High prevalence of multidrug resistant Escherichia coli isolated from fresh vegetables sold by selected formal and informal traders in the most densely populated Province of South Africa

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Abstract

Abstract

Contaminated fresh produce has increasingly been implicated in foodborne disease outbreaks. As microbiological safety surveillance in South Africa is limited, a total of 545 vegetable samples (spinach, tomato, lettuce, cucumber, and green beans) were purchased from retailers, street traders, trolley vendors and farmers' markets. *Escherichia coli*, coliforms and Enterobacteriaceae were enumerated and the prevalence of *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* determined. *E. coli* isolates were characterized phenotypically (antibiotic resistance) and genotypically (diarrheagenic virulence genes). Coliforms, *E. coli* and Enterobacteriaceae counts were mostly not significantly different between formal and informal markets, with exceptions noted on occasion. When compared to international standards, 90% to 98% tomatoes, 70% to 94% spinach, 82% cucumbers, 93% lettuce, and 80% green bean samples, had satisfactory (≤ 100 CFU/g) *E. coli* counts. Of the 545 vegetable samples analyzed, 14.86% (n = 81) harbored *E. coli*, predominantly from leafy green vegetables. Virulence genes (*lt*, *st*, *bfpA*, *eagg*, *eaeA*, *stx1*, *stx2*, and *ipaH)* were not detected in the *E. coli* isolates (n = 67) characterized, however 40.30% were multidrug-resistant. Resistance to aminoglycosides (neomycin, 73.13%; gentamycin, < 10%), penicillins (ampicillin, 38.81%; amoxicillin, 41.79%; augmentin, < 10%), sulfonamides (cotrimoxazole, 22.39%), tetracycline (19.4%), chloramphenicol (11.94%), cephalosporins (cefepime, 34.33%), and carbapenemases (imipenem, < 10%) were observed. This study highlights the need for continued surveillance of multidrug resistant foodborne pathogens in fresh produce retailed formally and informally for potential consumer health risks.

Practical Application

The results indicate that the microbiological quality of different vegetables were similar per product type, regardless of being purchased from formal retailers or informal street traders, trolley vendors or farmers' markets. Although no pathogenic bacteria (diarrheagenic *E. coli*, *Salmonella* spp. or *L. monocytogenes*) were isolated, high levels of multidrug-resistance was observed in the generic *E. coli* isolates. These findings highlight the importance of microbiological quality surveillance of fresh produce in formal and informal markets, as these products can

be a reservoir of multidrug resistant bacteria harboring antibiotic resistance and virulence genes, potentially impacting human health.

1 INTRODUCTION

Surveillance of the microbiological quality of fresh produce at retail level have been reported in various countries (de Oliveira, de Souza, Bergamini, & De Martinis, 2011; Kuan et al., 2017; Li et al., 2017; Roth, Simonne, House, & Ahn, 2018; Ryu, Kim, Kim, Beuchat, & Kim, 2014; Sair, Masud, Ayyaz, & Rafique, 2017; Tango et al., 2018), with increasing numbers being associated with fresh produce resulting in foodborne disease outbreaks (Denis, Zhang, Leroux, Trudel, & Bietlot, 2016). This highlights the need for effective foodborne disease outbreak surveillance and reporting systems in fresh produce supply chains. The South African food market is characterized by dualism; both well-developed, highly sophisticated and regulated formal—as well as the less regulated informal food systems that provide fresh produce to consumers throughout the country (Louw, Chikazunga, Jordaan, & Biénabe, 2006; Skinner & Haysom, 2016). Differences in the production and distribution systems raise the question of possible differences in microbiological quality of the retailed fresh produce (Verraes et al., 2015).

Enterobacteriaceae form part of the indigenous microbiota of vegetables (Blaak, van Hoek, Veenman, Docters van Leeuwen, & Lynch, 2014). Members of this family, that is, *Escherichia coli* and *Salmonella* spp., have often been associated with foodborne bacterial outbreaks following raw fresh produce consumption (Tope, Hitter, & Patel, 2016). This includes diarrheagenic *E. coli* strains, including enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC), enteroaggregative (EAEC), and enteroinvasive (EIEC) *E. coli* in foodborne disease outbreaks (Aijuka, Santiago, Girón, Nataro, & Buys, 2018; Canizalez-Roman et al., 2019). In addition to generic *E. coli*, diarrheagenic strains are also found in the intestinal tracts of mammals and are therefore often used as indicators of fecal contamination in fresh produce supply chains (Denis et al., 2016). Similarly, *Listeria monocytogenes* is increasingly linked to fresh produce associated foodborne disease outbreaks globally (Zhu, Gooneratne, & Hussain, 2017), but until recently, rarely reported in South Africa (SA), particularly associated with fresh produce (Kayode, Igbinosa, & Okoh, 2020).

As fresh produce is often consumed raw or minimally processed, no “kill step” occurs, leaving fewer barriers against microbial contamination (Mritunjay & Kumar, 2015). A previous study where the microbial quality of fresh produce sold in SA was investigated, reported that antibiotic resistant *E. coli* occurred in leafy green vegetables sold formally and informally in Johannesburg, SA (Du Plessis, Govender, Pillay, & Korsten, 2017). The importance of large-scale microbiological surveillance in the formal and informal supply chains were highlighted, focusing attention on the comparative safety levels of food sold in SA. The solitary focus on

foodborne pathogen prevalence in the world has expanded in the last decade to include more formal surveillance of antimicrobial resistance (AR) in microorganisms in agricultural production systems including fresh produce (Ben Said et al., 2016; Blaak et al., 2014; Ye et al., 2017). This follows after the World Health Organization (WHO) highlighted the need for a global AR surveillance system in various countries (WHO, 2015). It was further reported that members of the Enterobacteriaceae family form part of the priority pathogens for surveillance of AR (WHO, 2015). Environmental bacteria naturally harbor resistance genes to certain antimicrobials on their chromosomes (Blaak et al., 2014). However, the widespread use of antimicrobials in for example hospital settings and agricultural production (e.g., animal husbandry) has resulted in the selection of multidrug resistant microbes, posing a broader threat to the treatment of foodborne diseases (Doyle, 2015). Indeed, serious patient treatment complications may arise if multidrug resistant *E. coli* (or other foodborne pathogens) are ingested, even if no immediate or obvious health outcome arise (O'Flaherty, Solimini, Pantanella, De Giusti, & Cummins, 2019). This follows as transfer of antibiotic resistant genes to other bacterial species in the human gut may occur, increasing the risk of future antibiotic treatment options (O'Flaherty et al., 2019).

The aim of this study was to determine the microbiological safety (coliforms, *E. coli* and Enterobacteriaceae) and presence of potential human pathogenic bacteria (*E. coli*, *Salmonella* spp. and *Listeria monocytogenes*) in vegetables sold at formal retailers, informal street- and mobile trolley vendors, and from farmers' markets in the densest urban area in SA. The *E. coli* isolates from vegetables were characterized using phenotypic (antimicrobial resistance) and genotypic (*lt*, *st*, *bfpA*, *eaeA*, *eagg*, *stx1*, *stx2*, and *ipaH* virulence genes) analysis.

2 MATERIALS AND METHODS

2.1 Sample collection and processing of fresh produce

Ten suppliers in retail and 20 in informal markets (10 street traders and 10 mobile trolley vendors) as well as 13 stalls from two farmers' markets in Gauteng Province SA were selected for sampling. In total, 545 randomly chosen vegetable samples were purchased between September 2017 and May 2018. Depending on availability, spinach (bunches, baby leaves, or minimally processed ready-to-eat (RTE) pillow packs) and tomatoes, from retailers, street traders, trolley vendors and farmers' markets (n = 50 from each respective group), were analyzed. In addition, cucumbers (n = 45), lettuce (Iceberg lettuce heads or mixed salad leaf RTE pillow packs) (n = 50), and green beans (n = 50) were also included from the farmers' market vendors. All samples were transported cooled and stored at 4 °C until further processing within 24 hr.

A 50 g composite sample for each of the respective leafy vegetables were aseptically cut into a sterile polyethylene strainer stomacher bag containing 200 ml

buffered peptone water (BPW) (3M, Johannesburg, South Africa) in a 1:4 weight to volume ratio (Richter, Du Plessis, Duvenage, & Korsten, 2019). For the tomatoes and cucumbers (composite samples of at least three from each product), as well as green beans, 150 g samples were each placed into a sterile polyethylene stomacher bag containing 150 ml BPW in a 1:1 weight to volume ratio (Xu, Pahl, Buchanan, & Micallef, 2015). Individual vegetable samples were blended for 5 min at 230 rpm in a Stomacher® 400 Circulator paddle blender (Seward Ltd., London, UK).

2.2 Microbiological analysis

To enumerate coliforms and *E. coli*, a tenfold dilution series of each BPW sample mixture was plated in duplicate onto *E. coli*/coliform count plates and incubated for 24 hr at 37 °C according to the manufacturer's instructions (3M Petrifilm, 3M, St. Paul, MN, USA, ISO method 4832). Enterobacteriaceae were enumerated by plating in duplicate onto Violet Red Bile Glucose (VRBG) agar plates and incubated for 24 hr at 37 °C (Oxoid, Johannesburg, South Africa). The remaining sample in BPW was incubated for 24 hr at 37 °C for detection of *Salmonella* spp. and *E. coli*. After incubation, the samples in BPW were subsequently streaked onto Eosin methylene blue (EMB) media (Oxoid) for the detection of *E. coli*. The presence of *Salmonella* spp. was assessed using the iQ-Check *Salmonella* II Kit AOAC 010803 (BioRad, Johannesburg, South Africa) according to the manufacturer's instructions. Once positive results were obtained, the sample was streaked onto Xylose lysine deoxycholate (XLD) agar (Biolabs, Johannesburg, South Africa) and *Salmonella* Brilliance agar (Oxoid) and incubated for 24 hr at 37 °C. The presence of *Listeria* spp. was assessed by incubating an additional 25 g of each sample in 225 ml Buffered *Listeria* Enrichment Broth (BLEB) (Oxoid) at 30 °C for 24 hr and subsequently using the iQ-Check *Listeria monocytogenes* II Kit AOAC 010802 (BioRad) according to the manufacturer's instructions. Once positive results were obtained, the sample was streaked onto Agar Listeria Ottavani and Agosti (Biomèrieux SA, France) and Rapid'L.mono agar (BioRad) and incubated for 48 hr at 37 °C. All presumptive positive *E. coli*, *Salmonella* spp. and *L. monocytogenes* colonies were isolated and purified. Isolates were identified using matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF) (Bruker, Bremen, Germany) to species level as described by Standing, Du Plessis, Duvenage, and Korsten (2013) and AOAC-OMA#2017.09. Briefly, purified strains were transferred in duplicate onto the MALDI-TOF steel polished target plate, overlaid with the α-cyano-4-hydroxycinnamic acid matrix (Bruker) and analyzed using MicroFlex LT MALDI-TOF (Bruker) in conjunction with the Biotyper automation software and library (Bruker) following calibration with a bacterial standard according to the manufacturer's instructions (Bruker). The best organism match score values ranging between 2.30 to 3.00 were considered reliable for identification at species level, whilst the best organism match score values ranging between 2.00 to 2.29 were considered reliable for genus level, with

probable species identification, and values between 1.70 to 1.99 were considered as probable genus identification.

2.3 Antimicrobial susceptibility testing

A total of 67 isolates were selected which included one representative *E. coli* isolate per product type found from each supplier and tested further for antimicrobial resistance or susceptibility against seven antibiotic classes using the Kirby–Bauer disk diffusion technique (Clinical Laboratory Standard Institute [CLSI], 2018). The antibiotics included ampicillin (10 µg), amoxicillin-clavulanic acid/augmentin (20 µg/10 µg), amoxicillin (10 µg), trimethoprim-sulfamethoxazole/cotrimoxazole (1.25 µg/23.75 µg), cefoxitin (30 µg), cefepime (30 µg), imipenem (10 µg), neomycin (10 µg), tetracycline (30 µg), gentamycin (10 µg), and chloramphenicol (30 µg) (Mast Diagnostics, Randburg, South Africa) (CLSI, 2018). Break points measured were compared to those outlined by the CLSI (2018) for Enterobacteriaceae. Isolates resistant to three or more antimicrobial classes were regarded as multidrug resistant. *Escherichia coli* ATCC 25922 was included as a control (CLSI, 2018).

2.4 Molecular characterization of diarrheagenic *E. coli*

The presence of different diarrheagenic *E. coli* virulence genes for ETEC (*lt* and *st* genes), EPEC (*bfpA* and *eaeA* genes), Eagg (*eagg* gene), EHEC (*eaeA*, *stx1* and *stx2* genes), and EIEC (*ipaH* gene) (Table 1) were determined using Polymerase Chain Reaction (PCR) analysis and sequencing, with the *mdh* gene used as internal control in all reactions. Control strains for the PCR reactions included DSM 10973 and DSM 27503 (ETEC); DSM 8703 and DSM 8710 (EPEC); DSM 27502 (Eagg); *E. coli* O157:H7 and ATCC 25922 (EHEC); and DSM 9028 and DSM 9034 (EIEC).

Table 1. Primers used for screening of diarrheagenic *E. coli* isolated from fresh produce sold formally and informally

Diarrheagenic *E. coli*	Target genes	Primer sequences (5' to 3')	Thermocycling conditions	Expected amplicon size (bp)	Reference
Enterotoxigenic (ETEC)	*Lt*	F: GGC GAC AGA TTA TAC CGT GC	95 °C, 15 min; 35 cycles of 94 °C, 45 s; 55 °C, 45 s; 68 °C, 2.5 min; 72 °C 5 min	410	Omar & Barnard (2010)
		R: CGG TCT CTA TAT TCC CTG TT			
	St	F: TTT CCC CTC TTT TAG TCA		160	Omar & Barnard (2010)

Diarrheagenic *E. coli*	Target genes	Primer sequences (5' to 3')	Thermocycling conditions	Expected amplicon size (bp)	Reference
		GTC AAC TG			
		R: GGC AGG ATT ACA ACA AAG TTC ACA			
Enteropathogenic (EPEC)	*bfpA*	F: AAT GGT GCT TGC GCT TGC TGC	94 °C, 5 min; 35 cycles of 94 °C, 40 s; 68 °C, 60 s; 72 °C, 2 min; 72 °C 5 min	324	López-Saucedo et al. (2003)
		R: GCC GCT TTA TCC AAC CTG GTA			
	eaeA	F: CTG AAC GGC GAT TAC GCG AA	95 °C, 15 min; 35 cycles of (94 °C, 45 s; 55 °C, 45 s; 68 °C; 2 min	917	Omar & Barnard (2010)
		R: GAC GAT ACG ATC CAG			
Enteroaggregative (Eagg)	*eagg*	F: CTG GCG AAA GAC TGT ATC AT	94 °C, 5 min; 35 cycles of 94 °C, 40 s; 57 °C, 60 s; 72 °C, 2 min; 72 °C, 5 min	630	Aslani, Alikhani, Zavari, Yousefi, & Zamani (2011)
		R: AAT GTA TAG AAA TCC GCT GTT			
	eagg	F: CTG GCG AAA GAC TGA ATC AT	94 °C, 5 min; 35 cycles of 94 °C, 40 s; 53 °C, 60 s; 72 °C, 1 min; 72 °C, 5 min	630	Aslani et al. (2011)
		R: CAA TGT ATA GAA ATC CGC TGT T			
Enterohemorrhagic (EHEC)	*eaeA*	F: CTG AAC GGC GAT	95 °C, 15 min; 35 cycles of 94	917	Omar & Barnard (2010)

Diarrheagenic *E. coli*	Target genes	Primer sequences (5' to 3')	Thermocycling conditions	Expected amplicon size (bp)	Reference
		TAC GCG AA	°C, 45 s; 55 °C, 45 s; 68 °C; 2 min		
		R: GAC GAT ACG ATC CAG			
	stx1	F: ACA CTG GAT GAT CTC AGT GG	95 °C, 15 min; 35 cycles of 94 °C, 45 s; 55 °C, 45 s; 68 °C; 2 min	614	Omar & Barnard (2010)
		R: CTG AAT CCC CCT CCA TTA TG			
	stx2	F: CCA TGA CAA CGG ACA GCA GTT		779	Omar & Barnard (2010)
		R: CCT GTC AAC TGA GCA CTT TG			
Enteroinvasive (EIEC)	*ipaH*	F: GTT CCT TGA CCG CCT TTC CGA TAC CGT C	95 °C 5 min 35 cycles of 95 °C 60 s; 60 °C 90 s; 72 °C 2 min 72 °C 10 min	600	Aranda, Fagundes-Neto, & Scaletsky (2004)
		R: GCC GGT CAG CCA CCC TCT GAG AGT AC			

A single colony of each *E. coli* isolate was cultured aerobically under shaking conditions at 200 rpm in tryptone soy broth (TSB) (MERCK, Johannesburg, South Africa) for 24 hr at 30 °C. The cells were pelleted by centrifugation (12,500 *g* for 10 min), DNA was extracted using the Quick-gDNA Mini-Prep kit (Zymo Research, Irvine, CA, USA) and the DNA concentration was determined using the Qubit dsDNA Broad Range Assay and a Qubit 2.0 fluorometer (Life Technologies, Johannesburg, South Africa). PCR was performed using the 1x DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Johannesburg, South Africa) with 60 to 100 ng DNA, with specific primers and thermocycling conditions for each of the genes (Table 1). The PCR products were visualized on a 2% agarose gel using a molecular imager (Gel Doc XR+, Bio-Rad).

2.5 Statistical analysis

Data were analyzed using SAS version 9.3 statistical software (SAS Institute Inc., 2016). Analysis of variance was used to test for significant differences between group by product combinations. The Shapiro–Wilk test was performed on the standardized residuals to test for deviations from normality (Shapiro & Wilk, 1965). Student's protected t-LSD (least significant difference) were calculated at a 5% significance level to compare means of significant source effects (Snedecor & Cochran, 1980).

3 RESULTS AND DISCUSSION

3.1 Microbiological analysis

This study is the first to investigate the microbiological quality (including Enterobacteriaceae enumeration) and occurrence of multidrug resistant (MDR) generic *E. coli* in comparing fresh vegetables sold at retailers, street vendors, trolley vendors, and farmers' markets in Gauteng Province. Enumeration of coliforms, *E. coli* and Enterobacteriaceae showed similar ranges for the different vegetable types, regardless of the vendor groups where it was purchased (Figure 1). The microbiological quality of fresh produce, mainly leafy greens, sold at different markets have been studied worldwide (Du Plessis et al., 2017; Korir, Parveen, Hashem, & Bowers, 2016; Quansah, Kunadu, Saalia, Díaz-Pérez, & Chen, 2018; Roth et al., 2018). Leafy greens have previously been prioritized as the highest level of concern in terms of fresh produce safety from a global perspective (WHO, 2008). The WHO has further stated that commodities of second highest concern (level 2 priority) include tomatoes and green onions, whilst carrots and cucumbers amongst others were a level 3 priority. The coliforms enumerated from the different products across all vendor types in the current study ranged from 0.6 to 8.1 log CFU/g on spinach, 0.0 to 8.2 log CFU/g on tomatoes, 3.6 to 7.8 log CFU/g on lettuce, 0.0 to 6.5 log CFU/g on cucumber, and 0.7 to 6.8 log CFU/g on green bean samples (Figure 1; Supporting Information Table S1). The mean coliform counts on spinach from the formal and informal markets were not significantly different, with the exception of the mean coliform counts on spinach from the trolley vendors (5.1 log CFU/g), which were significantly lower (P = 0.0003) than that on spinach from the farmers' market vendors (6.0 log CFU/g) (Supporting Information Table S1). Similarly, the coliform counts on tomatoes from the formal and informal markets were not significantly different, with the exception of the mean coliform count on tomatoes from trolley vendors (4.4 log CFU/g) being significantly lower (P = 0.0003) than that on tomatoes from the farmers' market vendors (5.4 log CFU/g). Coliforms enumerated from cucumbers (4.1 log CFU/g) were significantly lower (P = 0.0003) than the coliforms enumerated from the leafy green vegetables (spinach and lettuce). The fresh produce samples from retailers, street traders, trolley vendors and farmers' markets collectively had a high prevalence of coliforms (≥90%), compared to the

52.0 to 75.6% coliform prevalence on vegetables from retailers and farmers' markets in Florida, United States (Roth et al., 2018), and 38.7% prevalence on vegetables from retail stores on the Eastern Shore of Maryland, United States (Korir et al., 2016). Regardless of the vegetable type, Roth et al. (2018) found produce from retailers to have constant lower coliform prevalence than the farmers' market vegetables. In contrast, the results from the current study were similar to a previous South African study where 100% of spinach samples from retailers as well as from street vendors were positive for coliforms (Du Plessis et al., 2017), with no significant difference in coliform counts observed in the vegetables from formal and informal markets.

Figure 1

Coliform, *E. coli* and Enterobacteriaceae counts (log CFU/g) on spinach, tomato, cucumber, green bean and lettuce samples purchased from formal and informal markets in Gauteng, South Africa.

The guidelines with regard to acceptable hygiene indicator bacteria counts on ready-to-eat (RTE) produce differ across the world (FSAI, 2016; FSANZ, 2001; Health Protection Agency, 2009). Moreover, the SA Department of Health's microbiological guidelines for fresh fruits and vegetables to be eaten raw are currently being revised. Other countries do not include coliform counts in the guidelines for interpretation of results of microbiological testing of RTE foods, which should be considered in the revision process of the SA guidelines. Naturally, coliform and Enterobacteriaceae counts of vegetables are often >4 log CFU/g. Coliforms include amongst other *Citrobacter*, *Klebsiella*, *Enterobacter*, and *E. coli*, that could potentially pose a threat to human health (Baylis, Uyttendaele, Joosten, Davies, & Heinz, 2011). Yet, as the coliform bacteria fall within the greater Enterobacteriaceae family, the significance of a high prevalence on vegetables is understandable and must be put into context due to the natural association with plants (Baylis et al., 2011). Enterobacteriaceae enumerated from trolley vendor spinach samples (4.6 log CFU/g) were significantly lower (P = 0.0082) than that of retailers (5.8 log CFU/g) and farmers' market vendors (5.9 log CFU/g) (Supporting Information Table S1). The Enterobacteriaceae counts on spinach ranged between 0.0 to 8.2 log CFU/g, on tomatoes between 0.0 to 8.1 log CFU/g, on lettuce between 4.2 to 8.3 log CFU/g, on cucumbers between 0.0 to 6.5 log CFU/g, and on green beans between 0.0 to 7.7 log CFU/g (Figure 1) (Supporting Information Table S1). The overall Enterobacteriaceae loads observed on the different vegetable types in the current study corresponded to results previously reported (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Al-Holy, Osaili, Alshammari, & Ashankyty, 2013; Al-Kharousi, Guizani, Al-Sadi, Al-Bulushi, & Shaharoona, 2016). The Enterobacteriaceae counts on different vegetables from formal and informal markets reiterated the natural bacterial

prevalence on the produce, regardless of food safety regulations being implemented or not in these contrasting points of sale with highly differing personal hygiene and sanitation standards and cold refrigeration capacity (Al-Kharousi et al., 2016; Grace, Dipeolu, & Alonso, 2019).

In the current study, *E. coli* was enumerated from all the different produce types and sampling points, however not all samples were positive for *E. coli* after enrichment. Except for the farmers' market spinach that had mean *E. coli* counts of 1.2 log CFU/g, the *E. coli* counts on different produce types in the current study were <10 CFU/g (Figure 1). This is similar to previous *E. coli* levels reported on spinach and cabbage from retailers and street vendors in SA (Du Plessis et al., 2017), and lower than *E. coli* counts on spinach from retailers (1.0 to 1.8 log CFU/g) in the United States (Korir et al., 2016). The mean *E. coli* levels on spinach from the farmers' market vendors (1.2 log CFU/g) were significantly higher (P = 0.0364) than that of spinach from street traders (0.3 log CFU/g). Interestingly, the *E. coli* occurrence (number of samples positive for *E. coli* enumeration) were higher on tomatoes than spinach for all groups, except for produce from farmers' markets (Supporting Information Table S1). Although the majority of *E. coli* counts on fresh produce was acceptable, some samples was of poor microbiological quality, which corresponds to previous reports of potential foodborne pathogen contamination in fresh produce in developing countries (Mir et al., 2018). Overall, 2% to 8% of the tomato samples from the different vendors had unsatisfactory *E. coli* counts (*E. coli* ≥ 1,000 CFU/g), according to the commission regulation on microbiological criteria for RTE precut fruit and vegetables (European Commission [EC], 2007). Spinach samples from all different vendors had unsatisfactory *E. coli* counts ranging between 12% from farmers' market vendors to 6, 4, and 2% from trolley vendors, retailers and street traders respectively. Similarly, 6, 4, and 2% lettuce, green beans, and cucumber samples respectively, had unsatisfactory *E. coli* counts. When evaluated against international guidelines as specified in the U.K. (20 to 100 CFU/g), Australia (3 to 100 CFU/g), and Canada (100 most probable number per g), 13.03% (n = 71) of the samples from the current study would not have been compliant (FSANZ, 2001; Health Canada, 2010; Health Protection Agency, 2009). This included 19.72% (n = 14) samples from the formal market and 80.28% (n = 57) samples from the informal market, respectively. The high percentage (50%) of the SA population that depend on informal trade, highlights the need to improve fresh produce safety in all the different markets (Petersen & Charman, 2018). In SA, 21.76 and 95.60% of the population purchasing from the informal sector consume raw and/or cooked spinach and tomatoes, respectively. The questionnaire survey results from the population purchasing from the formal sector, showed that 94, 29 and 94% of the respondents eat lettuce, beans, and cucumber raw, respectively Water Research Commission [WRC], 2018; Baloyi, 2020).

3.2 Detection of potential foodborne pathogens

In contrast to Du Plessis et al. (2017), no *Salmonella* spp. nor *L. monocytogenes* were detected from any of the vegetables in the current study after PCR confirmation. In the current study, 14.86% (81/545) of the vegetable samples analysed from all the different vendor types harboured *E. coli* after enrichment. This included 62/245 (25.30%) farmers' market samples, 6/100 (6.00%) street traders' samples, 3/100 (3.00%) trolley vendor samples, and 10/100 (10.00%) samples from retailers. The highest occurrence of *E. coli* isolates following enrichment was from the leafy green vegetable samples; 15/50 (30.00%) farmers' market spinach samples, 7/50 (14.00%) farmers' market lettuce samples, 4/50 (8.00%) street traders' spinach samples, 3/50 (6.00%) trolley vendor spinach samples, and 8/50 (16.00%) retailers' spinach samples. Previously, Scheinberg et al. (2017) reported that 29.00 and 17.00% of lettuce and spinach samples respectively, were positive for generic *E. coli* after enrichment from farmers' markets in Pennsylvania. In the current study, 14.00% and 30.00% of the farmers' market lettuce and spinach samples respectively, were positive for generic *E. coli*. *Escherichia coli* from tomatoes in the current study were isolated from 14.00% (7/50) of the farmers' market tomato samples and 2/50 (4.00%) street trader- and retailer tomato samples, respectively. From the farmers' market green bean samples (n = 50), 13 samples (26.00%) were contaminated with *E. coli*, whilst 9/45 (20.00%) of the farmers' market cucumber samples were contaminated with *E. coli*.

3.3 Phenotypic antimicrobial resistance profiling of *Escherichia coli* isolates

The natural occurrence of Enterobacteriaceae and higher microbial loads of potential pathogens such as *E. coli* becomes concerning when investigating the possibility of fresh produce aiding in dissemination of clinically important resistance genes (Vikesland et al., 2017). From the 67 selected *E. coli* isolates, resistance were observed against all the antibiotics screened for, with resistance against neomycin the highest (73.13%) followed by penicillins (ampicillin, 38.81% and amoxycillin, 41.79%), sulfonamides (cotrimoxazole, 22.39%), tetracycline (19.40%), and chloramphenicol (11.94%) (Figure 2). Less than 10.00% of the isolates were resistant to cefoxitin, imipenem, and gentamycin, respectively. Overall, multidrug resistance (resistance to ≥ 3 antibiotic classes) was observed in 40.30% of the *E. coli* isolates. This was similar to the 37.90% multidrug-resistance reported in *E. coli* isolates from spinach in another SA study (Du Plessis et al., 2017), but lower than the 100% multidrug resistance reported in *E. coli* from lettuce and cabbage in Ghana (Adzitey, 2018). Except for one cucumber *E. coli* isolate, the *E. coli* isolates from all product types were, similar to results reported by Du Plessis et al. (2017), susceptible to second generation cephalosporin antibiotics (cefoxitin). In addition, 34.30% of the isolates were resistant to fourth-generation cephalosporin antibiotics (cefepime) and <10% resistant to impenem (carbapenemase). The most frequent resistance patterns within the different antibiotic classes for the isolates included resistance to antibiotics in the penicillins–cephalosporins–aminoglycosides combination (13

MDR isolates), followed by the penicillins—aminoglycosides—sulfonamides–tetracyclines–chloramphenicol combination (5 isolates) and the penicillins–cephalosporins–aminoglycosides–sulfonamides (3 isolates) combination (Supporting Information Table S2). Environmental *E. coli* with multidrug-resistance phenotypes have similarly been described in previous reports, including in developing countries (Canizalez-Roman et al., 2019; Corzo-Ariyama et al., 2019; Du Plessis et al., 2017). With a rise in antimicrobial resistance in both commensal and pathogenic bacteria in different environments, subsequent treatment options to infections become limited (Freitag et al., 2018).

Figure 2
Open in figure viewerPowerPoint
Phenotypic antimicrobial resistance profiles of *E. coli* isolated from different fresh produce types sold at different vendors in Gauteng, South Africa.

3.4 Molecular characterization of diarrheagenic *Escherichia coli*

In contrast to other studies that have reported on spinach and lettuce contaminated with *E. coli* harbouring *stx2* and *eae* genes (Li et al., 2016) and *E. coli* isolates characterised as EAEC, EPEC, and ETEC positive strains (Waturangi, Hudiono, & Aliwarga, 2019), none of the 67 selected *E. coli* isolates for further characterisation from the current study harboured virulence genes. The presence of *E. coli* on fresh produce however remains significant, as these potential pathogens can be an additional reservoir of antimicrobial resistance genes (Luna-Guevara, Arenas-Hernandez, Martínez De La Peña, Silva, & Luna-Guevara, 2019). Antimicrobial resistance genes can readily be transferred to commensal bacteria, including nonpathogenic bacteria, that typically colonise the human gut and are therefore regarded as emerging environmental contaminants (Du Plessis et al., 2017).

4 CONCLUSION

This study showed that *E. coli* levels from spinach and tomatoes from the retailers, street traders, trolley vendors, and farmers' markets were not significantly different. Furthermore, the farmers' market lettuce samples also showed similar *E. coli* levels to the spinach from all the different groups tested. No *Salmonella* spp. nor *L. monocytogenes* were detected nor isolated from any of the vegetables sampled in this study. However, the prevalence of multidrug-resistant commensal *E. coli* highlights the need for improved food safety practices within the supply chains and identification of fresh produce contamination sources with antimicrobial resistant bacteria as a public health concern. The antimicrobial resistance levels observed in commensal *E. coli* isolated from fresh produce at the point of sale further highlights the need to include characterisation of Enterobacteriaceae (commensal and potential pathogenic bacteria) with expanded

spectrum antimicrobial resistance, as well as surveillance of fresh produce production systems from farm-to-retail, to identify potential sources of contamination which contribute to the presence and dissemination of antimicrobial resistant microorganisms and their genetic determinants.

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AUTHOR CONTRIBUTIONS

E.M. du Plessis, S. Duvenage and L. Korsten contributed to the conception and design of the study. L. Richter collected test data and proceeded to laboratory experiments, analysis and presentation. L. Richter, E.M. du Plessis and S. Duvenage contributed to results interpretation. All authors contributed to manuscript writing and approved the submitted version.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Supporting Information

REFERENCES