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A study on the prevalence of microbial contamination of cabbage (*Brassica oleracea* L.1753) in an urban area located in Daloa, Centre-West Côte d'Ivoire

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Abstract

Market gardeners in urban production are an essential source of fresh vegetables in the cities. Cabbages, like other urban market gardeners, are produced in difficult contexts, pushing producers to use wastewater for irrigation and animal manure as fertilizer. These practices would encourage soil contamination and production by various microorganisms often dangerous to human health. The aim of this study was to monitor production and assess microbial contamination of cabbage (*Brassica oleracea*) produced in the city of Daloa. An investigation was developed to determine the production of this vegetable. This survey revealed that the majority of actors were adults (> 30 years old) male dominated (63 %) and poorly educated (53 % illiterate). The main inputs were poultry manure, a mixture of dung and cow dung. The production process was empirical; which would increase the risk of contamination. The search for contamination flora as well as some potentially pathogenic bacterial species by reference microbiological standards was carried out on 18 cabbage samples from a production site. These microbiological analyzes revealed a high level of contamination by microflora, reflecting a deficit of good production and hygiene practices, particularly mesophilic aerobic germs, yeasts and molds, enterobacteria, fecal coliforms and fecal streptococci. All samples were contaminated with both *Escherichia coli, Staphylococcus aureus* and *Salmonella spp*. The CFU loads of microflora and bacterial species exceeded the microbiological standards for fresh vegetables. The cabbage produced on the study site investigated would represent a danger for the populations of Daloa, consumers of these fresh vegetables on the various public markets.

Keywords: Contamination, *Brassica oleracea*, Daloa

Résumé

Les maraîchères en production urbaine sont une source essentielle dans l'approvisionnement en légumes frais dans les grandes villes. Le chou comme d'autres maraichers urbains, sont produits dans des contextes difficiles, poussant des producteurs à utiliser les eaux usées pour l'irrigation et les déjections d'animaux comme fertilisants. Ces pratiques favoriseraient une contamination des sols et des productions par divers microorganismes souvent dangereux pour la santé de l'Homme. Cette étude avait pour objectif de suivre la production et d'évaluer la contamination microbienne du chou (*Brassica oleracea*) produit en pleine ville de Daloa. Une enquête a été élaborée pour cerner la production de ce légume. Cette enquête a révélé que la majorité des acteurs était des adultes (>30 ans), dominée par le genre masculin (63 %) et peu instruite (53 % d'analphabètes). Les principaux intrants étaient la fiente de volaille, un mélange de fiente et de la bouse de bœuf. L'itinéraire technique de production était empirique ; ce qui augmenterait le risque de contamination. La recherche de flores de contamination ainsi que certaines espèces bactériennes potentiellement pathogènes par utilisation des normes microbiologiques de référence a été réalisée sur 18 échantillons de chou d'un site de production. Ces analyses microbiologiques ont révélé une forte contamination par des microflores, traduisant un déficit de bonnes pratiques de production et d'hygiène. Cela s'est traduit par la mise en évidence de germes aérobies mésophiles, de levures de moisissures, d'entérobactéries, de coliformes fécaux et de streptocoques fécaux. Pire, tous les échantillons étaient contaminés à la fois par *Escherichia coli, Staphylococcus aureus et Salmonella spp*. Les charges en UFC des flores comme les espèces bactériennes dépassaient les normes microbiologiques prévues pour les légumes frais. Le chou produit sur le site d'étude investigué représenterait un danger pour les populations de Daloa, consommatrices de ces légumes frais sur les différents marchés publics.

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Introduction

Market gardening is an important part of agriculture and the economy of developing countries because it is an activity that responds to preferences and often to urban food demand (Bayendi et al., 2017). Cabbage is one of the most consumed vegetable products in the world (Aksoy et al., 2014). This leaf vegetable is of great importance in the diet of humans. It is a valuable source of vitamins A, C and E, mineral salts with iron dominance and sugars (Slavin & Beate, 2012). Furthermore, it has healing, digestive and nutritive properties (Gbekley et al., 2015). Cabbage generates substantial income for producers and other people involved in the marketing system, thereby reducing poverty and creating jobs (Kofie-Bikpo & Akoua, 2014; Groga et al., 2019). In Côte d'Ivoire, this agricultural sector plays a major role in the socio-economic life of the populations (CNRA, 2011). Food security in urban areas has become more worrying in large cities. In this context, urban and peri-urban agriculture has become an unavoidable source in the supply of fresh food in cities with high urbanization (Eigenbrod & Gruda, 2015). Men and women looking for jobs rush to the cultivable areas of the cities to make their activity (Tornaghi, 2014). Thus, in most cities in tropical Africa such as Côte d'Ivoire, this agricultural activity is clearly visible in some large cities (Nahmías et al., 2012; Kofie-Bikpo & Akoua, 2014). Daloa, the third most populous city in Côte d'Ivoire after Abidjan and Bouake, covering an area of 5,305 km2 with an estimated population of more than 319.427 inhabitants, contains several urban vegetable growing sites (INS, 2014; Kouassi et al., 2019). Unfortunately, cabbage crops like other market gardeners in these urban areas are practiced in difficult contexts, marked by a lack of financial means for the supply of safe water and synthetic fertilizer for soil fertilization. Market gardeners often use wastewater for irrigation and manure as fertilizer for the soil. These farming practices could promote high soil contamination and production by microorganisms that could be hazardous to human health (Koffi-Nevry et al., 2012; Pereira et al., 2013; Woldetsadik et al., 2017). Market gardening products such as cabbage resulting from these cultural practices would therefore be a source of infection for consumers (Koffi-Nevry et al., 2011; Allio et al., 2017). According to the work of (Petterson et al., 2010) and those of (Woldetsadik et al., 2017), consumption of such vegetables would be a potential risk factor for infection with enteropathogenic bacteria such as Salmonella and Escherichia coli O157. Cases of food poisoning related to the ingestion of contaminated vegetables have been identified around the world (Koffi-Nevry et al., 2012; Cuq et al., 2015). However, to our knowledge, no comprehensive study of the culture route and the risk of microbial contamination of Daloa's urban production cabbage have yet been studied. In addition, there is little data available on urban cabbage growers in this locality. The overall objective of this study was to evaluate the microbial contamination of cabbage produced in the city of Daloa. The information obtained can be used to sensitize urban producers to innovate production and to have a cabbage of good sanitary quality in the city of Daloa or in other cities where urban agriculture is practiced.

Materials and Methods

Presentation of the study site

The study area was the town of Daloa, located central Centre West Côte d'Ivoire between 6°3' north latitude and 6°27' west longitude. The study site is was a low-lying geographic coordinates 06°45'53.37'' west longitude and 06°90'42.92'' latitude north, located in the center of the city (Fig. 1).

Diagnosis and characterization of urban production of cabbage in Daloa

A survey with a questionnaire was developped to collect information on urban cabbage production sites in

the city of Daloa. This survey first gave information on the profile of the producers (gender, age, nationality, level of study) and then on the technical itinerary of the urban production of lettuce (source of irrigation water, type amendment). The survey was conducted from 11-01-2019 to 13-03-2018 at four urban and periurban production sites in the city of Daloa. That people surveyed were of two kinds, of all levels of study and of all social strata. In total, the survey covered 30 cabbage producers.

Cabbage sample collection

On the study site, three planks of cabbages constituted a study block. For each block, were selected three mature cabbages taken at random on the three planks. These harvested samples were packaged in stomacher sachets. Samples once taken were stored in a cooler with dry ice and transported to the laboratory for analysis. These analyses were done at the Agrovalorisation Laboratory of University Jean Lorougnon Guede. A total of 18 cabbage samples divided into 18 study blocks, (ie 54 planks).

Microbiological analysis

The preparation of stock solutions and decimal dilution, inoculation techniques for research and isolation of different microbial flora (enterobacteria, mesophilic aerobic flora, yeasts and molds, fecal coliforms and fecal streptococci) and bacterial species (*E. coli*, *S. aureus* and *Salmonella spp.*) were made according to Kouassi et al. (2019). Buffered Peptone Water (BPW) broth was used for stock solutions as described in ISO 6887-4: 2011. Decimal dilutions were performed with Tryptone Sel broth as recommended in ISO 6887-1: 1999. Plate Count Agar (PCA) was used to count mesophilic aerobic flora at 30°C for 72 hours as recommended in NF/ISO 4833: 2003. Enterobacteria count was performed at 37°C for 24 h on Violet Red Neutral Bile Glucose (VRBG) agar according to ISO 21528-2: 2004. Violet Neutral Bile Lactose (VRBL) agar was used for fecal coliforms count at 44°C for 24 h as described in ISO 4832: 2006. Typical Enterobacteria and fecal coliforms colonies were confirmed by oxidase test. Fecal streptococci were detected at 37°C for 24 h on Esculin Azide Bile Agar according to standard NF/ISO 7899-1: 1984. For Salmonella sp detection and enumeration of media Buffered Peptone Water (BPW), broth Rappaport of Vassiliadis Soya Broth and Hektoen Enteric Agar were used as described in the reference standard NF/ISO 6579:2002 Amd 1: 2007. Staphylococcus aureus was identified using Baird-Parker Agar containing Telluride Egg Yolk and 0.2% Sulphamethazine served the identification of at 37°C for 48 h according to the French standard NF/ISO 6888: 2004. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) were counted (15-150 characteristic colonies) according to dilution. Colonies were examined microscopically, tested for Gram and catalase reactions, and confirmed by coagulase activity (rabbit plasma-EDTA, Merck). Rapid'*E coli* 2 agar served for Escherichia coli isolation and enumeration at 44°C for 24 to 48 hours as recommended in standard NF/ISO 16140: 2013. Yeasts and molds were counted with Sabouraud agar containing chloramphenicol 25°C for 5 days according to the NF/ISO 16212: 2011 standard. Salmonella species were isolated in three stages. A pre-enrichment was performed by incubating the cabbage stock solution at 37°C for 24 h. 0.1 mL of stock solution (pre-enriched) was transferred to a tube containing 10 mL of Vassiliadis Rappaport. After homogenization, the tube was incubated at 42°C for 24 h. Finally the isolation was

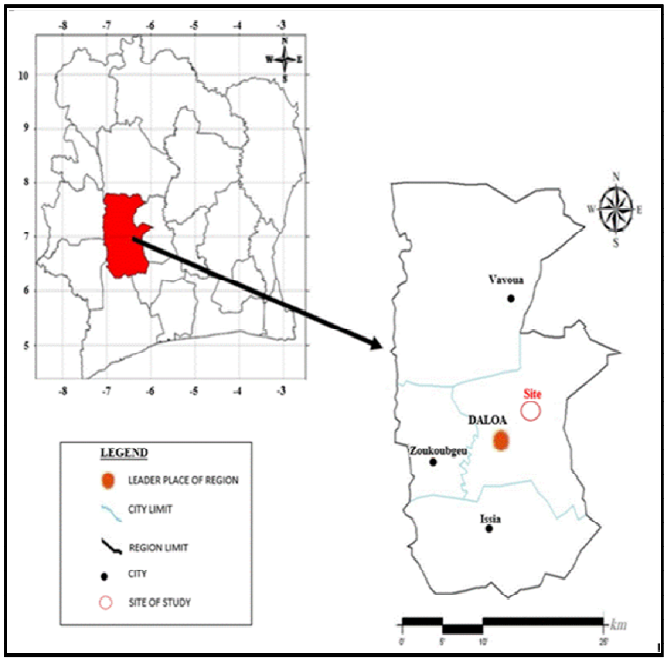

Figure 1: Map of the study site

carried out from the enrichment medium incubated on Hecktoen agar at 37°C for 24h. Typical Salmonella colonies were green or blue with a black center.

Colony enumeration

Colony Forming Units per milliliter of sample (CFU/g) were calculated according to standard NF/ISO 7218: 2007 using the following formula:

$$N = \frac{\Sigma Ci}{(N_1 + 0.1N_2)\, d \cdot V}$$

ΣCi: Sum of characteristic colonies counted on all retained Petri dishes;
N1: Number of Petri dishes retained at the first dilution;
N2: Number of Petri dishes retained at the second dilution;
d: Dilution rate corresponding to the first dilution;
V: Inoculated volume (mL);
N: Number of microorganisms (CFU/g).

Evaluation of cabbage sample quality

Cabbage microbiological quality assessment standards were taken from the "Microbiological Criteria for Foodstuffs Guidelines for Interpretation of 2015 of Luxembourg";supplemented by the normative reference of the microbiological criteria of human foods (C.E. n° 2073/2005). Moreover, probable source of cabbage contamination was made with reference to the Bourgeois index (1980) modified by Borrego & Romero (1982) based on the ratio of fecal coliforms / fecal streptococci (CF/SF).

Statistical analyses

Statistical analyzes were conducted with the Statistica, 99 Edition. The different parameters analyzed were then subjected to an analysis of variance (ANOVA) with the software Statistica, 99 Edition. For this purpose, a single-factor ANOVA and Duncan's multi-extended tests were used. ANOVA was used to test, on the one hand, the variability between the different samples. As for Duncan's test, he later made it possible to first locate the differences between the samples and then the differences between them. Statistical differences with P-values under 0.05 were considered significant.

Results

Characteristics of urban producers of cabbage and diagnosis of production process

The profile of the urban cabbage producers from the studied sites investigated is summarized in Table 1. Cabbage was produced by both genera. It was dominated by the male gender (63 %) against 37 % for the female gender. Producers ages varied from 30 to 60 years. They were mostly Ivorian (60 %) and were not well educated. As inputs, all these producers used surface water to water their crops. They were sumps and flows. Poultry excrement, beef droppings, food residues, sawdust and chemical fertilizers were also used as main soil fertilizers. The technical process of cabbage production from different study sites is summarized in the diagram below (Fig. 2). According to the survey, the quantities of chemical or natural fertilizer used were not quantified, so the earth planks for the crops were made by punching. In addition, animal droppings (poultry manure and beef dung) were used more than twice for one production cycle

Table 1: Profile of urban producers and characteristics of cabbage production

	Characteristics of production	Number of producers	Percentage (%)
Age (years)	[15-30 [years	10	33.33
	[30-60 [years	15	50
	> 60 years	5	16.67
Gender	Male	19	63.3
	Female	11	36.67
Nationality	Ivoirian	18	60
	Non Ivoirian	12	40
Level of study	Illiterate	16	53
	Primary	9	30
	Secondary	5	17
Agricultural inputs	Surface water	30	100
	Poultry manure + chemical fertilizers	11	36.67
	Poultry manure + cow dung + chemical fertilizers	19	63.33

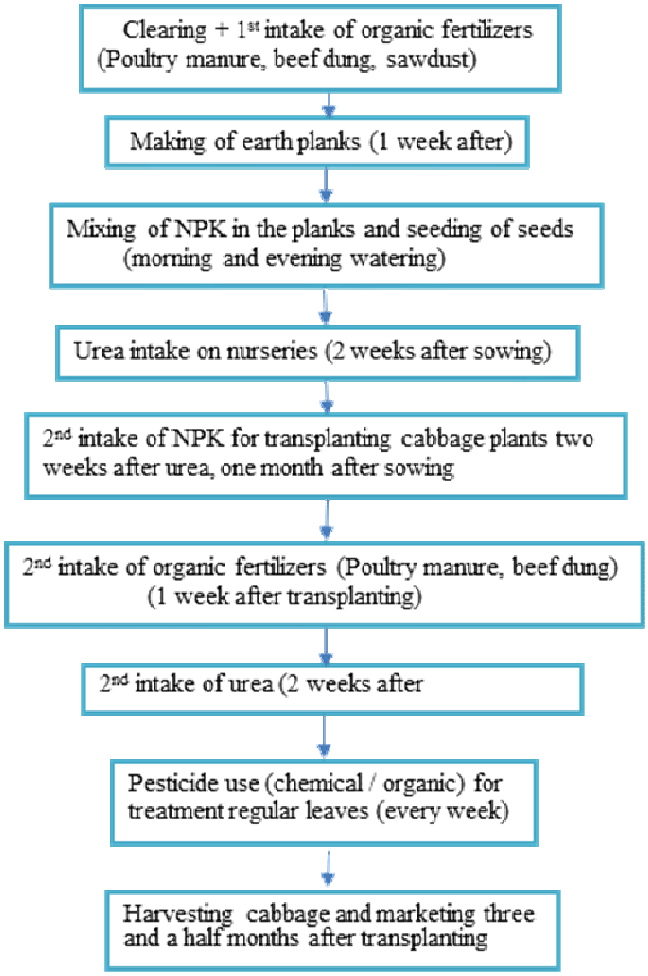

Figure 2: Urban production process of cabbage (*Brassica oleracea L.*)

The cabbage samples contained diverse microbial contaminants

Major alteration microfloras and/or floras including mesophilic aerobic germs, fecal coliforms, enterobacteria and fungal flora (yeasts/molds) were suggestive of a deficit in good production and hygiene practices of cabbage produced in urban areas in Daloa. Moreover, all charges (CFU/g) of the flora were above the expected microbiological quality standards. For example, concerning the mesophilic aerobic germs, the CFU/g load ranged from 1.8x107 to 3.6x107 while the standard indicated 3x106 (Fig. 3). The CFU/g load for yeasts and molds ranged from 5x105 to 8.8x105 while the standard predicts 104 (Fig. 4). The loads of the fungal flora of all samples were unequally distributed and therefore statistically different ($p > 0.05$) from one sample to another. As for enterobacteria, their loads ranged between 1.2x105 to 2.8x105 CFU/g while the standards were 104 CFU/g (Fig. 5). These microfloras varied from one sample to another and therefore statistically different ($p > 0.05$). The sample E13 was contaminated mostly by the mesophilic aerobic germs (3.6x107 CFU/g). The heaviest load of yeast and mold (8.3x104 CFU/g) was found in E8; sample E14 contained the largest enterobacteria load (2.08x106 CFU/g).

All cabbage samples from the investigated site were heavily contaminated with faecal coliforms and fecal streptococci. These loads ranged from 1.2x106 to 1.5x106 CFU/g for the first and from 6x105 to 9.2x105 CFU/g second (Fig. 6). However, the standards were 102 CFU/g. These two were unevenly distributed among the 18 cabbage samples. In addition, the ratio (R) fecal coliforms (CF)/fecal streptococci (SF) was 1.84. This translated fecal contamination of cabbage. However, the origin of this fecal contamination was diversified.

Cabbage from the investigated site was contaminated with pathogenic bacterial species including *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus*. Worse, all the samples contained all three species at the same time, with heavy loads exceeding the microbiology standards for fresh vegetables. The CFU/g number for *E. coli* ranged from 5.4x104 to 1.1x105, whereas the standard predicts 10 to 102 (Fig. 7). For S. aureus, the charges in CFU/g ranged from 9.3x103 to 5x105 while the standard indicated 102. There were the loads of ranged from1.4x103 to 1.5x104 CFU/g at different study blocks (Fig. 7). Moreover, E. coli, *Salmonella spp* and *S. aureus* charges from all samples were unevenly distributed from one sample another. These loads were therefore statistically different ($p > 0.05$) in the different samples.

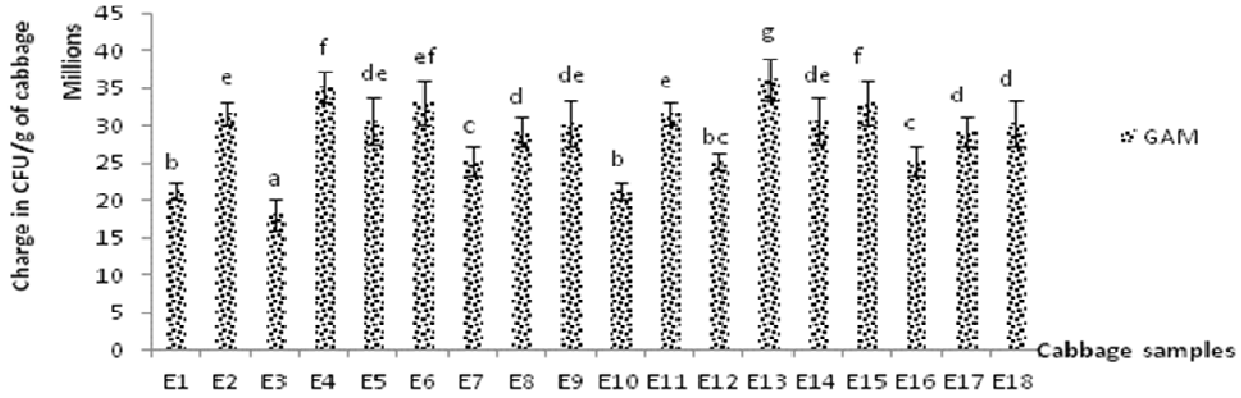

Figure 3: Numbers in CFU/g of mesophilic aerobic germs in cabbage samples. Values with the same letters are not significantly different (P>0.05).

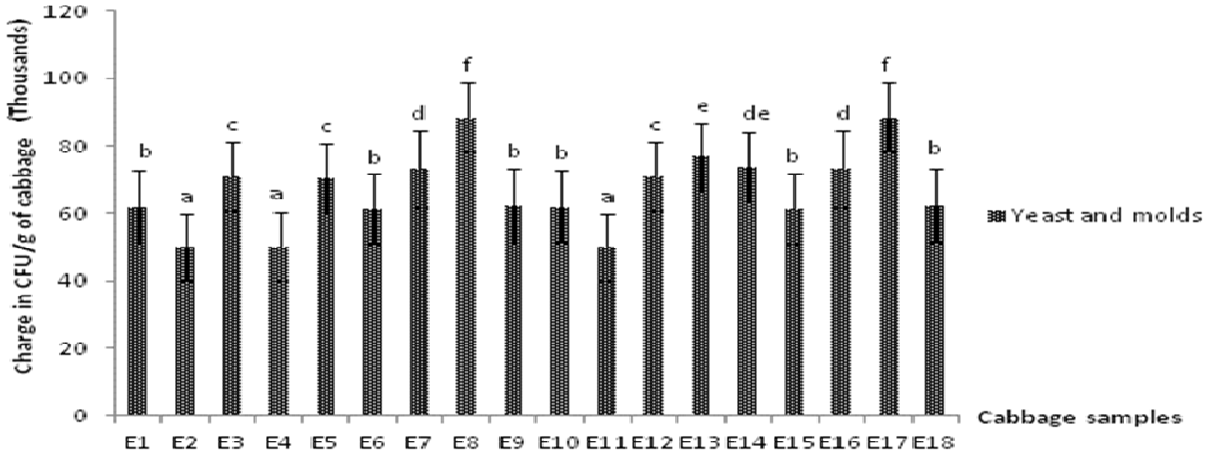

Figure 4: Charge of yeasts and molds in the cabbage samples. Values with the same letters are not significantly different (P>0.05).

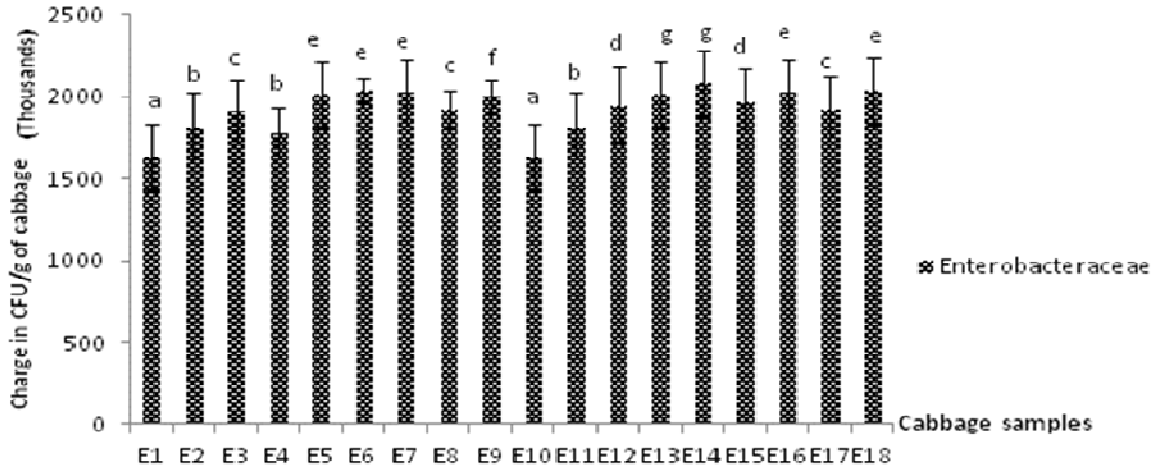

Figure 5 : Charge of Enterobacteriaceae in the cabbage samples. Values with the same letters are not significantly different (P>0.05).

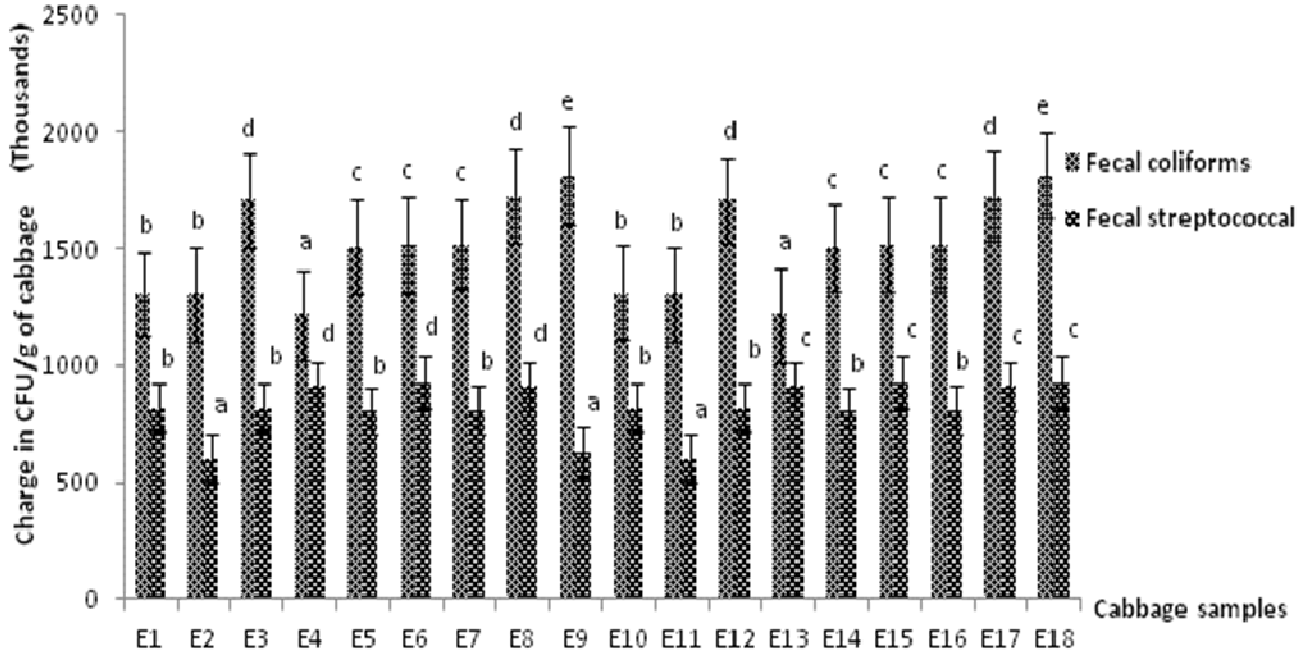

Figure 6 : Charge of bacterial flora of fecal contamination in the cabbage samples. Values with the same letters are not significantly different (P>0.05).

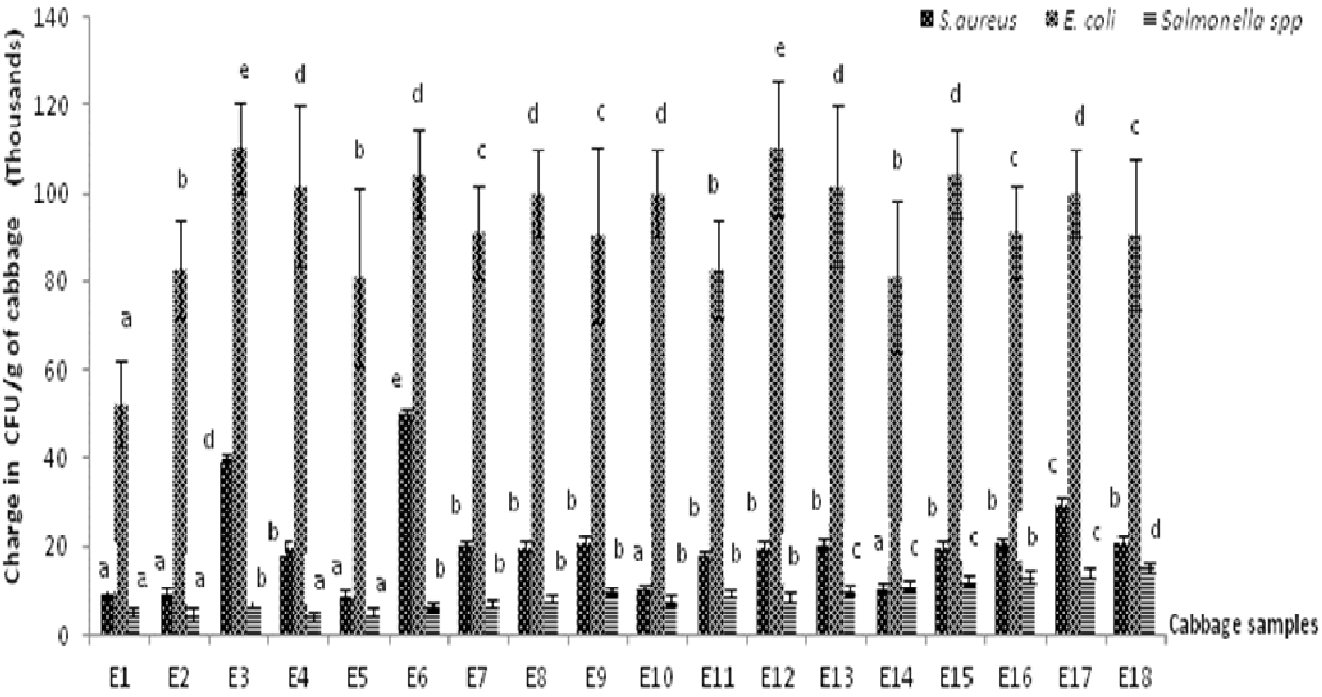

Figure 7: Charge of pathogenic bacterial species found in the cabbage samples. Values with the same letters are not significantly different (P>0.05).

Discussion

Market gardeners produced cabbage in the heart of downtown in lowlands, on inconstructible spaces and/or undeveloped spaces. Cabbage cultivation was practiced mainly by men (63 %) against (37 %) for women. The majority of these producers were adults over 30 (50 %), with an age range of 30 to 60 years. In addition, seniors (over 60) were also present (16.66 %). The predominance of the masculine gender could be explained by the arduousness of gardening work dominated by handicrafts. In fact, women were more involved in the post-harvest stage of cabbage production and responsible for cabbage transport and sale in various urban markets. This confirmed the results obtained by other authors in Cameroon and Côte d'Ivoire (Kenmogne et al., 2010, Koffi-Nevry et al., 2012; Wognin et al., 2013). Meanwhile, for less difficult gardening crops such as lettuce it was shown that women were the dominating gender in the same locality (Kouassi et al., 2019). The non-marginal senior producer rate could be explained by the fact that these people most often retired in a context of high unemployment among young people need additional income to meet the family needs (Ba et al., 2016).

Microbiological analyzes of the various cabbage samples revealed a high level of contamination by mesophilic aerobic germs, enterobacteria, yeasts and molds. As early evidenced in other urban vegetables including lettuce, green beans, onions, carrots, cucumbers elsewhere (Soendjojo et al., 2012; Soltan et al., 2015, Koffi-Nevry et al., 2011; Mngoli & Ng'ong'ola-Manani, 2014; Nwankwo et al., 2015). The origin of cabbage microbial contamination could be explained by a marked deficit of good hygienic practices at the study site. Indeed, this site was located a shallow, regularly receives, sewage and gutter water without any treatment. These waters were directly used for cabbage irrigation and could be the source of contaminations as shown elsewhere (Koffi-Nevry et al., 2012; Abbou et al., 2014; Holvoet et al., 2015). The samples were also contaminated with flora of fecal origin including fecal streptococci and fecal coliforms with loads far exceeding the prescribed microbiological standards. In addition, the bourgeois index of the site was 1.84 indicating fecal contamination of diverse origin that could be due to poor hygiene as early reported (Atidégla et al., 2015; Soncy et al., 2015). Moreover, numerous studies have demonstrated the link between the contamination of urban market gardeners and the immediate environment of production sites (Koffi-Nevry et al., 2012; Combbina et al., 2013; Woldetsadik et al., 2017; Kouassi et al., 2019). Concerning this study site, animal droppings such as poultry manure and cow dung were used as fertilizers without any treatment. Moreover, the site was located in a shallow, main wastewater receptacle of the city of Daloa with presence of garbage in places in the lowlands. Human excrement, farm animals (oxen, sheep) and other animals (lizards, migratory birds, dogs and cats) were also permanently found on this site. Their excreta were carried by rainwater runoff to soils, wells and other water sources used for watering. These factors could therefore increase the risk of cabbage contamination. All analyzed cabbage samples contained both potentially harmful bacterial species such as *Escherichia coli, Staphylococcus aureus*, and *Salmonella spp.* with loads exceeding the prescribed microbiological standards. *Staphylococcus aureus* has been reported in works in soups in Brazil (Cesar et al., 2015) in New Zealand (Wadamori et al., 2016) and Nigeria (Bishop & Okwori, 2017). *Escherichia coli* and Salmonella had previously been reported in several studies on vegetable contamination (Amponsah-Doku et al., 2010, Schikora et al., 2011, Koffi-Nevry et al., 2012, Jensen et al., 2013, Traore et al., 2015; Alio et al., 2017; Niguma et al., 2017; Kouassi et al., 2019).

The cabbages of the investigated urban site could be a source of microbiological hazards, which would cause multiple infectious diseases such as diarrhea, gastroenteritis, typhoid and paratyphoid fevers. Its consumption therefore could be a real risk of infection or a source of food poisoning which can lead to a public health problem.

Conclusion

Urban agriculture is an essential source of food supplies, including leafy vegetables in the city of Daloa. This study was devoted to assessing the risk of microbial contamination of cabbage produced in the city of Daloa. The microbiological analyzes of these cabbages revealed a high contamination by indicator flora of general pollution, fecal flora and also the presence of potential pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella spp.* Cabbage consumption could be a real danger to consumer health. The profile of producers, the upstream empirical production route and the difficult production conditions could increase the risk of microbial contamination. Thus, the competent authorities must raise awareness, health risks to consumers and establish regulations for urban agriculture in order to limit the risk of contamination.

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