unclear [25]. Another potential source for developing antimicrobial resistance in produce is through their traces in municipal water. Although water treatment has been shown to remove most of the antibiotics, studies show that traces of certain classes of antibiotics do remain [26]. A study conducted in Spain reported sulfonamides to be the most commonly detected antibiotics in sewage sludge and soil [27].

There are a number of reports of antimicrobial resistance in bacteria found in meat products [28,29]. However, there are relatively fewer such reports on produce [30].

In the current study, following objectives were pursued. (1) Determine the presence of Coliform and Enterobacteriaceae on produce from farms and packaged and loose varieties of produce from retail supermarkets as a risk factor for consumers; (2) Determine the antimicrobial resistance profiles of Coliform and Enterobacteriaceae members isolated from farm and retail produce samples.

Materials and Methods

Sample collection

Farm samples: During May to September 2014, twenty small farms were visited 1 to 3 times. Consenting small and limited- resource farmers were contacted and recruited through a mailing list from Kentucky State University's current outreach and extension programs, such as the Small Farm Program, Third Thursday Thing, the Socially Disadvantaged Farmer Outreach Project and the Organic Association of Kentucky. Each farmer was given a survey that explored details regarding certification status, fertilization practices (such as the type(s) of manure or compost and/or chemicals, age of the manure or compost and the time of application), the source of irrigation water, surrounding land use, handling practices during harvesting, post harvesting and handling practices such as washing, packaging and storage.

During each visit, 1 to 2 samples of produce were randomly picked from various locations on the field and immediately put into zip-lock bags that were wiped with 70% alcohol. The sample size for small vegetables and fruit was less than 100 grams. Samples from each were assigned a code ID number, and were labeled with the date, immediately placed in a cooler, and transported to the laboratory (Frankfort, KY) for analyses. Samples were kept in the refrigerator until analysis began. A total of 59 samples was collected from twenty farms, which included conventional and organic (certified and non-certified) farms.

Retail samples: During December 2014 to March 2015, three select varieties of vegetables (tomatoes, carrots and leafy greens), were collected at random, from 4 different grocery supermarkets, two of which were large chain supermarkets, and two were small supermarkets in a lower income area from Frankfort, KY. Each supermarket was visited thrice. From each supermarket, 50% of produce samples were packaged and remaining 50% samples were from loose varieties. During each visit, packaged and loose vegetables of three varieties were picked from the shelf and immediately put into zip-lock bags that were wiped with 70% alcohol. For supermarkets that did not offer both packaged and loose varieties of carrots, potatoes were substituted. The sample size for the vegetables was less than 100 grams. Samples from each were assigned a code ID number, and were labeled with the date, immediately placed in a cooler, and transported to the laboratory for analyses, which began within 24 h after collection. A total of 72 produce samples was collected from four supermarkets and each sample was tested in duplicate for detection of Coliforms and Enterobacteriaceae.

E. coli and Enterobacteriaceae identification

Using aseptic conditions, 10 grams of sample was then mixed with 100 mL of lauryl sulfate tryptose broth (LST; Fisher Scientific, Hanover, IL), and pummeled in the 400 circulator machine for 5 min, at 230 rotations per minute(RPM) (Seward Limited, London, UK). The mixture was then placed in 9 mL of LST, in serial dilutions up to 10^{-4}.

One milliliter of the serial dilution of 10^{-3} was plated onto *E. coli*, and Enterobacteriaceae Petrifilm plates (3M Company, Maplewood, MN) and 100 µL of the serial dilution of 10^{-3} was plated onto Eosin Methylene Blue (EMB; Fisher Scientific, IL) agar plates in duplicate, and placed into the incubator at 37ºC for 48 h. Predominant Enterobacteriaceae isolates in the produce samples were identified using API 20E kit for biochemical characterization (Bio-Merieux Inc., Marcy I'Etoile, France). The API 20E testing strips were read and final identification was made using API LAB PLUS computer software (Bio-Merieux Inc., France).

Analysis of *E. coli* O157:H7 was conducted for *E. coli* positive samples from the Petrifilm plates. The *E. coli* colonies were directly transferred from the Petrifilms into Nutrient broth (Remel, Lenexa, KS), and incubated at 37ºC, 24 h. One-hundred microliters of the sample was plated onto CHROMagar O157 (CHROMagar Microbiology, Paris, France) agar, and placed into the incubator at 37ºC, 48 h. *E. coli* O157:H7 was confirmed when characteristic mauve colored colonies in the background of blue or steel gray colonies were observed.

Isolation of Salmonella spp.

Colonies first identified through the API 20E biochemical testing kit to be positive for *Salmonella* were placed into 5 mL of nutrient broth and incubated at 37ºC, 24-48 h to stimulate growth. One milliliter of the nutrient broth cultures were transferred into 10 mL tetrathionate broth and incubated at 47ºC, 24 h for selective enrichment. Tetrathionate enrichment cultures (250 µL) were spread onto selective Xylose Lysine Tergitol 4 agar (XLT4, Difco, Becton Dickinson, Sparks, MD) and incubated at 37ºC, 24 h. The plates were evaluated for colonies typical of Salmonella species after 24 h of incubation. The colonies were also subjected to a second API biochemical testing for final confirmation.

Statistical analysis

IBM SPSS statistical software (Chicago, IL) and Microsoft Excel were used for statistical analyses. The Pearson Chi-Square was used to analyze the differences between groups. Statistical significance was defined at $P<0.05$.

Antimicrobial susceptibility

The antimicrobial susceptibility testing was performed by the standard CLSI (formerly known as NCCLS) using the Kirby Bauer disk diffusion technique with Mueller-Hinton agar (Fisher Scientific, IL) [31]. The antibiotics used in this study were amikacin (30 µg), amoxicillin (30 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftiofur (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), and trimethoprim (5 µg). Bacterial cultures were grown in 5 mL of nutrient broth at 37ºC, 24 h. Each overnight culture was spread evenly onto Mueller-Hinton agar and incubated at 37ºC, 48 h and the zones of inhibition were measured.

Results

Farm samples

The occurrence of all Enterobacteriaceae including Coliforms isolated from farms and their antimicrobial resistance profiles is presented in Table 1. The most common strain identified from Enterobacteriaceae was Pantoea spp. (27.3%). Other potentially pathogenic isolates include *Cronobacter sakazakii* (18.2%), *Serratia marcescens* (18.2%), and *Stenotrophomonas maltophilia* (9.1%). While *Cronobacter sakazakii* and *Serratia marcescens* were resistant to nine antibiotics, *Pantoea spp* isolates were resistant to thirteen of fourteen antibiotics tested.

Bacteria	% Detected	Antibiotic Resistance
Cronobacter sakazakii	18.2	Kanamycin, Ciprofloxacin, Nalidixic acid, Chloramphenicol, Gentamycin, Ceftiofur, Amikacin, Ceftriaxone, Trimethoprim.
Enterobacter cloacae	9.1	Amoxicillin, Ceftiofur, Amikacin, Ceftriaxone, Nalidixic acid, Chloramphenicol, Gentamycin, Ampicillin, Tetracycline, Kanamycin, Trimethoprim.
Pantoea spp.	27.3	Amoxicillin, Ceftiofur, Cefoxitin, Amikacin, Nalidixic acid, Chloramphenicol, Gentamycin, Ampicillin, Tetracycline, Ciprofloxacin, Streptomycin, Kanamycin, Trimethoprim
Proteus mirabilis	9.1	Ceftiofur, Ceftriaxone, Streptomycin, Trimethoprim.
Serratia marcescens	18.2	Amoxicillin, Nalidixic acid, Ciprofloxacin, Gentamycin, Ampicillin, Tetracycline, Streptomycin, Kanamycin, Trimethoprim.
Serratia liquefacians	9.1	Ampicillin, Trimethoprim
Stenotrophomonas maltophilia	9.1	Gentamycin, Trimethoprim

Table 1: Occurrence (%) and Resistance pattern of Enterobacteriaceae isolated from farm produce.

Retail Samples

Approximately 40.3% of the samples tested positive for presence of Enterobacteriaceae. Of the packaged produce samples, 61.1% tested positive for Enterobacteriaceae, while only 19.4% of the loose produce samples tested positive for Enterobacteriaceae, and this was statistically significant ($p \leq 0.03$) (Table 2).

Three different types of loose and packaged vegetables (leafy greens, tomatoes, and carrots) were collected during each experiment. These produce types were chosen based on a previous study in which higher contamination of *E. coli* was observed in produce samples that grew at or below the surface of the plant compared to produce samples that grew above the surface [32]. Each vegetable was chosen as representative of the placement, i.e. carrots as produce growing under the surface, leafy greens as produce growing at surface level, and tomatoes as produce growing above the surface level. As reported earlier [32], most Enterobacteriaceae were detected (18.5%) on carrots, followed by tomatoes and leafy greens.

Produce	Enterobacteriaceae positive samples[a] (%)	Enterobacteriaceae positive samples[a]	Total samples
Packaged	61.1 A	22	36
Loose	19.4 B	7	36
Total	40.3	29	72

Table 2: Detection of Enterobacteriaceae in packaged and loose produce samples from supermarkets [*]. [a]Pairs of data having different letters (A and B) were significantly different (P<0.05). [*]Pearson Chi-Square test was performed (TS=12.9906; df=1, p- value <0.05)

Bacteria	% Detected	Antibiotic Resistance
Acinetobacter baumannii	3.4	Amoxicillin, Ceftiofur, Amikacin, Cefoxitin, Ceftriaxone, Nalidixic acid, Chloramphenicol, Ciprofloxacin, Gentamycin, Ampicillin, Tetracycline, Streptomycin, Kanamycin, Trimethoprim.
Chryseobacterium indologenes	10.3	Amoxicillin, Ceftiofur, Cefoxitin, Ceftriaxone, Nalidixic acid, Chloramphenicol, Ciprofloxacin, Ampicillin, Tetracycline, Trimethoprim.
Proteus mirabilis	6.9	Ampicillin, Cefoxitin, Nalidixic acid, Trimethoprim.
Salmonella paratyphi A	3.4	
Serratia marcescens	17.2	Amoxicillin, Ceftiofur, Amikacin, Cefoxitin, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Gentamycin, Ampicillin, Tetracycline, Kanamycin, Trimethoprim.
Serratia liquefaciens	3.4	Ciprofloxacin, Gentamycin, Kanamycin, Nalidixic acid, Streptomycin, Trimethoprim
Stenotrophomonas maltophilia	31.0	Ceftiofur, Amikacin, Ceftriaxone, Trimethoprim.
Pasteurella pneumotropica	3.4	Ampicillin, Kanamycin, Trimethoprim
Pseudomonas luteola	3.4	Ceftriaxone, Nalidixic acid, Trimethoprim.
Citrobacter braakii	3.4	
Burkholderia cepacia	3.4	
Citrobacter freundii	3.4	
Pantoea spp.	6.9	

Table 3: Occurrence (%) Enterobacteriaceae from supermarket samples and their resistance profile.

The occurrence of individual *Enterobacteriaceae* member from the retail supermarkets along with their antimicrobial resistance profile is presented in Table 3. As shown, the most common type of bacterial strain (31%) was *Stenotrophomonas maltophilia*. Other potentially pathogenic isolates were *Acinetobacter baumannii* (3.4%), *Serratia marcescens* (17.2%), and *Citrobacter freundii* (3.4%). While *Stenotrophomonas maltophilia* strains were resistant to four antibiotics, the remaining two isolates, *Serratia marcescens* and *Chrysobacterium indologenes* were resistant to at least ten of the fourteen antibiotics tested.

No *E. coli* was detected in any of the retail produce samples that were tested. Only one sample tested positive for *Salmonella spp*. As mentioned earlier, the most common isolate was *Stenotrophomas maltophilia* (31.0%). *S. maltophila* are gram-negative bacteria often found in soil, water, and plants. They are opportunistic pathogens, becoming increasingly more virulent, especially in hospitalized patients, and is associated with mortality rates of 14-69% in patients with bacteremia [33].

Resistance patterns

The prevalence of drug-resistant Enterobacteriaceae isolated from farm produce is represented in Table 4. The results indicate that all the Enterobacteriaceae isolates were resistant to trimethoprim (100%).

Antimicrobial Agent	Concentration (µg)	% Resistant	% Susceptible
Amoxicillin	30	36.4	63.6
Ceftiofur	30	36.4	63.6
Cefoxitin	30	27.3	72.7
Amikacin	30	36.4	63.6
Ceftriaxone	30	36.4	63.6
Chloramphenicol	30	36.4	63.6
Kanamycin	30	54.5	45.5
Ampicillin	10	72.7	27.3
Gentamycin	10	36.4	63.6
Ciprofloxacin	5	27.3	72.7
Streptomycin	10	36.4	63.6
Nalidixic Acid	30	45.5	54.5
Tetracycline	30	54.5	45.5
Trimethoprim	5	100	0
DR[1] ≥ 1		100	
MDR[2] ≥ 3		63.6	
MDR[3] ≥ 10		18.2	

Table 4: Prevalence of drug-resistant *Enterobacteriaceae* (%) from produce sampled from farms. [1]Drug resistance to one or more antimicrobials. [2]Microbial drug resistance to 3 or more antimicrobials. [3]Microbial drug resistance to 10 or more antimicrobials.

All Enterobacteriaceae isolates from farms and supermarkets were resistant to at least one antibiotic. Overall, 63.6% of the isolates displayed MDR to at least five antibiotics, whereas 18.2% of the isolates displayed MDR to at least-ten antibiotics. No statistically significant differences were observed in the MDR profiles of bacterial isolates from organically and conventionally raised produce.

The prevalence of drug-resistant Enterobacteriaceae isolated from retail produce is represented in Table 5. Generally, the results indicate that the Enterobacteriaceae tested were resistant to nalidixic acid (86.2%) and trimethoprim (75.9%). Approximately 70% of the isolates displayed MDR to at least five antibiotics, whereas 41.4% of the isolates displayed MDR to at least ten antibiotics.

The intent of the present study was to provide some assessment on the Enterobacteriaceae count, vis-à-vis, and the microbiological quality of packaged and loose produce available at retail grocery supermarkets and offer a comparative evaluation with produce on the farm. Based largely on unfounded reports that loose produce poses a greater risk of foodborne illness than packaged produce [34], however, there are few scientific reports on the microbiological analysis of packaged and loose retail produce [35].

Antimicrobial Agent	Concentration (µg)	% Resistant	% Susceptible
Amoxicillin	30	44.8	51.1
Ceftiofur	30	48.3	51.7
Cefoxitin	30	51.7	48.3
Amikacin	30	55.2	44.8
Ceftriaxone	30	58.6	41.4
Chloramphenicol	30	37.9	62.1
Kanamycin	30	55.2	44.8
Ampicillin	10	58.6	41.4
Gentamycin	10	51.7	48.3
Ciprofloxacin	5	55.2	44.8
Streptomycin	10	41.4	58.6
Naladixic Acid	30	86.2	13.8
Tetracycline	30	58.6	41.4
Trimethoprim	5	75.9	24.1
DR[1] ≥ 1		100	
MDR[2] ≥ 3		70	
MDR[3] ≥ 10		41.4	

Table 5: Prevalence of drug-resistant Enterobacteriaceae (%) from produce sampled from supermarkets. [1]Drug resistance to one or more antimicrobials. [2]Microbial drug resistance to 3 or more antimicrobials. [3]Microbial drug resistance to 10 or more antimicrobials.

Among the sampled packaged produce, various members of Enterobacteriaceae were detected in 61.1% of the sample compared to 9.4% of the loose varieties of the same produce. Packaging adds another element to the food production chain, allowing for another avenue for contamination in the entire food production process. One of the most common types of packaging is Modified Atmosphere Packaging (MAP), as it can increase the shelf life of the product. MAP changes the air level of the produce inside the packaging, but often times the oxygen level is below 1%, even though the FDA recommends the oxygen level reach 1-3% to maintain safety and quality of the produce [36].

The results of this study indicate that packaged produce was more contaminated with Enterobacteriaceae than loose varieties. In addition, since a certain packaging type (MAP) affects the oxygen level in produce affecting the overall quality, a vigorous evaluation of efficacy of various packaging types is required.

In efforts to lower the chances of foodborne outbreaks from produce the FDA offers tips on how to handle fresh fruits and vegetables safely. Although washing raw produce by the consumer is encouraged, the FDA mentions that there is no need to wash the produce prior to consumption if the package indicates that the produce is pre-washed. [5]. However, in 2010 Consumer Reports published a report on tests performed on 208 bags of "pre-washed" greens from 16 different brands of salad greens. In that study, while the top foodborne pathogens such as *E coli* O157:H7 and *Salmonella* were not found, the study reported that 39% of the packaged greens exceeded safe levels for coliform bacteria, and 23% had exceeded safe levels for Enterococcus bacteria, which are the two indicators of poor sanitation methods and fecal contamination [37,38].

Overall, all Enterobacteriaceae members isolated from both farm and retail produce samples were resistant to trimethoprim. A one-year study evaluating impact of production environment on the prevalence of antimicrobial resistance reported isolating *E.coli*, 4.5% of which were resistant to multiple antibiotics, including trimethoprim and sulfamethoxazole [39]. In another study from Jamaica, antimicrobial resistance of *Pseudomonas aeruginosa* isolated from fresh produce from retail and supermarkets reported isolating strains, 5% of which were resistant to at-least three antibiotics, including trimethoprim and sulfamethoxazole [40].

The Enterobacteriaceae isolated from farm produce displayed greater resistance to ampicillin (72.7%) than the Enterobacteriaceae isolated from supermarket produce (58.6%). For the farm produce samples, the most common isolate was *Pantoea spp.* (27.3%), commonly found in soil and plants. As observed in the previous studies, most of the *Pantoea spp.* isolated in the current study was resistant to aminoglycosides as well as to ampicillin [41,42]. The Enterobacteriaceae isolated from supermarket produce displayed greater resistance to nalidixic acid (86.2%) compared to the resistance (45.5%) found in Enterobacteriaceae from farm produce. The most common isolate from the supermarket produce was *Stenotrophomonas maltophilia* (31%), commonly found in water, soil, and plant roots. *Stenotrophomonas maltophilia*, commonly found to be resistant to a number of quinolones, including nalidixic acid [43]. All the *Acinetobacter baumannii*, *Cronobacter sakazakii*, and *Salmonella spp.* isolates showed MDR. The MDR strains have arisen in Enterobacteriaceae, and this is a concern because of their potential for widespread complications in management of infected patients [44]. Studies show the use of antibiotics in agriculture can drive the extensive transmission of antimicrobial resistant bacteria [45,46].

All of the Enterobacteriaceae isolated from farm and supermarket produce displayed resistance to at least one of the 14 antibiotics tested. However, only 18.2% of the Enterobacteriaceae isolated from farm produce displayed MDR to ten antibiotics, compared to 41.4% of the Enterobacteriaceae isolated from supermarket produce. Steps involved in processing and packaging of the produce may have contributed toward this factor. A study conducted in North Carolina analyzed fresh produce from harvest and then from the conveyor belt after rinsing. The study reported coliform levels, including Enterobacteriaceae, nearly doubled during the conveyor belt step [47]. These bacteria could have entered the supply chain from multiple possible points, and possibly increased the amount of drug resistance present on the product.

In our yearlong study on fresh produce from organic and conventional small farms, E. coli was detected on 25.4% of the fresh produce samples. A correlation was observed with fields that fertilized with manure in the past 90 days or less with the frequency of *E. coli* detection [27]. Presence of antibiotic resistance from farm produce was consistent with the number of farms (9 out of 20) that had used manure as the primary fertilizer source for their produce fields. It is not uncommon to find *E.coli* in soil amended with animal manure, which can remain for many years [48,49].

Conclusion

To the best of our knowledge, this is the first report of evaluating antimicrobial resistance in Enterobacteriaceae isolated on farm, packaged and loose vegetables in Kentucky. The results of this study indicate that species of Enterobacteriaceae with MDR were detected in farm and retail produce. Resistant zoonotic bacteria reach the human population through a variety of pathways, including direct contact, manure use, and food consumption. Observance of hygiene can play an important role in ensuring food safety and controlling the transmission of resistant bacteria from produce.

Although this study reports the occurrence of antibiotic resistant Enterobacteriaceae on produce, further susceptibility test using larger sampling sizes is needed to verify the occurrence of MDR Enterobacteriaceae in farm and retail produce and to systematically evaluate the challenges it poses to human health.

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