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Insights on the prevalence of phyllo-epiphytic and endophytic pathogens on leafy vegetables from farms and retails in South Africa: A review

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Abstract

Leafy vegetables are a highly variable group of perishable food that broadly can be defined as vegetables grown for their edible leaves. This study was conducted to investigate the safety of commodities grown at farms and those sold in various retails globally, cascading to South Africa, and to determine the identification and characterization of the microbiota. Therefore, the authors conducted a desktop and literature review using popular trusted search engines with special keywords. For this study, the authors-maintained research material from 2010-2024, constituting research articles, reviews, book chapters, thesis, research short communications, and industrial short communications. From this study, it could be deduced that leafy green commodities differ from intact vegetables with regard to their physiology, handling, processing, and storage. In addition, various agronomic activities were identified as contributing to the contamination of leafy greens at various stages of minimal processing, including distribution. More studies have shown that various types of microorganisms are found in leafy vegetables purchased at small-scale farms and retail stores. Concerning that, characterise those that are pathogenic to human health and those that are beneficial and how to treat through antibiotics the diseases caused by those that are pathogenic to our health. Furthermore, the findings of this study revealed that some opportunistic pathogen communities in fresh leafy vegetables are diverse and can pose a health risk to consumers, leading to death. Finally, food safety and security remain a global challenge, and stunting continues to affect the majority of developing countries. Thus, future studies should focus on improving food safety management systems reducing food poisoning, recall incidents and outbreaks by controlling critical points and food security in primary horticulture production environments even at retail and determining which antimicrobials will inhibit the growth of specific bacteria causing a certain infection.

Key words: Proliferation, succession, coliforms, cold supply chain, opportunistic pathogens, antibiotic resistance.

Introduction

Myriad of fresh leafy green vegetables have been documented to provide ideal conditions for foodborne pathogens. Leafy green vegetables are perishable commodities and their phylloplane topography and natural apertures influence microbial community diversity (Qadri *et al*., 2015). Minimally processed fresh vegetables are generally defined as any vegetable that has been subjected to different processing stages, these include trimming, cutting, washing and disinfection, rinsing and storage (De Corato, 2020). The term phyllosphere has been utilised to describe the above-ground part of the plant environment and phylloplane is described as the leaf blade surface (Liu *et al*., 2023). Additionally, the term phylloplane has been utilised also, either instead of or in addition to the term phyllosphere.

Vegetables including cabbage (*Brassica oleracea* L. var. capitata), spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* subsp. vulgaris) and lettuce (*Lactuca sativa* L.) are not subjected to any lethal process which is mostly employed to kill pathogenic organisms effectively (Ampim *et al*., 2022). Precise identification of a potential source of contamination for produce is often difficult because contamination can occur anywhere in the farm-to-fork continuum (Whitney *et al*., 2021). Since the operations comprise several units, they are likely to provide opportunities for potential cross-contamination, for example, whereby a small lot of contaminated commodities may be responsible for the contamination of a large lot (Gil *et al*., 2015). Furthermore, these contaminated leafy green vegetables may cause pathogen transfer to the next large proportion of processed products which may lead to proliferation and succession (Castro-Ibanez *et al*., 2016).

Opportunistic pathogens can cross geographical barriers by attaching to natural apertures and thrive in new and different environments due to cross-contamination where they can multiply (Al-Kharousi *et al*., 2016). Jackson *et al*. (2015) reported highly diverse members of the *Proteobacteria* and *Bacteroidetes* in the leafy green phyllosphere group on ready-to-eat salads including spinach. The increased ratios of *Firmicutes* to *Bacteroidetes* species have been correlated with obesity and Type II diabetes as they are bad gut microbes (Kagele, 2015). Notable genera of *Firmicutes* include *Bacilli* such as *Bacillus, Listeria, Staphylococcus, Clostridia* such as *Clostridium* including *Acinetobacter* (Galperin, 2013).

Jongman and Korsten (2017) and Du Plessis and Korsten (2015) reported that several sources of surface water in South Africa are polluted, yet are still utilised for irrigation by commercial, small-scale, and homestead production systems. This is considered a potential factor and a risk exacerbating the prevalence of microorganisms, simply means that the viable count at the time of consumption may be elevated. Furthermore, it is highlighted that potential risks include the proximity of growing informal settlements without adequate sanitation and stormwater services, ill-functioning wastewater treatment plants, intensified urbanisation and the usage of animal manure as fertiliser.

Pathogen contamination and widespread may also originate from antibiotic residue through internalization and soil fertilizing mediums as they are utilised to control bacterial infection from plants (Van Pelt *et al*., 2018). The confirmed report shows that primary horticulture production environments are still challenges in South Africa and need to be tackled regarding potential risk factors and critical control points at integrated health standards. Therefore, this study was conducted to investigate the safety of commodities grown at farms and those sold in various retails globally, cascading to South Africa, and to determine the identification and characterization of the microbiota.

This review article was developed by examining studies on the prevalence, identification and characterisation of microbiota on vegetables at small-scale farms including their proliferation leading to succession at retail establishments worldwide and cascading to South African studies. Therefore, the authors conducted a desktop and literature review (Hartner, 2013) using Google Scholar, Scopus, Web of Science, ResearchGate and EBSCO-host with the following keywords: Prevalence of microbiota pathogens on vegetables at farms and retails; Identification of microbiota pathogens on vegetables at farms and retails; Characterisation of microbiota pathogens on vegetables at farms and retails; Antimicrobial susceptibility and succession on vegetables at farms and retails.

At most, the authors maintained research material from 2010 – 2024, constituting research articles, reviews, book chapters, thesis, research short communications, and industrial short communications. All material reviewed was analyzed and discussed based on the primary objective, with the conclusion leading to the understanding of contamination and potential risk parameters leading to the prevalence implicating leafy green vegetables in South Africa, and recommended future studies.

Enumeration of phyllo-epiphytic and endophytic pathogens on leafy vegetables from farms and retails: Each province in South Africa is unique in terms of suitable agricultural commodities that can be produced. Additionally, the Free State Agricultural Union reports that the province has 7.515 farming units, the highest in the country. Furthermore, it accounts for 26.4% of South Africa's field crops and 15.9% of all its livestock. Moreover, the Free State province is responsible for 15% of South Africa's gross agricultural income. The sector contributes approximately 7% to the provincial gross domestic product. Consumer demand put pressure on the fresh fruits and vegetables industries for all year-round supply.

Supermarkets have been spreading very rapidly in developing countries for the past decade. The rise in supermarkets was most significant in South Africa, Kenya, and Nigeria. The participation of urban consumers in the informal vegetable market forms a vital part and quantity regarding the urban economy as it offers easy access to food as informal traders source their supplies (Marumo and Mabuza, 2018).

In South Africa, most available studies on vegetables are based on indigenous leafy vegetables which are cultivated from home gardens and small plots in low-income rural villages (Maseko *et al*., 2017). Furthermore, they include their role in combating hunger and malnutrition is discussed in terms of their contribution to food security and nutritional status (Mavenghama, 2013). In a study in South Africa, Gauteng reported that spinach, cabbage including tomatoes (*Lycopersicon esculentum* L.) are considered popular vegetables particularly for low-income families as part of a sustainable daily diet (Methvin, 2015).

Processing of leafy greens: Pre-harvest and post-harvest prevalence: Pre-harvest and post–harvest sources as potentially hazardous activities and conditions that influence the survival and growth of pathogenic microorganisms on fresh vegetables. Holvoet *et al*. (2012) reported that *E. coli* can be found on weighing surfaces and convey belts in Belgian and these objects were highlighted as potential sources for cross-contamination due to poor manufacturing practices. Minimal operations are known to cause the onset of many physiological changes, reducing quality of a product. Spinach, cabbage, Swiss chard and lettuce are the kinds of fresh produce that were identified as commodity groups of highest concern from microbiological safety perspective because they are minimally processed with complex methods, which contributes to the increment of foodborne pathogens. Vegetables are minimally processed with the idea of improving the quality and the extending shelf- life including fulfilling consumers expectation (Corbo *et al*., 2010).

Fig. 1 depicts various stages of minimal processing. Harvesting of produce may be done through mechanical harvesters in large operational plants and by hands in small operational plants to the receiving point at the processing facility (James, 2010). The preparation procedure generally includes peeling, hand preparation, size reduction, defect sorting using different objects, washing, packaging and refrigeration but the first essential step is removing the outer leaf layer, especially in leafy greens such as cabbage (Siddiqui *et al*., 2011). Washing produce utilising potable water after harvest is an essential step to remove dirt and damaged tissues (Qadri *et al*., 2015). Due to favourable conditions and sufficient potential resources, pathogenic microbes are well able to thrive, contaminate and shift to the next phase and form a new niche and cause food poisoning.

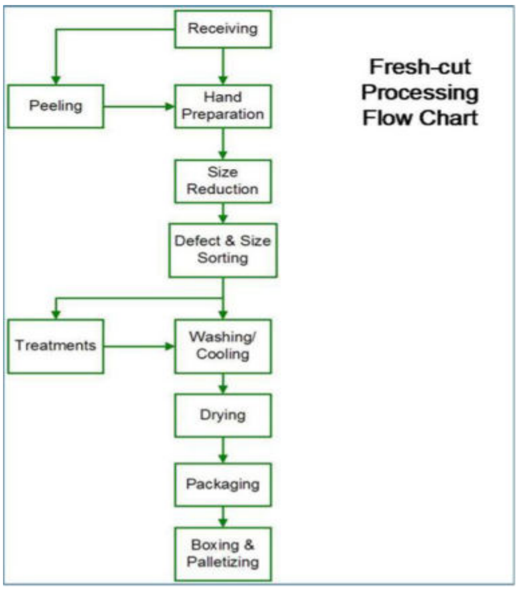

Fig. 1. Minimal processing operations

Microbiota persistent and colonisation on the phyllosphere: Attachment is the first step in the establishment of bacteria in the phyllosphere. They modify their habitat, divide, and increase in number utilising various mechanisms such as enhancing nutrients release from the plant phyllosphere and producing a polymer matrix for protection. The presence, attachment, colonisation, growth, and survival of microorganisms on produce depend on nutrients, heterogeneity traits of microflora, the environmental conditions and plant phenology (Jahid and Ha, 2012). Colonisation and attachment of foodborne pathogens on a produce-forming biofilm is a major cause of foodborne outbreaks infections including nosocomial infections (Jamal *et al*., 2018).

Composition and behaviour of bacteria diversity on the phyllosphere can be influenced by farming and storage conditions (Lopez-Velasco *et al*., 2011). Handling of leafy vegetables should be considered as critical control point to avoid cross contamination, pathogen colonisation and attachment on leafy greens as their interaction subsequent internalisation can incorporate into biofilm (Elhariry, 2011). Microbes' aggregates are associated with nutrient availability, interaction with other bacterial populations to more virulent pathogens, survival from environmental stress, and chances of infiltration into the plant as a mode of protection from disinfectant (Siddiqui *et al*., 2011). Preventative measures to limit the number of enteropathogens present on fresh produce relies on postharvest interventions (Gil *et al*., 2015).

Bacterial colonization steps strategies include modification of the phylloplane habitat including aggregation, ingression, and egression colonization forming external and internal microbial population. Produce has natural apertures such as stomata including veins, and cell wall junctions and often have punctures, cuts, splits, and cracks due to injury during pre-harvesting and post-harvesting handling, and bacteria can attach and assemble at the injury sites to form biofilms. A healthy phyllosphere may also support different numbers of epiphytic bacteria by passively leaking small amounts of metabolites such as carbohydrates, amino acids and organic acids to the leaf surface and assimilating atmospheric carbon dioxide into sugars (Sohrabi *et al*., 2023).

Bacteria can modify and manipulate the environment to enhance colonization and internalization, the interaction in the phyllosphere can affect physicochemical properties which can then affect the safety of crops for human consumption. Fig. 2 depicts ingression and egression colonization forming external and internal microbial populations.

However, phyllo-epiphytic bacteria can survive strenuous conditions and rapid fluctuations in environmental conditions that occur on a leaf surface by utilising major strategies: (i) Tolerance strategies to survive under direct exposure to environmental stresses and, (ii) Avoidance strategy through sites that are protected from those stresses.

Efflux pumps are another mechanism which allows the microorganisms to regulate their internal environment through toxic substances removal, including antimicrobial agents while quorum sensing is for communication and coordination for bacterial population also enhancing access to nutrients-rich niches, motility and plays a role in biofilm formation (Gaurav *et al*., 2023). Different pathogens broadly utilise three strategies to acquire the necessary nutrients for survival and replication from plant cells: (i) Manipulating host proteins to export nutrients to the apoplast where they reside (biotrophic), (ii) Causing the host cell to undergo programmed cell death and feeding on the remaining nutrients (necrotrophic), or (iii) A combination of the two approaches (hemi biotrophic)

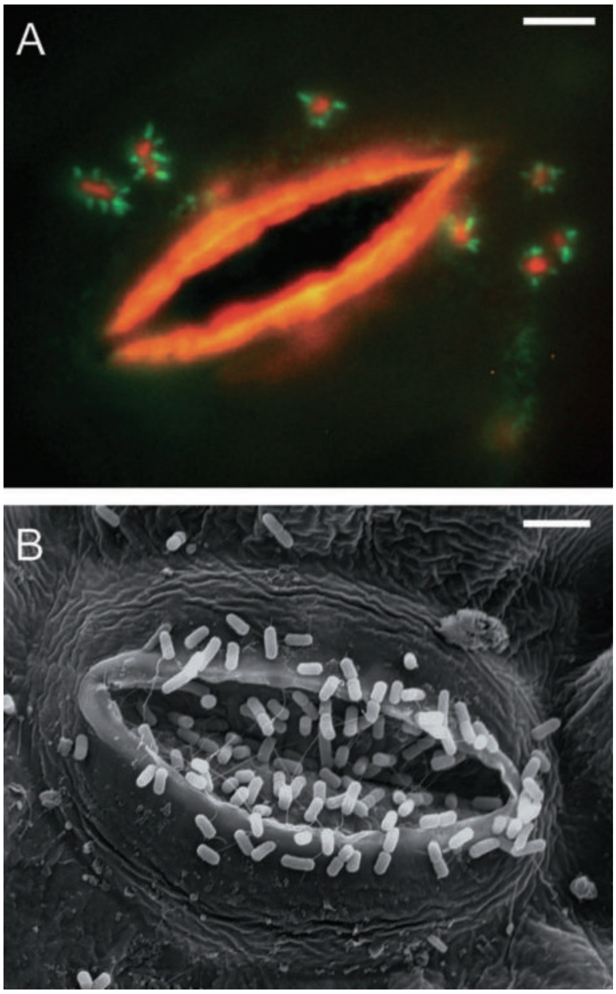

Fig. 2. Scanning electron micrograph showing binding of pathogenic *E. coli* to exterior and interior of vegetable leaf structure (stomata) (Berger *et al*., 2010)

Persistence of opportunistic pathogens in soil and soil amendments: Many environmental and management factors that are employed introduce enteric pathogens to soil, these include raw manure, wastewater, human biosolids, compost, and wild and domestic animal intrusion and other anthropogenic activities (Gutierrez-Rodriguez and Adhikari, 2018). Physicochemical properties found within soil as well as soil texture, pH, organic matter content, cation exchange capacity, porosity, and organic and inorganic nutrient sources influence the microbial ecology of all soilborne bacteria and enteric pathogens that are found in soil (Griffiths and Phillippot, 2013). Microbes capable of completing their entire life cycle in soil are capable of infecting humans through food consumption (Gutierrez-Rodriguez and Adhikari, 2018). *E. coli* O157:H7 strains can survive on manure-amended soil for more than two months (Tirado *et al*., 2010). Other studies also enlightened on the application of manure and the appropriate times for harvest after applying manure and the internalisation of microbes leading to biofilm (Woźniak-Karczewska *et al*., 2011).

Prevalence and persistence of enteric bacteria in irrigation water: Irrigation water is thought to be the leading pre-harvest source of contamination of fresh produce (Ijabadeniyi and Buys, 2012). In South Africa, different authors have highlighted and reported on the microbial contamination of surface water sources (Gemmell and Schmidt 2012; Chigor *et al*., 2013). The concern is that this contaminated surface water utilised for irrigation may contaminate fresh vegetables which may also negatively influence the trade of vegetables to the EU and USA due to phytosanitary measures (Ijabadeniyi and Buys, 2012). The deduction is that there is a correlation between increased numbers of pathogens on irrigated vegetables as a direct consequence of poor water quality utilised for irrigation (Decol *et al*., 2017).

Infiltration and internalization of endophytic bacteria: Internalization of foodborne bacteria into edible parts of fresh produce plants represents a serious health risk (Wright *et al*., 2017). Hirneisen *et al*. (2012) define internalization as the uptake of human enteric pathogens through wound space, vascular tissue, and damaged roots of the plant into the intracellular spaces. Type of plant, bacteria serovar, contamination route, effect of environmental stress, age and mechanism greatly influence the likelihood of internalization of a human pathogenic bacterium within a plant (Deering *et al*., 2012; Hirneisen *et al*., 2012). Temperature difference between produce, humidity and effectiveness of disinfectant water plays a role in infiltration resulting in internalization through hydrostatic pressure differential drawing bacteria into produce (Buchanan *et al*., 2017). Colonisation of bacteria following infiltration and internalisation is a dynamic process where various factors are included to promote contact, attachment, cell-cell interactions, defence from biocidal wash treatment and protection against stresses until the final stage which bacteria disperse new colonisation, this is illustrated in Fig. 3 (Srey *et al*., 2013). Microorganisms protect themselves from outside disturbance by an effective internal balance of physiological processes mechanism called homeostasis (Kumar *et al*., 2017). The waxy cuticles, internal leaf tissue of the phyllosphere and other polysaccharides serve as protective factors of pathogenic bacteria by keeping disinfectants and other environmental stresses away (Jahid and Ha, 2012).

Processing facility parameters influencing the prevalence of pathogens: Postharvest treatment of vegetables includes handling, workers, storage, washing, and transportation. Fresh produce susceptibility to survival and growth of pathogens is caused by poor handling which provides opportunities for contamination, growth, and ingress into plant tissues (Esmael *et al*., 2023). Attachment of pathogens onto shredders and slicers re-introduced *L. monocytogenes* contamination at vegetable processing plants (Francis *et al*., 2012). Cross-contamination can result from pathogen transfer from a contaminated leafy green vegetable to an uncontaminated leafy green vegetable through wash water (Gomba *et al*., 2017). The heaviest contamination was observed in the nets for soaking vegetables processed in cold water. Fresh vegetable storage at 4°C reduces the growth of psychrotrophic but organisms such as *L. monocytogenes* are capable of surviving at low temperatures (Jideani *et al*., 2017).

Storage facility area for cooling of leafy greens*:* The preservation and transportation of perishable foods are managed through the cold chain to slow biological decay processes and deliver safe and high-quality foods to consumers (Mercier *et al*., 2017). The composition of bacterial diversity and microbial quality of fresh produce can be influence by storage conditions (Lopez-Velasco *et al*., 2011). Many intrinsic factors such as water activity, pH, and nutrient content of fresh produce including extrinsic factors such as relative humidity and temperature can affect the stability of microorganisms affecting the quality and shelf life (James, 2010). Refrigeration temperatures will not retard microbial spoilage as these are favourable conditions for some microorganisms such as *Pseudomonas* spp. and *Listeria* spp. which rapidly grow under such temperatures (Mercier *et al*., 2017).

Succession of pathogens to retail: Small-scale growers who sell their produce locally to consumers mostly utilise their vehicles for almost all farm purposes (Sinkel, 2016). Holm *et al*. (2017) highlighted the lack of adequate water and sanitation infrastructure in the market as a contributing factor to bacteriological contamination of fresh vegetables with the prevalence of *E. coli* being the highest on leafy greens found in 74 (87 %) of the 85 samples. Insufficient precooling from the farm level can have a lasting effect on product temperature along the cold chain even if the subsequent steps are achieved at the correct ambient temperature at other stages of the cold chain (Nunes *et al*., 2014). Microbiological quality of fresh leafy greens and fruits collected from supermarkets in Istanbul, samples were analysed, in the study 261 food sampled, 10 (3.83%) *Salmonella* spp. and 17 (6.51%) thermotolerant *Campylobacter* sp. were detected with the highest count highlighting strict temperature control of cold chain (Buyukunal *et al*., 2015).

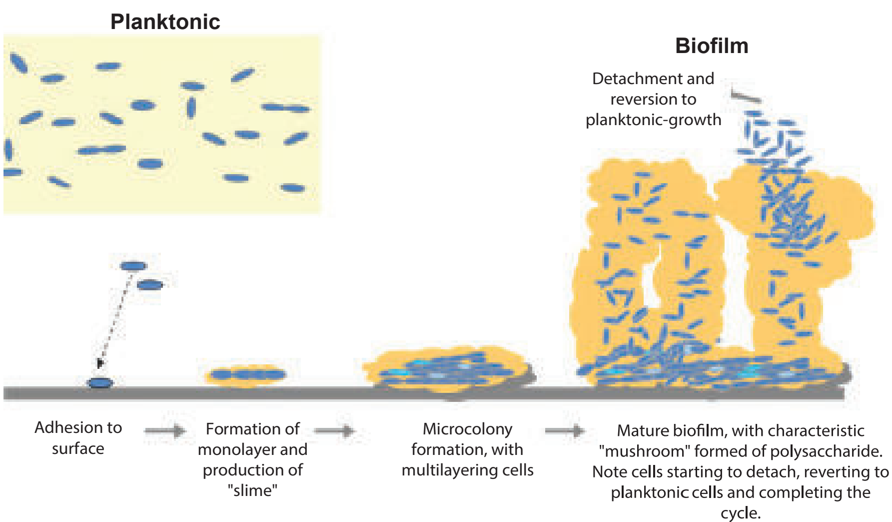

Fig. 3. Biofilm formation on a crop (Vasudevan, 2014)

Storage and farming operational practises influence the composition of bacterial groups on fresh cabbage, the microbiomes are highly diverse and complex and change dynamically during storage at refrigeration temperatures with the establishment of a dominant population (Lopez-Velasco *et al*., 2011). Transportation, distribution practices and storage conditions determine product quality and safety for future use and can all influence the diversity and composition of produce-associated microbial communities (He *et al*., 2018). At retail, fresh leafy vegetables must be refrigerated upon arrival, this is to prevent temperature from fluctuating. Many outbreaks occurred have implicated food workers as the main sources due to their infection, either because they were in the prodromic phase before symptoms develop or began or because they were asymptomatic carriers (Sumner *et al*., 2011).

Tolerated dose and infective dose: Different pathogenic species differ in the number of required cells or molecules to start an infection in a host (Table 1). Number of cells that are required to infect a host and start an infection is termed infective dose; they vary intensely across pathogens with each species strain which also depends upon the mode of action. In most instances, infection can be caused by an intake of a small dose of pathogenic cells including the type of pathogens causing the disease in important in determining the dose degree (Hara-Kudo and Takatori, 2011). The mode of infection and infection dose to cause a disease will then determine the level of risk and the severity of symptoms (Leggert *et al*., 2012). Infants, young children, pregnant women, the elderly, and those with an underlying illness are particularly vulnerable (WHO, 2017). In addition to that, every year 220 million children contract diarrhoeal diseases and 96,000 die. Furthermore, WHO reported that children under 5 years of age carry 40% of the foodborne disease burden, with 125,000 deaths every year.

Table 1. Pathogenic microorganisms associated with foodborne disease and their minimum infective dose

Bacteria sp.	Disease type	Tolerated dose	Foodstuffs source	References
Bacillus cereus	Diarrheal toxin Intoxication-emetic	($>10^5$/g)	Salads, vegetables	Granum (2017)
Escherichia coli 0157:H7	Infection	(10^1-10^2/g)	Contaminated fruits, vegetables	Schmid-Hempel and Frank (2017)
Listeria monocytogenes	Infection	($>10^2$/g)	Raw vegetables	Xu *et al*. (2023)
Staphylococcus aureus	Intoxication	(10^5/g)	Contaminated food source	CDC (2015)

Antibiotic residue as a threat to human health: The discovery and utilisation of various antibiotics have contributed significantly to the control of infectious diseases regarding reduction of the associated mortality and morbidity rate in both humans and animals. The growing antimicrobial resistance (AMR) phenomenon trait is generally linked to selective pressure which is normally caused due to improper use, overuse, or misuse of antimicrobials in humans and animals (Musoke *et al*., 2021). Fresh produce is reported as a source of exposure to various antimicrobial-resistant bacteria, and antibiotic resistance genes of clinical importance (Rahman *et al*., 2021). Few studies have reported and highlighted the presence of antibiotic resistance bacteria (ARB) and antibiotic-resistance genes (ARGs) in leafy greens (Kläui *et al*., 2024). Antibiotic resistance is highlighted as a global health crisis that best points out and elucidates "*one health approach*". The "*one health approach*" is outlined and expressed as a conjoined discipline to provide solutions for human health, animal health including environmental health (Djordjevic *et al*., 2024).

Antibiotic residue from soil amendment as a contaminant to crops: Antibiotic resistance genes (ARGs) originate from microorganisms in the environment and are incorporated into mobile genetic elements (MGEs) such as plasmids, transposons integrons and are mobilised through various biomes using various mechanisms (Colavecchio *et al*., 2017). Integrons, plasmids and transposons are genetic elements found in bacterial genomes carrying ARGs and are present in most gram-negative pathogens (Gilling, 2014). Different environmental biomes house bacteriophages and antibiotic resistance bacteria which harbour ARGs that can be mobilized from commensal bacteria to human pathogenic bacteria (Gilling, 2014). Commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. The movement of genes from one organism to the other through horizontal transfer can trigger a resistance phenotype (Sandberg and LaPara, 2016).

Soil has been investigated as a reservoir for bacteriophages carrying ARGs and at some point, regardless of the manure treatment used, bacteriophages have the potential for HGT in agricultural soil microbiomes (Ross and Topp, 2015). Mechanisms for antibiotic sorption to soil include amongst other things cation exchange, surface complexation and hydrogen bonding. About 30-90% of a parent compound and its breakdown metabolites are excreted via urine or faeces unchanged due to poorly absorbed antibiotics in animals' gut (Du and Liu, 2012). An *et al*. (2015) reported that antibiotic contamination in animal manure, soil and sewage sludge had a higher concentration of antibiotic residue on vegetables.

Utilising manure and biosolids as fertiliser are a common practice around the globe. Contamination of plants by antibiotic residue can also occur through different mediums employed for making the soil fertile such as the utilise of contaminated manure, sludge, and contaminated irrigation water (Lopez-Velasco *et al*., 2011). Feedlots are potential sources of antibiotics and about 75% of antibiotics provided to feedlot animals could be excreted in the environment as manure waste. Bacteria are capable of surviving for a longer time in the environment, their survival depends on species and environmental conditions however their genetic elements can persist regardless of cell viability. Plant uptake and bioaccumulation of antibiotics have received considerable interest due to issues of food safety and human health. Majority of gram-negative pathogens now have the integrons carrying resistance bacteria and known for dissemination of antibiotic resistance (Gilling, 2014).

Antibiotic residue uptake as a contaminant to the crop: The other study shared knowledge on plant uptake of most used antibiotics and their issues regarding food safety and human health (Yannarell *et al*., 2012). The plant is organ dependent, and the absorption of antibiotic residue is first by root from the soil through different mechanisms and the absorbed residues can be found in tissues (Migliore *et al*., 2010; Wang *et al*., 2015). Bioaccumulation and response of antibiotic residue from the plant vary depending on the type of species, antibiotic class and antibiotic concentration which occur through different mechanisms which include ionization, sorption properties, water solubility and others (Azanu *et al*., 2016; Minden *et al*., 2017). Moreover, Yannarell *et al*. (2012) reported on the entry of antibiotics into the environment and the chemical characteristics of antibiotics, behaviour and persistence in soil including the mechanism of degradation.

Antibiotic residue as a threat to human health: Indicator organisms have been utilised extensively for a very long time to assess the microbiological status of food products and are often termed safety or index indicators depending on their purpose. They may indicate the potential presence of various pathogens, a gap in adequate sanitation and improper hygiene practices and/or good hygiene practices or a process failure reflecting quality attributes that may influence consumer acceptability of a product (Costell *et al*., 2010). The *Enterobacteriaceae* are beneficial like coliforms as they are indicators of process integrity and for monitoring sanitation in food manufacturing plants. Frequently the presence of indicator organisms is a concern but, in most instances, it is the quantity that is significant as the count may reflect the time and conditions of storage. Since native microflora form part of a produce, the transition can be through contamination causing opportunistic to be part of an established niche, this occurs when the host mechanism fails and homeostasis is disrupted, this normal microflora becomes pathogenic, this also includes characters such as spatiotemporal present and specie abundance.

The biggest challenges faced by small-scale farmers are farm infrastructure, crop production and livestock breeding proximity to the crops due to shortage of space. Successional patterns exhibited by microbial communities in leafy greens have received relatively no attention in South Africa. Microbial assessments of all the pathogenic organisms that are a threat to the human species are significant in reducing unnecessary illnesses. Implementing a complete sanitation program that encompasses the entire processing and hygiene programme is essential to minimize the risk of contamination by pathogens and to assure consumers' safety.

Among the suggested future projects are enumerating microbiota and identifying microbial species isolated from common leafy vegetables in South Africa, especially at the small scale farm, retail and storage crates before purchase and distribution. It will help to identify dominant pathogen species. Antimicrobial susceptibility profiling of the pathogens isolated from these vegetables is also advised to assess the pathogens resistance to widely used antibiotics. A comparative evaluation based on epidemiological data, including the aetiology and related risks to human health, is also suggested to profile pharmaceutical antibiotics that could successfully treat infections caused by these pathogens.

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