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

Abbreviated running headline: Multidrug resistant *E. coli* in fresh produce

Authors: Tintswalo Baloyi[1,a], Stacey Duvenage[1,a,*], Erika Du Plessis[a], Germán Villamizar-Rodríguez[a] and Lise Korsten[a]

[1] Both authors contributed equally as first authors.

[a] *Department of Science and Innovation-National Research Foundation Centre of Excellence in Food Security, Department of Plant and Soil Sciences, University of Pretoria*

Co-Author 1: u13131975@tuks.co.za

Co-Author 1: stacey.duvenage@up.ac.za, https://orcid.org/0000-0002-5984-1491

Author 2: erika.duplessis@up.ac.za, https://orcid.org/0000-0002-5582-1434

Author 3: german.villamizar@gmail.com, https://orcid.org/0000-0002-3558-0573

Author 4: lise.korsten@up.ac.za, https://orcid.org/0000-0003-0232-7659

Corresponding Author: *Stacey Duvenage

Postal Address: Private Bag X20, Hatfield, Pretoria, South Africa, 0028

Email: stacey.duvenage@up.ac.za

Co-Author 1: Tintswalo Baloyi

Tintswalo Baloyi is a Masters graduate in plant pathology from the University of Pretoria, South Africa. She has broad knowledge on food safety, food security and consumer science studies. She has presented research papers that focuses on food safety of fresh produce sold and consumed in markets and households located in the informal settlements of South Africa in both local and international congresses. She is also

involved in a variety of community engagement projects that emphases the importance of food safety knowledge, attitude and practices. She has received several accolades in academia over the years. Her future aspirations include research that mainly focuses on food safety, security, waste and loses in the fresh produce agricultural sector.

Co-Author 1: Stacey Duvenage

Dr Stacey Duvenage a food safety expert with a passion for fresh produce safety within the formal and informal agroecosystems and supply chains, aspects of research include pathogen and antimicrobial resistant organism occurrence and organism characteristic resolution, including traditional and next generation technologies. Additionally, quality indicators within water and fresh produce is a big focus area with metadata analysis throughout South Africa. She is very passionate about next generation technology and has been instrumental in attaining the funding for two high impact, next generation and cutting edge technology platforms within the Plant Health and Safety laboratories, including a Matrix-Assisted Laser Desorption Time-of-Flight as well as a digital droplet PCR. She is currently a Senior Postdoctoral Research Fellow at the University of Pretoria. Research areas include food safety of fresh produce in the formal and informal sector, food genomics, and antibiotic resistance in the agroecosystem.

Author 2: Erika Du Plessis

Dr Erika du Plessis is a Senior Researcher in the Department of Plant and Soil Sciences at the University of Pretoria. She has 37 years research experience in food and water Microbiology/Biotechnology, published in international peer reviewed journals, co-authored two patents and presented at both national and international conferences. Her current responsibilities include research grant management, coordinating research activities and post graduate student mentoring. The main focus of her more recent research activities has been on the prevalence, dissemination and characteristics of antimicrobial resistant potential human pathogenic bacteria in the water-plant-food-public health interface. She has also been appointed to the Ministerial Advisory Committee on Antimicrobial Resistance since November 2019.

Author 3: Germán Villamizar-Rodríguez

Dr Germán Villamizar-Rodríguez (Caracas, 1978), received his PhD on Molecular and Functional Biology at the University of Oviedo with the dissertation: "Development of a multiple system for detection and enumeration of food-borne pathogens". He holds the authorship of indexed scientific publications regarding pathogen detection using molecular biology techniques as well as two registered patents. Dr. Villamizar-Rodriguez was awarded with a grant to conduct research in detection and characterisation of antibiotic resistant bacteria as a postdoctoral fellow in the University of Pretoria, under the supervision of Prof. Lise Korsten. His research interests have focused on the development and application of novel techniques, for detection of bacterial pathogens in different environments.

Author 4: Lise Korsten

Prof Korsten is the co-Director of the DSI/NRF Centre of Excellence Food Security, responsible for the food safety programme and actively interact with other researchers in various institutes. She is an editor for Food Security from Springer and Chair of the International Society for Plant Pathology Task Force on Global Food Security. Prof Korsten has addressed Parliament on Food Safety Control and has developed a national framework for Government to develop a Food Control Authority. Prof Korsten developed South Africa's first biocontrol agent for fruit and established a biocontrol research group at the University of Pretoria. She has also established a fruit health group that focuses on food safety of fresh produce and on Sanitary and Phytosanitary aspects related to international trade. Prof Korsten has focussed her research mainly on complementary fields of postharvest technology and food safety as related to international trade in fresh produce. As a team her group has been able to develop several innovative technologies to reduce disease and prevent product contamination. The value of her research programmes can best be illustrated by the sustained industry financial support, student training and publications. She has been able to attract extensive funding over her entire academic career.

Conflict of interest: None to declare.

Word count: 7246

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. 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* gene (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.

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 & 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).

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 & 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 & 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, -postharvest 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 & 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 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 ten informal street vendors (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 ten informal street vendors (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 24h at the Food Safety Laboratories as part of the University of Pretoria's diagnostic platform which operates on ISO 17025 management principles.

Samples of 150g (apple, carrots or tomato) were added to buffered peptone water (BPW) (3M, Johannesburg, South Africa) in a 1:1 weight: volume ratio (Xu et al. 2015). A 1:5 weight: volume ratio was used for 50g spinach and cabbage (Xu et al. 2015). The samples were macerated for 5 min at 230rpm 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 (3M) and incubated for 48h 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 24h 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 24h 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 (WHO AGISAR) (2018). This looked at 'critically important' (ampicillin-10µg, amoxicillin-10µg, nalidixic acid-30µg, streptomycin-10µg, cefotaxime-30µg, ciprofloxacin-5µg, gentamicin-10µg), 'highly important' (tetracycline-30µg and chloramphenicol-30µg, cephalothin-30µg and cotrimoxazole-25µg) and 'important' (nitrofurantoin-300µg) antimicrobials for human medicines. Inhibition zone diameters were measured and compared to breakpoints in the CLSI guidelines (CLSI, 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 40µL, using 1x DreamTaq PCR Master Mix (Life Technologies), 10-100µg of DNA, and 0,4µM 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).

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

Gene	Primer Sequence F / R	Size (bp)	Tm (˚C)	Ref.	Control
	Antibiotic Resistance Genes				
Aminoglycosides					
aac(6')-IB	5'-TTGCGATGCTCTATGAGTGGCTA3'/5'-CTCGAATGCCTGGCGTGTTT-3'	482	55	*	NO
strA-B	5'-TATCTGCGATTGGACCCTCTG-3'/5'-CATTGCTCATCATTTGATCGGCT-3'	538	60	†	NO
aadA1a	5'-GAGAACATACGCTTGCCTTGG-3'/5'-TCGGCGCGATTTTGCCGGTTAC-3'	198	48	†	
β Lactams (AmpC- β Lactamases)					
ACC	5'-CACCTCCAGCGACTTGTTAC-3'/5'-GTTAGCCAGCATCACGATCC-3'	346	60	‡	NO
FOX	5'-CTACAGTGCGGGTGGTTT-3'/5'-CTATTTGCGGCCAGGTGA-3'	162	60	‡	NO
MOX CMY	5'-GCAACAACGACAATCCATCCT-3'/5'-GGGATAGGCGTAACTCTCCCAA-3'	895	60	‡	NO
DHA	5'-TGATGGCACAGCAGGATATTC-3'/5'-GCTTTGACTCTTTCGGTATTCG-3'	997	60	‡	NO
LAT BIL CMY	5'-CGAAGAGGCAATGACCAGAC-3'/5'-ACGGACAGGGTTAGGATAGY-3'	538	60	‡	NO
ACT MIR	5'-CGGTAAAGCCGATGTTGCG-3'/5'-AGCCTAACCCCTGATACA-3'	683	60	‡	NO
β Lactams					
bla_{TEM}	5'-CATTTCCGTGTCGCCCTTATTC-3'/5'-CGTTCATCCATAGTTGCCTGAC-3'	800	60	‡	NCTC 13351
bla_{SHV}	5'-AGCCGCTTGAGCAAATTAAAC-3'/5'-ATCCCGCAGATAAATCACCAC-3'	713	60	‡	ATCC 700603
bla_{OXA}	5'-GGCACCAGATTCAACTTTCAAG-3'/5'-GACCCCAAGTTTCCTGTAAGTG-3'	564	60	‡	HWD 3.2 (14)
$bla_{CTX\text{-}M\ G1}$	5'-TTAGGAARTGTGCCGCTGYA-3'/5'-CGATATCGTTGGTGGTRCCAT-3'	688	60	‡	NO
$bla_{CTX\text{-}M\ G2}$	5'-CGTTAACGGCACGATGAC-3'/5'-CGATATCGTTGGTGGTRCCAT-3'	404	60	‡	NO
$bla_{CTX\text{-}M\ G9}$	5'-TCAAGCCTGCCGATCTGGT-3'/5'-TGATTCTCGCCGCTGAAG-3'	561	60	‡	HWD 3.2 (14)
$bla_{CTX\text{-}M\ G8/25}$	5'-AACRCRCAGACGCTCTAC-3'/5'-TCGAGCCGGAASGTGTYAT-3'	326	60	‡	NO
GES	5'-AGTCGGCTAGACCGGAAAG-3'/5'-TTTGTCCGTGCTCAGGAT-3'	399	57	‡	NO
PER	5'-GCTCCGATAATGAAAGCGT-3'/5'-TTCGGCTTGACTCGGCTGA-3'	520	60	‡	NO
VEB	5'-CATTTCCCGATGCAAAGCGT-3'/5'-CGAAGTTTCTTTGGACTCTG-3'	648	60	‡	NO
IMP	5'-TTGACACTCCATTTACDG-3'/5'-GATYGAGAATTAAGCCACYCT-3'	139	55	‡	NO
VIM	5'-GATGGTGTTTGGTCGCATA-3'/5'-CGAATGCGCAGCACCAG-3'	390	55	‡	NO
Cefotaxime					
ampC	5'-GTGACCAGATATGGCCACA-3'/5'-TTACTGTAGCGCCTCGAGGA-3'	822	55,8	§	NCTC 13406
Fluoroquinolones					
qnrD	5'-CGAGATCAATTTACGGGGAATA-3'/5'-AACAAGCTGAAGCGCCTG-3'	465	50	¶	NO
qnrS	5'-GCAAGTTCATTGAACAGGGT-3'/5'-TCTAAACCGTCGAGTTCGGCG-3'	428	54	¶	NO
Penicillin					
blaZ	5'-ACTTCAACACCTGCTGCTTTC-3'/5'-TGACCACTTTTATCAGCAACC-3'	173	56	**	ATCC 43300
Phenicols					
cat I	5'-AGTTGCTCAATGTACCTATAACC-3'/5'-TTGTAATTCATTAAGCATTCTGCC-3'	547	50	††	NO
cat II	5'-ACACTTTGCCCTTTATCGTC-3'/5'-TGAAAGCCATCACATACTGC-3'	543	50	††	NO
cat III	5'-TTCGCCGTGAGCATTTTG-3'/5'-TCGGATGAGTATGGGCAAC-3'	286	50	††	NO
Quinolones					
gyrA	5'-TACACCGGTCAACATTGAGG-3'/5'-TTAATGATTGCCGCCGTCGG-3'	648	64	‡‡	NO
parC	5'-AAACCTGTTCAGCGCCGCATT-3'/5'-GTGGTGCCGTTAAGCAAA-3'	395	64	§§	

Table 1 cont.

Gene	Primer Sequence F / R	Size (bp)	Tm (˚C)	Ref.	Control
Sulfonamides					
sulI	5'-TTCGGCATTCTGAATCTCAC-3'/5'-ATGATCTAACCCTCGGTCTC-3'	822	50	††	NO
sul II	5'-CGGCATCGTCAACATAACC-3'/5'-GTGTGCGGATGAAGTCAG-3'	722		††	NO
Tetracycline					
tet(A)	5'-GCTACATCCTGCTTGCCTTC-3'/5'-CATAGATCGCCGTGAAGAGG-3'	210	55	¶¶	NO
tet(B)	5'-TTGGTTAGGGGCAAGTTTTG-3'/5'-GTAATGGGCCAATAACACCG-3'	659	55	¶¶	NO
tet(C)	5'-CTTGAGAGCCTTCAACCCAG-3'/5'-ATGGTCGTCATCTACCTGCC-3'	418	55	¶¶	NO
tet(D)	5'-AAACCATTACGGCATTCTGC-3'/5'-GACCGGATACACCATCCATC-3'	787	55	¶¶	NO
tet(E)	5'-AAACCACATCCTCCATACGC-3'/5'-AAATAGGCCACAACCGTCAG-3'	278	55	¶¶	NO
	Diarrheagenic *Escherichia coli* virulence genes				
stx1	5'-ACACTGGATGATCTCAGTGG-3'/5'-CTGAATCCCCCTCCATTATG-3'	614	55	***	ATCC 35150
stx2	5'-CCATGACAACGGACAGCAGTT-3'/5'-CCTGTCAACTGAGCACTTTG-3'	779	55	***	ATCC 35150
eaeA	5'-CTGAACGGCGATTACGCGAA-3'/5'-GACGATACGATCCAG-3'	917	55	†††	ATCC 35150
mdh	5'-GGTATGGATCGTTCCGACCT-3'/5'-GGCAGAATGGTAACACCAGAGT-3'	300	55	‡‡‡	ATCC 35150
LT	5'-GGCGACAGATTATACCGTGC-3'/5'-CGGTCTCTATATTCCCTGTT-3'	410	55	***	DSM 10973, DSM 27503
ST	5'-TTTCCCCTCTTTTAGTCAGTCAACTG-3'/5'-GGCAGGATTACAACAAAGTTCACA-3'	162	55	***	DSM 10973, DSM 27503
ial	5'-GGTATGATGATGATGATGGGC-3'/5'-GGAGGCCAACAATTATTTCC-3'	630	50	***	DSM 9028, DSM 9034
ipaH	5'-GTTCCTTGACCGCCTTTCCGATACCGTC-3'/5'-GCCGGTCAGCCACCCTCTGAGAGTAC-3'	600	60	†††	DSM 9028, DSM 9034
AA PR	5'-CTGGCGAAAGACTGTATCAT-3'/AATGTATAGAAATCCGCTGTT-3'	630	57	†††	DSM 27502
	Phylogenetic grouping of *Escherichia coli*				
chuA	5'-ATGGTACCGGACGAACCAAC-3'/5'-TGCCGCCAGTACCAAAGACA-3'	288	59	§§§	ATCC 25922
yjaA	5'-CAAACGTGAAGTGTCAGGAG-3'/5'-AATGCGTTCCTCAACCTGTG-3'	211	59	§§§	ATCC 25922
TspE4.C2	5'-CACTATTCGTAAGGTCATCC-3'/5'-AGTTTATCGCTGCGGGTCGC-3'	152	59	§§§	ATCC 25922
arpA	5'-AACGCTATTCGCCAGCTTGC-3'/5'-TCTCCCCATACCGTACGCTA-3'	400	59	§§§	ATCC 25922
Group C Phylotyping confirmation					
trpA	5'-AGTTTTATGCCCAGTGCGAG-3'/5'-TCTGCGCCGGTCACGCCC-3'	219	57	§§§	NO
Group E Phylotyping confirmation					
arpA	5'-GATTCCATCTTGTCAAAATATGCC-3'/5'-GAAAAGAAAAAGAATTCCCAAGAG-3'	301	59	§§§	ATCC 35150

*: 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 2. 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 Tm according to primer pair (Table 1) for 30 sec 72°C for 60 sec	72°C for 5 min
Antibiotic Resistance Genes testing, without positive control			
94°C for 2 min	10x	94°C for 30 sec Tm+6°C for 30 sec 60 sec at 72° C	72°C for 5 min
	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 30sec 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 Tm according to primer pair (Table 1) for 30 sec 72°C for 2 min	72°C for 5 min
Phylogenetic grouping of *Escherichia coli*			
95°C for 4 min	30x	94°C for 5s Tm according to primer pair (Table 1) for 30 sec 72°C for 2 min	72°C for 5 min

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 of 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.

Table 3. 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-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	9	19.57%
KF30C-C30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-GM10C-S10C-TS25C-A10C-AP10C-T30C	1	2.17%
KF30C-C30C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	3	6.52%
KF30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-C30C-GM10C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-NI300C-TS25C-NA30C-CIP5C-T30C	1	2.17%
KF30C-C30C-S10C-TS25C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-TS25C-A10C-AP10C-CIP5C	1	2.17%
CTX30C-KF30C-S10C-A10C-T30C	1	2.17%
CTX30C-KF30C-TS25C-A10C-AP10C	1	2.17%
CTX30C-KF30C-TS25C-A10C-AP10C-T30C	1	2.17%
GM10C-S10C-NA30C-A10C-AP10C-T30C	1	2.17%
KF30C-S10C-NI300C-A10C-AP10C	1	2.17%
S10C-TS25C-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-TS25C-T30C	1	2.17%

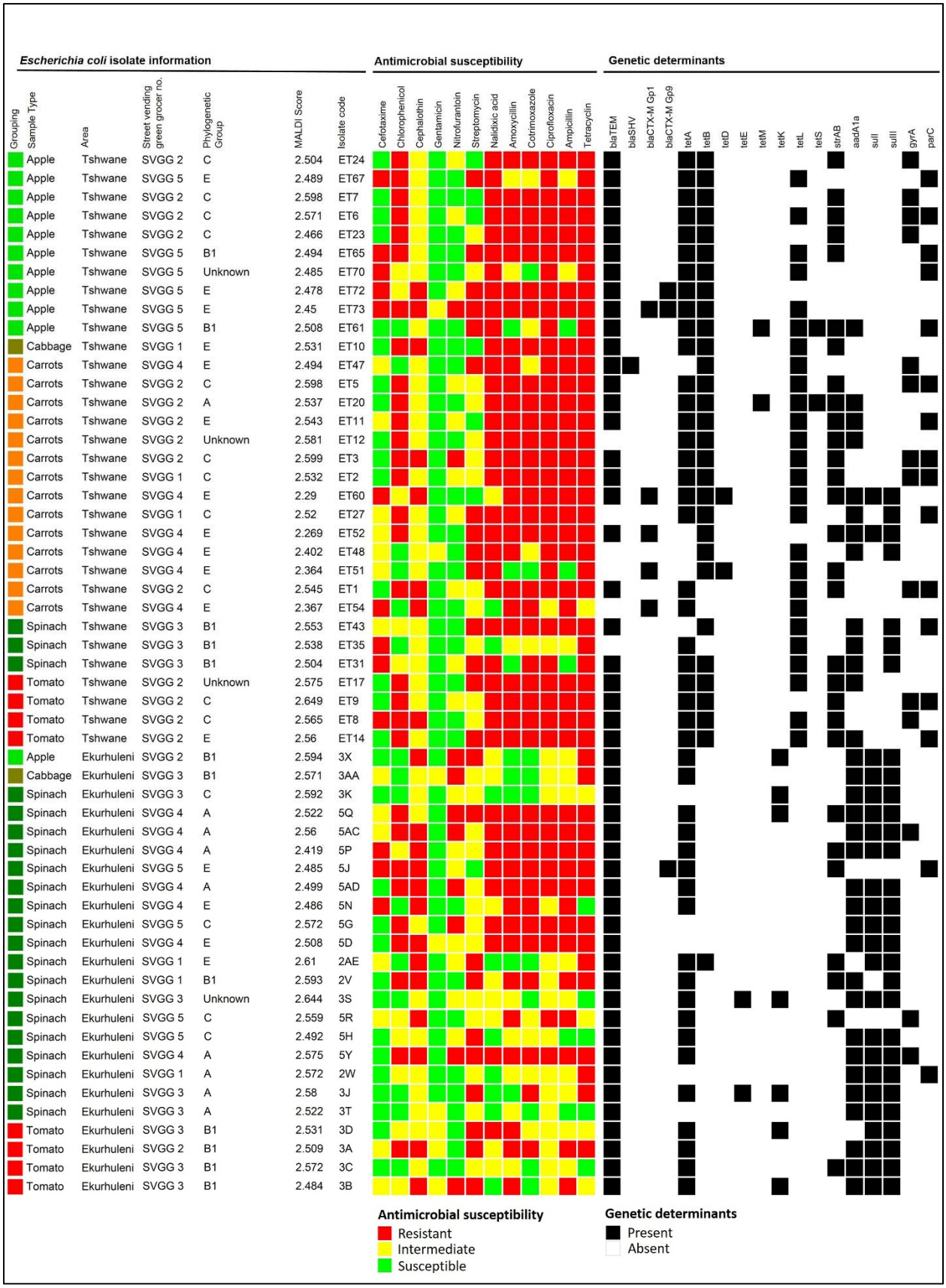
Escherichia coli isolate information
Antimicrobial susceptibility
Genetic determinants
Grouping
Sample Type
Area
Street vending green grocer no.
Phylogenetic Group
MALDI Score
Isolate code
Cefotaxime
Chloraphenicol
Cephalothin
Gentamicin
Nitrofurantoin
Streptomycin
Nalidixic acid
Amoxycillin
Cotrimoxazole
Ciprofloxacin
Ampicillin
Tetracyclin
blaTEM
blaSHV
blaCTX-M Gp1
blaCTX-M Gp9
tetA
tetB
tetD
tetE
tetM
tetK
tetL
tetS
strAB
aadA1a
sull
sulII
gyrA
parC
Apple Tshwane SVGG 2 C 2.504 ET24
Apple Tshwane SVGG 5 E 2.489 ET67
Apple Tshwane SVGG 2 C 2.598 ET7
Apple Tshwane SVGG 2 C 2.571 ET6
Apple Tshwane SVGG 2 C 2.466 ET23
Apple Tshwane SVGG 5 B1 2.494 ET65
Apple Tshwane SVGG 5 Unknown 2.485 ET70
Apple Tshwane SVGG 5 E 2.478 ET72
Apple Tshwane SVGG 5 E 2.45 ET73
Apple Tshwane SVGG 5 B1 2.508 ET61
Cabbage Tshwane SVGG 1 E 2.531 ET10
Carrots Tshwane SVGG 4 E 2.494 ET47
Carrots Tshwane SVGG 2 C 2.598 ET5
Carrots Tshwane SVGG 2 A 2.537 ET20
Carrots Tshwane SVGG 2 E 2.543 ET11
Carrots Tshwane SVGG 2 Unknown 2.581 ET12
Carrots Tshwane SVGG 2 C 2.599 ET3
Carrots Tshwane SVGG 1 C 2.532 ET2
Carrots Tshwane SVGG 4 E 2.29 ET60
Carrots Tshwane SVGG 1 C 2.52 ET27
Carrots Tshwane SVGG 4 E 2.269 ET52
Carrots Tshwane SVGG 4 E 2.402 ET48
Carrots Tshwane SVGG 4 E 2.364 ET51
Carrots Tshwane SVGG 2 C 2.545 ET1
Carrots Tshwane SVGG 4 E 2.367 ET54
Spinach Tshwane SVGG 3 B1 2.553 ET43
Spinach Tshwane SVGG 3 B1 2.538 ET35
Spinach Tshwane SVGG 3 B1 2.504 ET31
Tomato Tshwane SVGG 2 Unknown 2.575 ET17
Tomato Tshwane SVGG 2 C 2.649 ET9
Tomato Tshwane SVGG 2 C 2.565 ET8
Tomato Tshwane SVGG 2 E 2.56 ET14
Apple Ekurhuleni SVGG 2 B1 2.594 3X
Cabbage Ekurhuleni SVGG 3 B1 2.571 3AA
Spinach Ekurhuleni SVGG 3 C 2.592 3K
Spinach Ekurhuleni SVGG 4 A 2.522 5Q
Spinach Ekurhuleni SVGG 4 A 2.56 5AC
Spinach Ekurhuleni SVGG 4 A 2.419 5P
Spinach Ekurhuleni SVGG 5 E 2.485 5J
Spinach Ekurhuleni SVGG 4 A 2.499 5AD
Spinach Ekurhuleni SVGG 4 E 2.486 5N
Spinach Ekurhuleni SVGG 5 C 2.572 5G
Spinach Ekurhuleni SVGG 4 E 2.508 5D
Spinach Ekurhuleni SVGG 1 E 2.61 2AE
Spinach Ekurhuleni SVGG 1 B1 2.593 2V
Spinach Ekurhuleni SVGG 3 Unknown 2.644 3S
Spinach Ekurhuleni SVGG 5 C 2.559 5R
Spinach Ekurhuleni SVGG 5 C 2.492 5H
Spinach Ekurhuleni SVGG 4 A 2.575 5Y
Spinach Ekurhuleni SVGG 1 A 2.572 2W
Spinach Ekurhuleni SVGG 3 A 2.58 3J
Spinach Ekurhuleni SVGG 3 A 2.522 3T
Tomato Ekurhuleni SVGG 3 B1 2.531 3D
Tomato Ekurhuleni SVGG 2 B1 2.509 3A
Tomato Ekurhuleni SVGG 3 B1 2.572 3C
Tomato Ekurhuleni SVGG 3 B1 2.484 3B
Antimicrobial susceptibility
Resistant
Intermediate
Susceptible
Genetic determinants
Present
Absent

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.

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 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). 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).

Table 4. 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.

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% 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 & 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 et al. 2019). 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 & 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/DST 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.

Data availability statement

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

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410.

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.

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]. 00(00):1–11. https://doi.org/10.1080/09603123.2020.1769039

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2005. GenBank. Nucleic Acids Res. 33(Database Issue):D34–D38.

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.

Böckelmann U, Dörries H, Neus Ayuso-gabella M, de Marçay MS, Tandoi V, Levantesi C, Masciopinto C, Van Houtte E, Szewzyk U, Wintgens T, Grohmann E. 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.

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):32–8. 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.

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.

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.

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.

Clinical and Laboratory Standards Institute. 2018. Performance Standards for Antimicrobial Susceptibility Testing [Internet]. 28th editi. Pennsylvania, USA: Clinical and Laboratory Standrds Institute. 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.

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):96–104. https://doi.org/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.

Drlica K, Zhao X. 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 61(3):377–92.

El-Baky RMA, 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.

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.

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.

Jans C, Sarno E, Collineau L, Meile L, Stärk KDC, Stephan R. 2018. Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. Front Microbiol. 9(MAR).

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.

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.

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. https://doi.org/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.

Lynch M, Painter J, Woodruff R, Braden C. 2006. Surveillance for Foodborne: Disease Outbreaks: United States, 1998-2002. Morb Mortal Wkly Rep [Internet]. [accessed 2017 Mar 23] 55(SS10):1–34. http://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.

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.

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.

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.

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.

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.

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.

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.

Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P, Schwarz S. 2018. Antimicrobial Resistance in Escherichia coli. Antimicrob Resist Bact from Livest Companion Anim.: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.

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.

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.

Robert Koch Institute. 2011. Report: Final presentation and evaluation of epidemiological

findings in the EHEC O104:H4 outbreak [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.

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.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe R V., 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.

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.

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.

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.

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.

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.

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

Verma P, Saharan V V., 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.

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.

Vila J, Ruiz J, Goñi P, Jimenez De Anta MT. 1996. Detection of mutations in parC in quinolone-resistant clinical isolates of Escherichia coli. Antimicrob Agents Chemother. 40(2):491–493.

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.

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. https://doi.org/10.1186/s13104-019-4284-2

World Health Organisation Advisory Group on Integrated Surveillence of Antimicrobial Resistance. 2012. Critically Important Antimicrobials for Human Medicine. Geneva,

Switzerland.

Xu A, Pahl DM, Buchanan RL, Micallef S a. 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&issn=0362-028X&volume=78&issue=6&spage=1072