

# *Review Article*

# Microbial and Parasitic Contamination of Vegetables in Developing Countries and Their Food Safety Guidelines

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The safety of humans is of paramount importance in the vegetable production chain. Evidence of microbial and parasitic contamination of these products poses a great threat to consumers. This is an emerging issue the world is battling, and it is still in the process of unravelling. However, one of the contributing factors responsible for the rapid spread of these pathogens to millions of people among other factors is the distribution of food in our food systems. The purpose of this study was to draw the attention of producers, retailers, consumers, and various stakeholders to the occurrence and potential hazard of these organisms, their contamination origin, and food safety protocols. Among the food system, vegetables play a major role, and their consumption has increased as they form a larger portion of daily diets. This urge for healthy diets coupled with changing dietary habits and human population explosion has therefore accelerated their production. This has resulted in parasitic and microbial contamination gaining grounds in salad vegetables, and as such, a wide range of microbes such as Escherichia coli O157: H7, Listeria monocytogenes, Salmonella spp., Shigella, and Staphylococcus, and parasites such as Giardia lamblia, Entamoeba coli, Entamoeba histolytica, Cystoisospora belli, Toxoplasma gondii, Trichuris trichiura, and Ascaris lumbricoides have been isolated from them. Therefore, major routes for salad vegetable contamination and prevention methods have been pointed out in this review article. The topic of protective countermeasures will also be covered here in this review. Notwithstanding, several control measures have been reported to be effective and efficient in removing or eliminating pathogens, including treatment of irrigation water and fertilizers, use of disinfectants like vinegar and saltwater, irradiation, ozone, and bacteriophages. Though consumption of vegetables and salads is encouraged due to their nutritional advantage, appropriate systems should be put in place to ensure their safety.

# 1. Introduction

In recent years, there has been an upsurge in vegetable production and consumption throughout the whole world; this is said to be twice its initial production weight of 30 million metric tons in 2018 [1]. In developing countries like Ghana, the initiation of programmes such as the current "Planting for Food and Jobs" by the Government of Ghana has expanded the mass production of fresh vegetables [2]. This was anticipated to increase vegetable consumption by roughly 25% by 2020 [3]. Hence, the consumption of vegetables globally has increased due to their relatively low cost, high nutritious value, and convenience in preparation. Vegetables are either eaten raw or cooked, and are essential in human nutrition as a result of their nutrient composition such as dietary fibre, minerals (like iron, calcium, zinc, magnesium,

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and phosphorus), and vitamins (like vitamins B, C, and K) [4]. Furthermore, some of these vegetables are processed into ready-to-eat salads prepared by combining two or more vegetables such as cucumber, tomatoes, pepper, cabbage, carrots, spring onions, and radishes. They are usually sold by street vendors and in canteens, restaurants, and grocery stores as they are served with foods like "waakye," fried rice, jollof rice, and plain rice. Despite the numerous advantages vegetable consumption contributes to our well-being, the practice can adversely affect our health given if food safety practices are not carefully followed. This is because vegetables present numerous avenues for adherence and invasion of dangerous microbes or parasites during cultivation, harvesting, processing, and marketing [5]. A consequence of vegetable contamination is the associated outbreaks (such as giardiasis, amebiasis, cyclosporiasis, and cryptosporidiosis) which have been responsible for the higher rate of morbidity and mortality in various parts of developing countries [6]. While the rate of vegetable and salad consumption is accelerating, there is a tendency for increased microbial and parasitic contamination leading to many other foodborne illnesses. However, few reviews such as this have been conducted to integrate studies on this subject for proper surveillance and measures to combat the incidence. Hence, the need to draw the attention of producers, retailers, consumers, and various stakeholders to the epidemiology of microbes and parasites found in salad vegetables and vegetable salads, their contamination origin, and food safety protocols in developing countries have necessitated the conduct of this review.

# 2. Production, Distribution, and Consumption Trend of Vegetables and Vegetable Salads

Globally, the agriculture sector from 1980 to 2004 experienced a surge in vegetable production by 94%, with population increase being a significant contributing factor [7]. Since that period, there has been a continuous surge in production within the last eighteen years (2000 to 2018) from 682.43 to 1088.9 million metric tons though there was a slight drop between 2017 and 2018 (Figure 1) [8]. Among developing countries, interestingly, the production of vegetables is widely distributed in various continents including countries like India, Iran, Vietnam, Nigeria, Egypt, and Mexico, which form part of the top ten producers of vegetables in the world (Figure 2) [8]. Due to this increase in the production, the consumption of vegetables in recent years has also accelerated in different regions with Asian countries being the highest consumer, and then Europe, Northern America, Oceania, and Africa [1]. Additionally, the consumption rate of vegetables specifically in developing countries increased by 69% from 1961 to 2013 (Figure 3) [8]. However, due to a multitude of factors, many people in South Asian developing countries such as India prefer to consume raw vegetables as compared to fast foods [9]. In other African developing countries, vegetables such as cabbages, onions, tomatoes, and lettuce are affordable, accessible, and readily available in the markets; hence, their consumption have increased as compared to fruits [10].

According to the World Health Organization, vegetables constitute the majority (86%) of the total market streetvended foods during its survey [9]. They are processed into vegetable salads and served in restaurants, in canteens, and as street-vended foods [11]. A survey conducted in Ghana revealed that of the 15,000 street food vendors in the capital region, about one-third of them sell foods that utilize ready-to-eat vegetable salads (mainly lettuce and/or cabbage) as accompaniments [12]. This result corroborates with other studies which investigated foods sold in canteens and restaurants [13]. Since vegetable salads and salad vegetables play a major role within the food system, their security has become a priority globally as they are faced with the risk of contamination [7]. The increased demand has exerted pressure on everyone from the producers to the consumers to actively improve the safety of vegetable consumption [14]. Farmers in developing countries especially in Africa who are usually into vegetable farming are also faced with threats ranging from poor water quality and infertile soil to pest infestation, and hence the need for more research into microbial and parasitic contamination [15].

### 3. The State of Foodborne Illness Outbreak

With a surge in the supply and consumption of vegetable salads and salad vegetables in developing countries, there has been an increasing threat of microbial contamination as a result of the conditions under which they are been produced and sold due to poverty [17]. Street-vended food remains the topmost source of foodborne sickness due to the high prevalence of different microorganisms pointed out in numerous research works [12]. In South African regions, for instance, purchased and examined vegetables from the streets of Gaborone and Botswana harboured a significant level of Bacillus cereus, and this suggests a threat of foodborne illnesses [18]. Interestingly, around 1990, about 12% of food-related diseases were specifically attributed to fruit and vegetable consumption [7]. This is not different in the recent era (2018) where foodborne outbreaks in Ghana were linked to the presence of Vibrio spp., Salmonella spp., and other microbes in vegetables and salads [19]. In the subsequent year (2019), diarrhoeal diseases (many of which are foodborne) were the second top cause of death in low-income countries (Figure 4) [8].

In Saudi Arabia, numerous outbreaks of foodborne diseases have consistently been revealed. 31 foodborne disease outbreaks representing 251 cases were accounted for in 2006. From that report, a larger part (68.9%) of the affected individuals became ill after eating vegetables and salads that were commercially made, and *Salmonella* species was the highest pathogenic agent followed by *Staphylococcus aureus* [20]. Likewise, foodborne disease outbreaks (typhoid fever, food poisoning, meningitis, pneumonia, and gastroenteritis) in India from 1980 to 2016 were linked to ready-toeat foods harbouring *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli O157: H7, Yersinia enterocolitica*, and *Listeria monocytogenes*, with *Salmonella* species again

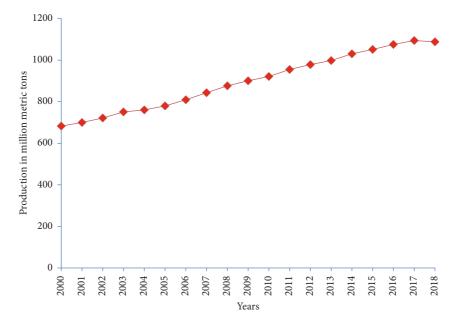


FIGURE 1: Volume (in million metric tonnes) of vegetables produced globally [8].

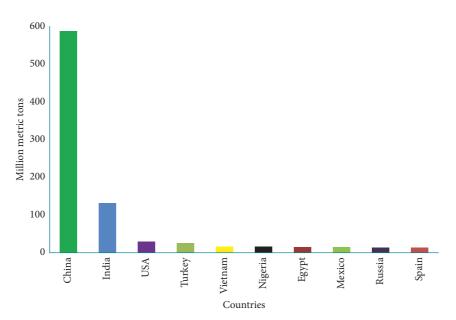


FIGURE 2: 2019 top ten leading producers of vegetables [8].

being the most common cause [21]. The epidemiological investigation also revealed the yearly prevalence of *Salmonella typhi* to be much higher in developing countries like Pakistan, India, and Vietnam, representing 451.7, 214.2, and 21.3 cases, respectively, per 10000 individuals as opposed to China (15.3 cases/100,000) [22]. A significant number of findings every year from Sri Lanka, Nepal, India, Maldives, Pakistan, Bhutan, and Bangladesh have indicated shigellosis as the most detrimental disease leading to high fatality and morbidity [22]. Concerning listeriosis, the disease initially occurred periodically in Asia [23]; however, it is presently spreading widely across developing regions [24]. This periodic disease is typically linked to consuming foods like milk and vegetables [25]. Though there are limited reports

and surveillance on listeriosis in Nigeria, research has shown its effect on pregnant women and infants [26]. South Africa recently experienced severe outbreaks of listeriosis (820 cases) [27] and salmonellosis thought to be related to vegetable consumption [28].

Furthermore, parasites have been shown to play a major role in the occurrence of foodborne illnesses. Foodborne parasites are endoparasites comprising protozoans, trematodes (flatworms or flukes), nematodes (roundworms), and cestodes (tapeworms). Parasitic diseases have been prevalent in tropical countries, but factors like climate change, global warming, and globalization of food supply are extending them to other non-tropical regions. *Entamoeba histolytica*, which is responsible for amebiasis, was

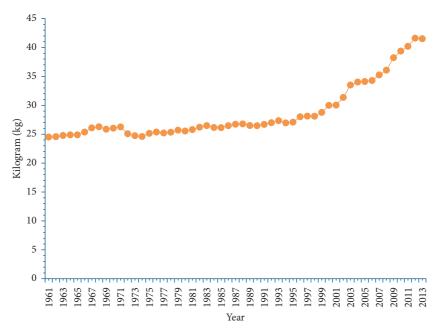


FIGURE 3: Trend in vegetable consumption per capita in the least developed countries, 1961 to 2013 [8].

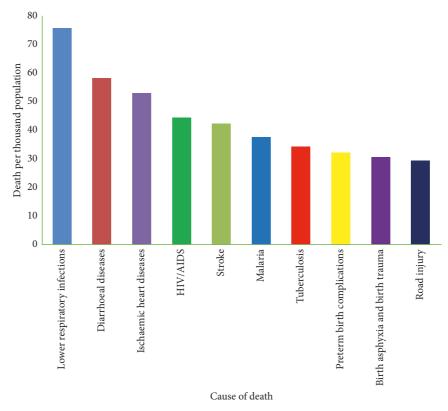


FIGURE 4: 2019 top ten leading causes of death in low-income countries [8].

reported to be the cause of illness among fifty million patients and also resulted in a hundred thousand deaths in developing countries across South America, Africa, and the Indian sub-continent [25]. Furthermore, toxoplasmosis (caused by *Toxoplasma gondii*), one of the most common foodborne diseases, sickens thousands globally each year [29]. It is thought that vegetables washed in contaminated water are a major contributing factor to foodborne parasite outbreaks [1]. Not only can these outbreaks be caused by familiar foes but there are also recent cases of emerging infections arising from contaminated vegetables. For instance, in 1977, the first human case of cyclosporiasis was

reported [30]. Similarly, twenty states of the USA in 1990 and 2004 were affected by cyclosporiasis, and this was caused by a new member of the coccidian family, *Cyclospora cayetanensis* [30]. Interestingly, these outbreaks were linked to lettuce, basil, and raspberry consumption [25]. It is observed that the outbreak of foodborne illness has been overlooked or un-investigated, and will gain attention after a major health incidence. Under such circumstances, identifying the risk factors and controlling the outbreak will be impossible [31]. Therefore, instituting foodborne disease observations would be essential as it will help to generate data on the issue, determine the extent of contamination, and determine the kind of food to be contaminated in the future, and in other preventive measures [25].

#### 4. Microbial Assessment of Salad Vegetables

4.1. Vegetables. Table 1 displays evidence of different species of microbes such as *Escherichia coli O157: H7, Listeria monocytogenes*, and *Salmonella* spp. isolated from salad vegetables including lettuce, cabbage, and cucumbers. In 2022, for instance, a study indicated the existence of multidrug-resistant *Listeria monocytogenes* in fruits and vegetables in South Africa [24].

4.2. Vegetable Salads. Due to the growing need for work and humans' busy schedules, most individuals in a bid to satisfy their nutritional requirements have resorted to the most convenient ways to access foods. They do not have enough time to cook, and that paves way for the consumption of already prepared foods which are usually served with readyto-eat vegetable salads. These are sold in open markets, in grocery stores, as street-vended foods, and in restaurants. Notwithstanding, an increased level of consumption is worth noting because these foods have been indicated as a source of several pathogenic infections with contamination originating from each point in the supply chain including retailers, processors, and/or consumers.

4.2.1. Vegetable Salads from Street Vendors and Open Markets. The street food industry is known to satisfy the everyday food needs of a large number of inhabitants of developing countries by serving different kinds of dishes that are affordable and easily accessible [43]. The preparation of these ready-to-eat street foods takes place either at home or on the street, and is sold along the street; hence, their consumption requires no further processing [44]. These foods are now popular in developing countries due to economic changes, population growth, and urbanization [44]. Consumption of street foods raised concern about potential food poisoning outbreaks due to improper handling and unhygienic practices among street food vendors [45]. One of the most essential sources of pathogen contamination is vegetable salads which are commonly served along with other foods [46]. This is because several microorganisms of public health concern have been identified in vegetable salads prepared in the streets [46-50]. The

presence of these microorganisms were also found when vegetables used for salad preparation and the salads itself were analysed concurrently [51, 52].

Contamination was reported in salads in some studies, and the total bacterial count was found to be above the national standard count of <5.0 log10 cfu/g [12, 53] as stipulated by the Ghana standard authority [16]. In those investigations, the bacterial count was 5.13 log10 cfu/g [53] and  $6.3 \pm 0.78 \log$  cfu/g [12], but a standard count was recorded in another study in Johannesburg [54]. Similarly, these pathogenic bacteria were found in an investigation in Ghana [12]. One of the major contributing factors to several microbial contaminations is the source of the vegetables used for salad preparation. Moreover, most street food vendors purchase vegetables for salads from open-aired markets and subject them to poor handling due to preferential differences of buyers as well as improper washing methods, thereby leaving some microbes at the point of consumption [55]. Hence, Penicillium spp., Staphylococcus aureus, Escherichia coli O157: H7, coagulase-positive staphylococci, Cladosporium, and Salmonella spp. were observed in fresh-cut vegetables and ready-to-eat salads purchased from open-air markets [52, 56].

4.2.2. Vegetable Salads from Restaurants and Canteens. Listeria spp. is a pathogen that causes listeriosis in animals and humans. It is transmitted to susceptible individuals when foods contaminated with manure from ruminants are ingested [7]. Listeria has been isolated from vegetable salads, salad vegetables, and coleslaw restaurants, and this may present a threat to human health [57]. In this study, 95.8% of the total 355 vegetable samples were contaminated with Listeria spp. Out of the Listeria spp., 4.1% were Listeria monocytogenes. In classifying the samples following the infection rate, 4.7% of salad vegetables showed contamination preceded by coleslaw (4.4%), with traditional vegetable salads being the lowest (1.7). Concerning the salad vegetables, cabbage (8.5%), lettuce (6.2%), and tomatoes (4.3%) were highly contaminated, with carrot and cucumber being the least (2.2%) [57].

A comparable study in India indicated the presence of *Listeria* spp. in 73% of salads studied, while 20% of the samples harboured *Yersinia* spp. A higher range of bacterial  $(10^6-10^8 \text{ cfu/gm})$  and mould  $(10^4-10^7 \text{ cfu/gm})$  counts were also observed in ready-to-eat salads. On the other hand, fresh vegetables used to prepare the salads exhibited the presence of *Listeria*, *Yersinia*, total bacteria, yeast, and moulds. However, higher bacteria and mould counts in salads than in freshly washed vegetable ingredients denote that cross-contamination into salads probably occurred during/after the preparation of salads [58].

Other important bacteria isolated from ready-to-eat salads from restaurants are *Clostridium difficile* and *Helicobacter pylori* [59, 60]. The study reporting the presence of *Helicobacter pylori* in both traditional and commercial vegetable salads was the first research paper in a developing country to make such a finding [60]. The prevalence rate of *Helicobacter pylori* in salads from these two sources was

Type of salad vegetable	Microbes isolated	Reference number
Lettuce	Escherichia coli O157:H7, Proteus spp., Enterobacter spp., Staphylococcus aureus, Salmonella spp., Klebsiella spp., Listeria monocytogenes, Pseudomonas spp., Acinetobacter spp., Micrococcus spp., Bacillus, yeast, and moulds	[32-35]
Cabbage	Escherichia coli O157:H7, Pseudomonas spp., Erwinia spp., Salmonella spp., Listeria monocytogenes, Staphylococcus, and Enterococcus	[33, 36, 37]
Carrot	Escherichia coli O157:H7, Erwinia spp., Staphylococcus aureus, Enterobacter spp., Citrobacter spp., Klebsiella spp., Lactobacillus spp., Pseudomonas spp., Vibrio spp., Proteus spp., Aspergillus spp., Rhizopus spp., and Enterococcus	[33, 34, 36, 38]
Cucumber	Escherichia coli O157:H7, Staphylococcus aureus, Enterobacter spp., Klebsiella spp., Lactobacillus spp., Pseudomonas spp., Vibrio spp., Aspergillus spp., and Shigella spp.	[34, 36, 38]
Onion	E. coli, Pseudomonas spp., Enterobacter spp., Staphylococcus spp., Streptococcus spp., and Cryptosporidium spp.	[36, 37, 39, 40]
Tomatoes	Citrobacter spp., Enterobacter spp., Klebsiella spp., Lactobacillus spp., Pseudomonas spp., s Staphylococcus spp., Vibrio spp., Aspergillus spp., Rhizopus spp., Penicillium spp., Shigella spp., Escherichia coli O157:H7, Salmonella spp., Listeria spp., and Enterococcus	

TABLE 1: Microbes isolated from notable vegetables.

Italicized words indicate scientific names of microrganisms (Genus and Species).

significantly variable, wherein improperly cleaned leeks, lettuce, and traditional salads were the highly contaminated products. 9.56% of salad samples were found contaminated when the culture method was applied for isolation, while the polymerase chain reaction (PCR) technique also revealed 10.86% contamination.

Furthermore, a study conducted in Nigeria, which took three months, revealed that salads from restaurants harboured twelve nonhemolytic bacterial pathogens, including *Bacillus* spp., *Klebsiella oxytoca*, and *Escherichia coli O157: H7* [61]. The prevalence of microbial contamination in some vegetables from Tamale in Ghana [62] reflected in the salads sold in that same area such that 73.3% and 76.7% of salad samples were contaminated with *Salmonella* spp. and *Shigella* spp., respectively [63].

In and around several University campuses in developing countries, pathogens causing several foodborne illnesses have been reported. Concerning E. coli and Salmonella, the majority (80%) of vegetable salads (Kachumbari) served in canteens around Egerton University, Kenya, contained an average of 3.047 log10 cfu/g Escherichia coli O157: H7, while 70% of the samples tested positive for Salmonella [64]. This confirms the findings of a similar study wherein vegetable salads (Kachumbari) harboured Escherichia coli O157: H7 but not Salmonella. Additionally, sampled vegetable salads were indicated as unsafe for consumption and hence detrimental to human health as a result of Campylobacter spp. and staphylococci contamination [65]. In another study, Escherichia coli O157: H7 was found in over 80% of the product, but the topmost contamination was observed in street food salads (4.23 log10 cfu/g) [66]. Salads from a University campus in Bangladesh were also contaminated with Escherichia coli O157: H7, Salmonella spp., and Staphylococcus spp. Monte Carlo simulation calculation on risk assessment showed that the risks of Staphylococcus spp., Salmonella spp., and E. coli infection yearly are 100% on average due to frequent salad consumption in this region [67, 68]. Furthermore, different E. coli strains (enterotoxigenic, enteropathogenic, and Shiga

toxin-producing E. coli pathotypes) have been identified in the vegetables [69]. In this study, vegetables were contaminated with E. coli strains, and less than half of the strains were classified as diarrhoea-causing E. coli pathotypes. 26.7% of salad samples were contaminated with E. coli pathotypes, 15% (two enterotoxigenic E. coli and one enteropathogenic E. coli) from spinach salads, and 25% (three enterotoxigenic E. coli, one enteropathogenic E. coli, and one Shiga toxin-producing E. coli) isolated from mixed salad [69]. These vegetables could be a route for the emergence of bloody diarrhoea and hemolytic uremic syndrome which have been of high prevalence in Africa, particularly in children and HIV patients, caused by these E. coli pathotypes [70]. Apart from the above-mentioned pathogens, P. aeruginosa, K. pneumoniae, Streptococcus spp., and Bacillus cereus were also isolated from readyto-eat salads offered with waakye on and across the University of Ghana campus, and from eating places around the campus. The majority of the vegetable salads harboured Bacillus cereus (93.3%) and Escherichia coli O157: H7 (96.7%), whereas Staphylococcus aureus was absent [71]. This prevalence on our campuses suggests the need for various departments in charge of food safety to strengthen safety guidelines in order to prevent foodborne illness among students.

4.2.3. Comparing Microbial Contamination of Vegetable Salads from Street Vendors and Restaurants/Cafeteria. There has been a notion that foods purchased from restaurants are much safer than those sold along the street. However, microbial contamination has been reported in salad prepared in restaurants and canteens though on a lower side than products from the street [72–75]. A study found street market salads to contain a higher bacteria load than those from fast food markets [72]. The vegetables used for the preparation of salad in the above study however harboured a lower number of bacteria, some of which include Staphylococcus aureus, Citrobacter freundii, and Neurospora crassa. However, Corynebacterium spp. found in salad samples originated from street markets [72]. Similarly, all fresh vegetable and ready-to-eat vegetable salads purchased from restaurants in another study were satisfactory regarding E. coli contamination, but street-vended samples yielded unsatisfactory levels [73]. Then again, 32% of salad samples had Salmonella spp. and 17% had helminth eggs and thermotolerant coliforms in street-vended and cafeteria salads in similar research [74]. Conversely, isolated Salmonella, Enterobacter aerogenes, Escherichia coli O157: H7, and Shigella spp. in another study were higher in salads from restaurants than in those from homes. Bacterial contamination reported was not surprising because the same vegetable salads analysed in another experiment isolated 36 bacterial species [76]. The presence of Shigella spp. in the above study corroborates another finding which detected Shigella spp. in tested salads before and after the enrichment [77]. The results found in restaurants and home-made salads are consistent with a lower state of hygienic practices of service workers, quality of water, and processing of vegetables as well as observance of other precautionary measures in the restaurants than the homes where enhanced care is taken [75, 76]. Therefore, there is a need to improve food safety knowledge attitude and practices, and foods served in restaurants and cafeterias where food products are considered to be highly safe.

4.2.4. Relationship between Microbial Prevalence in Vegetable Salads and Time. In developing countries, most individuals usually consume foods such as "waakye" (rice and beans) and plain rice in the morning in order to increase their energy for work [78]. However, the prevalence of microbes has been reported to vary at different times in a day. In several investigations, the highest level of pathogen contamination in vegetable salads has been in the afternoon. Moreover, a high load of bacteria was recorded in raw-mixed vegetable salads obtained in Ghana for 15 days [11], and this was above the standard (Log10 3.0 cfu/g) given by the World Health Organization (WHO) [79]. The bacteria identified comprised Streptococcus faecalis, Pseudomonas spp., Shigella spp., and Salmonella typhi. The rate of contamination was higher in afternoon samples than samples taken in the morning [11], and this confirms the findings of a similar investigation [49].

Other researchers [50, 61, 80] have added their finding that vegetable salads sold in the afternoon have higher contamination rate than those sold in the morning. Amala and Agha [80] isolated *Staphylococcus aureus* (45%), *Escherichia coli O157: H7* (36%), and *Bacillus* spp. (18%). In the study by Uwamere et al. [61], a substantial difference existed between morning and afternoon dishes in terms of the rate of bacterial contamination, but another investigation [50] had no significant difference although the afternoon bacteria load was higher. The two principal contributing factors for bacterial growth associated with fresh produce are storage temperature and pH. Hence, the low pH values and the short shelf life of morning salads could have been responsible for the low bacterial load [81].

However, the underlying factors that warrant the reduction and multiplication of pathogens should be further investigated.

4.2.5. Evidence of Antibiotic and Multidrug-Resistant Bacteria from Vegetables Salads. Ingestion of vegetables and salads contaminated with bacterial strains is associated with several diseases and can even lead to death. It is therefore paramount to use antibiotics for proper treatment; however, there has been an evolution of resistance against these antibiotics by most pathogenic bacteria, resulting in the loss of drug effectiveness. In previous years, the resistance of pathogenic microorganisms to multiple drugs was limited to hospitals due to the frequent application of antimicrobial agents. In the recent era, antimicrobial resistance is found in other environments including food products like vegetables. This situation is highly alarming, given that antimicrobial resistance can often spread via horizontal gene transfer [82]. Research in Nigeria revealed the existence of several bacterial antibiotic resistances in different fast food industries, and this has led to a spike in illness due to the consumption of contaminated raw vegetables [83].

Salads made from these vegetables and sold in fast-food industries harboured bacteria (25) of different species (6) which were highly (>50%) resistant against eleven of the twelve antibiotics tested. Among the antibiotics, the highest resistance rates against *Pseudomonas* spp., *Bacillus* spp., *Escherichia coli*, *Proteus* spp., *Enterobacter* spp., and *Aeromonas hydrophila* were Augmentin (96%), Cotrimoxazole (92%), and nitrofurantoin (88%), with streptomycin (44%) being the least [83], and this agrees with another study [72]. Furthermore, *Escherichia coli* O157: H7 found in vegetable salads and salads vegetables were resistant to more than five antibiotics, thus revealing the multidrug-resistant capacity of *Escherichia coli* O157: H7 [59, 69].

Apart from Escherichia coli O157: H7, two bacteria species, Salmonella spp. and Staphylococcus aureus, found in ready-to-eat vegetable salads showed a varied resistance rate (25.71% to 81.82%) against Chloramphenicol, Ofloxacin, Dovid, Siprosan, gentamicin, Tarcvid, Ciproxin, and pefloxacin [84] with an overall multiple resistance rate of 35.29%. Comparably, potential human pathogens, Staphylococcus aureus and Klebsiella aerogenes, from vegetable salads exhibited antibiotic resistance profiles at a range of 25.71% to 81.82% [85]. Whereas another study [86] revealed the ability of Staphylococcus aureus to show 83% resistance against co-amoxiclav and 75% against penicillin. Klebsiella aerogenes was fully (100%) ampicillin-resistant and against other antibiotics such as imipenem and amoxicillin/clavulanic acid [86]. Other pathogens isolated from vegetable salads that showed multidrug resistance were Listeria monocytogenes, Proteus vulgaris strain, Citrobacter freundii, Bacillus thuringiensis strain, Staphylococcus aureus, and Serratia marcescens strain [57, 87]. Specifically, *Listeria monocytogenes* were highly resistant to ampicillin (92.9%), oxacillin (85.7%), gentamicin (21.4%), and ciprofloxacin (14.3%). This resistance outcome of microbes in this study indicated that microbes were

resistant to more than one antibiotic and that most of them (64.3%) showed antimicrobial resistance against more than four antibiotics [57]. These results suggest that vegetables and salads may serve as a vehicle for bacteria that are resistant to multiple antibiotics.

## 5. Parasitic Contamination of Vegetables and Salads

5.1. Vegetables. Multiple types of research have assessed the occurrences of parasites in vegetables worldwide and have recovered several parasites such as Ascaris lumbricoides, Entamoeba histolytica, and Giardia intestinalis [88-90]. This contamination is also evident in various parasitic isolates from vegetable salads, as shown in Table 2. Many studies in Ethiopia, Ghana, and Iran also have consistently compared parasite prevalence in vegetables found in open-aired markets and supermarkets where the former recorded a high occurrence of human parasites [6, 104, 105]. The potential cause is the unhygienic nature of the environment which serves as a medium for the growth, multiplication, and transfer of parasites [106]. However, in some developing countries like Ghana, there are only a few available reports highlighting parasitic contamination of fresh vegetables with many of the studies centred on bacterial and chemical contamination.

5.2. Salads. A recent investigation into top causes of morbidity at the outpatient department by the Ghana Health service revealed intestinal worm infestation to be the 7<sup>th</sup> leading cause and has gradually increased from 2.1% to 3.6% [107]. Based on these reports, it was suspected that consumption of street-vended salad may be contributing to the increasing prevalence of helminthiasis in Ghana. In Ashanti Mampong, Ghana, field studies have reported risk to human health as a result of parasitic contamination in salad vegetables and ready-to-eat vegetable salads. In that work, parasite prevalence in the salad was the highest (66.0%), but the individual vegetables had a greater number (16) of species diversity than the salads (10) [2]. In the vegetables and salads, Ascaris lumbricoides and Giardia lamblia recorded varying levels of prevalence. While Ascaris lumbricoides was the highest (26.94%) succeeded by Giardia lamblia (19.93%) and Toxocara spp. (1.48%); in the vegetables, Giardia lamblia was more prevalent (24.17%) in the salad followed by Ascaris lumbricoides (19.17%) and Toxoplasma gondii (0.8%). Additionally, other parasites, namely, Fasciola spp., Moniezia, Toxocara spp., Trichuris trichiura, and Entamoeba histolytica, were recovered from both vegetables and salads.

The above study agrees with a similar finding in Ghana wherein *Giardia lamblia* in the salad was the also most prevalent but the overall prevalence of parasites in the salad was low (32.0%) [108]. In that report, out of 313 ready-to-eat vegetable salad samples analysed, 99 (32%) harboured more than one parasite. Twelve parasite species, including protozoa (*Giardia lamblia, Entamoeba coli, Entamoeba histolytica, Cystoisospora belli, and Toxoplasma gondii*), nematodes (*Enterobius vermicularis, Trichuris trichiura,* 

Ancylostoma duodenale, and Ascaris lumbricoides), and cestodes (*Taenia* spp., *Moniezia* spp., and *Fasciola* spp.) were identified. About a third (32%) was contaminated with at least one parasite. Among the twelve genera of parasites recovered, *Giardia lamblia*, *Entamoeba histolytica*, and *Moniezia* spp. were 6.7%, 6.4% and 4.2%, respectively. Other parasites found were *Trichuris trichiura* (3.8%) and *Entamoeba coli* (3.5%), with the rest recording low prevalence (<2%) [108].

Another report by Tefera et al. [109] revealed that 208 out of 360 vegetable salads and salad vegetables were infected with several strains of parasites, resulting in an overall contamination rate of 57.8%. The prevalence rate of the salad (16.8%) was greater than that of the vegetables. Concerning the parasites, the prevalence is arranged in descending order: *Strongyloides* (21.9%), *Toxocara* spp. (14.7%), *Cryptosporidium* spp. (12.8%), *H. nana* (8.3%), *G. lamblia* (7.5%), *A. lumbricoides* (6.7%), *E. histolytica/dispar* (5.3%), *Cyclospora* spp. (5.0%), and *H. diminuta* (1.4%). It was worthy to note that samples obtained from open markets were more frequently contaminated (84.6%) than those purchased from grocery stores (15.4%) [109].

#### 6. Major Sources of Contamination

#### 6.1. On-farm Routes

6.1.1. Irrigation Water Sources. (1) Wastewater: since accessibility to potable water for vegetable production is a big challenge among farmers, one other way to irrigate their produce is the reuse of wastewater [94]. This practice is rampant among farmers in developing countries where about 10% of the population is engaged in urban agriculture [110]. The wastewater comprises liquid wastes released from homes, farmlands, institutions, and commercial and industrial companies which are usually combined with surface water, groundwater, and rainwater [111]. However, wastewater that can be applied either in the raw, or partially or fully treated state may harbour pathogens, pesticides, toxins, organic particles, or inorganic particles [111].

In South Africa, a high load of *Escherichia coli O157: H7* and *Vibrio* spp. were recovered from effluents of two wastewater treatment facilities [112]. However, due to the costly and time-consuming nature of the treatment procedure, only about 10% of wastewater is adequately treated in developing countries [113].

Unfortunately, insufficiently treated wastewater harbours a high burden of pathogenic microbes [114]. One litre of community sewage can harbour thousands of pathogens such as *Entamoeba histolytica* (4500), *Salmonella* spp. (7000), *Shigella* spp. (7000), and *Ascaris lumbricoides* (600) [115]. Therefore, the application of wastewater during vegetable production should be critically looked at by the necessary stakeholders as this raises public health concerns, especially where vegetables are eaten uncooked or improperly cooked.

(2) Surface water, groundwater, and rainwater: surface water bodies include rivers, streams, ponds, and dams.

Type of salad vegetable	Parasites isolated	Reference number
Lettuce	Cryptosporidium spp., Microsporidia spp., Cyclospora spp., Ascaris spp., Toxocara spp., Giardia spp., Hymenolepis nana, Ostertagia circumcincta, Cooperia spp., Fischoederius cobboldi, Fasciola hepatica, Entamoeba histolytica, Giardia intestinalis, Entamoeba coli, Trichuris trichiura, Balantidium coli, Hookworm, and Trichostrongylus spp.	
Cabbage	Balantidium coli, Fischoederius cobboldi, Fasciola hepatica, Entamoeba histolytica, Diphyllobothrium, Ascaris lumbricoides, cryptosporidium spp., Giardia intestinalis, Strongyloides stercoralis, Giardia lamblia, Entamoeba coli, and Trichuris trichiura	
Carrot	Strongyloides stercoralis, Ascaris lumbricoides, Cryptosporidium spp., Entamoeba histolytica, Giardia lamblia, Entamoeba coli, Trichuris trichiura, and Cyclospora cayetanensis	[6, 93, 95, 99]
Cucumber	Fasciola hepatica, Entamoeba histolytica, and cryptosporidium spp.	[92, 93]
Green Onion	Cryptosporidium spp., Microsporidia spp., Ascaris spp., Toxocara spp., Trichuris trichiura, Hookworm eggs, and Taenia spp.	[91, 97]
Onion	Giardia lamblia, Entamoeba histolytica, Strongyloides stercoralis, Trichuris trichiura, Ascaris lumbricoides, Cyclospora cayetanensis, Cryptosporidium parvum, and Hookworm ova	
Tomatoes	Cryptosporidium spp., Entamoeba histolytica, Giardia lamblia, Entamoeba coli, Trichuris trichiura, Isospora belli, Cyclospora cayetanensis, Cryptosporidium parvum, Fasciolopsis buski, Hookworm ova, and Taenia spp.	
Radish	Ascaris spp., Trichuris trichiura, Hookworm eggs, Taenia spp., Taenia/Echinococcus, H. nana, Trichostrongylus spp., Giardia lamblia, Fasciola hepatica, Entamoeba coli, Blastocystis hominis, and Dicrocoelium dendriticum	
Spinach	Ascaris lumbricoides, Giardia intestinalis, Entamoeba histolytica, Giardia lamblia, Entamoeba coli, Trichuris trichiura, Entamoeba coli, Trichostrongylus spp., Toxocara spp., H. nana, Taenia spp., and Isospora spp.	[94, 95, 102]
Parsley	Ascaris spp., Trichuris trichiura, Hookworm eggs, Taenia spp., Hymenolepsis nana, Trichostrongylus spp., Entamoeba spp., Giardia lamblia, Trichostrongylus spp., Fasciola hepatica, Blastocystis hominis, and Dicrocoelium dendriticum	[97, 103]
Rocket	Entamoeba histolytica, Entamoeba coli, Giardia lamblia, Cocci spp., and Balantidium coli	[96]

TABLE 2: Parasites isolated from notable vegetables.

Globally, surface water is the topmost and most extensively exploited water for irrigation but is highly open to pathogenic infestation as opposed to other water sources [116]. Contaminants such as heavy metals, and carcinogenic and organic substances from rural, urban, and industrial facilities can enter bodies of water. Furthermore, these water sources can harbour several pathogenic microorganisms such as *Salmonella* spp. and *Listeria* spp. [117]. These can be released into farmlands and produced during irrigation, which therefore can affect the safety of food and consumers [118].

At a time when surface water bodies have dried up, groundwater is another alternative and inexpensive source of water for domestic and agricultural production. This is because the aquifers help to protect the water reserves during drought [119]. Groundwater is usually suggested as water for irrigation of farm produce as compared to surface water, and this is due to the lower rate of contamination arising from the enclosed nature. Microbes like *Clostridium* spp., *Staphylococcus*, and *E. coli* were identified from borehole water in Kenya [120]. Since the occurrence of contamination in groundwater is usually minimal, they are relatively safe microbiologically for agricultural purposes [119].

Research has elaborated that in rural regions especially where there are few or no pipe-borne or boreholes, collected rainwater is an extremely useful and most convenient source of water for domestic use and irrigation [121]. However, the method of collection, that is, from rooftops or runoff on the ground which is then collected into containers or tanks, can affect the quality of the water. For example, faeces from animals (such as bird's faeces) and other debris as well as pathogens found in the soil such as *Salmonella* spp. may contaminate the harvested rainwater [121].

(3) Prevalence of pathogens in irrigation water: water on farmland coming from irrigation systems or rainfall can serve as a potential vehicle for pathogen transmission onto fresh produce [122, 123]. These water sources are usually contaminated with enteric microbes or parasites which come from faeces, sewage, soil, and other material introduced into them [124]. Rainwater from rooftops has been reported to harbour pathogenic bacteria like Salmonella spp., Aeromonas spp., Listeria spp., Campylobacter spp., E.coli (faecal indicator), and Legionella spp. [125]. This was confirmed by Sánchez et al. [126], who asserted that the presence of these microorganisms is detrimental to the security and quality of vegetables which is not intensively treated before consumption. Twenty-three parasite species including eggs of Ascaris lumbricoides, Entamoeba histolytica, and Hymenolepis species, among others, were isolated from different irrigation waters located in five African regions [127].

Several studies conducted in Morocco [128], Ghana [129], and Ethiopia [94, 130] have demonstrated that irrigation water could affect the safety of fresh vegetables due to the high level of microbial and parasitic contamination. Thus, the application of irrigation water contaminated with faecal coliforms (CF), total coliform bacteria, *Bacillus cereus*, etc. was also observed to be present in vegetable samples [131, 132].

Furthermore, lettuce is a significant vegetable and forms a major part of vegetable salads. However, the application of irrigation water containing helminth eggs and larvae on lettuce resulted in their contamination. The helminth eggs identified included Ascaris lumbricoides, Trichuris trichiura, Hookworm, Enterobius vermicularis, Strongyloides larvae, and Taenia spp. [133]. Ascaris lumbricoides and Hookworm were the most prevalent microbe in both irrigation water and lettuce samples [133]. Compared with the World Health Organization (WHO) standards, the faecal coliform and helminth egg levels in irrigation water and lettuce samples exceeded the recommended levels [133]. Levels of helminth eggs were substantially reduced after washing in that water containing vinegar as compared to water without vinegar [133]. Similarly, protozoan parasites (E. histolytica cysts, microsporidia spores, and Cryptosporidium oocysts) were identified in vegetable washing water and vegetable irrigation water in a similar investigation [134]. In line with the above study, species of helminth eggs, Hymenolepis diminuta, Ascaris lumbricoides, Strongyloides larvae, and Fasciola hepatica, were also isolated from water and lettuce samples [135].

Treatment of wastewater is one of the reported strategies to minimize the risk of microbial contamination. This is because research found microbes (like faecal coliform, coliform bacteria, and E. coli) and parasites (such as Ascaris lumbricoides and Strongyloides stercoralis) in wastewater which was higher than that recorded on the vegetables (lettuce, Amaranthus, and cabbages) [62]. Also, significantly higher bacteria counts were observed in surface water which subsequently contaminated salad vegetables (spinach, tomato, green chilli, radish, and cabbage) than partially treated wastewater from sewage treatment plants [136]. However, wastewater treatment could not eliminate pathogens as Javanmard et al. [137] reported parasitic contamination of treated wastewater (53.8%) and vegetable (41.7%) samples irrigated with this water. Also, intestinal helminth ova (*Trichuris trichiura*, *Ascaris lumbricoides*, and hookworms) were isolated from raw wastewater (83.3%) and treated wastewater (68.2%), soil (68.6%), and vegetables (44.2%) in the study area [137].

Agricultural soil is highly susceptible to pathogenic contaminations originating from irrigation water, animal faeces, sewage, and effluents [138]. Then apart from the naturally contained microbes in the soil, Klutse [139] reported that soils irrigated with wastewater have an increased level of pathogenic microbes such as *Staphylococcus* spp., Enterobacteriaceae, *Clostridium* spp., *Pseudomonas* spp., and *Bacillus* spp. However, it has also been pointed out that though irrigation water is more contaminated, the soil remained a major route for

contamination. This is a result of the direct access farmers have to soil such that some (93%) worked barefooted, while others (86%) have direct hand contact with contaminated soil, which leads to oral contamination during hand-to-mouth events [66].

6.1.2. The Entry of the Pathogen into the Soil and Produce via Manure. The recent increase in population in developing countries has resulted in an upsurge in food production to ensure food security. However, vegetable production especially is facing many constraints like land pressure and low soil fertility [140]. Therefore, farmers have now intensified crop (vegetable) cultivation by introducing mineral and organic fertilizers to their crops in order to satisfy the increased demand by consumers. For centuries, the farmer has utilized animal manure of which the majority (60%) comes from poultry with the rest (40%) from cattle, and this is practiced even frequently in present days [141]. However, the intense application of manure from cattle, poultry, and other animal sources put the soil, water, crops, and humans at risk of pathogen infection [142]. This is because several disease outbreaks have been linked to the consumption of vegetables that had been directly or indirectly contaminated with infected animal manure [143]. The isolation of E. coli and faecal coliforms from sampled vegetables in three studies indicates faecal pollution [2, 142, 144]. Furthermore, several studies [136-138, 145] have also investigated how the type of manure applied determines the extent of contamination. Some of these studies [136-138] revealed that the ability of a pathogen to survive was greater in vegetables grown with manure from poultry as opposed to manure from cattle, whereas another study [145] found an opposite result. Contaminated manure has been reported to affect the quality of soil, and this subsequently contaminates the vegetables as pathogens found in soils reflected in the vegetables [146]. Strongyloides stercoralis, Taenia, and Entamoeba spp. were observed in raw manure of animal origin and vegetables [147]. Then also, fifteen different species of parasites, including nematode (9) species were concurrently identified from the soil, irrigation water, and vegetables produced, and these microbes emanated from animals and humans. From the soil samples were isolated Geo-helminth eggs, including Trichuris trichiura, Strongyloides, Ascaris lumbricoides, Enterobius vermicularis, and hookworms [2]. This suggests contamination from human faeces as a result of practices like open defecation among farmers and the use of improperly composted manure. Similarly, the recovery of Toxocara spp. and Moniezia spp. from vegetables, vegetable salads, and soils implicates the use of animal faeces as sources of fertilizer [148]. When bovine faeces was used as fertilizer, E coli was isolated from 17 samples (including 8 samples of soil and 9 samples of vegetables) [148].

Interestingly, several pathogenic microbes tend to thrive in the manure for a long-time, and this is due to specific elements including physiochemical properties (pH of soil and manure, moisture content, compost treatment techniques, air circulation, soil type, and the degree of fertilizer (manure) application) [149, 150]. *E. coli O157: H7* and *Salmonella* have been shown to live longer in farmyard manure under anaerobic conditions as well as in moist clay soil with a lower temperature [146, 151]. However, several methods such as composting, dry heating of pellets, and alkaline stabilization, among others, are accessible for the treatment of manure before they are being applied. At a temperature of 55°C for 3 days, composting is usually sufficient to destroy bacteria, whereas pelleting is more favourable for the treatment of poultry manure [138].

6.1.3. Intruding Animals on Farms. Domestic or wild animals moving into farmlands can result in vegetable contamination before harvest [152]. This is because several outbreak investigations have been associated with freemoving animals intruding into farms or cross-contamination originating from faeces of a neighbouring animal farm. Unfortunately, a lot of these wild and untamed animals harbour several parasites causing zoonotic diseases of human importance [153]. Similarly, houseflies have been found to transfer E. coli O157: H7 in cattle [154] and spinach farms [155]. The contamination of tomatoes by Salmonella was attributed to the movement of some of these animals such as mice and opossums onto production sites. It has been indicated that some bacteria such as *Campylobacter* spp. and E. coli O157: H7 originating from these animals can spread in the agricultural environment [156]. In Ghana, the identification of Toxocara spp., Giardia lamblia, and Moniezia spp. in farm soil and salad vegetables originating from droppings of dogs and ruminants is indicative of freemoving animals into production sites [2].

#### 6.2. Off-Farming Routes

6.2.1. Impact of Washing Procedure and Hygienic Practices. Washing is one of the hygienic practices done to remove dirt, bacteria, and parasites on vegetable surfaces. Washing of vegetables is of utmost importance and therefore encouraged. The level of microbial and parasitic contamination usually depends on the quality of water used, together with the kind of washing procedure performed. However, some of the vegetable sellers in some study areas use the same water in washing all vegetables [157]. This will lead to the transfer of pathogens to uncontaminated ones if the water used is not safe and the same wash water is repeatedly used. Most vegetables washed before sales and displayed on tables had more geohelminths contamination than those that were not washed [158]. Conversely, another study reported more parasites in unwashed produce before selling than in washed produce [159]. However, contamination in unwashed vegetables in this study was facilitated by the environment in which they were displayed. Most of them were on the floor, and it is known that bacterial and viral contamination can take place on the floor together with flies that can act as a vector for several microorganisms like Toxoplasma gondii and Cryptosporidium parvum [159]. Additionally, the source

of contamination in washed vegetables was linked to the medium (well and river water) in which the samples were washed, wherein they were contaminated with several parasites [160]. In the removal of pathogenic parasites, Alsubaie et al. [161] attributed running water from taps to perform a similar role to normal saline; therefore, the use of running taps and proper draining of the wash water should also be encouraged instead.

Normally, the water used for the washing procedure is also utilized for salad preparation, and if the water is not clean, there can be contamination in the prepared salad. Assessment of water quality and vegetable salads from a restaurant in a study revealed a high level of coliform, faecal coliform, Vibrio spp., and Salmonella spp. in both the water and salad samples. Salmonella spp. and Vibrio spp. were resistant to Cephalexin, Amoxicillin, and Cephradine [162]. The occurrence of coliforms in salads could be associated with cooks not engaging in enhanced sanitation, and the use of contaminated water to cleanse equipment like cutting or slicing machines resulting in cross-contamination. Most cooks also during salad preparation use their uncovered hands, potentially transferring harmful pathogens into the food. Similarly, the predominant population of coliforms, Salmonella spp., Listeria monocytogenes, E. coli, and Bacillus cereus, were found concurrently in prepared salads, worker's hands, and cooking equipment in a study [163]. This indicates the probable transfer of microbes between preparation surroundings and ready-to-eat foods. However, coliforms identified in vegetable salads are not surprising because vegetable irrigation water, as well as manure utilized as fertilizers, have been reported to harbour coliforms and other enterobacteria [164].

Furthermore, improperly washed vegetables can result in the contamination of vegetable salads, and this has been noticed in several studies [156, 157]. Among these studies, Orozco et al. [156] noticed contamination variation between vegetables that were cleaned with pure water, germicide, and vinegar as well as the uncleaned ones. Contamination of nematodes larvae was discovered in 82.5% of the uncleaned group. Additionally, 32.5%, 12.5%, and 0.5% of vegetables cleaned with pure water, vinegar, and germicide, respectively, were also infested with nematodes. This showed that the rate of parasite recurrence was significantly higher in vegetables cleaned with pure water than in the germicide and vinegar groups. Washing with soap has also been indicated to have a lesser effect on the reduction of microbial and parasitic contamination [165]. In that, the majority (43%) of parasites and bacteria were identified in vegetables used with soap plus water, whereas those washed with pure water were lesser (40.6%). Some isolated parasites and bacteria include Giardia lamblia, Taenia spp., Enterobacter aerogenes, Trichocephalus spp., and Ascaris lumbricoides [165].

In a one-year study, intestinal parasites (*Entamoeba* histolytica/dispar, Ascaris lumbricoides, Toxocara spp., Hymenolepis nana, Dicrocoelium, Echinococcus, Fasciola, Giardia lamblia, Entamoeba coli, and Trichostrongylus) were identified from unwashed (63.7%), traditionally washed (49.1%), and standard washed vegetables (36.9%) [166]. Comparing similar works in other developing countries, it

Country	Salad ingredients	Microbes/parasites identified	Reference number
Africa			
Ghana	Cabbage, tomato, onion, and lettuce	Shigella, Escherichia coli O157:H7, Bacillus spp., and Salmonella spp.	[63]
Ghana	Lettuce, cabbage, onion, cucumber, and tomato	Escherichia coli O157:H7, Staphylococcus aureus, Klebsiella spp., and Bacillus spp.	[48]
Ghana	Raw mixed vegetable salads	Salmonella typhi, Shigella spp., S. faecalis, and Pseudomonas spp.	[11]
Ghana	Salad (leaves and fresh vegetables)	Bacillus spp., Klebsiella spp., Aeromonas spp., Escherichia coli O157:H7, and Citrobacter spp.	[53]
Ghana	Salad (leaves and fresh vegetables)	Salmonella spp. and Helminth eggs Giardia lamblia, Entamoeba coli, Entamoeba histolytica,	[74]
Ghana	Cucumber, carrot, onions, tomatoes, and cabbage	Cystoisospora belli, and Toxoplasma gondii, Trichuris trichiura, Ascaris lumbricoides, Enterobius vermicularis, Ancylostoma duodenale, Taenia spp., Moniezia spp., and Fasciola spp.	[108]
Ghana	Cabbage, lettuce, carrot, and green pepper	Giardia lamblia, Ascaris lumbricoides, Moniezia spp., Entamoeba coli, Toxocara spp., Taenia spp., Fasciola hepatica, Enterobius vermicularis, Trichuris trichiura, and Toxoplasma gondii	[2]
Nigeria	Vegetable salad	Listeria monocytogenes	[57]
Nigeria	Carrot, lettuce, cabbage, and cucumber	Mucor spp., A. fumigatus, Aspergillus niger, Neurospora crassa, Proteus vulgaris, Staphylococcus aureus, and Pseudomonas	[72]
Vigeria	Vegetable salads	Escherichia coli O157:H7, Staphylococcus aureus, Salmonella spp.	[84]
Vigeria	Ready-to-eat vegetable salads	Bacillus spp., Pseudomonas spp., E. coli, Proteus spp., Enterobacter spp., and Aeromonas hydrophila Aeromonas spp., Serratia species, Pseudomonas spp.,	[83]
Nigeria	Fresh vegetable salads	Corynebacterium, Klebsiella spp., Escherichia coli, Lactobacillus, Staphylococcus, Aspergillus spp., and Rhizopus	[47]
Vigeria	Vegetable salads	spp. Enterobacter spp. and Streptococcus spp.	[46]
Vigeria	Cabbage, lettuce, carrot, and cream	Serratia spp., Citrobacter spp., Proteus spp., Staphylococcus spp., and Bacillus spp.	[87]
Vigeria	Vegetable salads	Acinetobacter spp., Bacillus spp., Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus epidermis, Micrococcus luteus, Enterobacter aerogenes, Serratia marcescens, Klebsiella pneumoniae, Klebsiella oxytoca, and Citrobacter freundii	[61]
Ethiopia vory Coast	Vegetable salads Tomatoes, onion, cucumber, and lettuce	Salmonella spp. and Escherichia coli O157:H7 E coli, Enterobacteriaceae, and Salmonella	[50] [75]
Kenya	Kachumbari (dhania, onions, tomatoes, and chilli)	Salmonella spp. and Escherichia coli O157:H7	[64]
Kenya	Onion, lettuce, carrot, tomatoes, cabbage, chilli, coriander, and green capsicum	Escherichia coli O157:H7, Campylobacter spp., and Staphylococcus spp.	[65]
Egypt	Cucumber, carrot, onions, tomatoes, cabbage, and parsley	Shigella, Salmonella, and Staphylococcus aureus	[167]
Egypt	Arugula, coriander, parsley, celery dill, lettuce, cucumber, tomato, green pepper, and mint	Escherichia coli O157:H7 and Salmonella	[73]
A <i>sia</i> Pakistan	Lettuce, tomato, onion, cucumber, and carrot	Escherichia coli O157:H7	[69]
Pakistan	Vegetable salads	Escherichia coli O157:H7, Shigella spp., Citrobacter spp., Pantoea spp., Bacillus species, Klebsiella spp., Enterobacter	[86]
ran	Lettuce, coleslaw, cucumber, carrot, and tomato	spp., and Pseudomonas aeruginosa Cladosporium spp., Penicillium spp., Alternaria, Escherichia coli O157:H7, Salmonella spp., Geotrichum, Trityracium, and Aspergillus	[56]
ran	Vegetable salads	Helicobacter pylori	[60]

TABLE 3: Highlights on pathogens identified among various developing countries.

Country	Salad ingredients	Microbes/parasites identified	Reference number
Iran	Lettuce, cabbage, tomato, cucumber, and carrot	Escherichia coli O157:H7	[59]
Iran	Vegetable salads	Yeast, <i>Staphylococcus aureus, Escherichia coli O157:H7</i> , and parasitic eggs	[68]
Iran	Lettuce	Shigella spp.	[77]
Iran	Mixed fresh cut vegetable salads	Yeast, mould, <i>E coli O157:H7</i> , <i>Salmonella</i> , and <i>Staphylococcus aureus</i>	[52]
India	Ready-to-eat green vegetable salads	Listeria spp. and Yersinia spp.	[58]
Saudi Arabia	Tabbouleh, Fattoush, Hummus, Mutabbel, and Caesar	Escherichia coli O157:H7, Shigella spp., Salmonella spp., and E. aerogenes	[76]
Saudi Arabia	Tabbouleh, Fattoush, Hummus, Mutabbel, and Caesar	Pseudomonas spp., Bacillus cereus, Acinetobacter, Serratia marcescens. Klebsiella spp., Enterobacter spp., Kluyvera cryocrescens, Enterobacter spp., Pantoea spp., and Escherichia coli O157:H7	[168]
Bangladesh	Cucumber, tomato, and carrot	E coli, Staphylococcus spp., and Salmonella spp.	[67]
Latin Amer	ica and the Caribbean		
Mexico	Vegetable salad samples	Salmonella	[169]
Mexico	Lettuce, tomato, onion, cucumber, carrot, spinach, mushrooms, watercress, wheat sprouts, and radish	<i>E coli</i> , faecal coliform, and diarrhoeagenic <i>E coli</i> pathotypes	[170]
Brazil	Lettuce, carrot, cabbage, spinach, collard green, and watercress	Staphylococcus aureus and Salmonella	[171]
Brazil	Kale, cabbage, lettuce, onion, parsley, chicory, spinach, arugula, watercress, carrot, collard green, and escarole	Listeria monocytogenes and Salmonella	[172, 173]
Brazil	Cabbage, kale, lettuce, spinach, parsley, and collard green	Cronobacter spp.	[174]
Brazil	Lettuce	Balantidium coli, Giardia spp., Ascaris lumbricoides, Entamoeba spp., and Strongyloides stercoralis	[175]
Chile	Vegetable salads	Listeria monocytogenes	[176]

TABLE 3: Continued.

Italicized words indicates scientific names of microrganisms (Genus and Species).

was found that the contamination rates in the above research are higher than the findings from other countries (Table 3), like Libya [88], Egypt [90], and Nigeria [177]. In Kenya, however, parasitic contamination of unwashed vegetables increased significantly (75.9%) as compared to the reduced level of contamination observed in washed vegetables [178]. In conformity with this research [166], soil-transmitted helminth infestation in standard washed vegetables (1.2%) and in traditionally washed vegetables (3.2%) was also indicated by Nawas et al. [162]. In contrast, Fallah et al. [179] isolated intestinal parasites in unwashed (32.6%), traditionally washed (1.3%), and standard washed (0%) vegetables in Shahrekord, Iran. Despite the findings of these investigations, Salavati et al. [166] explained that standard washing procedures cannot utterly eliminate the parasite from the vegetables. It should be noted that the identification of parasites in standard washed vegetables is probably because disinfectant solutions are capable of completely killing worm eggs, cysts, and larvae, but the remaining dead agents in the corresponding vegetables are still detectable via light microscopy [166, 180].

6.2.2. Processing. Manipulation of vegetables after harvest is a major concern in the farm-to-fork chain. During processing, there are several ways through which pathogenic contamination can enter humans. Pathogenic microorganisms could be transferred from contaminated soil or produced in workers' hands. In other instances, workers harbouring infectious microbes could transfer them to produce [181]. Then also, harvesting knives can serve as contamination vectors during cross-contamination of microbes from infected soil to vegetables. For example, trimming and coring of lettuces after harvest can lead to plant contamination [182]. Moreover, the environment where these vegetables are handled including machinery for processing could also be an important route of vegetable contamination [183].

In an observational study, vegetables were displayed on unhygienic sacks, and unsold ones were stored in uncontrolled states in the market or backyard of homes, resulting in bacterial and parasitic contamination of these vegetables [184]. Six bacteria species were isolated in the previous study, with *Staphylococcus aureus* being the highest, while *Serratia marcescens* was the least. Furthermore, ten parasitic protozoans and helminths were discovered, with *Balantidium coli* being frequently detected and found in all samples together with *Entamoeba* spp. Interestingly, lettuce (25.4%) recorded the greatest level of infestation, with the lowest being green pepper (8.5%) [184]. 6.2.3. Storage and Distribution. Several risk factors are associated with the presence of enteric pathogens on vegetables during storage. Some of which include relative humidity, gaseous composition of the atmosphere, storage temperature, and the presence of nutrients, bacteria, or antimicrobial compounds [185, 186]. Hence, at the point of storage, an intervention to reduce the multiplication and activity of human pathogens on the products is by storing them at an appropriate refrigerated temperature. This is because depending on the temperature range, the shelf life and survival of microbes on the surface of vegetables can extend *Listeria monocytogenes*, for instance, multiply slowly at temperatures above 3.8°C [187].

At home, cross-contamination is expected to occur if domestic refrigerated vegetables are stored together with other foods that have been contaminated. In developing countries, consumers usually store vegetables in all-purpose refrigerators together with other foods that have varying temperature ranges due to financial constraints [188]. However, if they are not routinely cleaned, they may serve as a source of harmful organisms. Furthermore, the spread of bacteria and mould is also predicated on refrigeration units located in warehouses; therefore, routine disinfection of air filters and refrigeration systems is required [189]. If contamination takes place in the storage area, the whole facility should be cleaned and disinfected to reduce any potential pathogens [190].

6.2.4. Retail Food Services and Consumer Handling. The foodservice industry receives the majority (60%) of freshcut produce, while the remaining (40%) goes into the retail market. A significant cause of foodborne diseases has been attributed to contamination by food service workers during outbreak studies [191]. This is thought to be a result of the frequency with which workers have an unguarded hold on fresh produce during preparation and distribution in food service and retail facilities [163, 192]. Higher bacterial contamination was observed in takeout vegetable salads kept in packages than in those prepared and served at the restaurant, suggesting potential cross-contamination [163, 192]. Those studies recorded a high prevalence of Gram-negative and positive bacteria in salads, and this was in correlation with bacteria found on cooking utensils, cutting boards, and serving spoons [163, 192]. Furthermore, most vendors do not take precautions during vegetable salad preparation as consumers reported the occurrence of food poisoning after consumption [11]. Since these places do not utilize techniques designed to kill microorganisms, the occurrence of microbes in fresh produces is expected to be influenced by the hygienic conditions of food workers. Hence, retailers should have a defined procedure to examine and accept only fresh produce that attains the ideal standard.

Apart from the contamination originating from retailers in retail joints, consumers also play a major role in the contamination of vegetables. During sales, consumers deliberate on buying fresh vegetables by touching them, and this process could lead to cross-contamination if the buyer is contaminated as a result of poor hygienic practices [184]. Furthermore, the temperature of cars and the cooling period tend to proliferate pathogens when fresh vegetables are transported by consumers [193]. At home, undertaking food hygiene practices like hand-washing before handling fresh produce is important [188]. Kitchen sink, sponges, and dishrags are another substantial and familiar reserve for pathogenic microorganism [188]. Therefore, the number one point of call should be educating retailers on excellent food safety practices together with consumers on how to handle and process vegetable and vegetable salads in other to protect produce from contamination.

## 7. Food Safety Guidelines

7.1. Strategies of Minimizing Microbial and Parasitic Contamination. Whole vegetable and vegetable product contamination occurs at any point from planting to consumption. Unfortunately, there have been no potent antimicrobial treatments to efficiently eliminate pathogenic microbes and parasites [14]. However, the extent of contamination is usually linked to the environmental conditions under which the produce is grown and produced. Therefore, programs such as "Good Agricultural Practices (GAP) and an in-plant food safety program such as Hazard Analysis and Critical Control Point (HACCP)" at the preharvest level are major techniques to minimize threats to food safety [194].

Julien-Javaux et al. [181] and Gil et al. [190], from a food safety standpoint, suggested key factors that should influence the choice of cultivation sites at the preharvest level. Factors that should be considered include the closeness of the farm site to livestock farms or industrial facilities, prior land use, and landscape as a potential for water and soil runoff [195]. Another important factor is the establishment of sanitary facilities that are positioned away from the production environment coupled with producers' education on proper hygiene is essential to avoid cross-contamination of pathogens onto produce [190, 195]. Then again, the safety of irrigation water is necessary to prevent contamination of produce, soil, and working equipment and machinery that should be maintained in sanitized condition [190, 195]. However, plants cultivated close to the soil from which we get fresh vegetables consumed raw should not be irrigated with sewage or untreated water. This is because it could be a route through which Escherichia coli O157: H7 enters the fruit via the leaves [196]. The use of either manual or mechanical harvest during processing together with containers for collecting produce should be done in a way to reduce the risk of potential contamination coming from soil, workers, water, or machine surfaces [190, 195].

During the postharvest period, good handling practices during processing are critical. Processing tends to facilitate the growth of pathogens when there is an alteration of vegetable colour, nutrients, and water loss as a result of mechanical injury, and hence the need for proper treatment [172]. This indicates the importance of a well-controlled layout across every production point. At this stage, one must consider several interventions consisting of physical, chemical, and biological treatments that minimize contamination, prolong life span, and enhance product quality TABLE 4: Food safety legislation in some developing countries (Nigeria, South Africa, Ghana, Ethiopia, and Ivory Coast) [215].

Country	Legislation	Ministries, departments, and agencies involved in enforcement and monitoring (MDAs not in any particular order concerning the enforcement of food laws for each country. Mandates and functions overlap)
Nigeria	Counterfeit and Fake Drugs and Unwholesome Processed Food Decree (Act No. 25 of 1999)	Federal Ministry of Health
	National Agency for Food and Drug Administration and Control (Amendment) Decree 1999 (No. 19 of 1999)	Food and Drug Agency
	Food and Drugs (Amendment) Decree 1999 (No. 21 of 1999)	Food and Drug Administration and Control (NAFDAC)
	National Agency for Food and Drug Administration and Control Decree 1993 (No. 15 of 1993)	Standards Organization of Nigeria (SON)
	Public Health Ordinance Cap 164 of 1958	National Codex Committee
	The Standards Organisation of Nigeria Decree, No. 56 of 1971 The Animal Disease Control Decree, No. 10 of 1988	Standards Organization of Nigeria (SON)
	The Marketing of Breast Milk substitute Decree, No. 41 of 1990	Federal Ministry of Agriculture
	Regulations relating to the labelling of alcoholic beverages (No. 109 of 2005)	Ministry of Health
	Regulations governing general hygiene requirements for food premises and the transport of food of 12 July 2002	Ministry of Health
	Agreement between the European Community and the Republic of South Africa on trade in spirits - November 2002 Meat Safety Act, 2000	Dept. of Health and SABS
	Foodstuffs, cosmetics and Disinfectant, Act No. 54 of 1972	Dept. of Agriculture
South	Health Act No. 63 of 1977	Ministry of Health
Africa	Standards Act No. 29 of 1993 Food, Drugs and Disinfectant, Act No. 13 of 1929	SABS Dept. of Trade and Industry
	Trade Metrology Act No. 77 of 1973	Dept. of Trade and Industry
	Regulations on Food Establish & Export/Import	Customs and Excise Division
	The International Health Regulations Act, 1974 (Act 28 of 1974)	Ministry of Health
	The Medicines and Related Substances Act, 1965 (Act 101 of 1965) Wine and Spirits Control Act Regulations governing general hygiene requirements for food premises and the transport of food (G. N. No. R.918 of 1999)	Ministry of Health
	Food and Drugs Law (P.N.D.C.L. 305B, 1992)	Food and Drugs Board
	Standards Decree (N.R.C.D. 173, 1973)	Ghana Standards Board
Ghana	General Labelling Rules, 1992 (L.I. No. 1541, 1992)	Food and Drugs Board
	Ghana Standards Board Certification Mark Rule, LI 662,1970	Ghana Standards Board
	Pest and Plant Disease Act 307, 1965 (L.I. No. 1541, 1992) Pesticides Act 528, 1997	Plant Protection and Regulatory Services Ministries of Health, Food and Agriculture and Trade
Ethiopia	Quality and Standards Authority of Ethiopia Establishment Proclamation (No. 102/1998) Emergency Food Security Reserve Administration Establishment Council of Ministers Regulations (No. 67/2000)	Emergency food security reserve administration
Ivory Coast	Decree No. 86–454 establishing the power of the government to the municipalities and to the city of Abidjan on veterinary public hygiene measures	Ministries of Finance, Rural Development

as well as its safety during processing vegetables into vegetable salads [197, 198]. Physical decontamination techniques involving irradiation, ultraviolet (UV), and high pressure have been used to deactivate microbes and parasites. Studies have revealed UV's efficacy to destroy and/or slow down the development of microorganisms, and increase shelf life, quality, and nutritional benefit of food products [199]. The application of biological treatments such as bacteriocins, bacteriophages, or protective cultures has also been reported [198]. The use of bacteriophages in recent years has gained ground to suppress microbes present in carrots and tomatoes [200, 201]. High doses of chlorine, chlorine-based compounds, ozone, electrolyzed water, organic acids, modified atmosphere packaging, refrigeration, and atmospheric cold plasma discharges or plasma-activated water have been employed in intervention in recent times [202, 203]. Washing is an important process that removes dirt and soil, reduces microbial load, and prevents crosscontamination to some extent; however, the addition of sanitizers such as chlorine has facilitated the action [204]. Chlorine is the most commonly used sanitizer in washing procedures due to its inexpensiveness and effectiveness against several pathogens. Notwithstanding, pathogen sensitivity varies; *Listeria monocytogenes* have higher resistance against chlorine than *E. coli O157: H7* and *Salmonella* spp. [205]. It has also been reported that it is detrimental to human health; hence, other alternatives such as chlorine dioxide, electrolyzed water, hydroxide peroxide, and ozone have been employed [70, 196]. The efficacy of ozone to eliminate microbes has been reported to be higher than treatments that make use of chlorine and organic acid [206]. Additionally, it helps to improve the sensory qualities of produce such as lettuce [206]. Increased ozone concentration in the atmosphere is harmful to human health; therefore, more research is needed regarding the efficient use of ozone treatment [207].

7.2. Biochemical and Molecular Methods for Isolating, Identifying, and Characterizing Pathogens. Applying robust techniques to identify, count, and characterize microorganisms is a major step toward understanding the risk associated with microbial and parasitic contamination as well as further mitigating measures to ensure food safety. Diagnostic techniques employed in molecular biology and microbiology such as ELISA, magnetic immunity isolation (IMS), polymerase chain reaction (PCR), microarrays, and biochip technologies have been extensively proven to be reliable in the said action [208]. PCR analysis including conventional, real-time, and more digital droplet PCR is used to detect microbial load and harmful pathogens in vegetables including Listeria monocytogenes, Salmonella enterica, and pathogenic Escherichia coli [209]. Other biochemical techniques like liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and Raman spectroscopy have been utilized for bacterial, virus, pesticide, and pathogen detection, quality, and freshness assessment in vegetables and other food products to ensure their safety [210, 211].

Additionally, technological advancements in recent years have paved for the emergence of newer isolation detection techniques such as digital droplet PCR (ddPCR) [212], whole-genome sequencing (WGS) [213], and nextgeneration sequencing (NGS) [214]. These technologies make it possible to detect pathogens at a lower rate in vegetables, and they are more precise. Harnessing healthy vegetable consumption is a necessity and can only be attained when these technologies are integrated into the various production and inspection chain of vegetable production chain.

7.3. Food Safety Legislations. Developing countries are now working harder to develop working systems to achieve good food safety standards; these countries include South Africa, Nigeria, and Ghana. The table below shows the good safety guidelines adopted in these countries. Specifically, Table 4 presents food safety legislation in Nigeria, South Africa, and Ghana.

# 8. Conclusion

Several studies have pointed out the increased production and consumption of vegetables in today's world. This can be

partially attributed to changes in increased dietary habits leading, to increased patronage of foods sold outside homes of which vegetable and vegetable products form a greater part. Microbes are found everywhere, but vegetables have demonstrated their higher susceptibility to being contaminated with these microbes. As such, almost any readyto-eat vegetable and salads have been infested with microbes originating from the field of cultivation, produce processing, handling, or storage. This has accelerated the outbreak of several foodborne diseases. Since there is an increased need for healthy and quality products, it is essential and possible to minimize pathogens in vegetable and vegetable salads by utilizing various methods. At the preharvest level, the first step is to educate farmers on the threat posed by using contaminated water, fertilizers, human faeces, and untreated fertilizers. Therefore, treatment of sources of irrigation water and organic fertilizers should be done to reduce microbe contamination at the highest level. Then also individuals involved in the production continuum from cultivation to consumption comprising farmers, packers, and carriers should be trained on hygienic customs for contamination to reduce. During the processing and handling of vegetables and vegetable salads at the postharvest stage, issues regarding workers' hygiene and health, proper washing and disinfection procedures (making use of vinegar and salt but avoiding the utilization of one cycle of water to prevent cross-contamination), and equipment (use of smooth contact surfaces) should be addressed. This is because some retailers and consumers in developing countries like Ghana do not wash vegetables with disinfectants. Hence, these operations should be undertaken in a way that will guarantee the safety of products for consumption. Governmental stakeholders should have detectable limits always associated with pathogens' infectious doses, particularly in developing countries. To this end, various stakeholders should put in place various systems to survey when, where, how, and what preventive measures to take when there is contamination to address the issue of when and where it is essential to give attention to the "five major risks associated with vegetable production proposed by the European Commission. This comprises environmental and wildlife factors, irrigation water, usage of fertilizers and pesticides, hygiene of workers, equipment, and contact levels" [216].

## **Data Availability**

All data used for the study have been included in the article.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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