Microbiological quality assessment of fresh produce: potential health risk to children and urgent need for improved food safety in school feeding schemes

Running title: Fresh produce safety in schools

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Abstract

About 388 million school-going children worldwide benefit from school feeding schemes which make use of fresh produce to prepare meals. Fresh produce, including leafy greens and other vegetables were served at 37% and 31% of school feeding programmes respectively in Africa. This study aimed at assessing the microbiological quality of fresh produce grown onsite or supplied to South African schools that are part of the national school feeding programmes that benefits over 9 million school-going children.

Coliforms, *Escherichia coli*, Enterobacteriaceae, and *Staphylococcus aureus* were enumerated from fresh produce (n=321) samples. The occurrence of *E. coli*, *Listeria monocytogenes*, *Salmonella* spp., and extended-spectrum-β-lactamase producing (ESBL-) Enterobacteriaceae was determined. Presumptive pathogens were tested for antimicrobial resistance. *Escherichia coli* was further tested for diarrheagenic virulence genes. Enterobacteriaceae on 62.5% of fresh produce samples (200/321) exceeded previous microbiological guidelines for ready-to-eat food, while 86% (276/321 samples) and 31.6% (101/321 samples) exceeded coliform and *E. coli* criteria, respectively. A total of 76 Enterobacteriaceae were isolated from fresh produce including *E. coli* (n=43), *Enterobacter* spp. (n=15) and *Klebsiella* spp. (n=18). Extendedspectrum-β-lactamase production was confirmed in 11 *E. coli*, 13 *Enterobacter* spp., and 17 *Klebsiella* spp. isolates. No diarrheagenic virulence genes were detected in *E. coli* isolates. However, multidrug resistance (MDR) was found in 60.5% (26/43) of the *E. coli* isolates, while all (100%; n=41) of the confirmed ESBL- and AmpC Enterobacteriaceae, showed multidrug resistance.

Our study indicates the reality of the potential health risk that contaminated fresh produce may pose to school-going children, especially with the growing food safety challenges and antimicrobial resistance crisis globally. This also shows that improved food safety approaches to prevent foodborne illness and the spread of foodborne pathogens through food served by school feeding schemes are necessary.

Keywords: microbiological quality, school-going children, potential health risk, school feeding, fresh produce

Introduction

There are 388 million school children, in 161 countries receiving meals at schools globally (World Food Programme, 2021). The largest school feeding programmes are in India (90 million children), Brazil and China (40 million children), the United States of America (30 million children), and Egypt (11 million children) (World Food Programme, 2021). South

Africa provides meals to over 9.6 million school-going children (Department of Basic Education, 2019). These school feeding schemes make use of vegetables to prepare meals for the school children. In addition, the school children normally also get fruit with their meals (Department of Basic Education, 2019).

Fresh produce is associated with health benefits, and thus a desirable component of any meal (Weichselbaum and Buttriss 2014). However, fresh produce has also been linked to foodborne disease outbreaks (Park *et al*. 2012). Globally an estimated 600 million foodborne disease cases occur every year, resulting in over 400 000 deaths mostly caused by bacterial pathogens [Havelaar *et al.*, 2015; World Health Organization (WHO), 2015]. *Escherichia coli* are often a harmless commensal organism, however pathogenic strains cause diarrhoea and other serious gastrointestinal diseases (Hamilton *et al*., 2010). Other major foodborne pathogens include *Salmonella* spp. and *Listeria monocytogenes* [Centers for Disease Control and Prevention (CDC) 2020]. In addition to these pathogens, extended-spectrum-β-lactamase (ESBL-) producing Enterobacteriaceae have also been detected in food linked to foodborne disease outbreaks (Calbo *et al.*, 2011; Lavilla *et al.*, 2008). These ESBL- as well as AmpC βlactamase-producing Enterobacteriaceae have also been detected in fresh vegetables (Berner *et al.*, 2015; Richter *et al.*, 2019; Blaak *et al.*, 2014; Li *et al.*, 2018a) and are therefore a reason for concern especially with the global drive to increase consumption of fresh produce. Moreover, with increasing antibiotic resistance in bacterial pathogens, general treatment of foodborne diseases is a growing concern in health care [Centers for Disease Control and Prevention (CDC), 2013; WHO, 2016]. In addition to illness, foodborne diseases could result in death and long-term chronic ailments (James, 1997). Anxiety, an indirect effect of foodborne diseases can also exist in communities that have experienced outbreaks and further lack trust in the food system (Bryan, 1978). Foodborne diseases also put extreme pressure on the public health system as well on health care workers (Bryan, 1978). For school going children, who are classified in the most vulnerable group (Kirk *et al*. 2017), foodborne disease also means loss of learning time and negatively impacting on their growth and development (Sibanyoni and Tabit 2016).

The National Institute for Communicable Diseases (NICD) reported 31 cases of foodborne and/or waterborne disease outbreaks in the first six months of 2017 in South Africa (SA), nine of which were recorded from schools (NICD, 2017). Fresh produce was implicated in two of these outbreaks, where *Salmonella* spp. and *Clostridium perfringens* were detected (NICD, 2014; Msomi, 2017).

The safety of fresh produce used to make meals and served at schools, globally, is therefore a concern and warrants for further investigations. Moreover, as far as the authors are aware, the potential food safety risk associated with fresh produce in schools has not been explored in South Africa. This study investigated the microbiological safety of fresh produce (spinach, Chinese spinach, carrots, cabbage, onions, tomatoes, lettuce, and apples) grown at or supplied to schools to prepare meals.

Methods and materials Sample collection

Fresh produce was collected from six schools in Gauteng Province (schools 1-3 in Ekhuruleni district and schools 4-6 in Tshwane district) and from six schools in the Mpumalanga Province (schools 7-9 in Nkangala district and schools 10-12 in the Gert Sibande district) after permission was granted by the provincial Departments of Basic Education, each school was visited twice. Fresh produce (growing in gardens on the school premises) available at the time of sampling, were collected aseptically at five points in the school garden per crop planted (n=186) and from three different packages in the kitchen storage area per produce item (n=135). Each sample consisted of an equal number of three different fresh produce units. Samples included spinach (Swiss chard), Chinese spinach, lettuce (Iceberg), onions, cabbage, apples, tomatoes, and carrots. These samples were transported in cool boxes to the Plant Pathology laboratories, University of Pretoria and kept refrigerated (4^0C) until processing was done, usually within 48h. **Microbiological analysis**

Fresh produce (50g of spinach, lettuce and cabbage, 150g of apples, onions, carrots and tomatoes) was macerated in buffered peptone water (BPW) (Merck, Johannesburg, SA) [200 ml for spinach, cabbage and lettuce (1:4 ratio), 250 ml for apples, tomatoes, onions and carrots (1:5 ratio)] (Xu *et al.*, 2015) in Seward stomacher 400 circulator strainer bags (Lasec, Johannesburg), using the Seward Stomacher (Lasec) at 230 g for 5 min. A dilution series of each sample was done using 0.1% BPW and spread plated onto Violet Red Bile Glucose agar (Oxoid, Johannesburg) (ISO 21528 and ISO 11133:2014) in duplicate to enumerate Enterobacteriaceae, onto Staph Express Count Plates and *E. coli*/coliform Count Plates (3M, Johannesburg) to enumerate *Staphylococcus aureus* and *E. coli* and coliforms, respectively. Agar plates and count plates were incubated at 37°C for 24h.

Fresh produce samples in BPW were incubated at 37°C, following 4h of incubation, 1ml was transferred to 9ml of Enterobacteriaceae Enrichment Broth (EE Broth) (Oxoid) and incubated at 30°C for 24h. Samples in BPW were then further incubated at 37°C for 24h. Subsequently, samples in BPW were streaked onto Eosin methylene blue agar (Oxoid) for the detection of *E.*

coli, Baird-Parker agar (Merck) and Mannitol Salt agar (ThermoFisher Scientific, Johannesburg) for *S. aureus*. The incubated EE broth was streaked onto chromID ESBL agar (Biomeriuex, Johannesburg) to detect ESBL-producing Enterobacteriaceae. For the detection of *L. monocytogenes* and other *Listeria* species, $1ml^{-1}$ of the overnight incubated sample in BPW was transferred to $9ml^{-1}$ of Buffered Listeria Enrichment Broth (Oxoid) and incubated at 37°C for 48h and then streaked onto Agar *Listeria* according to Ottaviani and Agosti (BioRad, AEC Amersham, Johannesburg) and Rapid L. mono agar (BioRad). *Salmonella* spp. detection was done from samples incubated in BPW using the BioRad iQ check *Salmonella* kit (AEC Amersham), following the manufacturer's instructions (AOAC OMA 2017.06). All presumptive positive isolates were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF) in conjunction with the Bruker MALDI Biotyper software (Bruker, Johannesburg) (Standing *et al*. 2013).

Antimicrobial resistance testing and virulence gene screening of *Escherichia coli* isolates

All 43 *E. coli* isolates were subjected to antimicrobial resistance screening using the KirbyBauer Disc Diffusion method (Bauer *et al*. 1966). *Escherichia coli* isolates were cultured in brain heart infusion broth (BHI) and plated onto Mueller Hinton agar plates (ThermoFisher Scientific). *Escherichia coli* isolates were tested against cefotaxime (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), cephalothin (30µg), gentamicin (100µg), nitrofurantoin (300µg), streptomycin (10µg), nalidixic acid (30µg), amoxycillin (10µg), ampicillin (10µg), trimethoprim/sulfamethoxazole (1.25/23.75µg) and tetracycline (30µg). Zone diameters were measured (mm) and analysed according to the Clinical & Laboratory Standards Institute (CLSI) (CLSI, 2018) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2013) guidelines. Break points measured were recorded as susceptible or resistant, with isolates demonstrating intermediate resistance classified as susceptible, in order to avoid overestimation of resistance (Ta *et al*., 2014). Isolates with resistance to more than three antibiotic classes were classified as multidrug resistance (MDR).

Additionally, *E. coli* isolates were cultured in Tryptone Soy Broth (Merck) at 37°C for 24h, followed by genomic DNA extraction using the Quick gDNA Mini-Prep Kit (Zymo Research) according to manufacturer's instructions. The concentration of the DNA extracts were determined using the Qubit broad range double stranded DNA assay and the Qubit fluorometer (Life Technologies). For the detection of enterotoxigenic, enteropathogenic, enteroaggregative, enterohemorrhagic, enteroinvasive and shiga-toxin virulence genes in the *E. coli* isolates, specific primers and primer concentrations as indicated in Table 1 were used

for PCR reactions. *Escherichia coli* strains ATCC 35150 (*E. coli* O157:H7) and ATCC 25922 (generic non-pathogenic *E. coli*) were used as positive and negative controls, respectively. In addition, PCR grade water was used as the no template control. The PCR reactions (25 µl) contained between 100-120ng of the template DNA, the forward and reverse primer (Table 1) as well as 1x DreamTaq Green PCR Master Mix (Thermofisher Scientific). PCR cycling conditions were as follows: an initial denaturation at 95°C for15min, followed by 35 cycles of 94°C for 45s, primer specific annealing temperature (Table 1) for 45s and 68°C for 2 min, and a final extension for 7 min at 72°C. Products of the PCR reaction were electrophoresed on a 2% agarose gel (ThermoFisher Scientific), prepared according to manufacturer's instructions, at 120V for 90 min and thereafter visualised using the GelDoc system (BioRad) in conjunction with Image Lab software (version 4.0.1).

Confirmation of extended spectrum β-lactamase and AmpC production in presumptive extended spectrum β-lactamase Enterobacteriaceae isolates

Forty-four presumptive ESBL- producing Enterobacteriaceae isolates including *E. coli* (n=11), *Klebsiella* spp. (n=18) and *Enterobacter* spp. (n=15) were cultured as previously described in BHI and on Mueller Hinton agar plates to screen for ESBL and AmpC production. The doubledisk synergy test (DDST), using cefotaxime (30µg), ceftazidime (30µg) and cefpodoxime (10µg) alone and in combination with clavulanic acid (10µg) (Mast Diagnostics, Johannesburg) [European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2013]. The agar plates were incubated at 37°C for 24h. Additionally, ESBL-production in presumptive ESBL-*Enterobacter* spp. was confirmed using cefepime (30µg) alone and in combination with clavulanic acid (10µg) (Mast Diagnostics). The confirmation of AmpCproduction in all isolates was done using the AmpC detection set (Mast diagnostics). Zone diameters were measured (mm) and analysed according to the CLSI (2018) and EUCAST (2013) guidelines.

Antimicrobial resistance screening of extended spectrum β-lactamase- and AmpC-producing Enterobacteriaceae isolates

Confirmed ESBL- and AmpC- producing *E. coli* (n=11), *Enterobacter* spp. (n=13) and *Klebsiella* spp. (n=17) isolates were then subjected to additional antimicrobial screening against amoxicillin (10µg), ampicillin (10µg), amoxicillin/clavulanic acid (20µg/10µg), cefoxitin (30µg), cefepime (30µg), cefpodoxime (10µg), ceftazidime (30µg), imipenem (10µg), tetracycline (30µg), neomycin (10µg), gentamicin (10µg), chloramphenicol (30µg), cefotaxime (30µg), ciprofloxacin (5µg) and nitrofurantoin (300µg). *Klebsiella pneumonia*

ATCC 700603, *E. coli* NTCC, *E. coli* ATCC 25922 and *E. cloacae* NCTC 1406 were used as controls (CLSI, 2018). Zone diameters were measured, and results recorded as previously described.

Results Coliform, *Escherichia coli*, Enterobacteriaceae and *Staphylococcus aureus* counts

For fresh produce obtained from school gardens, cabbage samples had the highest mean Enterobacteriaceae and coliform counts at 5.12 log cfu g^{-1} and 4.46 log cfu g^{-1}, respectively (Table 2). However, spinach had the highest mean *E. coli* counts (1.06 log cfu g^{-1}) while mean *S. aureus* counts were highest in Chinese spinach samples (3.49 log cfu g^{-1}) (Table 2). Enterobacteriaceae, coliform, *E. coli* and *S. aureus* counts for fresh produce obtained from school storerooms were highest in tomatoes (5.65 log cfu g^{-1}), carrots (4.28 log cfu g^{-1}), tomatoes (0.61 log cfu g^{-1}) and onions (1.49 log cfu g^{-1}), respectively (Table 3). Mean indicator organism counts for carrot samples obtained from the school storerooms were higher than for carrot samples obtained from the school gardens. Mean *E. coli* counts were the highest in spinach growing in the school gardens at 1.06 log cfu g^{-1} (Table 2). Whilst the highest *S. aureus* counts were observed in Chinese spinach growing in the school gardens (Table 2). Apples in the storeroom contained the lowest *E. coli* counts for fresh produce in the storerooms. Whilst the lowest *S. aureus* count was observed in carrots growing the school gardens (Table 2). No *E. coli* were enumerated from the lettuce samples.

Detection of Enterobacteriaceae and *Listeria monocytogenes*

A total of 73 Enterobacteriaceae isolates were detected on fresh produce obtained from schools in the Gauteng and Mpumalanga Provinces. These included *E. coli* (n=43) (both generic and ESBL-producing), *Enterobacter* spp. (n=13) as well as *Klebsiella* spp. (n=17). *Escherichia coli* was detected in 10.0% of cabbage (2 out of 20 samples), 11.8% carrots (2 out of 17 samples) and 11.1% Chinese spinach (2 out of 18 samples), respectively as well as 3.7% onions (1 out of 27 samples) and 20.0% spinach (21 out of 105 samples) obtained from the school gardens. From fresh produce obtained from the storerooms, *E. coli* was detected in 7.1% onions (3 out of 42 samples) and 16.7% tomatoes (2 out of 12 samples).

Of these *E. coli* isolates, 25.6% (11 out of 43) of them were found to be ESBL and/or AmpC-producing and were detected in 5.6% Chinese spinach (1 out of 18 samples), 7.4% onions (2 out of 27 samples) and 4.8% spinach (5 out of 105 samples) from the school gardens whereas in the storerooms they were detected from 4.8% onions (2 out of 42 samples), 8.3% tomatoes (1 out of 12 samples) as well as 22.2% carrots (2 out of 9 samples).

Klebsiella pnuemoniae and *Klebsiella oxycota* (n=17) isolates were of found to be ESBL- and/or AmpC- producing and were detected in 5.6% Chinese spinach (1 out of 18 samples), 11.1% onions (3 out of 27 samples) as well as 6.7% of spinach (7 out of 105 samples) obtained from the school gardens. Extended spectrum beta-lactamase *Klebsiella* spp. were also detected from 11.9% onions (5 out of 42 samples), 22.2% carrots (2 out of 9 samples) and 2.6% cabbages (1 out of 39 samples) from the school storerooms.

Enterobacter spp. (n=13) producing ESBL's were detected from 15% cabbage (3 out of 20 samples), 11.1% Chinese spinach (2 out of 18 samples), 3.7% onions (1 out of 27 samples), 6.8% of spinach (7 out of 105 samples) and 20% of lettuce (1 out of 5 samples) samples obtained from the school gardens. In the storerooms, ESBL- *Enterobacter* spp. were detected in 2.4% onions (1 out of 42 samples) and 3.7% apples (1 out of 27 samples), 16.7% of tomatoes (2 out of 12 samples) and 7.7% of cabbage samples (3 out of 39 samples).

Listeria monocytogenes and *Salmonella* spp. were not detected in any of the fresh produce samples obtained from schools in the Gauteng and Mpumalanga Provinces. **Antimicrobial resistance and virulence gene screening of *Escherichia coli* isolates** No virulence genes were detected in the *E. coli* isolates that were screened. However, MDR was found in 60.5% of these 43 *E. coli* isolates (Table 4). *Escherichia coli* isolates (n=43) displayed resistance to amoxicillin (62.8%), ampicillin (60.5%), trimethoprim (55.8) and tetracycline (55.8%). This was followed by resistance to cephalothin (51.2%), nitrofurantoin (46.5%), streptomycin (46.5%), nalidixic acid (46.5%), ciprofloxacin (44.2%) and cefotaxime (41.9%) with the least resistance to chloramphenicol (20.9%) and gentamicin (20.9%). Six (18.6%) *E. coli* isolates obtained from spinach collected from schools 9 and 10 and two *E. coli* isolates obtained from carrot samples collected from school 11 showed resistance to eight antibiotic classes (Table 4). Similarly, six of the *E. coli* isolates found on two spinach samples from schools 11 and 12, as well as on two onion samples from schools 7 and 8, and two tomato samples from schools 10 and 11 were resistant to seven classes of antibiotics. *Escherichia coli* isolates that were resistant to nine classes of antibiotics were found on one carrot sample and two spinach samples, all obtained from school 7.

Antimicrobial resistance screening of extended spectrum β-lactamase- and AmpCproducing *Escherichia* coli, *Enterobacter* and *Klebsiella* species

Of the 41 ESBL and/or AmpC-producing isolates, 47.8% were AmpC producers, 78.0% were ESBL- producers, while 24.4% were both AmpC and ESBL producers. These included *E. coli* (n=11), *Enterobacter spp.* (n=13), *Klebsiella* spp. (n=17). Of these 41 AmpC and/or ESBL-

238 producing isolates, 97.6% were resistant to neomycin and nitrofurantoin followed by 95.1% of
239 the isolates showing resistance to both ampicillin and amoxicillin. Resistance to tetracycline
240 and trimethoprim was seen in 82.9% and 87.8% of the *E. coli* isolates, respectively, whereas
241 resistance to ciprofloxacin and amoxicillin/clavulanic acid was seen in 78.1% of the isolates.
242 Resistance to cefoxitin, gentamicin and chloramphenicol was seen in 39.0%, 34.2% and 22.0%
243 of the isolates, respectively. Only 14.6% of the isolates were resistant to imipenem, an antibiotic
244 belonging to the carbapenem class of antibiotics. Resistance against the third generation
245 cephalosporins, cefotaxime, ceftazidime and cefpodoxime was found seen in 78.1%, 82.9% as
246 well as 97.6% of ESBL and/or AmpC- producing Enterobacteriaceae. About 90.0% of these
247 isolates were resistant to cefepime, a fourth-generation cephalosporin. Multidrug resistance
248 was seen in 100% of the ESBL- and/or AmpC-producing
249 *Enterobacter*iaceae isolates, with up to 46.3% of these isolates resistant to eight classes of
250 antibiotics (Table 5).

251 **Discussion**

252 Fresh produce is included in global and the national school feeding menus in addition to the
253 starch and protein component to ensure that learners get the required vitamins, minerals and
254 nutrients daily (Rendall-Mkosi *et al*. 2013). Most vegetables are cooked and fruit such as
255 apples, bananas and oranges are served raw. However, the present study has shown that fresh
256 produce grown and supplied to schools in the Gauteng and Mpumalanga Provinces are not
257 always compliant with food safety criteria (based on previous SA Department of Health
258 guidance, under review) Public Health England and the Hong Kong Centre for Food Safety
259 criteria (Department of Health 2010; Public Health England 2013; Hong Kong Centre for Food
260 Safety 2014) due to the presence of MDR *E. coli* and ESBL and/or AmpC- producing
261 Enterobacteriaceae as well as coliforms, *E. coli* and *S. aureus.*
262 In this study, 86.0% and 31.0% of the fresh produce (from the school gardens and those
263 delivered to the school), exceeded the coliform and *E. coli* guidelines respectively based on the
264 previous Department of Health guidelines, (Department of Health 2010). Keeping in mind that
265 fresh produce are grown on smaller scale at schools and are mostly supplied to the school by
266 independent suppliers based on the Department of Basic Education procurement processes
267 (Rendall-Mkosi *et al*. 2013).
268 Du Plessis *et al.* (2017) described mean coliform counts of 4.0 log cfu/g^{-1} and 3.3 log cfu/g^{-1}
269 for cabbage samples obtained from vendors and retailers, respectively. These were comparable
270 to the mean coliform counts observed from cabbage samples in this study. While the mean *E.*

coli count for spinach in this study, did not exceed 1.1 log cfu/g^{-1}, similar to those reported by Du Plessis *et al.* (2017) (0.8 log cfu/g^{-1} and 0.4 log cfu/g^{-1}). Similarly, an *E. coli* mean count of 0.7 log cfu/g^{-1} for spinach was reported by Johnston *et al.* (2005), also lower than the mean *E. coli* count for spinach in the present study.

Escherichia coli (n=43) isolates were detected on fresh produce samples from the garden and storeroom of the schools. Moreover, 20.0% of spinach samples indicated the presence of *E. coli* isolates in the present study. In a study by Jongman and Korsten (2016), *E. coli* was found on 18.0% of baby spinach, 20.0% of lettuce and 27.0% of cabbage samples. The *E. coli* prevalence on spinach was similar to that of *E. coli* found in our study. However, no *E. coli* was found on the lettuce samples, while *E. coli* were found on 10% of cabbage samples in this study. In contrast to our study, *E. coli* was found on up to 73.3% and 100% of spinach samples as well as 3.3% and 6.7% cabbage samples from retailers and street vendors respectively in SA (Du Plessis *et al*., 2017). These authors also found an *E. coli* prevalence of 8.3% on onion samples from a farm, which was higher than the 3.7% found in our study for onions obtained from the garden. For onions obtained from the storeroom, the prevalence of *E. coli* was 7.1%. Due to the general lack of cold room storage facilities at schools visited and subsequent results found in this study, it is considered important to assess the influence of storage on the microbiological quality of fresh produce in schools.

When compared to the SA Department of Health, Public Health England, and Hong Kong's Centre for Food Safety Microbiological Guidelines, levels of coliform, *E. coli*, *Enterobacter*iaceae and *S. aureus* on fresh produce in this study, were found unsatisfactory (Department of Health 2010; Public Health England 2013; Hong Kong Centre for Food Safety 2014). This highlights the importance of mitigation through proper washing and cooking (Bacon *et al*., 2003). Cooking may decrease the levels of bacteria on food (Wang *et al*., 2012). However, this does not apply to *S. aureus*, toxins (Bintsis 2017). The bacteria may be susceptible to heat, but the toxins may survive and be able to cause disease (Bintsis 2017). Cross contamination after cooking may also occur (Murray *et al.*, 2017). Therefore, it is important that proper hygiene practices are followed to prevent foodborne diseases (Bacon *et al., 2003)*. Not all fresh produce at the schools is washed and cooked before consumption. Apples were found not to harbor any pathogens in this study, and were the main fruit served at the schools visited. The washing of fresh produce with adequate sanitisers is also important in decreasing potential pathogen contamination. (Gil *et al.* 2009; Olaimat and Holley 2012). Allende *et al*. (2008) demonstrated in their study the need for wash water sanitisers to

effectively eliminate pathogens in water. The schools visited did not use water sanitisers and relied on only using potable water to wash the apples (observation). However, potable water was not always available at these schools due to lack of resources or water cuts in their respective areas, further posing a challenge to maintaining adequate facility and personal hygiene in food preparation facilities.

In contrast to the present study where *E. coli* isolates detected did not harbour the diarrheagenic virulence genes that were screened for, other studies have detected pathogenic *E. coli* on fresh produce. Castro-Rosas *et al.* (2012) found *stx1*, *stx2* and *ial* virulence genes in *E. coli* isolated from spinach, tomato and lettuce, whereas du Plessis *et al.* (2015) was able to detect the *stx1* gene in *E. coli* detected on onions. Although no diarrheagenic virulence genes were detected in *E. coli* isolates in the present study, 60.4% and 62.8% of the *E. coli* isolates displayed resistance to ampicillin and amoxicillin, respectively. Furthermore, 55.8% of these *E. coli* isolates in our study were resistant to tetracycline and trimethoprim/sulfamethoxazole. Ampicillin resistance has also been reported in previous studies to be high among *E. coli*. Rasheed *et al.* (2014) found the dominant type of resistance to be to ampicillin and amoxycillin, followed by tetracycline, cotrimoxazole and streptomycin. Tetracycline resistance in this study was found to be 55.8%, higher than that reported by Faour-Klingbeil *et al.* (2016) which was 42.0%.

Multidrug resistance was seen in 67.0% of the *E. coli* isolates detected on fresh produce in the study carried out by Faour-Klingbeil *et al.* (2016) similar to our study where 60.5% of the *E. coli* isolates were multidrug resistant. The most used antibiotics in animal production systems are tetracyclines, aminoglycosides and penicillin's (Kimera *et al*., 2020). Similarly, penicillin's and tetracyclines as well as sulfonamides (trimethoprim) are also widely used in the SA public health sector (Schellack *et al*., 2017). *Escherichia coli* isolates in this study were mostly resistant to penicillin's, trimethoprim and tetracyclines, indicating that the widespread use of these antibiotics may be contributing to and may be leading to MDR development in bacterial pathogens. The implications for particularly immunocompromised people, who may be exposed to these resistant bacteria through fresh produce handling is of concern due to obvious more limited treatment options (Schellack *et al*., 2017).

Our study also indicated that ESBL and/or AmpC- producing *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. are present on fresh produce. Furthermore, these isolates were resistant to cefotaxime (78.1%), ceftazidime (82.1%), cefpodoxime (97.6%) third-generation cephalosporins as well as cefepime (90.2%), a fourth-generation cephalosporin. Kim *et al.* (2015) and Zurfluh *et al.* (2015) reported 100% and 88.3% resistance to cefotaxime in

ESBLproducing *Enterobacter*iaceae isolates, higher than in our study. However, resistance to ceftazidime (15.8%) and cefepime (10.2%) was lower in the study by Kim *et al*. (2015).

Resistance to non-β lactam antibiotics was found in this study, with resistance to nitrofurans and aminoglycosides antibiotic classes being dominant. Similar to our study, Richter *et al.* (2019) also reported that 94.8% of Enterobacteriaceae isolates were resistant to the aminoglycoside class. A 100% resistance to ampicillin was reported by Mesbah Zekar *et al.* (2017). However, in our study, 95.1% of the ESBL and/or AmpC- producing isolates were resistant to ampicillin. In contrast to our study, a 100% of ESBL producing Enterobacteriaceae isolates were susceptible to ampicillin.

Carbapenem resistance has come under the spotlight in SA as carbapenem resistant Enterobacteriaceae have caused outbreaks in hospitals (NICD, 2019; SAnews, 2020). Although, these outbreaks were not related to food, these bacteria are able to genetically transfer their antimicrobial resistance to other related bacteria. The present study found carbapenem resistance in 14.6% of the ESBL and/or AmpC- producing Enterobacteriaceae, higher than the 0% and 10.6% resistance previously reported in similar studies (Kim *et al*., 2015; Singh *et al*., 2017). Multidrug resistance was reported in 100% of the ESBL and/or AmpC- producing Enterobacteriaceae isolates in this study, whereas in other studies it was reported to be 96.1% (Richter *et al*., 2019) and 78.3% (Zurfluh *et al*., 2015). The CDC (2013) and WHO (2016) have described carbapenem resistant *Enterobacter*iaceae as a huge threat. These bacteria are resistant to almost all antibiotics and cause death in half of the patients infected with them. Therefore, antimicrobial resistance, moreover, carbapenem resistance in Enterobacteriaceae isolates found on fresh produce at schools is concerning.

Raw fresh produce samples obtained from surveyed schools in this study were found to not always comply with generally considered levels of coliform, *E. coli*, Enterobacteriaceae as well as *S. aureus*. Thus, a need for a national improved food safety strategy is needed to prevent foodborne disease outbreaks at schools and to better monitor produced and procured fresh produce. Forthcoming studies should focus on investigating the implementation of good food safety management principles at schools to ensure food is safe for consumption. Future studies should seek to determine the potential link between the microbiological quality of fresh produce grown and served at schools to the production and handling practices. The training of food handlers at these schools is imperative and should be conducted on a regular basis. Similarly, the state of food safety at schools should also be monitored and audited as part of a food safety assurance system. Additionally, quantitative microbial risk assessment studies should be done

to determine the risk involved when school children are exposed to certain foods provided through the school feeding scheme or sold in or near school premises. We envisage that the results of this study will be considered by international and national governments to develop new policies and guidelines that will help to safeguard the safety of food provided in the national school feeding programme.

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Conflict 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 upon reasonable request.

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Table

1. Genes that were screened for, primers used and cycling conditions.

Gene	Primer type	Primer (5' – 3')	Primer concentration (µM)	Positive controls	Primer specific annealing	Amplicon size (bp)	Reference
stx 1	Forward	ACA CTG GAT GAT CTC AGT GG	30	ATCC 35150	55°C	614	1
	Reverse	CTG AAT CCC CCT CCA TTA TG					
stx 2	Forward	CCA TGA CAA CGG ACA GCA GTT	30	ATCC 35150	55°C	779	1
	Reverse	CCT GTC AAC TGA GCA CTT TG					
eaeA	Forward	CTG AAC GGC GAT TAC GCG AA	60	ATCC 35150	55°C	917	1
	Reverse	GAC GAT ACG ATC CAG					
bfpA	Forward	AAT GGT GCT TGC GCT TGC TGC	21	DSM 8703, DSM 8710	68°C	324	2
	Reverse	GCC GCT TTA TCC AAC CTG GTA					
lt	Forward	GGC GAC AGA TTA TAC CGT GC	40	DSM 10973 DSM 27503	68°C	410	3
	Reverse	CGG TCT CTA TAT TCC CTG TT					
st	Forward	TTT CCC CTC TTT TAG TCA GTC AAC TG	20	DSM 10973	68°C	160	3
	Reverse	GGC AGG ATT ACA ACA AAG TTC ACA		DSM 27503			
pCVD4321AA probe	Forward	CTG GCG AAA GAC TGA ATC AT	30	DSM 27502	53°C	630	4
	Reverse	CAA TGT ATA GAA ATC CGC TGT T					
ipaH	Forward	GTT CCT TGA CCG CCT TTC CGA TAC CGT C	42	DSM 9028, DSM 9034	60°C	600	5
	Reverse	GCC GGT CAG CCA CCC TCT GAG AGT AC					

Table

ial	Forward	GGT ATG ATG ATG ATG ATG GGC	20	DSM 9028, DSM 9034	55°C	630	6
	Reverse	GGA GGC CAA CAA TTA TTT CC					

1: Omar and Barnard (2010); 2: López-Saucedo *et al.* (2003); 3: Pass *et al*. (2000); 4: Schmidt *et al.*(1995); 5: Sethabutr *et al.* (1994); 6: da Cruz *et al.* (2014). *ATCC 25922 was used as a negative control and PCR grade water additionally used the non-template control.

2: Enterobacteriaceae, coliforms, *Escherichia coli* and *Staphylococcus aureus* counts (log cfu/g^{-1}) on fresh produce obtained from school gardens

Fresh produce type (n)	**Enterobacteriaceae**		**Coliforms**		***Escherichia coli***		***Staphylococcus aureus***	
	Range, min-max[a]	**Mean±SD[b]**	**Range, min-max**	**Mean±SD**	**Range, min-max**	**Mean±SD**	**Range, min-max**	**Mean±SD**
Cabbage (20)	0.00-6.90	5.12±1.80	3.53-5.16	4.46±0.49	0.00-3.00	0.80±0.96	0.00-3.54	1.74±1.16
Carrots (17)	3.59-5.69	4.74±0.59	1.35-4.75	2.92±1.04	0.00-1.079	0.26±0.41	0.00-3.28	0.97±1.08
Chinese spinach (13)	2.19-6.15	4.73±1.26	1.64-5.01	3.55±1.33	0.00-3.32	0.76±1.21	2.00-5.02	3.49±1.05
Lettuce (5)	4.12-5.10	4.62±0.36	3.09-4.72	4.08±0.60	0.00-0.00	0.00±0.00	0.78-1.88	1.25±0.38
Onions (27)	2.52-5.77	4.48±1.00	0.00-5.39	3.63±1.12	0.00-3.05	0.39±0.95	0.00-3.60	1.44±1.21
Spinach (110)	0.00-6.97	4.73±1.17	0.00-5.97	3.89±0.94	0.00-4.57	1.06±1.27	0.00-4.51	2.34±1.35

a: The range indicates the minimum (min) and maximum (max) log cfu/g^{-1} for each fresh produce type. b: SD represents the standard deviation.

Table 3: Enterobacteriaceae, coliforms, *Escherichia coli* and *Staphylococcus aureus* counts (log cfu/g^{-1}) on fresh produce obtained from school storerooms

Fresh produce type (n)	**Enterobacteriaceae**		**Coliforms**		***Escherichia coli***		***Staphylococcus aureus***	
	Range, min-max[a]	**Mean±SD[b]**	**Range, min-max**	**Mean±SD**	**Range, min-max**	**Mean±SD**	**Range, min-max**	**Mean±SD**
Apples (27)	0.00-6.49	3.37±1.66	0.00-5.62	2.46±1.44	0.00-1.44	0.07±0.28	0.00-3.08	0.98±0.94
Cabbage (39)	0.00-6.81	5.02±1.84	0.00-6.14	3.68±1.75	0.00-3.60	0.50±1.00	0.00-3.86	1.23±1.30

Table

Carrots (9)	4.73-6.63	5.38±0.76	3.60-4.75	4.28±0.35	0.00-2.85	0.50±0.97	0.78-2.78	1.14±0.81
Onions (42)	1.49-5.35	3.56±1.13	1.20-4.88	3.04±1.16	0.00-4.26	0.36±0.94	0.00-2.95	1.49±1.00
Tomatoes (12)	4.42-6.43	5.65±0.68	0.00-4.85	3.11±1.83	0.00-2.43	0.61±0.91	0.00-2.70	1.47±1.06

a: The range indicates the minimum (min) and maximum (max) log cfu/g^{-1} for each fresh produce type. b: SD represents the standard deviation

4. Antimicrobial resistance patterns of *Escherichia coli* found on fresh produce obtained from schools in the Gauteng and Mpumalanga Provinces

Number of classes resistant to	Number (percentage) of isolates resistant to antibiotic classes	Most frequent antibiotic resistance pattern displayed by isolate (number of isolates)	Fresh produce types
0	10 (23.26)		Spinach (9), Chinese spinach (1)
1	3 (6.98)	*	Spinach (2), carrots (1)
2	3 (6.98)	CIP5C, S10C (2)	Spinach (3)
3	1 (2.33)	*	Carrots
4	3 (6.98)	A10C, AP10C, TS25C, T30C (2)	Spinach (2), cabbage (1)
5	2 (4.65)	KF30C, N300C, A10C, AP10C, TS25C, T30C (2)	Spinach (1), tomatoes (1)
6	4 (9.30)	KF30C, N300C, NA30C, A10C, AP10C, TS25C, T30C (4)	Cabbage (1), Carrots (1), tomatoes (1), spinach (1)
7	6 (13.95)	CTX30C, CIP5C, KF30C, S10C, A10C, AP10C, T30C (5)	Spinach (2), onions (2), tomatoes (2)
8	8 (18.60)	CTX30C, CIP5C, KF30C, N300C, S10C, A10C, AP10C,TS25C, T30C (8)	Spinach (6), carrots (2)
9	3 (6.98)	CTX30C, CIP5C, C30C, KF30C, GM10C, N300C, S10C, NA30C, A10C, AP10C, TS25C, T30C (3)	Spinach (2), carrots (1)

* Antibiotic resistance patterns for isolates demonstrating resistance to the same number of classes were all different.

Table

Table 5. Antimicrobial resistance patterns of extended spectrum β-lactamase *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. found on fresh produce obtained from schools in the Gauteng and Mpumalanga Provinces

Number of antibiotic	Number of isolates resistant to the number	Most frequent antibiotic resistance pattern displayed by isolate (number of isolates) classes	Fresh produce types

	of antibiotic classes (%)		
4	1 (2.44)	*	Spinach
5	4 (9.76)	*	Spinach (1), cabbage (1), tomatoes (2)
7	10 (24.39)	AP10C, A10C, CPM30C, TS25C, T30C, NE10C, GM10C, CPD10C, CAZ30C, CTX30C, CIP5C, NI300C (2)	Spinach (5), onions (3), cabbage (1), carrots (1)
8	19 (46.34)	AP10C, A10C, AUG30C, CPM30C, TS25C, T30C, NE10C, CPD10C, CAZ30C, CTX30C, CIP5C, NI300C (4)	Spinach (8), onions (4), carrots (3), tomatoes (2), lettuce (1), cabbage (1)
9	7 (17.07)	AP10C, A10C, AUG30C, FOX30C, CPM30C, TS25C, T30C, NE10C, GM10C, C30C, CPD10C, CAZ30C, CTX30C, CIP5C, NI300C (3)	Spinach (6), cabbage (1)

* Antibiotic resistance patterns for isolates demonstrating resistance to the same number of classes were all different.