International Journal of Environmental Health Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cije20

Multidrug resistant *Escherichia coli* from fresh produce sold by street vendors in South African informal settlements

Tintswalo Baloyi, Stacey Duvenage, Erika Du Plessis, Germán Villamizar-Rodríguez & Lise Korsten

To cite this article: Tintswalo Baloyi, Stacey Duvenage, Erika Du Plessis, Germán Villamizar-Rodríguez & Lise Korsten (2021): Multidrug resistant *Escherichia coli* from fresh produce sold by street vendors in South African informal settlements, International Journal of Environmental Health Research, DOI: 10.1080/09603123.2021.1896681

To link to this article: https://doi.org/10.1080/09603123.2021.1896681

INTERNATIONAL JOURNAL OF ENVIRONMENTAL HEALTH RESEARCH
https://doi.org/10.1080/09603123.2021.1896681

Taylor & Francis
Taylor & Francis Group

ARTICLE

Multidrug resistant *Escherichia coli* from fresh produce sold by street vendors in South African informal settlements

Tintswalo Baloyi*, Stacey Duvenage*, Erika Du Plessis, Germán Villamizar-Rodríguez and Lise Korsten

Department of Science and Innovation-National Research Foundation Centre of Excellence in Food Security, Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa

ABSTRACT

The aim of this study was to assess the prevalence of commensal and pathogenic *Escherichia coli* on informally sold fresh produce in South Africa, who harbour and express antimicrobial resistance genes and therefore pose indirect risks to public health. The majority (85.71%) of *E. coli* isolates from spinach, apples, carrots, cabbage and tomatoes, were multidrug resistant (MDR). Resistance to Aminoglycoside (94.81%), Cephalosporin (93.51%), Penicillin (93.51%) and Chloramphenicol (87.01%) antibiotic classes were most prevalent. Antibiotic resistance genes detected included bla_{TEM} (89.29%), *tetA* (82.14%), *tetB* (53.57%), *tetL* (46.43%), *sulI* (41.07%), *sulII* (51.79%), *aadA1a* (58.93%) and *strAB* (51.79%). A single isolate was found to harbour *eae* virulence factor. Moreover, *E. coli* isolates were grouped into the intra-intestinal infectious phylogenetic group E (28.57%), the rare group C (26.79%), the generalist group B1 (21.43%) and the human commensal group A (16.07%). Presence of MDR *E. coli* represents a transmission route and significant human health risk.

ARTICLE HISTORY
Received 9 September 2020
Accepted 23 February 2021

KEYWORDS
Antimicrobial resistance; antimicrobial resistance genes; phylogenetic grouping; diarrheagenic virulence genes

Introduction

Globally, there has been a general shift towards consumption of fresh raw food specifically fruit and vegetables. In South Africa, a recent study revealed that 97% of South Africans eat apples, 98% carrots and tomatoes, 91% spinach and 89% cabbage, either raw or cooked (WRC Project No K5/2706/4, Deliverable 5, 2018). Fresh produce is often locally traded through informal supply chains which includes street-vending green grocers (Du Plessis et al. 2017), which are unregulated with no formal registered operating certificate or food safety training. The risk to the consumer thus increases due to a lack of knowledge in proper handling – storage and poor personal hygienic conditions. Contamination can occur due to poor personal hygiene of food handlers as well as poor facility sanitation and limited space that can lead to cross contamination of fresh fruit and vegetables (Khalil et al. 2015). In fact, 82% of consumers in South Africa were not confident about the safety of fresh produce originating from these vendors (Du Rand and Coundouris 2017). Globally, the increase in fresh produce consumption has been linked to an escalation in the number of foodborne pathogen associated outbreaks (Callejón et al. 2015). Additionally, the presence of commensal and pathogenic bacteria who harbour and express antimicrobial resistant genes pose direct and indirect public health risks, respectively (Verraes et al. 2013).

CONTACT Stacey Duvenage stacey.duvenage@up.ac.za Private Bag X20, Hatfield, Pretoria, 0028, South Africa
*Both authors contributed equally as first authors.

Escherichia coli are mostly commensal (Waturangi et al. 2019). However, some strains contain and express virulence genes that allow them to cause disease, including Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAgg), Enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) (Nataro and Kaper 1998; Waturangi et al. 2019). Shiga toxin producing *E. coli* are the most common strains implicated in foodborne outbreaks (Carstens et al. 2019). Additionally, non-O157 STEC cause more than 112,000 cases of foodborne disease annually in the United States of America (USA) (Scallan et al. 2011). Moreover, ETEC is responsible for more than 18,000 cases of foodborne infection annually in the USA and other diarrheagenic *E. coli* are responsible for a further 12,000 cases (Scallan et al. 2011). Disease outbreaks linked to the consumption of *E. coli* contaminated cabbage, spinach, apples and tomatoes have been reported in the USA [Centres for Disease Control and Prevention (CDC) 2020]. Two of largest *E. coli* outbreaks recorded thus far both occurred in 2011, one involved the consumption of romaine lettuce contaminated with EHEC *E. coli* O157:H7 in the USA, where 58 people died (CDC 2011); the second involved the consumption of sprouts contaminated with enteroaggregative hemorrhagic *E. coli* O104:H4 in Germany in 2011, where more than 53 people died (Robert Koch Institute 2011).

The surveillance of antimicrobial resistant bacteria and their genetic determinants has become more common within food research (Ben Said et al. 2016). *Escherichia coli* has increasingly been reported as a reservoir of antimicrobial resistance genes, many of which were acquired through horizontal gene transfer (Poirel et al. 2018). Both pathogenic and commensal *E. coli* can be a reservoir of antibiotic resistance genes (Du Plessis et al. 2017; Poirel et al. 2018). The presence of virulence and resistance genes increases the pathogenicity of microorganism and therefore the severity of the infection (El-Baky et al. 2020). *Escherichia coli*, both pathogenic and generic, and multidrug resistant (MDR) microorganisms can be present on fresh produce (Jongman and Korsten 2016; Du Plessis et al. 2017; Kilonzo-Nthenge et al. 2018) and readily acquired through food and water (Collignon 2009). Emergence of multidrug resistant *E. coli* can be considered a public health concern (Sa´enz et al. 2004). Often, the presence of foodborne pathogens and multidrug resistant bacteria on fresh produce is due to contamination during production – post-harvest practices (Lynch et al. 2006) or, at the point of sale. Given the scale of consumption of fresh produce, it is imperative to establish a baseline of *E. coli* occurrence and prevalence in the informal sector. In order to determine the ecological niche, disease causing ability and tracking of *E. coli* Clermont et al. (2013) developed a phylogenetic typing method which groups *E. coli* into eight phylogenetic groups (A, B1, B2, C, D, E, F and *E. coli* cryptic clade I). Group A strains include mainly commensal *E. coli*, whilst most virulent extraintestinal strains belong to group B2 and D (Johnson and Stell 2002). Additionally, group B1 is dominated by plant associated *E. coli* and groups A and B2 contain many animal associated strains. Group C is closely related to Group B1 (Escobar-Páramo et al. 2004). Group E has been found to be associated with human and animal sources, for example, *E. coli* O157:H7 is grouped in this phylogenetic group (Clermont et al. 2011).

As such, this scoping study was performed primarily to determine the prevalence of antimicrobial resistant *E. coli* on fresh produce (cabbage, spinach, tomato, apple and carrots) sold at street vendors in the informal settlements of Gauteng Province and to secondarily, characterize virulence and phylogenetic grouping of these isolates.

Materials and methods

Site selection, description, sampling strategy and processing

This study was carried out in Gauteng Province, South Africa between March 2016 and June 2017. The street vendors (SVs) were selected based on the informal nature of the vendor, which typically had semi-permanent wooden structures (with or without cloth coverings) in open-air markets where fresh produce were sold. Moreover, fresh produce were displayed either directly on wooden

planks supported by crates or cardboard boxes, or were kept in reused plastic plates or in plastic bags. In total, 250 fresh produce samples were collected from a total of 10 informal SVs, from two of the largest informal settlements in Gauteng Province [Tshwane (TSV) and Ekurhuleni (ESV)]. Five samples of five different vegetable types (apples, carrots, tomatoes, spinach and cabbage) were collected from each of the 10 informal SVs. Samples at each SV were collected based on what was available and on display that day. All fresh produce were transported and stored cooled. Samples were analysed within 24 h at the Food Safety Laboratories as part of the University of Pretoria's diagnostic platform which operates on ISO 17,025 management principles.

Samples of 150 g (apple, carrots or tomato) were added to buffered peptone water (BPW) (3 M, Johannesburg, South Africa) in a 1:1 weight: volume ratio (Xu et al. 2015). A 1:5 weight: volume ratio was used for 50 g spinach and cabbage (Xu et al. 2015). The samples were macerated for 5 min at 230 rpm in a Stomacher® 400 Circulator (Seward Ltd., London, UK).

Microbiological analysis

In order to enumerate the population of *E. coli*, a tenfold dilution series of each macerated sample was prepared using 0.1% BPW. Suspensions were plated onto Petrifilm *E. coli*/coliform count plates (3 M) and incubated for 48 h at 37°C, according to manufacturer's instructions. Single colonies were counted, recorded and converted to $\log_{10}$ cfug^{-1}.

For the detection of *E. coli*, the macerated sample was incubated at 37°C for 24 h and was subsequently manually streaked onto Eosin methylene blue media (Merck, Johannesburg). Presumptive colonies based on morphology were isolated and identified using Matrix Assisted Laser Desorption Ionisation- Time of Flight (MALDI), in conjunction with the Bruker MALDI Biotyper software and default database (Bruker, Johannesburg) (Standing et al. 2013).

Phenotypic antimicrobial susceptibility screening

Antimicrobial susceptibility testing was done on each of the isolates by culturing in 9 ml brain heart infusion broth (Merck) followed by incubation for 24 h at 37°C; subsequently, suspensions were plated onto Mueller-Hinton agar plates (Merck) according to the Kirby–Bauer disc-diffusion method as outlined in Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). Antibiotics tested were determined based on three categories of important antimicrobials for human medicine, as categorized by the World Health Organisation Advisory Group on Integrated Surveillence of Antimicrobial Resistance (2018). This looked at 'critically important' (ampicillin-10 µg, amoxicillin-10 µg, nalidixic acid-30 µg, streptomycin-10 µg, cefotaxime-30 µg, ciprofloxacin-5µg, gentamicin-10 µg), 'highly important' (tetracycline-30 µg and chloramphenicol-30 µg, cephalothin-30 µg and cotrimoxazole-25 µg) and 'important' (nitrofurantoin-300 µg) antimicrobials for human medicines. Inhibition zone diameters were measured and compared to breakpoints in the CLSI guidelines (Clinical and Laboratory Standards Institute 2018) with a modification that classified intermediate resistance as susceptible (Ta et al. 2014). Strains resistant to three or more antimicrobial classes were defined as MDR. In addition, the multiple-antimicrobial -resistance indices (MARI) were calculated based on Krumperman (1983). *Escherichia coli* ATCC25922 was included as a negative control.

Virulence and antibiotic gene screening and phylogenetic grouping of the Escherichia coli isolates

Isolates (n = 56) were screened for 1) the presence antibiotic resistance genes (45 genes selected based on phenotypic antimicrobial resistance profiles), 2) presence diarrheagenic *E. coli* virulence genes *stx1, stx2, eae* (EHEC), *lt, st* (ETEC), plasmid portion pCVD4321AA probe (Eagg), ial and ipaH (EIEC), with an internal amplification control (*mdh*) and 3) phylogenetic groups were

assigned to *E. coli* isolates using the Clermont *E. coli* phylo-typing method (Clermont et al. 2013). All primers and thermocycling conditions are outlined in Table 1 and Table 2, respectively. Additionally, *Escherichia coli* ATCC25922 was included as a negative control and type cultures used for positive controls are outlined in Table 1, with molecular grade water used as a no template control.

Genomic DNA was extracted using the Zymo Quick-gDNA Mini-Prep kit (Inqaba Biotech, Pretoria, South Africa) and DNA quantification was performed using the Qubit Broad Range dsDNA kit and a Qubit 2.0 fluorometer (Life Technologies, Johannesburg). PCR mixtures were prepared to a final volume of 40 µL, using 1x DreamTaq PCR Master Mix (Life Technologies), 10–100 µg of DNA, and 0,4 µM of primer concentration (Table 1), with PCR cycling conditions as outlined in Table 2. All reactions were performed on a Bio-Rad T100 thermal cycler (Bio-Rad, Johannesburg) and the products were visualised in 1,5% agarose gel stained with Roti®-Safe (Carl Roth GmbH & Co, Germany) using a Bio-Rad GelDoc XR in conjunction with the Image LabTM software (BioRad). Positive amplicons were sequenced in the forward and reverse direction for confirmation of identity by. Inqaba Biotechnical Industries (Pty) Ltd. all sequences were editing and aligned using Chromas 2.6.6 software (Technelysium). To confirm the specificity of the PCR products, sequences were aligned against the GenBank database (Benson et al. 2005) using the Blastn alignment tool (Altschul et al. 1990). The *gyrA* and *parC* required a deeper analysis to detect changes in the amino-acid chain of the DNA gyrase and Topoisomerase IV enzymes encoded by those genes. Following PCR and sequencing, as outlined previously, sequences were translated and then aligned with the corresponding tool included in the BioEdit Sequence Alignment Editor 7.2.6 (Hall 1999).

Data analysis

The statistics were done using SAS for the enumeration of quality indicators and prevalence of foodborne pathogens was using SAS 1999 version 9.4 statistical software. The data was analysed using analysis of variance. The Shapiro–Wilk test was performed to test for deviations from normality. Student's protected t LSD (least significant difference) values were calculated at a 5% significance level to compare means of significant source effects.

Results

Mean *E. coli* counts on spinach purchased from TSV (1.13 ± 0.36 log cfug^{-1}) were significantly higher than those from ESV (0.15 ± 0.11 log cfug^{-1}) (Supplementary Table S1). Spinach purchased from two TSV were found to be contaminated with *E. coli* (60% TSV 2 and 100% TSV 4), ranging from 2.3 to 5.24 log cfug^{-1} (Supplementary Table S1). Only one ESV had spinach samples (40%) contaminated with *E. coli* with a range from 0.00 to 2.10 log cfug^{-1} (Supplementary Table S1). Mean *E. coli* counts from carrot samples from Ekurhuleni and Tshwane did not differ significantly (Supplementary Table S1), with only one ESV demonstrating *E. coli* contamination on 100% of samples and two TSV's having *E. coli* contamination on 20% of carrot samples. These *E. coli* counts ranged from 0.89 to 2.37 log cfug^{-1} (Supplementary Table S1). It was not possible to enumerate *E. coli* from cabbage and apple samples from Tshwane or Ekurhuleni (Supplementary Table S1). In total, 56 *E. coli* isolates were used for antimicrobial resistance testing, virulence testing and phylogenetic grouping. *Escherichia coli* was detected from 24.8% of all samples, with 17.6% from ESV and 32% from TSV.

Overall, *E. coli* isolates retrieved in this study demonstrated high levels of antimicrobial resistance, with 85.71% of all *E. coli* isolates demonstrated MDR (n = 48) and 82.00% exhibited a MARI value of more than 0.2 (Krumperman 1983). *Escherichia coli* retrieved demonstrated resistance to tetracycline (80.36%, n = 45), amoxicillin (73.21%, n = 41), ampicillin (71.43%, n = 40), trimethoprim-sulfamethoxazole (66.07%, n = 37), cephalothin (64.29%, n = 36), nalidixic acid and

Table 1. Primers used for screening of enterohemorrhagic *Escherichia coli* virulence genes, phylogrouping and antimicrobial resistance genes.

Gene		Primer Sequence 5'-F-3'/5'-R-3'	Size (bp)	Tm (°C)	Ref.	Control
Antibiotic Resistance Genes						
Aminoglycosides						
	aac(6')-IB	TTGCGATGCTCTATGAGTGGCTA/CTCGAATGCCTGGCGTGTTT	482	55	*	NO
	strA-B	TATCTGCGATTGGACCCTCTG/CATTGCTCATCATTTGATCGGCT	538	60	†	NO
	aadA1a	GAGAACATACGCTTGCCTTGG/TCGGCGCGATTTTGCCGGTTAC	198	48	†	
β Lactams (AmpC- β Lactamases)						
	ACC	CACCTCCAGCGACTTGTTAC/GTTAGCCAGCATCACGATCC	346	60	‡	NO
	FOX	CTACAGTGCGGGTGGTTT/CTATTTGCGGCCAGGTGA	162	60	‡	NO
	MOX CMY	GCAACAACGACAATCCATCCT/GGGATAGGCGTAACTCTCCCAA	895	60	‡	NO
	DHA	TGATGGCACAGCAGGATATTC/GCTTTGACTCTTTCGGTATTCG	997	60	‡	NO
	LAT BIL CMY	CGAAGAGGCAATGACCAGAC/ACGGACAGGGTTAGGATAGY	538	60	‡	NO
	ACT MIR	CGGTAAAGCCGATGTTGCG/AGCCTAACCCCTGATACA	683	60	‡	NO
β Lactams						
	bla_{TEM}	CATTTCCGTGTCGCCCTTATTC/CGTTCATCCATAGTTGCCTGAC	800	60	‡	NCTC 13,351
	bla_{SHV}	AGCCGCTTGAGCAAATTAAAC/ATCCCGCAGATAAATCACCAC	713	60	‡	ATCC 700,603
	bla_{OXA}	GGCACCAGATTCAACTTTCAAG/GACCCCAAGTTTCCTGTAAGTG	564	60	‡	HWD 3.2 (14)
	$bla_{CTX\text{-}M\ G1}$	TTAGGAARTGTGCCGCTGYA/CGATATCGTTGGTGGTRCCAT	688	60	‡	NO
	$bla_{CTX\text{-}M\ G2}$	CGTTAACGGCACGATGAC/CGATATCGTTGGTGGTRCCAT	404	60	‡	NO
	$bla_{CTX\text{-}M\ G9}$	TCAAGCCTGCCGATCTGGT/TGATTCTCGCCGCTGAAG	561	60	‡	HWD 3.2 (14)
	$bla_{CTX\text{-}M\ G8/25}$	AACRCRCAGACGCTCTAC/TCGAGCCGGAASGTGTYAT	326	60	‡	NO
	GES	AGTCGGCTAGACCGGAAAG/TTTGTCCGTGCTCAGGAT	399	57	‡	NO
	PER	GCTCCGATAATGAAAGCGT/TTCGGCTTGACTCGGCTGA	520	60	‡	NO
	VEB	CATTTCCCGATGCAAAGCGT/CGAAGTTTCTTTGGACTCTG	648	60	‡	NO
	IMP	TTGACACTCCATTTACDG/GATYGAGAATTAAGCCACYCT	139	55	‡	NO
	VIM	GATGGTGTTTGGTCGCATA/CGAATGCGCAGCACCAG	390	55	‡	NO
Cefotaxime						
	ampC	GTGACCAGATATGGCCACA/TTACTGTAGCGCCTCGAGGA	822	55,8	§	NCTC 13,406
Fluoroquinolones						
	qnrD	CGAGATCAATTTACGGGGAATA/AACAAGCTGAAGCGCCTG	465	50	¶	NO
	qnrS	GCAAGTTCATTGAACAGGGT/TCTAAACCGTCGAGTTCGGCG	428	54	¶	NO
Penicillin						
	blaZ	ACTTCAACACCTGCTGCTTTC/TGACCACTTTTATCAGCAACC	173	56	**	ATCC 43,300
Phenicols						
	cat I	AGTTGCTCAATGTACCTATAACC/TTGTAATTCATTAAGCATTCTGCC	547	50	††	NO
	cat II	ACACTTTGCCCTTTATCGTC/TGAAAGCCATCACATACTGC	543	50	††	NO
	cat III	TTCGCCGTGAGCATTTTG/TCGGATGAGTATGGGCAAC	286	50	††	NO
Quinolones						
	gyrA	TACACCGGTCAACATTGAGG/TTAATGATTGCCGCCGTCGG	648	64	‡‡	NO
	parC	AAACCTGTTCAGCGCCGCATT/GTGGTGCCGTTAAGCAAA	395	64	§§	

Table 2. Continued

Gene	Primer Sequence 5'-F-3'/5'-R-'3	Size (bp)	Tm (°C)	Ref.	Control
Sulfonamides					
sulI	TTCGGCATTCTGAATCTCAC/ATGATCTAACCCTCGGTCTC	822	50	††	NO
sul II	CGGCATCGTCAACATAACC/GTGTGCGGATGAAGTCAG	722		††	NO
Tetracycline					
tet(A)	GCTACATCCTGCTTGCCTTC/CATAGATCGCCGTGAAGAGG	210	55	¶¶	NO
tet(B)	TTGGTTAGGGGCAAGTTTTG/GTAATGGGCCAATAACACCG	659	55	¶¶	NO
tet(C)	CTTGAGAGCCTTCAACCCAG/ATGGTCGTCATCTACCTGCC	418	55	¶¶	NO
tet(D)	AAACCATTACGGCATTCTGC/GACCGGATACACCATCCATC	787	55	¶¶	NO
tet(E)	AAACCACATCCTCCATACGC/AAATAGGCCACAACCGTCAG	278	55	¶¶	NO
Diarrheagenic *Escherichia coli* virulence genes					
stx1	ACACTGGATGATCTCAGTGG/CTGAATCCCCCTCCATTATG	614	55	***	ATCC 35,150
stx2	CCATGACAACGGACAGCAGTT/CCTGTCAACTGAGCACTTTG	779	55	***	ATCC 35,150
eaeA	CTGAACGGCGATTACGCGAA/GACGATACGATCCAG	917	55	†††	ATCC 35,150
mdh	GGTATGGATCGTTCCGACCT/GGCAGAATGGTAACACCAGAGT	300	55	‡‡‡	ATCC 35,150
LT	GGCGACAGATTATACCGTGC/CGGTCTCTATATTCCCTGTT	410	55	***	DSM 10,973, DSM 27,503
ST	TTTCCCCTCTTTTAGTCAGTCAACTG/GGCAGGATTACAACAAAGTTCACA	162	55	***	DSM 10,973, DSM 27,503
ial	GGTATGATGATGATGATGGGC/GGAGGCCAACAATTATTTCC	630	50	***	DSM 9028, DSM 9034
ipaH	GTTCCTTGACCGCCTTTCCGATACCGTC/GCCGGTCAGCCACCCTCTGAGAGTAC	600	60	†††	DSM 9028, DSM 9034
AA PR	CTGGCGAAAGACTGTATCAT/AATGTATAGAAATCCGCTGTT	630	57	†††	DSM 27,502
Phylogenetic grouping of *Escherichia coli*					
chuA	ATGGTACCGGACGAACCAAC/TGCCGCCAGTACCAAAGACA	288	59	§§§	ATCC 25,922
yjaA	CAAACGTGAAGTGTCAGGAG/AATGCGTTCCTCAACCTGTG	211	59	§§§	ATCC 25,922
TspE4.C2	CACTATTCGTAAGGTCATCC/AGTTTATCGCTGCGGGTCGC	152	59	§§§	ATCC 25,922
arpA	AACGCTATTCGCCAGCTTGC/TCTCCCCATACCGTACGCTA	400	59	§§§	ATCC 25,922
Group C Phylotyping confirmation					
trpA	AGTTTTATGCCCAGTGCGAG/TCTGCGCCGGTCACGCCC	219	57	§§§	NO
Group E Phylotyping confirmation					
arpA	GATTCCATCTTGTCAAAATATGCC/GAAAAGAAAAAGAATTCCCAAGAG	301	59	§§§	ATCC 35,150

*: Park *et al*., 2006; †: Sunde and Norström, 2005; ‡: Dallenne *et al*., 2010; §: Böckelmann *et al*., 2009; ¶: Li *et al*., 2012; **: Martineau *et al*., 2000; ††: Maynard *et al*., 2004; ‡‡: Oram and Fisher, 1991; §§: Vila *et al*., 1996; ¶¶: Ng *et al*., 2001; ***: Omar and Barnard, 2010; †††: Aranda *et al*., 2004; ‡‡‡: Tarr *et al*., 2002; and §§§: Clermont *et al*., 2013.

Table 3. PCR cycling conditions for screening of *Escherichia coli* antimicrobial resistance genes, virulence genes and phylogenetic grouping.

Initial denaturation		Cycles	Final Extension
Antibiotic Resistance Genes testing, with positive control			
94°C for 2 min	35x	94°C for 30 sec	72°C for 5 min
		Tm according to primer pair (Table 1) for 30 sec	
		72°C for 60 sec	
Antibiotic Resistance Genes testing, without positive control			
94°C for 2 min	10x	94°C for 30 sec	72°C for 5 min
		Tm+6°C for 30 sec	
		60 sec at 72°C	
	10x	30 sec at 94°C	
		Tm+4°C for 30 sec	
		60 sec at 72°C	
	10x	30 sec at 94°C	
		Tm+2°C for 30 sec	
		60 sec at 72°C	
	5x	30 sec at 94°C	
		Tm for 30 sec	
		60 sec at 72°C	
		Tm according to primer pair (Table 1)	
Diarrheagenic *Escherichia coli* virulence genes			
94°C for 2 min	35x	94°C for 2 min	72°C for 5 min
		Tm according to primer pair (Table 1) for 30 sec	
		72°C for 2 min	
Phylogenetic grouping of *Escherichia coli*			
95°C for 4 min	30x	94°C for 5s	72°C for 5 min
		Tm according to primer pair (Table 1) for 30 sec	
		72°C for 2 min	

ciprofloxacin (57.14%, n = 32), chloramphenicol (50%, n = 28) to, streptomycin (46.43%, n = 26) to, nitrofurantoin (41.07%, n = 23) and gentamicin (10.71%, n = 6) (Figure 1, Table 3). Therefore, multidrug resistant *E. coli* was detected from 12.8% of samples, with 11.2% from ESV and 14.4% from TSV (Figure 1).

The frequency of the detected antimicrobial resistance genes are shown in Figure 1, Table 4. The following β-lactamase encoding genes were detected from the 56 isolates: bla_{TEM} (89.29%; n = 50), $bla_{CTX\text{-}M\ Gp1}$ (8.93%; n = 5), $bla_{CTX\text{-}M\ Gp9}$ (5.36%; n = 3) and bla_{SHV} (1.79%; n = 1). The following tetracycline encoding genes were detected: *tetA* (82.14%; n = 46), *tetB* (53.57%; n = 30), *tetL* (46.43%; n = 26), *tetK* (12.50%; n = 7), *tetD* (3.57%; n = 2), *tetE* (3.57%; n = 2), *tetM* (3.57%; n = 2) and *tetS* (3.57%; n = 2). Gene conferring resistance to aminoglycosides were detected with aadA1a and strAB present in 58.93% (n = 3) and 51.79% (n = 29) of isolates, respectively (Figure 1; Table 4). Genes *sulI* and *sulII* conferring resistance to Sulfonamides were detected from 41.07% (n = 23) and 51.79% (n = 29) of isolates, respectively (Table 4, Table 5). No AmpC β-lactamase, *ampC*, Fluoroquinolones (*qnrD* and *qnrS*) and Phenicol (*catI, catII* and *catIII*) resistance encoding genes tested for were detected from the 56 isolates. Comparative analysis of the *gyrA* and *parC* encoding sequences of the isolates with reference sequences (APC52470.1-GyrA/M58408), showed that three isolates from ESV's spinach (Figure 1) had substitutions in both enzyme encoding genes: Ser83 and Asp87 (Ser83→Leu/Asp87→Asn) in *gyrA*; and Ser80 (Ser80→Ile) in *parC*. Moreover, 11 isolates from Tshwane had substitutions in Ser83 and Asp87 in *gyrA* (Ser83→Leu/Asp87→Asn), while 12 isolated had substitutions in Ser80 to Ile in *parC*, and two in Ser80 to Thr in *parC* (Figure 1). Only six isolates from Tshwane have shown substitutions in both enzymes (Figure 1).

Out of the 56 *E. coli* isolates screened, only one retrieved from tomato purchased from ESV was positive for *eae* virulence factor; no other virulence genes were detected. The main phylogenetic groups identified were E (28.57%, n = 16), C (26.79%, n = 15) and B1 (21.43%; n = 12), with 16.07%

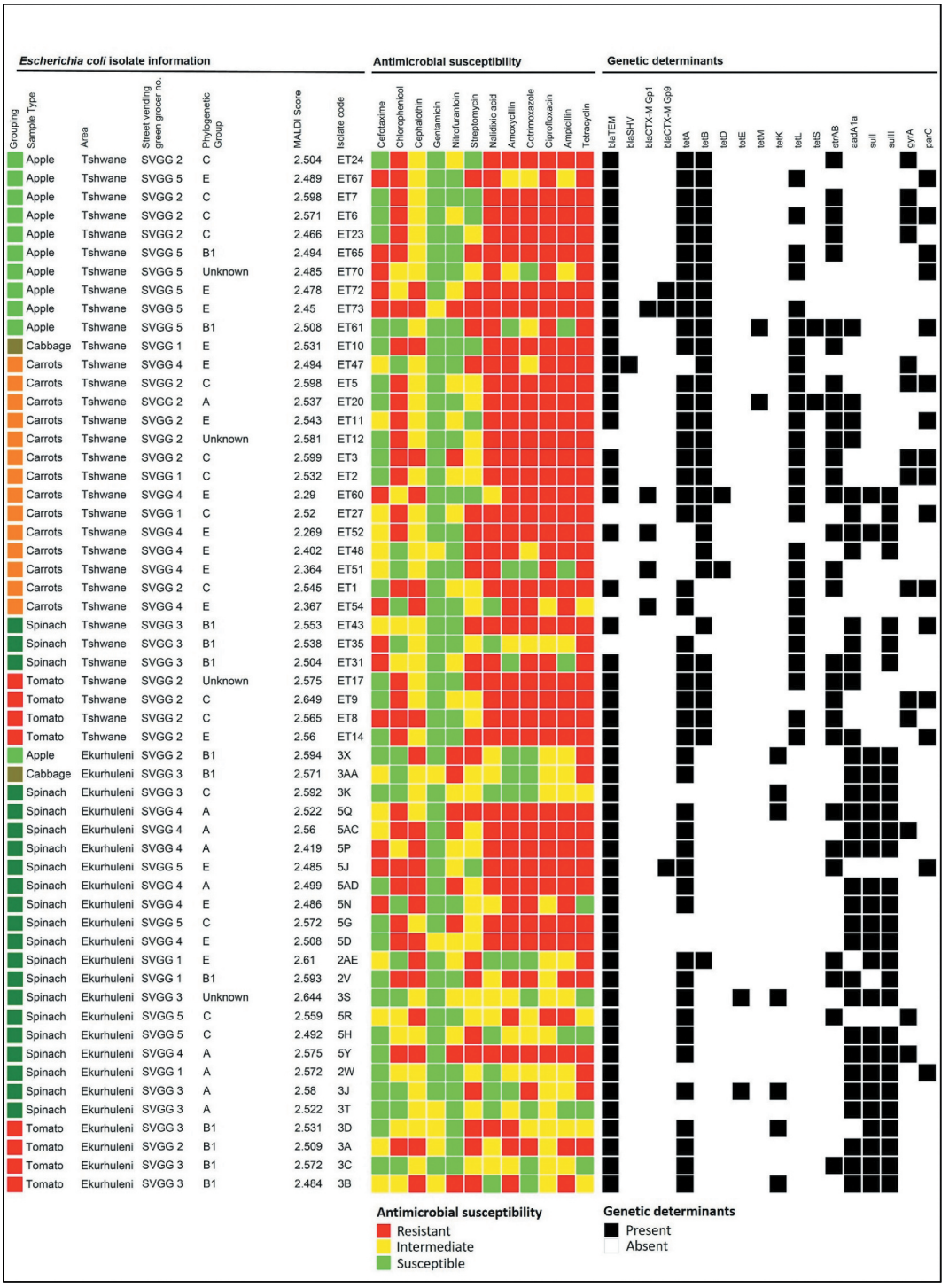

Figure 1. Antimicrobial resistance profiles and antimicrobial genetic determinants present in *Escherichia coli* isolated from fresh produce sold in informal vendors in South Africa. Red blocks represent an isolate is resistant to the specified antibiotic, a yellow block indicates intermediate resistance to the specified antibiotic and a green block represents susceptibility to the specified antibiotic. A black block represents the presence of the antimicrobial resistance genes screened for and a white block indicates the absence of the gene.

Table 4. Antibiotic resistance profiles of *Escherichia coli* associated with fresh produce sampled.

Multidrug resistant *Escherichia coli* profiles	No. Isolates	% with same profiles
CTX30C-KF30C-C30C-GM10C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-C30C-GM10C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	3	6.52%
C30C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-NI300C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-S10C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-NI300C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	9	19.57%
KF30C-C30C-S10C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-S10C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-GM10C-S10C-TS25 C-A10C-AP10C-T30C	1	2.17%
KF30C-C30C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	3	6.52%
KF30C-S10C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-TS25 C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-C30C-GM10C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-NI300C-TS25 C-NA30C-CIP5C-T30C	1	2.17%
KF30C-C30C-S10C-TS25 C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-TS25 C-A10C-AP10C-CIP5C	1	2.17%
CTX30C-KF30C-S10C-A10C-T30C	1	2.17%
CTX30C-KF30C-TS25 C-A10C-AP10C	1	2.17%
CTX30C-KF30C-TS25 C-A10C-AP10C-T30C	1	2.17%
GM10C-S10C-NA30C-A10C-AP10C-T30C	1	2.17%
KF30C-S10C-NI300C-A10C-AP10C	1	2.17%
S10C-TS25 C-NA30C-CIP5C-T30C	1	2.17%
KF30C-A10C-AP10C-T30C	1	2.17%
KF30C-S10C-NI300C-T30C	1	2.17%
KF30C-S10C-NI300C-T30C	1	2.17%
S10C-NA30C-CIP5C-T30C	1	2.17%
KF30C-NI300C-T30C	1	2.17%
KF30C-S10C-T30C	1	2.17%
S10C-TS25 C-T30C	1	2.17%

Table 5. Prevalence of antimicrobial resistance genes in *Escherichia coli* isolated from fresh produce.

Antimicrobial resistance gene tested	Number of isolates positive for the gene	Percentage of isolates containing the gene
bla_{TEM}	50	89.29%
tetA	46	82.14%
aadA1a	33	58.93%
tetB	30	53.57%
strAB	29	51.79%
sulII	29	51.79%
tetL	26	46.43%
sulI	23	41.07%
parC	16	28.57%
gyrA	14	25.00%
tetK	7	12.50%
$bla_{CTX\text{-}M\ Gp1}$	5	8.93%
$bla_{CTX\text{-}M\ Gp9}$	3	5.36%
tetD	2	3.57%
tetE	2	3.57%
tetM	2	3.57%
tetS	2	3.57%
bla_{SHV}	1	1.79%

bla_{OXA}, $bla_{CTX\text{-}M\ Gp2}$, $bla_{CTX\text{-}M\ Gp8\text{-}25}$, *VEB, PER, GES, blaZ, ACC, FOX, MOX, DHA, CIT, EBC, ampC, tetC, tetO, tetP, tetQ, tetX, aac(6')-Ib, qnrD, qnrS, catI, catII* and *catIII* were not detected in the 56 isolates.

of isolates belonging to phylogenetic group A and 7.14% of isolates not grouped into a phylogenetic group and classified as unknown (Figure 1).

Discussion

Street vendors in the informal settlements are confronted with lack of infrastructure such as potable water, ablution, storage and cooling facilities, that can impact on the microbiological safety of fresh produce (Marutlulle 2017; Du Plessis et al. 2017). Moreover, due to the unregulated system in informal street vending, implementation of food safety standards can be challenging. Food, including fresh produce, for human consumption contaminated with multidrug resistant bacteria is a cause for concern in public health (Soufi et al. 2009; Thanner et al. 2016). This is the first study investigating the diarrheagenic virulence and antimicrobial resistance genes of multidrug resistant *E. coli* from fresh produce within the South African informal retail sector. In this study a high proportion of the commensal *E. coli* found in fresh produce purchased from street vendors from the informal sector were MDR. In contrast, Du Plessis et al. (2017) found that 37.93% *E. coli* isolates from informal street vendors from cabbage and spinach were MDR and Corzo-Ariyama et al. (2019) found that only 20% of *E. coli* isolates from tomatoes in the supply chain were found to be MDR. Globally the resistance of fresh produce and environmental organisms is unknown and this information is required for further risk assessment, therefore it is essential to determine the role that fresh produce plays in the dissemination of MDR organisms within vulnerable and poor communities (Richter et al. 2020).

In this study, 80.36% of isolates were resistant to the Tetracyline class of antibiotics with 92.86% of isolates containing at least one *tet* gene. Tetracycline is not used for the treatment of *E. coli*, however the presence of *tet* genes and resistance to tetracycline has epidemiological importance (Badi et al. 2020). In contrast, a study on the antimicrobial resistance of *E. coli* isolated from cabbage and spinach, found that only 6.67% of isolates were resistant to the Tetracyline class (Du Plessis et al. 2017). Similarly, only 2.7% of *E. coli* isolated from fresh produce (spinach, tomatoes, carrots, cucumber, radish and cantaloupe) from India were found to be resistant to the Tetracycline class of antibiotics, which is in contrast to this current study (Verma et al. 2018). However, Corzo-Ariyama et al. 2019 found that 76.67% of *E. coli* isolates from tomatoes were resistant to tetracycline which was in agreement with what this study has shown. Badi et al. (2020) found high levels of *E. coli* (65%) to harbour *tet* genes detected for, although lower than the current study.

Resistance to the Sulfonamide and Penicillin classes were seen in 73.21% of isolates whilst resistance to the Cephalosporin class was seen in 67.86% of isolates, with genes conferring resistance to sulfonamides tested being detected from 51.79%, *ampC* β-Lactamase genes detected from 92.86%. Extended spectrum β-Lactamases and β-Lactamases genes were found in none of isolates, a finding that was also seen by Badi et al. (2020). In contrast, only 2.7% of *E. coli* isolated from fresh produce in India were resistant to Sulfonamides, with 6.8% resistant to the Penicillin class and between 0 and 2.7% resistant to the Cephalosporin class (Verma et al. 2018). Du Plessis et al. (2017) found that 40% of *E. coli* isolates from leafy greens were resistant to the Sulfonamide class and 13.33% of *E. coli* isolates to the Penicillin class. Whilst higher levels of resistance (76.4%) to the Penicillin class were observed for *E. coli* isolated from fresh produce (lettuce, spinach, carrots, parsley, cilantro and tomatoes) from small-acreage farms in Tennessee (Kilonzo-Nthenge et al. 2018). A similar observation of 66.67% of resistance to ampicillin was seen on isolates from tomatoes within the supply chain (Corzo-Ariyama et al. 2019).

A low percentage of *E. coli* isolates (4.1%) from fresh produce in India had resistance towards the Phenicol class of antibiotics (Verma et al. 2018), whereas in the current study, half the *E. coli* isolates (50%) demonstrated resistance to Phenicol. Similarly, Kilonzo-Nthenge et al. (2018) found 38.2% of the *E. coli* isolated from fresh produce in Tennessee showed resistance to the Phenicol class. *Escherichia coli* resistance towards Aminoglycosides (48.21%) in this study was more than that reported by Verma et al. (2018), but less than that found by Du Plessis et al. (2017). Similar levels of resistance (33.33%) to Aminoglycosides were observed from tomatoes from Northern Mexico (Corzo-Ariyama et al. 2019). In the current study, resistance to gentamycin (10.71%) was lower than that of streptomycin (46.43%). A similar pattern was seen by Kilonzo-Nthenge et al. (2018)

where no *E. coli* isolates demonstrated resistance to gentamycin and 34.5% of isolates demonstrated resistance to streptomycin.

Escherichia coli isolates from tomatoes (36.67%) in Northern Mexico were found to be resistant to nalidixic acid (Corzo-Ariyama et al. 2019), in contrast, only between 0 and 2.7% of *E. coli* isolated from fresh produce in India were resistant to the Synthetic Quinolone class (Verma et al. 2018). In this study, just over half (57.14%) of isolates were resistant to the Synthetic Quinolone class, with mutations in the *gyrA* and *parC* genes detected in 42.86% of isolates. Mutations in *gyrA* and *parC* genes, encoding the DNA gyrase and topoisomerase IV enzymes, have been described as one of the genetic bases in the resistance to quinolone family of antibiotics (Drlica and Zhao 1997). Due to the DNA gyrase, Gram negative bacteria are more susceptible to the action of Quinolones. Changes in the amino acid sequence of the DNA gyrase in these bacteria is related to the resistance to Quinolones. Also, mutations on *parC* can further increment the level of resistance against those antibiotics (Jacoby 2005).

Therefore, resistance of the *E. coli* isolates in this study to all classes tested were far higher than those reported in other similar studies (Du Plessis et al. 2017; Verma et al. 2018) and similar to some (Kilonzo-Nthenge et al. 2018; Corzo-Ariyama HA, García-Heredia A, Heredia N, García S, León J, Jaykus LA, Solís-Soto L. 2019. Phylogroups, pathotypes, biofilm formation and antimicrobial resistance of Escherichia coli isolates in farms and packing facilities of tomato, jalapeño pepper and cantaloupe from Northern Mexico. Int J Food Microbiol [Internet]. 290(August 2018). The presence of these antimicrobial resistant commensal and environmental *E. coli* strains is considered a high-risk (Krumperman 1983) due to the potential that these organisms have to transmit antimicrobial resistance conferring genes to other environmental and human gut bacteria (Marshall et al. 2009). Therefore, the spread of antimicrobial resistant bacteria from plants to humans via the food chain as well as the potential spread of antimicrobial resistant genes requires a holistic 'One-Health' approach in order to control and mitigate the risk of exposure (Jans et al. 2018).

However, with the lack of effective policies and regulation, as well as sector specific food safety standards, it is difficult to evaluate how safe the fresh produce really is and what the actual level of risk to the consumer is. In this study, spinach had the highest level of *E. coli* (44%), followed by carrots (22%), apples (22%), tomatoes (16%) and finally cabbage (8% ESV; 32% TSV). In contrast Verma et al. (2018) found that only 3.5% of spinach samples purchased in India were contaminated with *E. coli*. However, 16.3% of spinach samples from small-scale farms in Tennessee, USA had *E. coli* (Kilonzo-Nthenge et al. 2018). In the current study, 22% of the carrots and apples and 16% of the tomatoes were contaminated with *E. coli*, which was higher than the 2.3% of carrots and 1.5% of tomatoes reported in India (Verma et al. 2018). However, similar to our study, 60% of carrots from Tennessee and 4.9% of tomatoes were contaminated with *E. coli* (Kilonzo-Nthenge et al. 2018). In contrast to the current study, Kilonzo-Nthenge et al. (2018) found that apples were free of *E. coli* contamination. Du Plessis et al. (2017) found that only 3.33% of cabbage samples from informal vendors and 6.66% of cabbages sold in formal retailers had detectable *E. coli*. In this study, 8% of cabbages purchased from ESV and 32% from TSV were contaminated with *E. coli*. Moreover, *E. coli* levels from spinach were the highest throughout the study with levels ranging from 0 to 5.3 log10cfug-1 and an average of 0.15 log10cfug-1 (ESV) and 1.10 log10cfug-1 (TSV). These levels were similar to those reported from spinach by Kilonzo-Nthenge et al. (2018). These significantly higher values were then followed by *E. coli* levels on carrots which were contaminated with 0.47 log10cfug-1 (ESV) and 0.39 log10cfug-1 (TSV), which was similar to those found by Kilonzo-Nthenge et al. (2018). Average *E. coli* concentration on tomatoes [of 0.16 log10cfug-1 (TSV)] were significantly lower than spinach in this study and were enumerated from only 20% of the samples, while Kilonzo-Nthenge et al. (2018) reported a higher average of 0.7 log10cfug-1.

The diversity of phylogenetic groups in this study were higher than reported by Du Plessis et al. (2017) who found that *E. coli* isolated from spinach and cabbage sold at retailers and informal vendors in South Africa belonged mainly to phylogenetic group A (86%), followed by group E (7%). Similar results were seen from tomatoes, jalapeño peppers and cantaloupe where the majority of *E. coli* isolates from fresh produce belonged to phylogenetic group A (Corzo-Ariyama et al. 2019). In this study, a total of 28.57% of *E. coli* retrieved from apples, cabbage, carrots, spinach and tomatoes, were phylogenetically grouped into group E, which has predominantly been associated with intra-intestinal infections (Clermont et al. 2011). A further 26.79% of *E. coli* isolates in this study were grouped into the phylogenetic group C, which is far rarer and has previously been shown to demonstrate the potential for gut colonization, transmission and virulence (Moissenet et al. 2010). Interestingly, Du Plessis et al. (2017) found that 3% of *E. coli* isolates from informal vendors in South Africa were retrieved from cabbage and spinach samples were grouped into this rare phylogenetic group. Therefore these two phylogenetic groups are present in all fresh produce types from informal vendors in South Africa. Phylogenetic group B1 which previously been shown to be predominantly associated with plants (Méric et al. 2013) and herbivorous animals (Carlos et al. 2010) were associated with apples, spinach, cabbage and tomatoes, but not carrots. These 'generalist' and commensals (Bingen et al. 2009) were found to have the ability to persist in the environment (Walk et al. 2007) and were found to contain less virulence factors than groups B2 and D (Johnson and Stell 2002). On the other hand, Pupo et al. (1997) found that phylogenetic group B1 could be associated with intra-intestinal pathogens. Corzo-Ariyama et al. 2019 found that 16.7% of isolates from jalapeño peppers and 40% of isolates from cantaloupe during distribution belonged to phylogenetic group B1.

Acknowledgements

The financial assistance of the Department of Science and Innovation (DSI) – National Research Foundation (NRF) Centre of Excellence in Food Security who funded this research under the Food Safety Programme's 'Safe Food for the Food Insecure' project (Project 160301 and 160302). This work is based on the research supported in part by the NRF of South Africa (Grant specific unique reference number (UID). 74426). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF. The Water Research Commission (WRC) funded project 'Measurement of water pollution determining the sources and changes of microbial contamination and impact on food safety from farming to retail level for fresh vegetables' (WRC Project No K5/2706/4, WRC Knowledge Review 2017/18) and the Partnerships for Enhanced Engagement in Research (PEER) a USAID/DSI funded project 'Characterizing and tracking of antimicrobial resistance in the water-plant-food public health interface' (Grant no. 48) are also acknowledged. Authors would like to acknowledge the DSI-NRF Centre of Excellence in Food Security and PEER for financial assistance. The authors would like to acknowledge Ms L. Richter and Ms Z. Zulu for laboratory assistance, Mr M. Jongman, Mrs A. Lombard and Mrs D. Muller for logistical support and Dr J. Gokul for editorial support. Additionally, the support of Ms Liesl Morey from the Agricultural Research Council of South Africa's Biometry Unit is hereby acknowledged with sample design and statistical analysis.

Disclosure of interest

The authors declare no conflict of interest.

Funding

This work was supported by the Department of Science and Innovation – National Research Foundation Centre of Excellence in Food Security [Project 160301 and 160302]; USAID Partnerships for Enhanced Engagement in Research (PEER) [Grant no. 48]; Water Research Commission [WRC Project No K5/2706/4]; National Research Foundation of South Africa [Grant specific unique reference number 74426].

Data availability statement

The data that support the findings of this study are available from the corresponding author, S. Duvenage, upon reasonable request.

ORCID

Stacey Duvenage http://orcid.org/0000-0002-5984-1491
Erika Du Plessis http://orcid.org/0000-0002-5582-1434
Germán Villamizar-Rodríguez http://orcid.org/0000-0002-3558-0573
Lise Korsten http://orcid.org/0000-0003-0232-7659

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215 (3):403–410. doi:10.1016/S0022-2836(05)80360-2.

Aranda KRS, Fagundes-Neto U, Scaletsky IC. 2004. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic *Escherichia coli* and *Shigella* spp. J Clin Microbiol. 42(12):5849–5853. doi:10.1128/JCM.42.12.5849-5853.2004.

Badi S, Salah Abbassi M, Snoussi M, Werheni R, Hammami S, Maal-Bared R, Hassen A. 2020. High rates of antibiotic resistance and biofilm production in *Escherichia coli* isolates from food products of animal and vegetable origins in Tunisia: a real threat to human health. Int J Environ Health Res [Internet]. 1–11. doi:10.1080/09603123.2020.1769039.

Ben Said L, Klibi N, Dziri R, Borgo F, Boudabous A, Ben Slama K, Torres C. 2016. Prevalence, antimicrobial resistance and genetic lineages of *Enterococcus* spp. from vegetable food, soil and irrigation water in farm environments in Tunisia. J Sci Food Agric. 96(5):1627–1633. doi:10.1002/jsfa.7264.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2005. GenBank. Nucleic Acids Res. 33:D34–D38. doi:10.1093/nar/gki063.

Bingen E, Picard B, Brahimi N, Mathy S, Desjardins P, Elion J, Denamur E. 2009. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J Infect Dis. 177(3):642–650. doi:10.1086/514217.

Böckelmann U, Dörries H, Neus Ayuso-gabella M, De Marçay MS, Tandoi V, Levantesi C, Masciopinto C, Van Houtte E, Szewzyk U, Wintgens T, et al. 2009. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Appl Environ Microbiol. 75 (1):154–163. doi:10.1128/AEM.01649-08.

Callejón RM, Rodríguez-Naranjo MI, Ubeda C, Hornedo-Ortega R, Garcia-Parrilla MC, Troncoso AM. 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathog Dis [Internet]. 12(1):32–38. http://online.liebertpub.com/doi/full/10.1089/fpd.2014.1821

Carlos C, Pires MM, Stoppe NC, Hachich EM, Sato MIZ, Gomes TAT, Amaral LA, Ottoboni LMM. 2010. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol. 10:161. doi:10.1186/1471-2180-10-161

Carstens CK, Salazar JK, Darkoh C. 2019. Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. Front Microbiol. 10:(November):1–15. doi:10.3389/fmicb.2019.02667.

Centers for Disease Control and Prevention. 2011. Outbreak of Shiga toxin-producing *E.coli* O104 (STEC O104: H4) infections associated with travel to Germany (FINAL UPDATE) [Internet]. [accessed 2019 Apr 30]. https://www.cdc.gov/ecoli/2011/travel-germany-7-8-11.html

Centres for Disease Control and Prevention. 2020. National Outbreak Reporting System (NORS) [Internet]. [accessed 2020 Apr 1]. https://wwwn.cdc.gov/norsdashboard/

Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 5(1):58–65. doi:10.1111/1758-2229.12019.

Clermont O, Olier M, Hoede C, Diancourt L, Brisse S, Keroudean M, Glodt J, Picard B, Oswald E, Denamur E. 2011. Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. Infect Genet Evol. 11 (3):654–662. doi:10.1016/j.meegid.2011.02.005.

Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing [internet]. 28th editi. Pennsylvania, USA: clinical and laboratory standrds institute. [accessed 2019 Apr 15]. http://www.emeraldinsight.com/doi/10.1108/08876049410065598

Collignon P. 2009. Resistant *Escherichia coli*— we Are what we eat. Clin Infect Dis. 49(2):202–204. doi:10.1086/599831.

Corzo-Ariyama, HA, García-Heredia, A, Heredia, N, García, S, León, J, Jaykus, L, Solís-Soto, L. 2019. Phylogroups, pathotypes, biofilm formation and antimicrobial resistance of *Escherichia coli* isolates in farms and packing facilities of tomato, jalapeño pepper and cantaloupe from Northern Mexico. Int. J. Food Microbiol. 290:96-104. doi:10.1016/j.ijfoodmicro.2018.10.006

Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 65 (3):490–495. doi:10.1093/jac/dkp498.

Drlica K, Zhao X. 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 61 (3):377–392. doi:10.1128/.61.3.377-392.1997.

Du Plessis EM, Govender S, Pillay B, Korsten L. 2017. Exploratory study into the microbiological quality of spinach and cabbage purchased from street vendors and retailers in Johannesburg, South Africa. J Food Prot. 80 (10):1726–1733. doi:10.4315/0362-028X.JFP-16-540.

Du Rand G, Coundouris E 2017. Food safety knowledge, attitudes and practices of South African consumers toward fresh produce. In: Cape Town, South Africa: 22nd Biennial International Congress & Exhibition, 3 to 6 September 2017.

Escobar-Páramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguénec C, Denamur E. 2004. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. Mol Biol Evol. 21 (6):1085–1094. doi:10.1093/molbev/msh118.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.

Jacoby GA. 2005. Mechanisms of resistance to quinolones. Clin Infect Dis. 41(Supplement 2):S120–6. doi:10.1086/428052.

Jans C, Sarno E, Collineau L, Meile L, Kdc S, Stephan R. 2018. Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. Front Microbiol. 9(MAR). doi:10.3389/fmicb.2018.00362.

Johnson JR, Stell AL. 2002. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 181(1):261–272. doi:10.1086/315217.

Jongman M, Korsten L. 2016. Assessment of irrigation water quality and microbiological safety of leafy greens in different production systems. J Food Saf. 37:(May):1–12.

Khalil RKS, Gomaa MAE, Khalil MIM. 2015. Detection of shiga-toxin producing *E.coli* (STEC) in leafy greens sold at local retail markets in Alexandria, Egypt. Int J Food Microbiol. 197:58–64. doi:10.1016/j.ijfoodmicro.2014.12.019.

Kilonzo-Nthenge A, Liu S, Hashem F, Millner P, Githua S. 2018. Prevalence of Enterobacteriaceae on fresh produce and food safety practices in small-acreage farms in Tennessee, USA. J fur Verbraucherschutz und Leb [Internet]. 13(3):279–287. doi:10.1007/s00003-018-1172-y.

Krumperman PH. 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foodst. Appl Environ Microbiol [Internet]. 46(1):165–170. http://link.springer.com/article/10.1007/s11356-014-3887-3.

Li J, Wang T, Shao B, Shen J, Wang S, Wu Y. 2012. Plasmid-mediated quinolone resistance genes and antibiotic residues in wastewater and soil adjacent to swine feedlots: potential transfer to agricultural lands. Environ Health Perspect. 120(8):1144–1149. doi:10.1289/ehp.1104776.

Lynch M, Painter J, Woodruff R, Braden C. 2006. Surveillance for foodborne: disease outbreaks: United States, 1998-2002. Morb Mortal Wkly Rep [Internet]. 55(SS10):1–34. [accessed 2017Mar23http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5510a1.htm?_cid=ss

Marshall BM, Ochieng DJ, Levy SB. 2009. Commensals: underappreciated reservoir of antibiotic resistance. Microbe. 4(5):231–238.

Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette M, Michel G. 2000. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis* correlation between the resistance genotype determined by multiplex PCR assay. Antimicrob Agents Chemother. 44(2):231–238. doi:10.1128/AAC.44.2.231-238.2000.

Marutlulle NK. 2017. Causes of informal settlements in Ekurhuleni metropolitan municipality: an exploration. Africa's Public Serv Deliv Perform Rev. 5(1):11.

Maynard C, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Larivière S, Harel J, Larivie S. 2004. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal escherichia coli I. J Clin Microbiol. 42(12):5444–5452. doi:10.1128/JCM.42.12.5444-5452.2004.

Méric G, Kemsley EK, Falush D, Saggers EJ, Lucchini S. 2013. Phylogenetic distribution of traits associated with plant colonization in *Escherichia coli*. Environ Microbiol. 15(2):487–501. doi:10.1111/j.1462-2920.2012.02852.x.

Moissenet D, Salauze B, Clermont O, Bingen E, Arlet G, Denamur E, Mérens A, Mitanchez D, Vu-Thien H. 2010. Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum β-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. J Clin Microbiol. 48 (7):2459–2463. doi:10.1128/JCM.00529-10.

Nataro JP, Kaper JB. 1998. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev [Internet]. 11(1):142–201. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=121379&tool=pmcentrez&rendertype=abstract

Ng LK, Martin I, Alfa M, Mulvey M. 2001. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes. 15(4):209–215. doi:10.1006/mcpr.2001.0363.

Omar KB, Barnard TG. 2010. The occurrence of pathogenic *Escherichia coli* in South African wastewater treatment plants as detected by multiplex PCR. Water SA [Internet]. 36(2):172–176. http://www.scopus.com/inward/record.url?eid=2-s2.0-77954231151&partnerID=tZOtx3y1.

Oram M, Fisher LM. 1991. 4-Quinolone resistance mutations in the DNA gyrase of *Escherichia coli* clinical isolates identified by using the polymerase chain. Antimicrob Agents Chemother. 35(2):387–389. doi:10.1128/AAC.35.2.387.

Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. 2006. Prevalence in the United States of aac(6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother. 50(11):3953–3955. doi:10.1128/AAC.00915-06.

Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P, Schwarz S. 2018. Antimicrobial resistance in *Escherichia coli*. In: Antimicrob resist bact from livest companion anim. Washington, DC: American Society for Microbiology; p. 289–316.

Pupo GM, Karaolis DKR, Lan R, Reeves PR. 1997. Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. Infect Immun. 65(7):2685–2692. doi:10.1128/IAI.65.7.2685-2692.1997.

Richter L, Plessis EM, Duvenage S, Korsten L. 2020. Occurrence, phenotypic and molecular characterization of extended-spectrum- and AmpC- β -lactamase producing enterobacteriaceae isolated from selected commercial spinach supply chains in South Africa. Front Microbiol. 11:(April):1–10. doi:10.3389/fmicb.2020.00638.

Rma E-B, Ibrahim RA, Mohamed DS, Ahmed EF, Hashem ZS. 2020. Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic *E.coli* isolated from egyptian patients with different clinical infections. Infect Drug Resist. 13:1221–1236. doi:10.2147/IDR.S241073.

Robert Koch Institute. 2011. Report: final presentation and evaluation of epidemiological findings in the EHEC O104: h4outbreak [Internet]. [place unknown]: Robert Koch-Institute (RKI), Berlin - Germany. https://www.rki.de/EN/Content/infections/epidemiology/outbreaks/EHEC_O104/EHEC_final_report.pdf?__blob=publicationFile

Sa´enz Y, Brin˜as L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C. 2004. Mechanisms of resistance in multiple-antibiotic-resistant. Antimicrob Agents Chemother. 48(10):3996–4001. doi:10.1128/AAC.48.10.3996-4001.2004.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States-major pathogens. Emerg Infect Dis. 17(1):7–15. doi:10.3201/eid1701.P11101.

Soufi L, Abbassi MS, Sáenz Y, Vinué L, Somalo S, Zarazaga M, Abbas A, Dbaya R, Khanfir L, Ben Hassen A, et al. 2009. Prevalence and diversity of integrons and associated resistance genes in *Escherichia coli* isolates from poultry meat in Tunisia. Foodborne Pathog Dis. 6(9):1067–1073. doi:10.1089/fpd.2009.0284

Standing T-A, Du Plessis E, Duvenage S, Korsten L. 2013. Internalisation potential of *Escherichia coli* O157: H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* in lettuce seedlings and mature plants. J Water Health. 11(2):210. doi:10.2166/wh.2013.164.

Sunde M, Norström M. 2005. The genetic background for streptomycin resistance in *Escherichia coli* influences the distribution of MICs. J Antimicrob Chemother. 56(1):87–90. doi:10.1093/jac/dki150.

Ta Y, Nguyen T, To PB, Pham DX, Le HT, Thi GN, Alali WQ, Walls I, Doyle MP. 2014. Quantification, serovars, and antibiotic resistance of *Salmonella* isolated from retail raw chicken meat in Vietnam. J Food Prot. 77(1):57–66. doi:10.4315/0362-028X.JFP-13-221.

Tarr CL, Large TM, Moeller CL, Lacher DW, Tarr PI, Acheson DW, Whittam TS. 2002. Molecular characterization of a serotype O121: H19 clone, a distinct Shiga toxin-producing clone of pathogenic *Escherichia coli*. Infect Immun. 70(12):6853–6859. doi:10.1128/IAI.70.12.6853-6859.2002.

Thanner S, Drer D, Walsh F. 2016. Antimicrobial resistance in agriculture. Nationla Public Heal Serv Wales. 7(2): e02227–15.

Verma P, Saharan VV, Nimesh S, Singh AP. 2018. Phenotypic and virulence traits of *Escherichia coli* and *Salmonella* strains isolated from vegetables and fruits from India. J Appl Microbiol. 125(1):270–281. doi:10.1111/jam.13754.

Verraes C, Van Boxstael S, Van Meervenne E, Van Coillie E, Butaye P, Catry B, De Schaetzen MA, Van Huffel X, Imberechts H, Dierick K, et al. 2013. Antimicrobial resistance in the food chain: a review. Int J Environ Res Public Health. 10(7):2643–2669. doi:10.3390/ijerph10072643

Vila J, Ruiz J, Goñi P, Mt JDA. 1996. Detection of mutations in parC in quinolone-resistant clinical isolates of *Escherichia coli*. Antimicrob Agents Chemother. 40(2):491–493. doi:10.1128/AAC.40.2.491.

Walk ST, Alm EW, Calhoun LM, Mladonicky JM, Whittam TS. 2007. Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. Environ Microbiol. 9(9):2274–2288. doi:10.1111/j.1462-2920.2007.01341.x.

Waturangi DE, Hudiono F, Aliwarga E. 2019. Prevalence of pathogenic *Escherichia coli* from salad vegetable and fruits sold in Jakarta. BMC Res Notes [Internet]. 12(1):1–10. doi:10.1186/s13104-019-4284-2.

World Health Organisation Advisory Group on Integrated Surveillence of Antimicrobial Resistance. 2018. Critically important antimicrobials for human medicine. Switzerland:Geneva.

Xu A, Pahl DM, Buchanan RL, Micallef SA. 2015. Comparing the microbiological status of pre- and postharvest produce from small organic production. J Food Prot [Internet]. 78(6):1072–1080. http://openurl.ingenta.com/content/xref?genre=article&=0362-028X&volume=78&issue=6&spage=1072