Antimicrobial resistance in fresh produce production in Africa

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Microbiological safety and antimicrobial resistance in fresh produce production in Africa

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1. Introduction

Fresh produce, consisting of fruits and vegetables, are increasingly recognised, and promoted as essential components of a healthy diet. There is increasing evidence that regular consumption of fresh produce reduces the incidence of chronic diseases including, cardiovascular disease, type 2 diabetes, obesity, dementia, and the risk of cancer (Boeing *et al*., 2012; Boffetta *et al*., 2010; Gonzalez *et al*., 2012; Hodder *et al*., 2020; Hu *et al*., 2014; Oyebode *et al*. 2014; Slavin and Lloyd, 2012). These health benefits have been attributed to the high amounts of vitamins, minerals, fibres, and antioxidants present in these foods (Pennington and Fisher, 2010).

The World Health Organisation (WHO) recommends consuming at least five portions (400 g) of fruits and vegetables per day for a healthy diet (WHO, 2004, 2020). However, fresh produce consumption relies on the availability, access, and consumer socio-demographic characteristics such as age, gender, income, and educational level (Gustat *et al*., 2015). Several studies have noted that considerable numbers of the global population, particularly in low-income settings, have yet to achieve the WHO recommendation (Kabwama *et al*., 2019; Lutfiyya *et al*., 2012; Miller *et al*., 2016). However, global production and consumption of fresh produce are increasing (Balali *et al*., 2020). For example, a recent modelling study by Mason-D'Croz *et al*. (2019) estimated that between 1965 and 2015, the global population achieving the WHO recommendation had increased from 17% to 55%.

At the same time, the role of fresh produce as a vehicle for the transmission of pathogenic and antimicrobial-resistant bacteria has become well established (Allen *et al*., 2013; Callejon *et al*., 2015; Holzel *et al*., 2018; Willis *et al*., 2020; Zhang *et al*., 2020). Some contributory factors include the increased demand for fruit and vegetables as ready-to-eat (RTE) products which require minimal or no processing before consumption, thus removing a significant hurdle for eliminating or inactivating foodborne pathogens (Olaimat and Holly, 2012). In addition, to ensure the availability of fresh produce year-round, supply chains have become increasingly global and complex (Lynch *et al*., 2009; Carstens *et al*., 2019). The global food trade is also becoming recognised as a route for the transmission of pathogenic bacteria (Dada *et al*., 2021; Somorin *et al*., 2021). Fresh produce trade has been established through geographical routes culminating in the import or export of varieties of tropical fruits and vegetables across the globe. Sub-Saharan Africa is the hub for the cultivation and processing of tropical fruits and vegetables, with many countries harnessing the opportunities for fresh produce export. The highlight of fresh produce export is providing off-season fresh fruits and vegetables to countries with temperate climates, principally the European Union, as one of their most important export markets (Huang, 2004).

Fruit and vegetables are increasingly implicated in foodborne outbreaks globally (Stephan *et al*., 2015). In the United States, 37% (85) of the multistate foodborne outbreaks between 2010 and 2017 were linked to the consumption of contaminated fresh produce. These led to 4501 cases of illness, of which 1117 required hospitalisation, and 55 deaths (Carstens *et al*., 2019). Some notable examples include the 2017 multistate *Salmonella* infections outbreak linked to contaminated papaya. Two hundred and twenty (220) cases across 23 states were recorded, leading to 68 hospitalisations and one fatality (CDC, 2017). More recently, 40 people across 19 states were infected with *Escherichia coli* 0157:H7 after consuming leafy greens (CDC, 2020).

A similar trend has also been observed in Europe. For example, between 2006 and 2016, the proportion of foodborne outbreaks associated with non-animal-based foods increased from 5 % to 13 % (Machado-Moreira *et al*., 2019). In 2011, one of the largest outbreaks of enterohaemorrhagic *Escherichia coli* (EHEC) serotype O107:H4 occurred in Germany (Robert Koch Institute, 2011). There were over 4000 cases of illness and 55 deaths, including visitors from 15 other countries, and a smaller outbreak in France. The vehicle implicated in the outbreak was contaminated fenugreek sprouts produced from imported seeds (Kockerling *et al*., 2017; Machado-Moreira *et al*., 2019). Recently, a multi-national outbreak of listeriosis linked to frozen sweetcorn involved 54 cases across the United Kingdom, Australia, Finland, Sweden, Denmark, and Austria (McLauchin *et al*., 2021). These examples demonstrate that fresh produce can be considered a major vehicle for transmitting pathogenic bacteria, and this remains a significant challenge even in high resource settings.

In Africa, fruit and vegetable consumption has increased significantly over the last 30 years (Mensah *et al*., 2021). However, there is limited reliable information about the prevalence of pathogenic bacteria in fresh produce and epidemiology studies linking these to foodborne illness outbreaks on the continent (Aworh, 2021; Imathui, 2018). The consumption of food contaminated with microbial hazards remains a significant health threat in the African region (WHO, 2015). Contributory factors include inadequate physical infrastructure (e.g. clean water, storage facilities, transport networks), poor awareness of food safety issues and good manufacturing practices among relevant stakeholders (farmers, distributors, manufacturers, handlers and consumers), and limited capacity for developing and enforcing food safety regulations (Anyogu *et al*., 2021; Jaffee *et al*., 2019).

In addition, the presence of antimicrobial-resistant (AMR) pathogenic microorganisms in food is a risk to public health. Antibiotic-resistant bacteria have been observed worldwide in surface water often used as irrigation water for fresh produce (Blaak *et al.*, 2015), making fresh produce a frequent vehicle of AMR bacteria, including multidrug-resistant ones. There are reports on the prevalence of Extended-Spectrum β-Lactamase (ESBL)-producing *Enterobacteriaceae* (3rd-generation resistant *Enterobacteriaceae*) in retail vegetables produced in the Netherlands, USA, and Denmark (Van Hoek *et al*., 2015). According to Cantón *et al*. (2008), the prevalence of ESBL producers in Europe is higher than in the USA but lower in South America and Asia. A study by Nüesch-Inderbinen *et al*. (2015) reported that ESBL producers are mostly found in the irrigation water used for fresh produce rather than on the fresh produce itself.

Thus, this chapter provides a comprehensive overview of current information about pathogenic and antimicrobial-resistant bacteria in fresh produce in Africa. It examines relevant factors contributing to the microbial contamination of fresh produce, identifies data gaps and discusses recommendations to support stakeholders in taking appropriate steps to improve the safety of the fresh produce chain in Africa.

2. Microorganisms of public health significance in fresh produce in Africa

Pathogenic bacteria have multiple entry routes into the fresh produce supply chain. These have been identified as soil, faeces, irrigation water, unhygienic processing plants and human handling (Iwu and Okoh, 2019; Olaimat and Holley, 2012). In addition, studies investigating the presence of contaminants along the farm-to-fork fresh produce continuum in Africa have identified bacteria of public health concern. For example, Jongman and Korsten (2016) established a link between *Escherichia coli* in irrigation water sources and vegetables grown in home gardens and commercial farms. However, contrary observations were reported by van Dyk *et al*. (2016) as there was no significant overlap of microbial species in irrigation water samples, the processing environment, or the tomatoes at retail sale. Despite this, they also reported a high level of coliforms in tomatoes at the market.

The most commonly reported pathogenic bacteria associated with fresh produce supply chains in Africa include *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Table 1). This observation is not surprising and correlates with investigations from other regions (Ramirez-Hernandez *et al*., 2020; Townsend *et al*., 2021; Vital *et al*., 2014). However, there is sparse data on the prevalence of pathogenic microbes, especially antimicrobial-resistant bacteria, in fruits and vegetables along the supply chain compared to other parts of the world. In addition, in most studies, identifying indicator and pathogenic bacteria has been undertaken using phenotypic methods, which are limited in providing reliable information. The microbiological quality of fruits and vegetables has been of increasing interest to researchers (Aiyedun *et al*., 2021; Machado-Moreira *et al*., 2019).

However, more studies are required to better understand how current food systems influence the microbial profile of fresh produce as it relates to safety. In addition, the role of fresh produce as vehicles for antimicrobial resistance requires consideration.

Table 1: Studies on the significant indicator and pathogenic bacteria in fresh produce in Africa

Microorganisms	**Number of positive samples/Number of samples (Prevalence)**	**Food product**	**Sample collection stage**	**Country**	**Reference**
Enterobacteriaceae	117/160	Mixed vegetables (baby corn, beans, carrots, chilli Fresno, mangetout, peas, and patty pan) and green beans	Processing plant	Zambia	Nguz *et al*. (2005)
Listeria monocytogenes	16/80				
Salmonella spp.	37/160				
Staphylococcus aureus	54/80				
Listeria monocytogenes	63/120	Leafy vegetables	Markets	Nigeria	Nwachukwu *et al*. (2010)
Listeria monocytogenes	104/160	Leafy vegetables	Markets	Nigeria	David and Odeyemi (2007)
Listeria spp.	17/20	Cabbage, lettuce, carrots, green peas	Markets	Nigeria	Ikeh *et al*. (2010)
Campylobacter spp.	7/128	Leafy vegetables	Farms	Benin	Kougblenou *et al*. (2019)
Salmonella spp.	16/86	Ready to eat vegetable salad	Not mentioned	Kenya	Imathui, (2018)
Staphylococcus aureus	63/86				
Listeria monocytogenes	56/86				
Salmonella spp., *Enterococcus* spp., Faecal coliforms	ND	Leafy greens	Farms and markets	Ghana	Quansah *et al*. (2018)
Staphylococcus aureus, *Salmonella* spp.	ND	Lettuce, Cabbage, Cucumber	Markets	Nigeria	Abdullahi and Abdulkareem (2010)

ND: Not determined

Table 1 (contd.): Studies on the significant indicator and pathogenic bacteria in fresh produce in Africa

Microorganisms	Number of positive samples/Number of samples	Food product	Sample collection stage	Country	Reference
Escherichia coli	64/96	Lettuce, Strawberries	Farm, Markets	Egypt	Uyttendaele *et al*. (2014)
Salmonella spp.	34/96				
Listeria monocytogenes	1/99	Vegetables and fruits	Farm market	Rwanda	Ssemanda *et al*., (2018a)
Thermotolerant *Campylobacter* spp.	3/99				
Salmonella spp.	5/99				
Pathogenic *Escherichia coli*	6/99				
Bacillus cereus, Clostridium perfringens, *Staphylococcus* spp., *Salmonella* spp.	ND	Spinach, Kenaf, Lettuce, Pepper, Okra	Farms	Ghana	Adetunde *et al*., (2015)
Salmonella spp.	10/20	Lettuce	Garden	Burkina Faso	Traore *et al*., (2015)
Staphylococcus aureus	30/150	Lettuce, Cabbage, Tomato, Carrot	Farms	Ethiopia	Weldezgina and Muleta (2018)
Salmonella spp.	24/150				
Enterococcus spp.	73/100	Vegetables	Farms	Tunisia	Ben-Said *et al*., (2016)
Escherichia coli	ND	Tomatoes	Fields, Markets	Nigeria	Shenge *et al*., (2015)

ND – Not determined

Table 1 (contd.): Studies on the significant indicator and pathogenic bacteria in fresh produce in Africa

Microorganisms	Number of positive samples/Number of samples	Food product	Sample collection stage	Country	Reference
Escherichia coli O157:H7	4/180	Vegetables	Shops, Supermarkets, Open-air markets	South Africa	Abong'O *et al*., (2008)
Bacillus cereus, *Staphylococcus aureus*, *Listeria monocytogenes*	ND	Frozen vegetables	Supermarkets	Botswana	Manani *et al*. (2006)
Escherichia coli	ND	Tomatoes	Fields, Markets	Nigeria	Shenge *et al*. (2015)
Escherichia coli O157:H7	4/180	Vegetables	Shops, Supermarkets, Open-air markets	South Africa	Abong'O *et al*., (2008)
Escherichia coli 0157	28/486	Leafy vegetables	Markets and supermarkets	Egypt	Khalil *et al*. (2015)
Shiga-toxin producing *Escherichia coli*	6/486				
Escherichia coli	81/545	Vegetables	Street traders, Trolley vendors, Farmer's market	South Africa	Richter *et al*. (2021)
Salmonella spp.	10/106	Lettuce	Farms and markets	Senegal	Ndiaye *et al*. (2011)

3. Antimicrobial resistance (AMR) in fresh produce in Africa

Gram-negative bacteria are predominantly implicated in many outbreaks linked with fresh produce consumption (Blaak *et al*., 2014; Vital *et al*., 2017). Furthermore, some of these Gram-negative bacteria have been reported to increasingly become resistant to multiple antibiotics, making the choice of antimicrobial therapy difficult (Oliphant & Eroschenko, 2015a). Fresh produce is minimally processed and consumed raw for the most part. Hence, humans may be infected with antimicrobial-resistant bacteria through fresh produce consumption (Blaak *et al*., 2014; Van Hoek *et al*., 2015).

Antimicrobial resistance genes can be disseminated into the environment through faecal matters, contaminating surface waters and soils (Said *et al*., 2015; Thanner *et al*., 2016). The resistance genes may then be transferred to the bacteria found in the environment via contaminated irrigation water, inadequately treated manure, and soils. Resistant microorganisms transmitted to crops proliferate or survive in the growing stems and may remain on crops until consumed (Schwaiger *et al*., 2011; Van Hoek *et al*., 2015; Holzel *et al*., 2018).

The increasing occurrence of antibiotic-resistant bacteria, particularly *Enterobacteriaceae*, in healthcare systems, the environment and fresh produce is of serious concern globally. Microorganisms can develop resistance to certain classes of antibiotics through chromosomal genes mutation (Munita and Arias, 2016; Partridge, 2015). In *Enterobacteriaceae,* resistance mainly occurs due to resistance genes carried on various mobile genetic elements (Munita and Arias, 2016; Partridge, 2015). Resistance genes on plasmids can be transferred between cells of different bacteria and species (horizontal transfer of genes) and can also be transferred during cell division – vertical transfer (Munita and Arias, 2016; Partridge, 2015).

Some antimicrobial classes used in health care include penicillins, cephalosporins, carbapenems, aminoglycosides, vancomycin, tetracyclines, fluoroquinolones and sulfonamides (Oliphant & Eroschenko, 2015b). These antimicrobial agents have different mechanisms through which they act against bacteria. For instance, some agents like penicillins and cephalosporins are β-lactams that counteract bacterial infections by inhibiting the bacterial cell wall synthesis (Oliphant & Eroschenko, 2015b).

However, bacteria like the *Enterobacteriaceae* produce the β-lactamases, which render β-lactams inactive (Oliphant & Eroschenko, 2015a). Members of the *Enterobacteriaceae* family have emerged with resistance to penicillin and the broad-spectrum cephalosporins, resulting from the extended-spectrum β-lactamases (ESBLs) production, therefore, becoming a threat globally (Pitout & Laupland, 2008; Blaak *et al.*, 2014; Zurfluh *et al.*, 2015). Contaminated fresh produce may represent a route of human exposure to the ESBLs-producing bacteria (Van Hoek *et al.*, 2015). The dissemination of resistant bacteria between irrigation water and fresh produce can be related to a study done in South Africa by Du Plessis *et al.* (2015) which reported the transfer of bacteria from river water used for irrigation to irrigated onions.

In Switzerland, ESBL-producing *Enterobacteriaceae* have been isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam, with 25% of the isolates being ESBL-producing *Enterobacteriaceae*, and 78% of them were identified with multidrug resistance (Blaak *et al.*, 2014; Zurfluh *et al.*, 2015). In Nigeria, there have been reported cases of ceftazidime and cefuroxime-resistant bacteria from street-vended fruits and vegetables (Adekanle *et al.*, 2016). Furthermore, 3rd generation cephalosporins (3GC)-resistant *Enterobacteriaceae* have been reported on some vegetables bought from Dutch stores (van Hoek *et al.*, 2015). *Escherichia coli* and *Klebsiella pneumonia* isolates have been reported as the predominant ESBL-producing organisms globally (Pitout & Laupland, 2008 van Hoek *et al.*, 2015). However, other *Enterobacteriaceae* species such as *Citrobacter* spp., *Enterobacter* spp., *Kluyvera*, *Serratia* and *Rahnella* also carry the ESBL genes and are found in agricultural soils, animal manure and faecal contaminated water (Blaak *et al.*, 2014; Van Hoek *et al.*, 2015) thereby standing a chance of transferring the ESBL genes to the fresh produce. In Africa, AMR in fresh produce has been under-reported with limited data available. Reports on the incidence of AMR bacteria from produce from various African regions are summarised in Table 2.

Microbial drug resistance is one of the most pressing public health issues of our time, but its significance in public health is underappreciated in many countries. Bacteria resistant to various antibiotics have been discovered in both hospital and community settings; thus, there is no longer any localisation of drug resistance gene reservoirs to a specific setting. Migratory birds, domestic animals, travellers, and the global movement of commercial food are among the natural forces responsible for spreading bacteria with multidrug-resistance properties (Islam *et al*., 2021).

Multiple drug resistance in bacterial isolates causing diarrhoea has severe implications for empiric therapy against pathogenic isolates and the possible co-selection of antimicrobial-resistant Plasmids. Bacteria from clinical settings are known to harbour plasmids of various molecular sizes; it has also been widely reported that bacteria harbour antibiotic-resistant genes that can be horizontally transferred to other bacteria. In addition, antibiotic resistance among enteric pathogens has been reported to be increasing in developing countries, which could be due to environmental factors, geographic differences, or different antibiotic usage patterns (Ayukekbong *et al*., 2017).

Multidrug-resistant *E. coli* isolates from spinach, apples, carrots, cabbage, and tomatoes from both the formal and informal fresh produce sector of South Africa have been reported to show resistance to aminoglycosides, cephalosporins, penicillins, and amphenicol antibiotic classes (Baloyi *et al*., 2021).

Table 2: AMR in fresh produce from Africa regions

Southern Africa region

Country	Produce	Microorganisms	AM Type resistance	Genome/Plasmid -mediated resistance	AMR genes	Reference
South Africa	Variable fruits	*Salmonella* spp.	Chloramphenicol Kanamycin Trimethoprim Sulfamethoxazole Streptomycin Ampicillin Amikacin Amoxicillin-Clavulanic acid			Gomba *et al.*, 2016
	Spinach Lettuce Cucumber Tomato Green beans	*E. coli*	Neomycin Gentamycin Ampicillin Amoxicillin Augmentin Cotrimoxazole Tetracycline Chloramphenicol Cefepime Imipenem			Richter *et al.*, 2021
	Lettuce	*E. coli*	Ceftazidime, Cefotaxime, Ceftriaxone Cefpodoxime, Aztreonam	Plasmid		Njage & Buys, 2014
	Spinach	*Serratia fonticola* *Escherichia coli* *Klebsiella pneumoniae* *Rahnella aquatilis*	Amoxicillin Ampicillin Augmentin Cefoxitin			Richter *et al.*, 2020

		Imipenem Cotrimoxazole Tetracycline		
Spinach Cabbage	*Escherichia coli*	Neomycin; Trimethoprim-sulfamethoxazole; Gentamicin; Kanamycin; Amoxicillin-clavulanate; Aztreonam; Streptomycin; Ampicillin; Florfenicol; Chloramphenicol; Cefoxitin; Ciprofloxacin; Cefepime; Tetracycline; Enrofloxacin; Nalidixic acid.		du Plessis *et al*., 2017
Spinach Tomatoes Cucumbers Lettuce Green beans	*E. coli,* *Enterobacter cloacae* *Serratia fonticola*	Cefoxitin Cefoxitin, Ceftazidime, Cefotaxime Cefoxitin	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA} bla_{ACC}, bla_{FOX}, bla_{MOX}, bla_{DHA}, bla_{CIT}, bla_{EBC}	Richter *et al*., 2019

East and Central Africa region

Country	**Produce**	**Microorganism**	**AM Type resistance**	**Genome/Plasmid-mediated resistance**	**AMR genes**	**Reference**
Kenya	Vegetable salad	Pseudomonas spp. *Citrobacter freundii*	Streptomycin Gentamycin Amoxicillin Tetracycline Chloramphenicol Trimethoprim + Sulphamethoxazole Cefotaxime Nalidixic acid			Muriuki *et al*., 2020
Ethiopia	Lettuce Cabbage Tomato Carrot	*S. aureus* *Salmonella* spp.	Ampicillin Gentamycin Chloramphenicol Tetracycline Erythromycin Cotrimoxazole Cefuroxime-sodium Penicillin Norfloxacin			Weldezgina and Muleta, 2016

Avocado Potato	*E. coli* *S. aureus* *Salmonella* spp. *Proteus* spp. *Klebsiella* spp.	Ampicillin Clindamycin Cloxacillin Erythromycin Oxacillin Penicillin G Vancomycin Cefotaxime Ceftriaxone Chloramphenicol Ciprofloxacin Gentamycin Kanamycin Doxycycline Nalidixic acid Norfloxacin Trimethoprim sulphamethoxazole	Eromo *et al.,* 2016
Lettuce Garlic Carrot	*Bacillus* spp. *E. coli* *Streptococcus* spp. *Corynebacterium* *Neisseria* spp. Salmonella spp. *S. aureus* *Lactobacillus* spp.	Vancomycin Penicillin Ampicillin Chloramphenicol Perfloxacilin Erythromycin	Belay *et al*., 2020

North Africa region

Country	Produce	Microorganism	AM Type resistance	Genome/Plasmid-mediated resistance	AMR genes	Reference
Algeria	Cucumber Tomato Watermelon Celery Mint Parsley Lettuce Chili Nectarine Pear Carrot Grape Peach Apple	*Enterobacter cloacae* *Enterobacter asburiae* *Klebsiella pneumonia* *Citrobacter murliniae* *Citrobacter murliniae*	Ampicillin Ticarcillin Amoxicillin-clavulanic acid Cephalothin Cefamandole Cefuroxime Cefoxitin Cefotaxime Ceftazidime Ceftriaxone Cefepime Aztreonam Ertapenem Gentamicin Kanamycin Streptomycin Tetracycline Tigecycline Ciprofloxacin Pefloxacin Nalidixic acid Trimethoprim-sulfamethoxazole Trimethoprim Sulfonamides; Chloramphenicol Temocillin			Mesbah Zekar *et al.,* 2017

	lettuce, carrot, onions, tomatoes, potatoes	*Escherichia coli* *Klebsiella pneumoniae*	Streptomycin Kanamycin Gentamicin Tobramycin Chloramphenicol Florfenicol Tetracyclines Sulfonamides Trimethoprim Nalidixic acid Enrofloxacin		$bla_{CTX-M-1}$ $bla_{CTX-M-15}$ $bla_{CTX-M-14}$ $bla_{CTX-M-2}$ bla_{SHV-2}	Yaici *et al.*, 2017
Tunisia	Barley Tomato Parsley Fennel Radish Apricot	*E. coli* *Enterobacter hormaechei* *Citrobacter freundii* *Klebsiella pneumoniae*	Cefotaxime Chloramphenicol Ciprofloxacin Cefoxitin Gentamicin Nalidixic acid Streptomycin Sulphonamides Trimethoprim-sulfamethoxazole Tetracycline Tobramycin	Plasmid-mediated resistance	$bla_{CTX-M-1}$ $bla_{CTX-M-15}$ $bla_{CTX-M-14}$ bla_{SHV-12}	Ben Said *et al.*, 2016

West Africa region

Country	Produce	Microorganism	AM Type resistance	Genome/Plasmid mediated resistance	AMR genes	Reference
Ghana	Lettuce Cabbage	*E. coli*	Ofloxacin Ampicillin Erythromycin			Adzitey, 2018
		Salmonella enterica	Ofloxacin Erythromycin			
Nigeria	Lettuce Cabbage	*E. coli* O157 serogroups	Tetracycline Cephalothin	Plasmid	Quinolone resistance-determinant (*gyrA*)	Abakpa *et al*., 2015
	Cucumber Guava Pineapple Tomatoes Tangerine Lemon Eggplant leaf African spinach *Worowo* Jute leaf	*Citrobacter freundii* *Citrobacter braakii* *Citrobacter youngae*	Ceftriaxone Amoxicillin Piperacillin Gentamycin Cefotaxime Tetracycline Cotrimoxazole Nitrofurantoin Augmentin		$\mathit{bla}_{\mathrm{CTX}}$, $\mathit{bla}_{\mathrm{SHV}}$ *aac6*	Adigun *et al.*, 2019
	Amaranth Fluted pumpkin leaves Scarlet eggplant leaf Water leaf	*E. coli*	Cefuroxime Chloramphenicol Ciprofloxacin Norfloxacin			Chigor *et al.*, 2020
	Pineapples Watermelon	*E. coli* *Shigella flexneri* *Enterobacter hormaechei* *Enterobacter sichuanensis*	Tetracycline Ampicillin Sulbactam Gentamicin			Oyedele *et al.*, 2020

4. Sources of antimicrobial-resistant pathogen contamination

Antimicrobial-resistant bacteria contaminating fresh produce enter the produce value chain at various stages of production, from farm to the fork. Therefore, the sources of contamination in fresh produce vary depending on the production system and scale of cultivation. The sources of contamination of fresh produce in Africa by antimicrobial-resistant pathogens could be categorised as on-farm/field sources, sources during processing, sources during distribution, retail, and food preparation.

4.1 On-Farm Sources of Contamination

4.1.1. Soil

This is the primary resource for growing crops in Africa, including fruit and vegetables. However, the soil is a complex environment harbouring indigenous populations as well as pathogenic bacteria. Some foodborne pathogens, such as *Listeria monocytogenes* and *Bacillus cereus,* are natural contaminants of soil (Weis and Seeliger, 1975; Dowe *et al*., 1997; Vilain *et al*., 2006; Stenfors Arnesen *et al*., 2008; Locatelli *et al*., 2013). Furthermore, some others that are not natural contaminants of soil have been reported to survive for extended periods in the soil. They include *E. coli* (Mukherjee *et al*., 2006; Ibekwe *et al*., 2007; Somorin *et al*., 2016); *Salmonella* (Uyttendaele *et al*., 2014) and *Campylobacter* (Donnison and Ross, 2009). In addition, many African soils used for cultivating fresh produce are often contaminated by irrigation water and manure, animal grazing, municipal solid wastes, and other effluents (Santamaria and Toranzos, 2003; Amoah *et al*., 2005). When crops are grown in such contaminated soils, pathogens could be transmitted through the seeds or roots to other parts of the plant where they could become internalised and persist in vegetables (Ibenyassine *et al*., 2006; Ávila-Quezada *et al*., 2010; Solomon *et al*., 2002; Wright *et al*., 2013).

4.1.2. Seeds

Contaminated seeds constitute an important contamination route for some fresh produce, particularly edible sprouts (Proctor *et al*., 2001), as the pathogens could become internalised during the germination process (Liu *et al*., 2017). The internalisation of pathogens promotes the transmission of the pathogens within the plant, which makes the pathogens challenging to eliminate (Wright *et al*., 2013). For example, contaminated fenugreek seeds from Egypt were implicated in a large multi-country *E. coli* STEC O104:H4 outbreak in Europe and North America in 2011 (EFSA, 2011).

4.1.3. Organic manure/soil amendment

The use of raw or poorly treated manure and compost is a significant source of direct contamination of fresh produce with *E. coli* O157:H7 and *Salmonella enterica* (Beuchat, 2002). It has been shown that the application of manure increases the population of antimicrobial-resistant bacteria in soil (Fatoba et al., 2021; Fatoba et al., 2022; Udikovic-Kolic *et al*., 2014). Smallholder farmers commonly use poultry manure as a fertiliser in Africa because it is relatively cheap and readily available (Amoah *et al*., 2007; Orji *et al*., 2005). It is used by over 70% of producers of irrigated lettuce in Ghanaian cities (Amoah *et al*., 2007) and in maize and pepper farms in Nigeria (Omeike *et al*., 2020).

Live chicken, cattle and other animals in many Africa countries harbour antimicrobial-resistant pathogens, including *E. coli* O157 (Abdalla et al., 2021; Abakpa *et al*., 2015); extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing (CPE)-*E. coli* (Abdallah *et al*., 2015); ESBL-*Salmonella* (Abdel–Maksoud *et al*., 2015; Abd-Elghany 2015; Abdellah, *et al*., 2009); *Aeromonas caviae* **(**Abu-Elala *et al*., 2015); *Enterocococcus* (Molechan et al., 2019); *Campylobacter* (Sithole et al., 2019), and *Listeria monocytogenes*, which they shed in their faeces (Arrus *et al*., 2006; Abakpa *et al*., 2015; Delahoy *et al*., 2018).

These pathogens can survive in manure slurry (Arrus *et al*., 2006), and if the faecal materials are not adequately composted before use as organic manure/fertiliser, they could transmit pathogens to the developing fruit or vegetable. In addition, pathogenic bacteria deposited into the soil by organic fertilisers and soil amendments could be transferred to fresh produce by water droplets splashing from rain and irrigation (Girardin *et al*., 2006; Monaghan and Hutchison, 2012; Cevallos-Cevallos *et al*., 2012; Ndiaye *et al*., 2011a).

4.1.4. Exposure to contaminated water

4.1.4.1. Irrigation

Using contaminated irrigation water could be a source of contamination of fresh produce. Shallow groundwater, wastewater and well water are often used for irrigating fresh produce in Africa (Ndiaye *et al*., 2011a). Of the types of water used for irrigation, wastewater and surface waters have poor microbial quality. Surface water sources are often polluted by water runoff, animal faecal material or sewage effluent. Irrigation water could be contaminated by faecal coliforms and pathogens like cephalothin-resistant *E. coli* O157 and *Salmonella* spp. (Ndiaye *et al*., 2011b; Abakpa *et al*., 2013, 2015; Uyttendaele *et al*., 2014).

The occurrence of plasmid-mediated multidrug-resistant *E. coli* O157 in surface waters used for irrigating fresh produce has been reported (Chigor *et al*., 2020). Furthermore, groundwater and rainwater are important sources of irrigation water, which have better microbial quality than surface water. For example, groundwater is used for 78% of irrigation in South Africa (Water Research Commission, 2019). However, urbanisation and increasing population size have further put pressure on the scarce land and water resources, and most farmers have limited land for farming in African countries. This situation increases the risk of contamination of fresh produce by pathogenic organisms (Jongman and Korsten, 2018). In addition, many other countries depend on rainwater for irrigation. However, climate change is causing reduced rainfall in many parts of Africa (World Meteorological Organization, 2020), thus leading to greater dependence on other water sources, which may be of poor microbiological quality, for irrigation. This consequently results in more frequent contamination of fresh produce.

The irrigation method also plays a vital role in the contamination of fresh produce. For example, irrigation using sprinkling water over the plants with watering cans resulted in higher crop contamination compared to when drip irrigation was used (Ndiaye *et al*., 2011a; Keraita *et al*., 2007). However, when furrow irrigation was used, there was lower contamination with *E. coli* in the irrigated vegetables even when there was a high *E. coli* concentration in the irrigation water (Ensink *et al*., 2007; Ndiaye *et al*., 2011a). On the other hand, irrigation using watering cans results in higher contamination of vegetables due to splashes causing deposition of pathogens from animal manure in the soil.

4.1.4.2. Sewage Sludge/Runoff water

Home gardens and smallholder farms situated close to sewage pits may experience leakage in their sewage system, leading to seepage of sewage into nearby soils and contamination with faecal pathogens. Domestic sewage and runoff are used to water vegetables such as lettuce, cabbage, and cucumber in Nigeria (Abdullahi and Abdulkareem, 2010), and the frequent use of wastewater is mainly due to water scarcity (Ndiaye *et al*., 2011a).

4.1.5. Faecal contamination from livestock and wild animals

Livestock and wild animals could be natural habitats for antimicrobial-resistant pathogens. Many animals harbour enteric pathogens which are shed along with faeces (Moriya *et al*., 1999; Corrente *et al*., 2004; Foster *et al*., 2006; Kaufmann *et al*., 2006; Sproston *et al*., 2006). For example, norfloxacin-resistant *E. coli* O157 was found in the faeces of sheep and goats (Abreham *et al*., 2019). Similarly, multidrug-resistant *Listeria monocytogenes* was reported in cow faeces (David and Odeyemi, 2007), while methicillin-resistant *Staphylococcus aureus* (MRSA) harbouring the *mecA* gene and *SCCmec* mobile genetic element was detected in faeces of healthy chicken (Amoako *et al*. 2019). Besides the direct contact of faecal materials with the soil upon which fresh vegetables are produced, insect pests could also serve as vectors of transmitting antimicrobial-resistant pathogens from faecal materials to fresh produce (Ignasiak and Maxwell, 2017).

4.1.6. Farm workers

Individuals involved in the primary production of fruits and vegetables could also be a source of contamination. Unsanitary practices by farm workers such as open defaecation and not washing hands properly after defaecation could lead to transmission of enteric pathogens to fresh produce. Farm workers could harbour methicillin-resistant *Staphylococcus aureus* (Amoako *et al*. 2019), and this could be transmitted to leafy vegetables when they sneeze without using face covering. This becomes particularly worrying during harvest when farm workers sneeze into their palms and use the same hands (without washing) to harvest fresh produce manually.

4.1.7. Farm implements

Farm tools, such as hand trowels and rakes that have come in contact with contaminated soil or manure could be vehicles for transmitting antimicrobial-resistant pathogens to fruits, leafy and root vegetables during crop husbandry, such as transplanting and harvesting (Amoako *et al*., 2020). In addition, contaminated knives and other tools used to harvest leafy vegetables and buckets, baskets and sacks used to transport the produce from the farm could contaminate the fresh produce.

The on-farm sources of contamination of fresh produce are summarised in Figure 1 below.

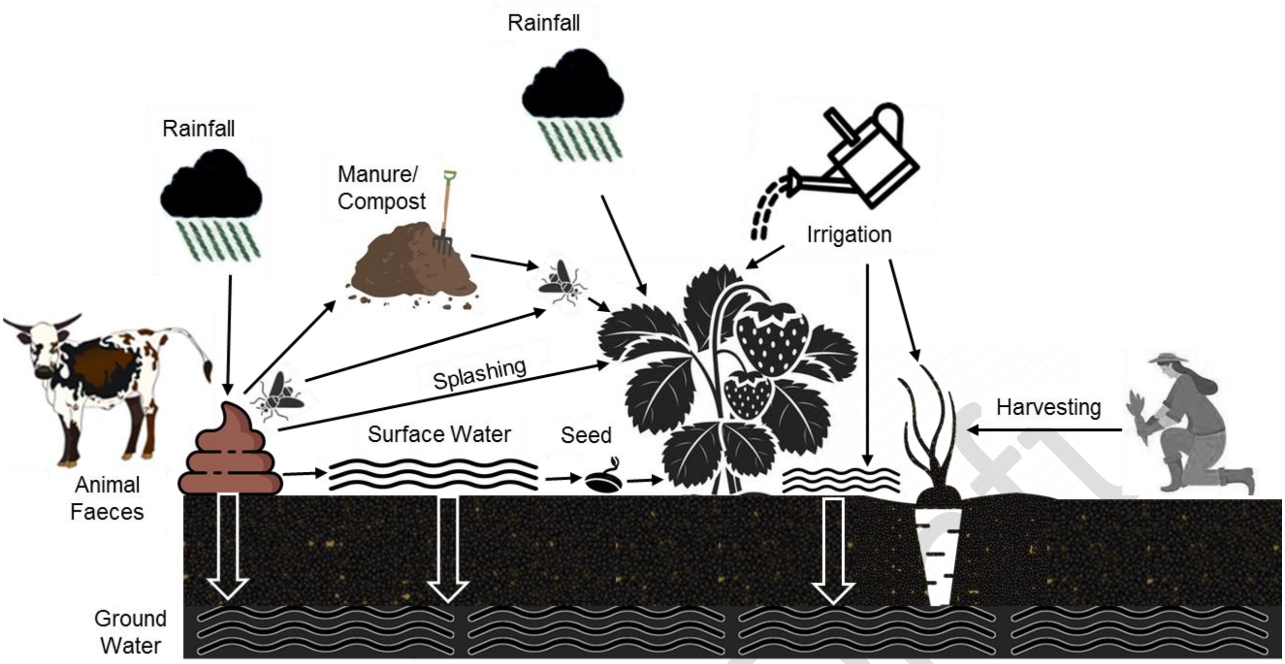

Figure 1: On-farm sources of antimicrobial-resistant pathogen contamination in fresh produce production in Africa (please cite the source if you did not design this originally)

4.2. Processing/Post-Harvest Sources of Contamination

4.2.1. Washing

Vegetables are sometimes washed with water on/around the farm to remove dirt before being transported to distribution centres or retail markets (Figure 2). However, washing fruits and vegetables with contaminated water could transmit antimicrobial-resistant pathogens to the fruit and vegetables. For example, washing vegetables in the field played a crucial role in contaminating lettuce with *Salmonella* (Ndiaye *et al*., 2011a).

4.2.2. Handling during processing

Poor hygiene, lack of handwashing and other unsanitary practices have been reported among individuals handling fresh produce (Akoachere *et al*., 2018). In addition, handling fresh produce without proper personal protection equipment could expose the fresh produce to pathogens from the handler's saliva and mucus. Cracks on surfaces in food processing facilities could also be a source of persistent pathogen contamination during processing (Leong *et al*., 2017).

4.2.3. Transport, Distribution and Storage equipment

Transportation of fruits and vegetables to market centres and other retail points in open and non-refrigeration conditions (Figure 2) exposes the produce to contaminants and environmental conditions that promote the proliferation of pathogens (Akoachere *et al*., 2018). Fresh produce transported under these conditions is further exposed to pathogen-harbouring insects and faecal materials from animals or humans sitting on them. Transport vehicles and crates have been reported to be sources of *S. aureus* contamination (Amoako *et al*., 2020), while bags/sacks, previously used for collecting poultry waste, have been reported to be used to transport fruits and vegetables (Omeike *et al*., 2020).

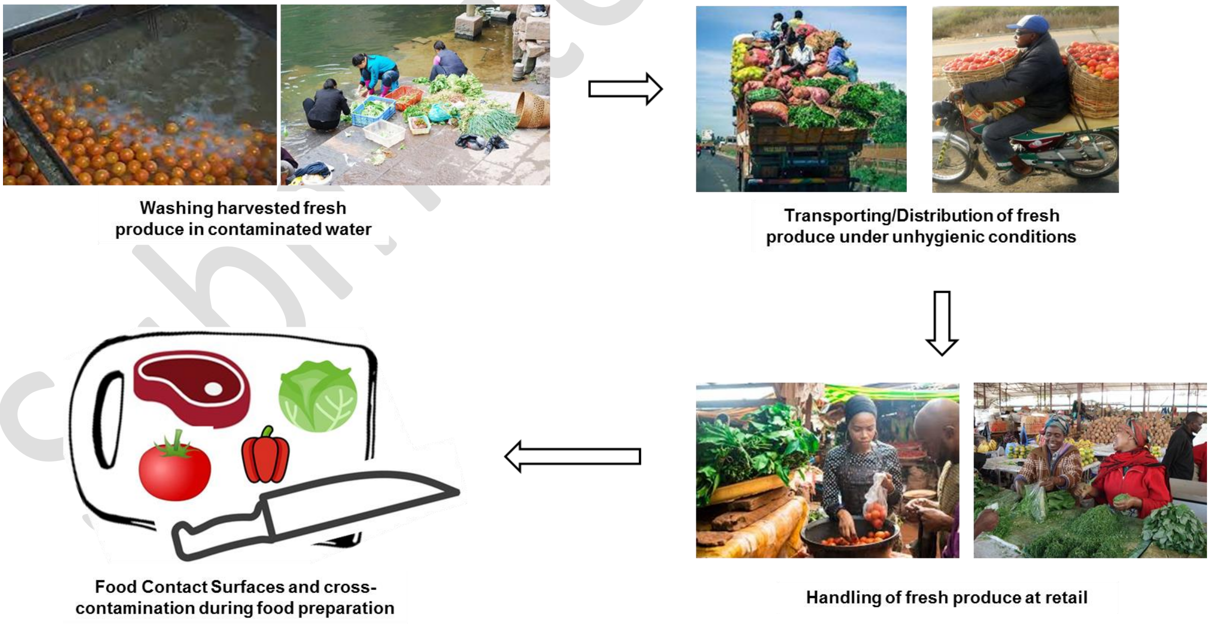

Figure 2: Postharvest sources of antimicrobial-resistance pathogen contamination in fresh produce production

4.2.4. Handling during retail

In Africa the sale of fresh produce occurs mostly in open markets with limited sanitary facilities. Fruits and vegetables are not protected from environmental contaminants and are often touched with bare hands (Figure 2). Retail fresh produce has been reported to be more contaminated than those collected directly from primary production/farms (Uyttendaele *et al*., 2014; Quansah *et al*., 2018), thus suggesting that postharvest handling contributes significantly to microbial contamination of fresh produce. Vegetables are often cut by retailers so that it can be easier for consumers to use. However, chopping boards are rough and cannot be thoroughly cleaned or washed and the knives used are not cleaned and/or disinfected. *E. coli* O157:H7 has been found on cutting boards in retail shops (Abdissa *et al*., 2017). Furthermore, cut vegetables are sometimes placed in dirty bags during sales, and unsold vegetables are kept in the market or left uncovered in the backyard of houses (Akoachere *et al*., 2018), where they are exposed to further contamination.

4.2.5. Cross-contamination during food preparation

Cross-contamination of fruit and vegetables from contaminated foodstuff during preparation could be another source of contamination. In addition, food processing surfaces and other foodstuffs which are not adequately washed or separated during food preparation may be a source of contamination (Abayneh *et al*., 2019).

Foods often prepared alongside fresh produce have been reported to be contaminated by antimicrobial-resistant pathogens. For example, several meat products (mutton, beef, chevon, guinea fowl and chicken) in different parts of Africa have been reported to carry antimicrobial-resistant *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and *Shigella* spp. (Abeyneh *et al*., 2019; Adzitey, 2020; Adzitey *et al*., 2020; Ahmed *et al*., 2016; Amoako *et al.,* 2020; Afnabi *et al*., 2019; Ahmed and Shimamoto, 2014; Al-Gallas *et al*., 2013). Antimicrobial-resistant *Vibrio* spp. were found in shrimps, crabs, and cuttlefish, with 18% of the isolates resistant to all (10) antibiotics tested (Adeleye *et al*., 2008). *V. parahaemolyticus* and *V. cholerae* were found in crab and shrimp (Ahmed *et al*., 2018a), while antimicrobial-resistant *Salmonella* was detected in seafood (Al-Gallas *et al*., 2013). *Aeromonas hydrophila* in fish (Ahmed *et al*., 2018b; Algammal *et al*., 2020). Dairy products, such as cheese, often used to make salads and vegetables, could be contaminated with MRSA (Ahmed *et al*., 2019). Antimicrobial-resistant and MRSA have also been detected in indigenous cheeses in Africa, such as Damietta and Kareish cheese (Al-Ashmawy *et al*., 2016).

Since some of the pathogens found in these food items harbour integrons and gene cassettes that could transfer multiple-drug resistance to other pathogens (Ammar *et al*., 2016), their presence in foods and food preparation environments could become a source of antimicrobial resistance in fresh produce.

5. Strategies for controlling antimicrobial-resistant pathogens in fresh produce

To minimize contamination with antimicrobial-resistant foodborne pathogens in fruit and vegetables, it is important to create awareness of the practices that promote bacterial contamination throughout the supply chain and take necessary actions to reduce or possibly avoid them. Therefore, all actors within the fresh produce value chain (from the farmers/farm workers through the processors, retailers to the consumers) must be continuously educated on good agricultural, handling and storage practices, some of which include:

a. **Disinfecting seeds before planting**: Disinfection of seeds with 5% commercial bleach (hypochlorite) and dry heating at 45°C can remove bacterial infections without affecting the germinability of the seeds (Taški-Ajdukovic and Vasic, 2005). Other treatment options for reducing or removing bacterial pathogens from vegetable seed surfaces include chlorine solutions, acid, and hot water treatment (Li *et al*., 2014; Saunders and Everis, 2014)

b. **Organic soil amendments**: The use of organic amendments has been shown to reduce the population of soil-borne pathogens (Noble, 2011). Organic manure must be stored and treated in such a way as to ensure proper composting before application to soil. The heat and microbial community generated during the composting process inactivate foodborne pathogens and reduce the risk of fresh produce contamination (Gurtler *et al*., 2018).

c. **Irrigation**: Groundwater and rainwater should be used to irrigate fresh produce as they are of better microbial quality than ponds, lakes, and rivers. Irrigating fresh produce with river and marshland water should be avoided as they have a high prevalence of pathogens (Ssemanda *et al*., 2018a). Furthermore, irrigation using sprinklers and watering cans should be avoided because of their higher likelihood of depositing pathogens on fresh produce crops through splashing. Alternatively, drip and furrow irrigation should be encouraged (Ndiaye *et al*., 2011a)

d. **Avoiding faecal contamination from animals**: Vegetable farms should not be located near poultry farms as faecal materials from these farms could be carried by runoff to the vegetable farms. Also, mixed farming, where livestock is reared or allowed to roam around vegetable farms, should be discouraged. This is because faecal droppings from the animals could be carrying pathogens, which could be readily transferred to the vegetables. Barriers should be erected around vegetable farms to prevent access by livestock and wild animals.

e. **Handwashing and Hygienic Practices:** Adequate sanitation facilities must be provided for employees on the farm, processing plants, distribution points, and retailers. Good hygienic practices such as hand washing with soap should be regularly practised in farms and produce handling environments. Hand sanitisers should be provided where soap and water are unavailable (de Aceituno *et al*., 2015).

f. **Farm implement**: Tools used in fresh produce cultivation should be regularly disinfected to avoid transmission of pathogens. Tools, such as boots, shovels, and spades used on raw sewage or manure could transfer enteric pathogens to fresh produce. Hence, separate tools should be used for other farming practices. Where not possible, such tools should be thoroughly cleaned and disinfected before use in other farm operations.

g. **Washing harvested produce:** Washing fresh produce in clean water, with the addition of other disinfectants, reduces pathogen contamination and cross-contamination of fresh produce through wash water. Appropriate addition of disinfectants to water used for washing fresh produce is an important strategy for controlling pathogens in fresh produce. Chlorine is widely used for washing fresh produce and has been demonstrated to significantly reduce *E. coli* O157:H7 cross-contamination in fresh produce through wash water (Luo *et al*., 2011; Tomas-Callejas *et al*., 2012). Washing of fresh produce in atmospheric cold plasma (ACP)-treated water was found to reduce bacteria contamination on fresh produce and inactivate bacteria in wash water (Patange *et al*., 2019). Plasma treatment of wash water reduces the survival of *E. coli* compared to tap water (untreated water) (Fridman *et al*., 2021). This allows re-use of processing water for washing and reduces contamination of subsequent batches of fresh produce from washing water. UV is also effective in reducing *E. coli* O157:H7 on spinach leaves surfaces and in fresh produce wash water (Cossu *et al*., 2016) and reducing *Salmonella* on blueberry, tomato, and lettuce (Huang and Chen, 2020)

h. **Safe Transportation, Distribution and Storage:** Fresh produce should be transported, distributed, and stored under sanitary conditions that prevent microbiological contamination. Vehicles used to transport waste and manure should not be used to transport fresh produce. Transport vehicles should have clean floors and walls, and fresh produce should not be sat upon. Furthermore, fresh produce should be maintained at lower (refrigerated) storage temperatures during transportation, distribution, and display for retail to ensure microbiological safety (Zhang *et al*., 2020; Rapusas and Rolle, 2009). The use of reusable plastic containers (RPC) for transporting and handling fresh produce has been shown to reduce postharvest losses, although some pathogens, such as *Salmonella* spp., can survive on some RPC materials, and there is a risk of cross-contamination (López-Gálvez *et al*., 2021). Hence, RPCs and bags, sacks, and other containers used for transporting and storing fruit and vegetables should be cleaned and sanitised after each use to prevent cross-contamination with pathogenic microorganisms.

i. **Handling during processing:** Proper hygiene and handwashing are important for reducing microbial contamination of fresh produce from farm workers/handlers. Toilet and handwashing facilities should be provided for use by farm workers/handlers. Using a two-step alcohol-based hand sanitiser significantly reduces soil and bacterial contamination (coliforms, *Escherichia coli*, and *Enterococcus* spp.) on the hands of farmworkers (de Aceituno *et al*., 2015, 2016). This is especially useful in farms and produce handling environments where soap and water are unavailable, such as is prevalent in many agricultural contexts in Africa. Regular education and training on basic sanitation and hygienic practices should be provided to postharvest and processing operations personnel. This would improve their knowledge and practice on safe fresh produce handling. The use of personal protective equipment (PPE) should be ensured to prevent contamination of fresh produce by handlers. These include suitable aprons, coveralls, hair nets, beard guards, hand gloves, face masks and footwear cover. Training in the appropriate use of these PPE should also be ensured.

j. **Handling at retail and household levels:** Good handling practices at postharvest, retail and consumers are important to minimise pathogen contamination. Retailers and customers buying fresh produce should avoid touching the fresh produce displayed for sale. Washing and rinsing vegetables with clean water are important to reduce microbial contamination (James, 2006), although this might be a challenge in countries with limited access to potable water. Washing fresh produce with river and marshland water should be avoided as they have a higher prevalence of pathogens than ground water (Ssemanda *et al*., 2018a). Washing vegetables with sanitizers, organic acids, lemon juice, and hot water reduces the load of *Listeria* spp., *E. coli* and aerobic plate counts (Ssemanda *et al*., 2018b). Furthermore, cross-contamination should be avoided during food preparation. For example, raw meat and chicken products and vegetables should not be cut on the same chopping board, with the same knife, as pathogens could be transmitted through these food preparation surfaces (Gorman *et al*., 2002; Redmond *et al*., 2004).

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