Journal of Food Research; Vol. 12, No. 2; 2023
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Foodborne Pathogens in Leafy Vegetables Grown and Consumed Locally in Yaounde, Cameroon: A Public Health Concern

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Received: January 27, 2023 Accepted: February 28, 2023 Online Published: March 3, 2023

doi:10.5539/jfr.v12n2p11 URL: https://doi.org/10.5539/jfr.v12n2p11

Abstract

This study sought to understand the health risks of foodborne pathogens in fresh leafy vegetables that are grown and consumed locally in Yaounde, Cameroon. Through a survey, 200 respondents were recruited to relate possible food-related illnesses to leafy vegetable consumption. Additionally, a total of 168 vegetable samples consisting of six leafy vegetables and 15 irrigated water samples from five water sources were collected from farms and local markets for microbiological analysis. Using a high-fidelity DNA polymerase, five potential bacterial pathogens, namely, Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* were also examined. The mean counts of total viable count and total coliforms followed decreasing trends from vegetables obtained on the farms to the local markets, and these ranged from 4.98-8.74 log cfu/g and 1.77-7.42 log cfu/g respectively. All pathogens detected were of significant concern to public health showing high occurrence in some vegetables: STEC (20%) and *Yersinia enterolitica* (13%) in cabbage, *Campylobacter* spp. (21%) in lettuce, *Listeria monocytogenes* (15%) in African nightshade, and *Salmonella* spp. (15%) in amaranth. Importantly, 42% of respondents highlighted that they frequently got sick from eating leafy vegetables from the study area. These microbiological and qualitative results along with certain vegetable farming and vending practices (such as the use of untreated sewage water for crop irrigation, the sales of physically dirty, muddy, and unpackaged vegetables) indicated that foodborne diseases could be occurring among leafy vegetable-consuming populations in Cameroon.

Keywords: leafy vegetables, foodborne pathogens, local practices, foodborne diseases, public health risks

1. Introduction

The World Health Organisation recommends the consumption of at least 400g of fruits and vegetables per day (Dias, 2011; FAO, 2020a; Varadaraju & Patel, 2019). Specifically, leafy vegetables are recommended at atleast 116g per day (Dhaliwal, 2017). Leafy vegetables play an important role in a healthy diet, providing essential vitamins, minerals, and phytonutrients (CDC, 2022; Eni et al., 2010; Taban & Halkman, 2011; Yafetto et al., 2019). They maintain healthy body tissues and prevent certain chronic conditions such as heart diseases, some cancers, diabetes, and obesity (Asongwe et al., 2014; Dias, 2019; Eni et al., 2010; Maseko et al., 2018; Varadaraju & Patel, 2019). To acquire these nutritional and health benefits, leafy vegetables should be consumed either raw as in ready-to-eat salads and/or after minimal processing.

Although millions of people around the world have safely consumed leafy vegetables, foodborne illnesses caused by microbial pathogens have been increasingly reported in their consumption in recent years (Buyukunal et al., 2015; CDC, 2021, 2022; Johnson, 2019; Taban & Halkman, 2011). For example, between 1998 and 2016, the consumption of contaminated leafy vegetables accounted for 20 to 40% of foodborne diseases in the United States (Johnson, 2019). Each year within this period, single and multistate foodborne disease outbreaks ranged from 10 to 20 and sickened between 100 and 1100 people (CDC, 2021; Johnson, 2019). The vulnerable, including children, the elderly, pregnant women, and immunocompromised individuals, are the most at risk and often develop complications that sometimes lead to death (CDC, 2021; El-Sayed et al., 2013; Taban & Halkman, 2011).

In Cameroon, leafy vegetables are currently the third product of the gardening sector, that is, after onions and tomatoes (Bayi et al., 2020). Market gardening employs over 60% of Cameroon's rural and peri-urban population and an estimated 2,832,000 tons of vegetables are produced annually (Abang et al., 2013; Asongwe et al., 2014; FAO, 2020b; Ntangmo et al., 2012). Several research (Akoachere et al., 2018; Tsama et al., 2015) have demonstrated that leafy vegetables cultivated and consumed in Cameroon could be highly infected with pathogenic microorganisms. Notably, Tsama et al. (2015) enumerated high concentrations of faecal coliforms (6092 cfu/100 ml), faecal streptococci (3390 cfu/100 ml), and helminth eggs (43 eggs/100 g) in irrigated water and lettuce (*Lactuca sativa L.*), cultivated along the Avo'o river in Nomayos, a peri-urban area in Yaounde, Cameroon. However, there is inadequate information on the microbial contamination of abundantly available and readily consumed leafy vegetables in Cameroon (Akoachere et al., 2018), and the extent to which farmers, vendors and consumers of leafy vegetables are expose to foodborne pathogens during vegetable farming, handling and consumption is unknown. Furthermore, data on the most commonly foodborne diseases-causing pathogens, that is, Shiga-toxin producing *Escherichia coli* (STEC), *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes, Yersinia* spp. among others are inexistent. This information gap has hindered food safety measures and fruitful interventions aimed at improving public health in the country.

To understand the health risks of foodborne pathogens in fresh leafy vegetable handling and consumption, and thus guide food safety policies in Cameroon, we examined for the presence of selected bacteria foodborne pathogens in the following.

1) Six types of leafy vegetables grown on five farms (Note 1) and sold in six local markets (Note 2) in Yaounde (Cameroon), and

2) Five water sources (Note 3) used in the irrigation of leafy vegetables cultivated in the five farms mentioned above.

A survey-based approach was also used to provide some potential information on foodborne diseases that could be occurring among leafy vegetable-consuming populations in Cameroon.

2. Materials and Method

To achieve the purpose of this research, we used two approaches: 1) qualitative data collection to obtain background information on potential foodborne diseases in leafy vegetable farming, handling, and consumption, and 2) microbiological analysis to enumerate total viable counts (TVC) and total coliforms (TC) and detect selected foodborne pathogens in sampled selected leafy vegetables and irrigated water.

2.1 Study Area

The study was carried out between September 2021 and February 2022 in Yaounde, the main town of the centre region in Cameroon. Covering a total area of 256 km^2, Yaounde is situated at an altitude of 750 m between latitude 3°47′ to 3°56′N and longitude 11°10′ to 11°45′E. Its annual rainfall pattern is bimodal, with an average of 1600 mm. As in many parts of the country, Yaounde is made up of a humid forest (Abang et al., 2013). It has an average annual temperature of 24 ℃, and this has favoured an all-year abundant vegetable production of about 134,808 tons per year (Abang et al., 2013; Kamda et al., 2021; Kamga et al., 2013). Also, it has an estimated population of 1,817,524 people in high demand for vegetables in the region (BUCREP, 2005; Kamda et al., 2021; Kamga et al., 2013).

2.2 Qualitative Data Collection

To identify locations with significant leafy vegetable production and sales activities, we initially visited 20 local markets and 15 vegetable farms mainly located in the valleys of the study area. Our choice of these locations was guided by field observations and previous research (Abang et al., 2013; Kamda et al., 2021; Kamga et al., 2013), which demonstrated the significant participation of this population in vegetable farming, vending and consumption activities. Also, the identified vegetable farms were observed to use diverse sources of irrigated water, such as, rivers, streams, marshes, boreholes, and groundwater.

During these field visits, ten (10) closed questionnaires were completed by randomly selected two hundred (200) inhabitants who weekly visited these local markets and farms to purchase food items for their families. Respondents were required to highlight their 1) frequency of leafy vegetable consumption, 2) perceptions of possible microbial contamination in leafy vegetable farming, handling, and vending practices, and to 3) highlight any occurrence of food-related illnesses, either experienced individually or as a family, that could have led them to reflect on the food safety of leafy vegetable farming to consumption practices. Participation was voluntary, and confidentiality was maintained through anonymous responses.

2.3 Microbiological Analysis

2.3.1 Sampling of Leafy Vegetables and Irrigated Water

A total of 168 leafy vegetable samples (28 samples per six different types of leafy green vegetable, namely, African nightshade or huckleberry (*Solanum scabrum Miller*), amaranth (*Amaranthus* spp.), celery (*Apium graveolens*), cabbage (*Brassica oleracea var. capitata*), leaves of jute mallow (*Corchorus olitorius L.*) or simply jute leaves, and lettuce (*Lactuva sativa*)) were obtained aseptically in the early hours of 9 am, each week from vegetable farms and local markets. According to Abang et al. (2013), these leafy vegetables are among the 10 most important crops of the vegetable breeding and seed system of The World Vegetable Center in Cameroon (AVRDC), and are significantly cultivated in the study area especially during the dry seasons. About one kilogram of each type of vegetable was purchased from selected farmers and market vendors on the farms and local markets respectively.

For irrigated water, one litre each of separate 15 water samples (three per vegetable farm including five water sources) were collected simultaneously with leafy vegetables from the farms on three different sampling dates.

Each labelled sample was placed in a sterile polythene bag and transported to the laboratory in ice packs at temperatures between 4-6^{0}C. All samples were analysed within an hour.

2.3.2 Sample Preparation

Samples for microbiological analyses included irrigated water and leafy vegetable wastewater. Leafy vegetable wastewater was obtained as described by Akoachere et al. (2018). Here, 25g of each leafy vegetable was completely immersed in 225 ml of sterile distilled water for 15 min. It was vortexed at 8000 g for 2 min, and the wastewater was then collected and stored at 4^{0}C. Subsequent experiments were carried out within one hour in a biosafety cabinet, and each sample was briefly vortexed prior to each microbiological analysis.

2.3.3 Enumeration of Total Viable Count and Total Coliforms

Using aseptic techniques, 1ml of each prepared vegetable wastewater and irrigated water sample was transferred into 9 ml distilled water and serial dilutions of up to 10^{-6} were prepared (Eni et al., 2010). 50 µl of each dilution was streaked on Nutrient Agar no. 2 (Oxoid, UK), for total viable counts (TVC), and on Violet Red Bile Agar (Oxoid, UK) (El-Sayed et al., 2013), for total coliform (TC). The plates were incubated at 37^{0}C for 20 h, and 30-250 colonies were visually counted using a 6X LED illuminated linen tester (Magnifiers, NZ). The colony counts were then converted to $\log_{10}$ cfu/g.

2.3.4 PCR Detection of Selected Foodborne Pathogens

DNA was extracted using the PowerWater$^@$ DNA isolation kit (Qiagen Company, USA), as detailed in the manufacturer's guidelines. Subsequently, DNA extracts were examined for Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica*, using previously described PCR primers and cycling conditions (Tanyitiku et al., 2022). In brief, each reaction mixture was prepared to a total volume of 20µl and consisted of 2µl of a 1 in 100 diluted DNA extract, 6µl of distilled water, 1µl each of the forward and reverse primers (100μM prepared working solution), and 10µl of Quantabio repliQa Hifi toughmix. Specifically, Quantabio repliQa Hifi toughmix consisted of 2x reaction buffer containing optimized concentrations of $MgCl_2$, dNTP's and proprietarily formulated HiFi polymerase, hot start antibodies and toughmix chemistry (repliQa Hifi toughmix: Quantabio, MA, USA). As shown in Figure 1, previously validated in-house authenticated reference strains of *Escherichia coli* NZRM 4396 (0178:H7, stx1 positive), *E. coli* NZRM 4397 (0171:H2, stx2 positive), *Listeria monocytogenes* NZRM 44, *Campylobacter jejuni* NZRM 2397, *Salmonella Enterica* serovar Menston NZRM 383 and *Yersinia enterocolitica* NZRM 2603 were used to evaluate the different cycling protocols. The 16S rRNA gene served as the positive control while *Pseudomonas marincola* LU P2 served as a negative control for all experiments and was included in each run. All experiments were carried out using a 96-well GenePro thermocycler (BIOER technology, England). The PCR products were detected by electrophoresis with 0.8% agarose, ran for 40 min at 100V and stained with SYBR Safe (Invitrogen, MA, USA). Electrophoresed gels were visualised using a UV-fluorescence Bio-Rad imaging system (Bio-Rad laboratories, USA).

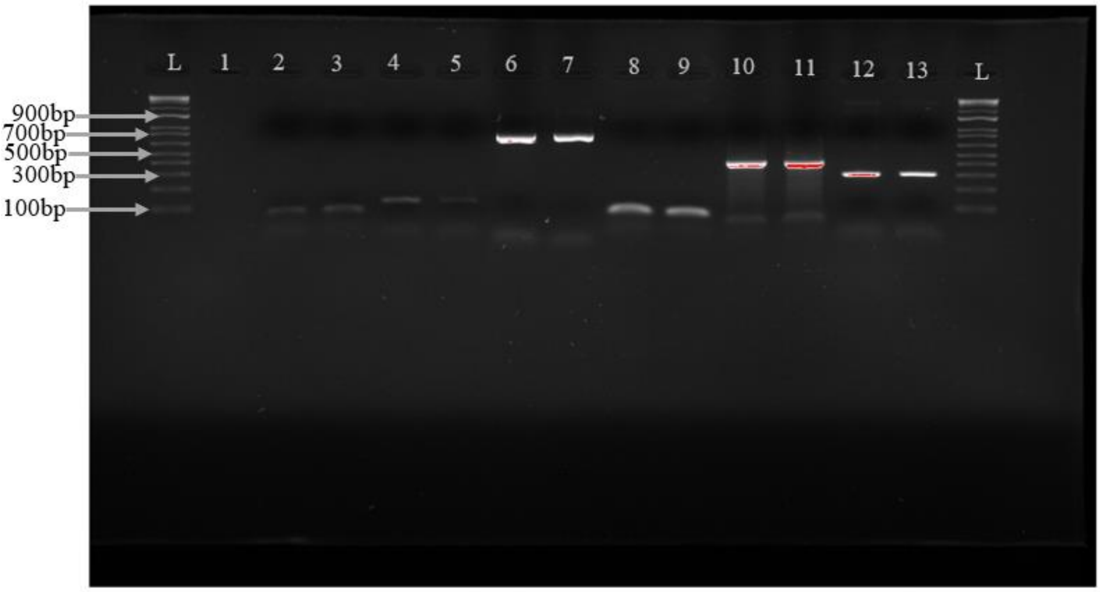

Figure 1. Specific bands at optimized PCR conditions. Lane L: 1kb plus DNA, ladder 1: *Pseudomonas marincola* isolate (*Salmonella* spp. assay, negative control for all assays), lane 2/3: *E. coli* Stx1, lane 4/5: *E. coli* Stx2 gene; lane 6/7: *Campylobacter jejuni*; lane 8/9: *Listeria monocytogenes*; lane 10/11: *Salmonella Enterica* serovar *Menston*; lane 12/13: *Yersinia enterocolitica*

2.3.5 Statistical Analysis

All experiments were conducted in three replicates. Statistical Package for Social Sciences (SPSS, Version 26 SPSS Inc., Chicago, USA) was used to analyse the results of this research. The mean values obtained from plate counting were analysed using one-way Analysis of variance (ANOVA) and expressed as mean ± standard deviation. Significance was tested at the 5% probability level. Duncan's multiple-range test was used to determine any statistically significant difference ($p< 0.05$) among the means. For each PCR assay, the presence of an amplicon of the appropriate size was recorded as a positive result to detect *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica.* For Shiga-toxin producing *Escherichia coli* (STEC), a positive result required the detection of both Stx1 and Stx2 genes.

3. Results and Discussion

From the background information presented in Table 1, more than half of the respondents (52%) consumed a vegetable meal at least twice a week. Indeed, this frequency of leafy vegetable consumption is essential in improving the health and wellbeing of the population.

However, as presented in Figure 2 (a, b, c, d, and e), the findings of the survey showed that vegetable farmers and consumers could be routinely exposed to pathogenic microorganisms. Over half of the respondents (59%) agreed that the local methods used in farming and vending could introduce foodborne pathogens to leafy vegetables (Figure 2a). Specifically, a majority (78%) accepted that irrigated water sources contain human faeces and could be the main cause of pathogenic contamination of leafy vegetables (Figure 2b). When describing the state of vegetables sold in various farms and local markets, 56% of the respondents highlighted that leafy vegetables were usually presented in physically dirty and muddy conditions (Figure 2c). Typical in the study area, climbing leafy vegetables (such as *Telfairia occidentalis*) were observed to be grown near domestic gates or fences, while others (African nightshade, celery, lettuce, cabbage) were grown on land bounded by rivers, streams, marshes, lakes, and irrigated by these water sources. These leafy vegetable farming practices are ancestral, and the scarcity of water for irrigation have constraint many farmers to these irrigated water sources. Regrettably, in such farming conditions, vegetables are frequently overflooded (with mud and domestic waste) during the raining seasons (March to October) (Akoachere et al., 2018; Ntangmo et al., 2012) or directly exposed to dust and/or domestic waste during the dry seasons (mid-October to March). Ntangmo et al. (2012) further reported that several pit latrines and septic tanks are designed in such a way that their contents are emptied directly into the above-mentioned rivers and streams. It should be noted that excreta and wastewater generally contain high concentrations of excreted foodborne pathogens (WHO, 1989), especially in countries such as

Cameroon where diarrhoeal diseases and intestinal parasites are particularly relevant (Kuitcha et al., 2010; Kuitcha et al., 2008; WHO, 1989). Microbiological contamination of vegetables can occur through contact with soil, dust, water or by penetrating punctures or open cuts in vegetable tissues (Lehto et al., 2011; Yafetto et al., 2019). The absence of wastewater treatment plants for the reuse of such nutrient-rich water sources for crop irrigation clearly indicated that leafy vegetable farming and handling in this research setting could be an unsafe practice which directly exposes vegetable farmers and consumers to foodborne pathogens.

Furthermore, most of the respondents (81%) were limited to certain farms and local markets in their purchase of leafy vegetables for two main reasons: 1) the proximity of vegetable farms or markets to their homes and 2) reduced costs of leafy vegetables at the time of purchase. With this, consumers often paid little or no attention to the food safety of the vegetables displayed for sales. Among this research respondents, 74% of them highlighted that they often got sick from eating these leafy vegetables (Figure 2d). Food-related illnesses such as diarrhoea, vomiting and fever were found to occur among 29 to 42% of the sampled population (Figure 1e), and mainly affected families of two to four persons (as highlighted by 49% of the respondents in Table 1) or a single person in a household (28% in Figure 1e). Similarly to these research findings, Shrestha and Rai (2014) stated that 75% of the Kathmandu population (Nepal) got sick from eating vegetables that were occasionally found to contain dirt, mud and plant diseases. In the same study area of Yaounde, Kuitcha et al. (2008) reported that 24% of households suffered enormously from food-related diarrhoeal diseases. According to the Centers for Disease Control and Prevention (CDC), a foodborne disease outbreak occurs when two or more people get the same illness from the same contaminated food or drink (Johnson, 2019). These findings thus indicated that foodborne disease outbreaks, still unidentified, could be occurring among the leafy vegetable-consuming population in Yaounde, Cameroon.

Table 1. Background of the respondents highlighting vegetable procurement and frequency of consumption

Background questions	**Number of responses from 200 respondents**
Age (years)	Less than 20 (11/5.5%), 20-30 (64/32%), 30-40 (86/43%), greater than 40 (39/19.5%)
Level of education	No formal education (0), Primary (0), Secondary (7/3.5%), University degree (193/96.5%)
Household size	less than 2 (34/17%), 2-4 (98/49%), 5-8 (49/24.5%), greater than 8 (19/9.5%)
Vegetable procurement	at the market (27/13.5%), farm (11/5.5%), both market and farm (162/81%)
Consumption frequency	Once a week (38/19%), 2-3 times a week (104/52%), greater than 4 times a week (55/27.5%), Not at all (3/1.5%)

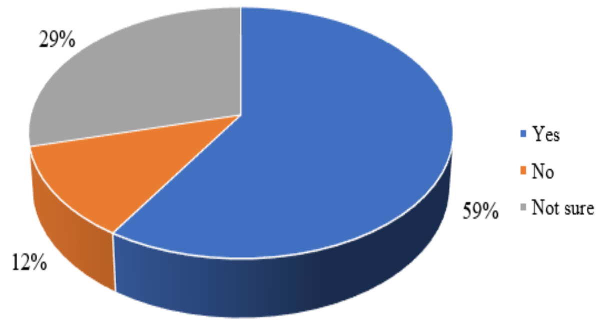

Figure 2a. Microbial safety involving leafy vegetable farming and vending practices

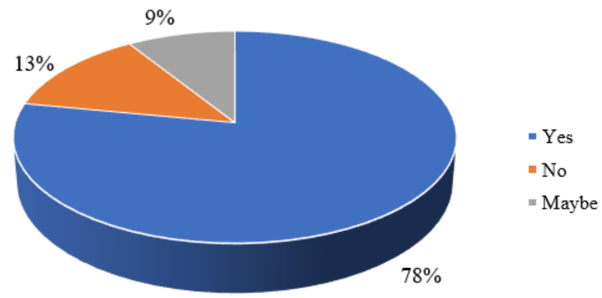

Figure 2b. Occurrence of human faeces in irrigated water as highlighted by research participants

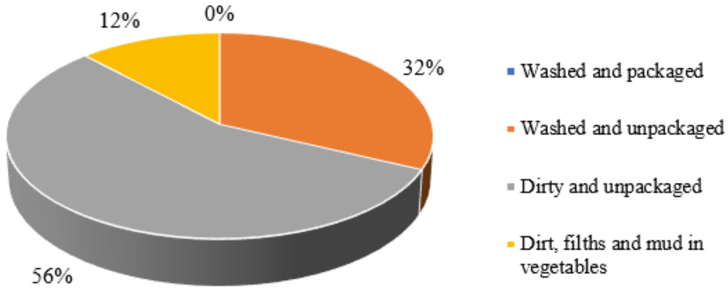

Figure 2c. Physical appearance of leafy vegetables sold in various farms and local markets

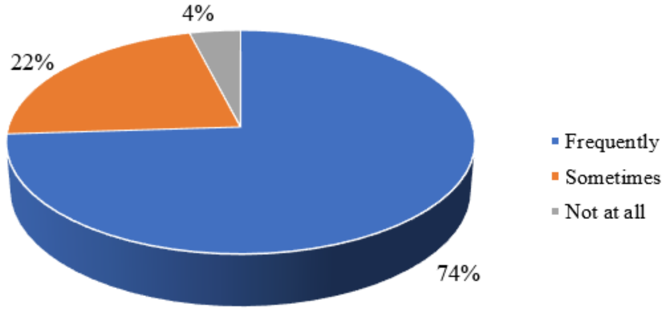

Figure 2d. Respondents getting sick from eating leafy vegetables

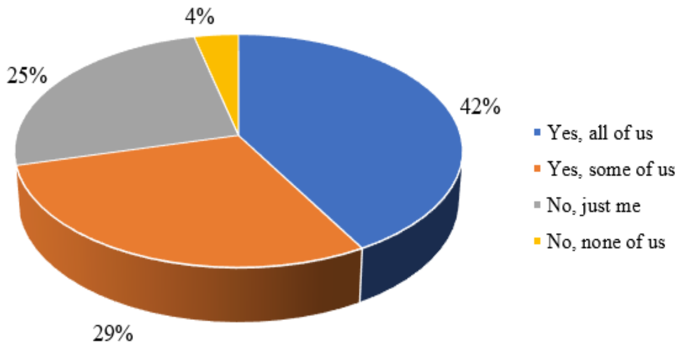

Figure 2e. Evidence of households getting sick after a vegetable meal

Figure 2 (a-e). Responses of the respondents showing possible foodborne pathogenic contamination and diseases in leafy vegetable farming to consumption activities

The microbial counts (log cfu/g) of six types of leafy vegetables collected from five farms and six local markets are presented in Table 2. Statistical analysis showed that there was significant difference ($p<0.05$) in mean TVC and TC between the same types of leafy vegetable obtained from various farms and local markets. The mean TVC and TC for market vegetables were significantly lower ($p<0.05$) than farmed leafy vegetables. It should be noted that freshly cut leafy vegetable samples obtained from the local markets were wet (with some containing drops of water) due to market vendors' continuous sprinkling of water on the leafy vegetables during sales. This research observation agrees with Figure 2c, where 32% of the respondents highlighted that leafy vegetables were sold washed and unpackaged at the local markets. Even though the continuous sprinkling of leafy vegetables by market vendors was to maintain its freshness, it could have contributed to this significant reduction in the microbial counts of market leafy vegetable wastewater samples. With this reduction in microbial counts following the vendor's continuous watering, further research is needed to establish that a proper washing of leafy vegetables from the study area could effectively eliminate the microorganisms enumerated in this research.

The mean TVC was high at 7.27 log cfu/g (market vegetables) and 7.67 log cfu/g (farmed vegetables), with a range of 4.98 to 8.74 log cfu/g. The mean TC was 4.72 log cfu/g (market vegetables) and 6.07 log cfu/g (farmed vegetables), with a range of 1.77 to 7.42 log cfu/g. While the highest TVC (8.74 log cfu/g) was observed in farmed cabbage, the highest TC (7.42 log cfu/g) was detected in farmed lettuce. Market celery had the lowest TVC (4.98 log cfu/g) and total coliforms (1.77 log cfu/g). The HACCP-TQM (Total Quality Management) Technical Guidelines (2000) laid down the microbial quality of raw foods: food containing less than 10^4 cfu/g is rated as 'good', 10^4-$5x10^6$ cfu/g as 'average', $5x10^6$–$5x10^7$ cfu/g as 'poor' and food containing more than $5x10^7$ cfu/g is 'spoil' (Anonymous, 2000; Lehto et al., 2011). The high microbial counts in this research thus indicated that all vegetable samples were microbiologically unsafe for handling by farmers and then for consumption by consumers. Similarly, a study in Singapore carried out by Seow et al. (2012) reported unacceptable high bacterial counts ranging from 5.8-7.3 log cfu/g in selected salad vegetables consumed as ready-to-eat foods. Likewise, Nwankwo et al. (2015) reported total viable counts ranging from $2.35x10^5$ to $9.50x10^5$ cfu/ml with the highest incidence in cabbage (9.50×10^5 cfu/ml) and other salad vegetables sold by vendors at the Umuahia main market, Nigeria. Specifically in Cameroon, Akoachere et al., (2018) reported higher mean total aerobic counts (9.5×10^6 cfu/g) and total coliforms (1171.6±117.16 cfu/g) in salad vegetables, including lettuce sold in various markets in Fako division, located southwest of Cameroon.

Furthermore, the microbial loads in the leafy vegetable samples were similar with those isolated in the different sources of irrigated water (Table 3). Its mean TVC ranged from 4.55 log cfu/g (borehole samples) to 9.74 log cfu/g (river samples) while its mean TC ranged from 2.13 log cfu/g (borehole samples) to 9.52 log cfu/g (river samples). The presence of high concentration of coliforms in the samples indicated the unhygienic status of the water used for vegetable irrigation, and coliforms strongly indicated fecal contamination, thereby, making the water sources a credible source of contamination of leafy vegetables cultivated in the study area. Kuitcha et al. (2010) reported similar results of faecal coliforms (34053±94225.5 cfu/100ml) and faecal streptococci (15107.6±50515cfu/100 ml) in water points: Mbankolo, Etoa Meki, Ngousso, Nkol-Eton, Rue Manguier, Etoudi, and Tongoloa, across Yaounde (Cameroon). In the same study, the level of bacterial load pollution was higher in rivers (127866.67±155254.57cfu/100ml) than in wells or boreholes (2966.10±3786.15 cfu/100ml) and springs (340.67±557.98 cfu/100ml) (Kuitcha et al., 2010). Similar findings in Ghana (Adetunde et al., 2015; Yafetto et al., 2019), Nigeria (Eni et al., 2010; Nwankwo et al., 2015; Oyinlola et al., 2017) and Rwanda (Ssemanda et al., 2018) have also demonstrated the potential of irrigated water to contaminate locally grown salad vegetables.

Table 2. Total microbial counts of leafy vegetables

Sampling location	Leafy vegetables	Total Viable Count (log cfu/g)	Total coliforms (log cfu/g)
Farms	African nightshade	7.44 ± 0.65^a	6.77 ± 0.01^a
	Amaranth	6.67 ± 0.03^b	5.45 ± 0.43^b
	Cabbage	8.74 ± 0.02^c	5.64 ± 0.08^b
	Jute leaves	8.33 ± 1.04^c	5.66 ± 0.30^b
	Celery	7.45 ± 0.08^a	5.49 ± 0.57^b
	Lettuce	7.32 ± 0.04^a	7.42 ± 0.09^c
	Mean	**7.67±0.31**	**6.07±0.25**
Local markets	African nightshade	8.61 ± 0.64^c	7.28 ± 0.20^c
	Amaranth	6.84 ± 1.21^b	4.55 ± 0.02^d
	Cabbage	8.60 ± 0.28^c	4.95 ± 0.82^d
	Jute leaves	5.97 ± 0.08^d	3.89 ± 0.97^e
	Celery	4.98 ± 0.88^e	1.77 ± 0.98^f
	Lettuce	8.64 ± 0.75^c	5.85 ± 0.01^b
	Mean	**7.27±0.64**	**4.72±0.50**

Note. cfu: colony forming units. Values are mean ± standard deviation of three replicates. Different superscripts (a, b, c) within the same column are significantly different ($p < 0.05$)

Table 3. Total microbial counts in irrigated water collected in five farms

Source of irrigated water	Total Viable Count (log cfu/g)	Total coliforms (log cfu/g)
Rivers	9.74 ±1.42[a]	9.52 ±0.08[a]
Streams	8.54 ±0.03[b]	7.67 ±0.12[b]
Marshes	9.23 ±0.88[a]	6.42 ±0.33[c]
Groundwater	6.25 ±1.07[c]	6.73 ±0.02[c]
Boreholes	4.55 ±0.06[d]	2.13 ±0.44[d]
Mean	**7.67 ±0.70**	**6.36 ±0.20**

Note. cfu - colony forming units. Values are mean ± standard deviation of three replicates. Different superscripts (a, b, c) within the same column are significantly different ($p < 0.05$)

The results from identified bands of PCR and gel detection (Figure 1) showed that *Campylobacter* spp. was highly present in farmed lettuce (21%), *Listeria monocytogenes* in farmed African nightshade (15%), and STEC and *Yersinia enterolitica* in farmed cabbage at 20% and 13% respectively (Figures 3). On the contrary, *Salmonella* spp. was highest in market amaranth (15%) (Figure 4). A high foodborne pathogenic prevalence in these specific leafy vegetables could be due to the fact that lettuce just like cabbage, are leafy vegetables with large surface areas consisting of open leaves and folds. This makes them more susceptible to microbial contamination since they directly come in contact with soil and irrigation water, thus trapping dirt and microorganisms in their folds (Aycicek et al., 2006; Seow et al., 2012). This further explained why STEC, *Salmonella* spp., and *Campylobacter* spp. were lowest in these thin-leafy vegetables: amaranth and celery at 2% and 1% respectively. *Yersinia enterocolitica* was not detected in all jute leaves and amaranth samples.

In Figure 5, irrigated water from rivers was highest in STEC (21%), *Campylobacter* spp. (17%) and *Listeria monocytogenes* (18%), marshes were highly contaminated with *Salmonella* spp. (5%) and *Yersinia enterolitica* (6%). *Campylobacter* spp. was not detected in all borehole samples, *Salmonella* spp. and *Yersinia enterolitica* were not detected in both groundwater and borehole samples. Ndiaye et al. (2011) obtained contrasting results in urban agricultural sites cultivated with lettuce in Dakar, Senegal, where *Salmonella* spp. was detected in more than half of shallow groundwater (57%) and 27% of well water. However, wastewater was found Salmonella-free (Ndiaye et al., 2011). In Cameroon, market gardening activities are generally labour intensive and carried out manually with little or no personal protective equipment, such as boots and gloves. Irrigated water is supplied to vegetables by hand watering using small plastic buckets and cans. Berinyuy and Fontem (2011) reported that the poorest agricultural wage labourers in Cameroon were observed in vegetable farms. With the main purpose of generating an income, most farmers do not have sufficient information on hygienic production and good agricultural practices (Buyukunal et al., 2015). Such farming practices could lead to human exposures to the foodborne pathogens isolated in this research thus directly causing foodborne diseases among rural farmers and consumers.

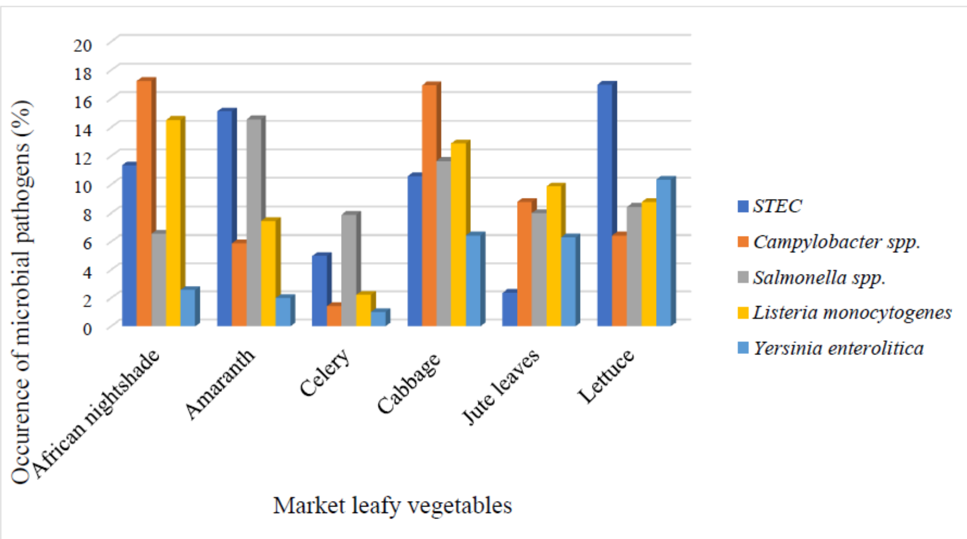

Figure 3. Occurrence of pathogens in leafy vegetables sold at six local markets

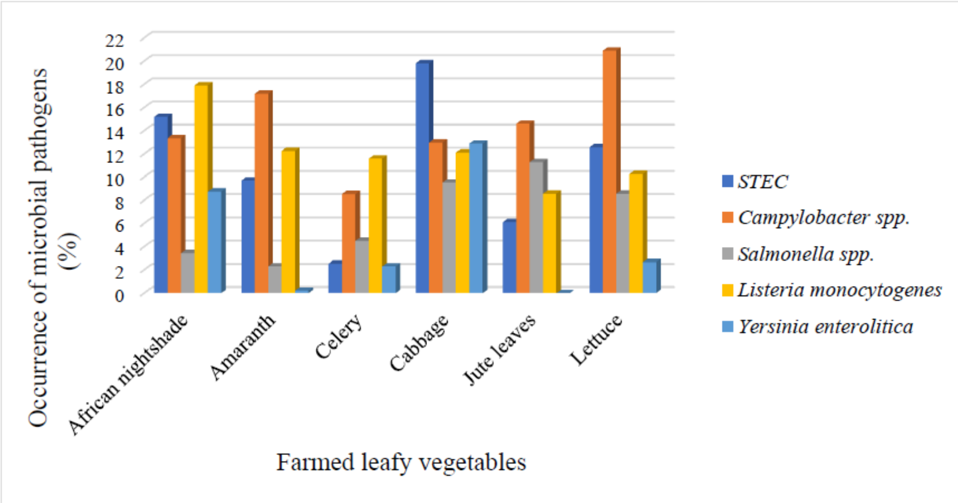

Figure 4. Occurrence of pathogens in leafy vegetables sampled from five farms

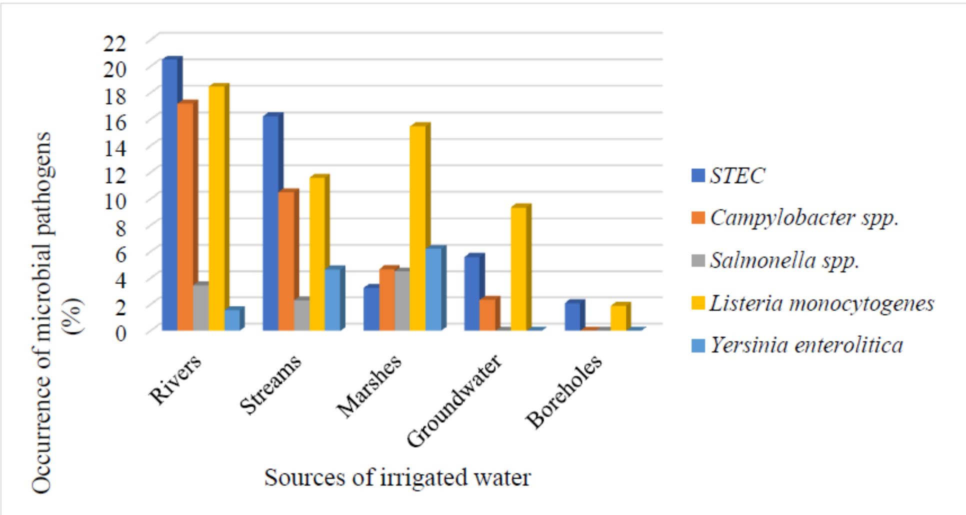

Figure 5. Occurrence of pathogens in five sources of irrigated water collected from five farms

4. Conclusion

To our knowledge, this research is the first to examine selected foodborne pathogens in leafy vegetables grown and consumed locally in Yaounde, Cameroon. Our findings have shown that these leafy vegetables contained foodborne pathogens of significant public health concern. Irrigated water was found to be the main source of leafy vegetable contamination, and 29 to 42% of respondents who participated in the study indicated that they have frequently suffered from food-related illnesses after eating a vegetable meal. Further research could be carried out: 1) to understand the survival characteristics of these foodborne pathogens in leafy vegetables, 2) to establish clinical cases that further provide evidence that the local practices of farming, handling and consumption of leafy vegetables is a credible source of transmission of food-related illnesses as suffered by respondents in this research survey, and 3) to investigate the incidence of foodborne disease outbreaks among the studied population. In addition to this research findings, this further research could strongly confirm that outbreaks of foodborne diseases are occurring among leafy vegetable consuming populations in Cameroon.

Acknowledgment

The authors wish to thank placement students at The Biotechnological Center (BTC) in Nkolbission, Yaounde, who assisted in carrying out the DNA extraction of several samples analysed in this research.

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Notes

Note 1. Vegetable farms cultivated in the rural and peri-urban areas of Nkolbisson, Ahala, Awae, Biyem assi, and quartier Carriere

Note 2. Namely, the Mendong, Acacia, 8eme, Ekounou, Nsam and Mokolo local markets

Note 3. Rivers, streams, marshes, boreholes, and groundwater

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