

Assessment of the Risk of Microbial Contamination of an Urban Crop in the City of Daloa (Côte d'Ivoire): Case of Lettuce (*Lactuca sativa* L.)

Clément Kouassi Kouassi^{1,2}, Athanase Kra Kouassi^{1,2}, Mathurin Konan Yao^{2,3}, Gustave Adingra Kouassi¹ & Rose Koffi-Nevry²

¹Université Jean Lorougnon GUEDE, Département Biochimie-Microbiologie, Laboratoire Agrovalorisation, BP 150 Daloa, Côte D'Ivoire

²Université Nagui Abrogoua, Département des Sciences et Technologies des Aliments, Laboratoire de Biotechnologie et Microbiologie alimentaire, 02 BP 801 Abidjan 02, Côte d'Ivoire

³Université Peleforo Gon COULIBALY, Département de Biochimie-Général, Unité de formation et de Recherche des sciences Biologiques BP 1328 Korhogo, Côte d'Ivoire

Correspondence: Kouassi Kouassi Clément, Université Jean Lorougnon GUEDE, Département Biochimie-Microbiologie, Laboratoire Agrovalorisation, BP 150 Daloa, Côte D'Ivoire. E-mail: clemankoici@yahoo.fr

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Abstract

The growing population of the city of Daloa, together with its growing urbanization, has a diversified need for food resources. Urban and periurban production of lettuce is a food resource for its people. This study was designed to assess the risk of microbial contamination. For the conduct of the study, first a survey was carried out on different sites of urban production to know the technical process of production. Then, mature lettuce, ready for sale and then consumed from a site, was subjected to microbiological characterization according to the standards in force. Microbiological analyzes revealed a high contamination. For mesophilic aerobic germs, the charges in CFU/g ranged between 1.7×10^7 and 6.7×10^7 . The loads in CFU/g for yeasts and molds ranged from 3.4×10^5 to 9.3×10^5 . As for fecal coliforms and enterobacteria, their loads ranged between 1.2×10^5 to 2.8×10^5 CFU/g for the first and 7.2×10^5 to 10^6 CFU/g for the second. These samples were contaminated with both *Escherichia coli*, *Staphylococcus aureus* c + and *Salmonella* sp. The loads in *E. coli* and *S. aureus* c+ ranged between 9.4×10^4 and 1.8×10^5 for the first and 4×10^3 to 1.1×10^4 CFU/g for the second. *Salmonella* sp. was found with loads ranging from 6.1×10^4 to 8.2×10^4 CFU/g. Empirical production process would increase the risk of microbial contamination. It is necessary to produce healthy lettuce for Daloa's consumers.

Keywords: contamination, *Lactuca sativa*, Daloa, Côte d'Ivoire

1. Introduction

Rapid urbanization and the strong concentration of urban populations have spawned a new form of agriculture, practiced in the urban and periurban areas of the major cities of West Africa. In Côte d'Ivoire, like other African countries, this new agriculture, dominated by market gardening, is booming (Dongmo *et al.*, 2005; Soro *et al.* 2008; Ba & Aubry, 2011). This urban and periurban agriculture is a revenue and employment generating activity practiced by urban vulnerable groups; thus contributing to the food security of the populations (Gomiero *et al.*, 2011; Koffi *et al.*, 2012; Loudit *et al.*, 2017; Ba & Cantoreggi, 2018). Daloa, the third most populous city in Côte d'Ivoire after Abidjan and Bouake, covering an area of 5.305 km² and an estimated population of more than 288 000 inhabitants, with its many administrative changes (commune, sub-prefecture, department and region, university town), is also confronted with this new form of agriculture (RGPH, 2014; Zah, 2015). This agricultural activity often practiced in the city center, faces enormous difficulties such as the thorny problem of urban pollution and the proximity of garbage and waste disposal sites of all kinds. Unfortunately, crops are being grown in difficult contexts, marked in particular by the lack of financial means for the supply of drinking water and synthetic fertilizers for soil fertilization; this often pushes market gardeners to use wastewater for irrigation and animal manure as fertilizer for the soil. These farming practices could favor a high contamination of soil and

production tools by microorganisms, some of which may be harmful to human health according to several studies (Koffi *et al.*, 2012; Pereira *et al.*, 2013; Woldetsadik *et al.*, 2017). Productions resulting from these cultivation practices, especially market garden produce, contaminated by pathogens can be dangerous for the consumer (Koffi *et al.*, 2011; Alio *et al.*, 2017). Based on the work of Patterson *et al.* (2010) and those of Blaak *et al.* (2015), the consumption of such cultures constitutes a potential risk factor for infection. Contamination of vegetables is one of the potential risks of infection with enteropathogenic bacteria such as *Salmonella* and *Escherichia coli*. This contamination occurs from an environmental, animal or human source at the time of planting, harvesting or handling vegetable prior to consumption (Cobbina *et al.*, 2013; Wognin *et al.*, 2013). Considered as a contamination of plant surfaces, recent work has shown that certain pathogens such as *salmonella* are able to infect and multiply in the mesophyll of certain plants such as lettuce (Kroupitski *et al.*, 2009; Guchi *et al.*, 2010; Pelletier *et al.*, 2011; Schikora *et al.*, 2011). In Daloa, lettuce is cultivated in urban, peri-urban areas on marshy sites and in shallows at the very heart of some of the central districts of the city. Thanks to its incomparable nutritional richness and the fibers it contains, lettuce (*Lactuca sativa L.*) is of paramount importance in the dietary habits of the population and also for the proper functioning of the organism (Zhang *et al.*, 2009; Berger *et al.*, 2010; Pereira *et al.*, 2013). It is one of the most popular and most consumed vegetables in the world, often the main ingredient in salads (Koffi *et al.*, 2011; Maffei *et al.*, 2013). However, to our knowledge, no exhaustive study of the production route, the microbiological quality of the urban production lettuce in Daloa has not been the subject of any scientific study. In addition, there is little data available on urban lettuce producers. The overall objective of this study was to assess the risk of microbial contamination of lettuce produced in the city of Daloa. The information obtained can be used to sensitize producers to improve the route and sanitary quality of lettuce (*Lactuca sativa L.*) produced in the city of Daloa or in other cities where urban and periurban agriculture were practiced.

2. Method and Material

2.1 Presentation of the Study Site

The study area is the town of Daloa, located in the central west of Côte d'Ivoire between 6°3' north latitude and 6°27' west longitude. The study site is a low-lying geographic coordinates 06°27'33.52" west longitude and 06°53'35.92" latitude north, located in the center of the city. **Figure 1** below shows the geographical location of the study area.

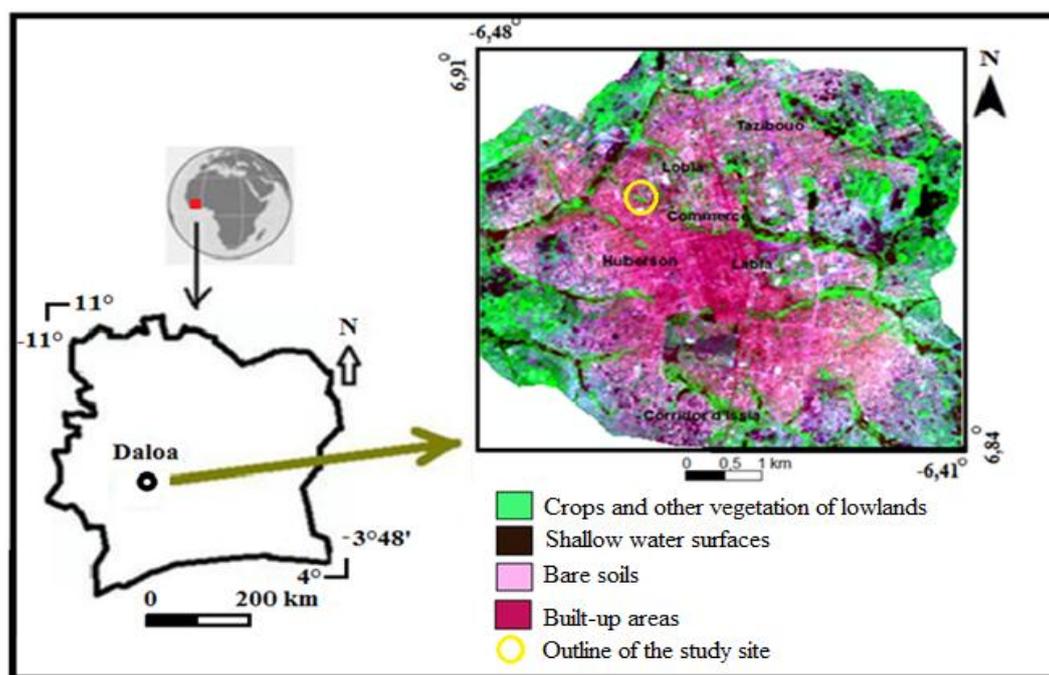


Figure 1. Map of the city of Daloa presenting the study site

2.2 Diagnosis and Characterization of Urban Production of Lettuce in Daloa

A survey with a questionnaire was developed to collect information on urban lettuce 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 25-10-2017 to 28-12-2017 at four urban and periurban production sites in the city of Daloa. The people surveyed are of two kinds, of all levels of study and of all social strata. In total, the survey covered 31 lettuce producers.

2.3 Microbiological Characterization

2.3.1 Sampling

On the site, three different lettuce beds are a study block. For each block, were selected three different samples composed of three feet of lettuce at maturity taken at random on the three boards. These harvested samples are packaged in stomacher sachets. Samples once taken are stored in a cooler with dry ice and transported to the laboratory for analysis. These analyses were done at the Laboratory of Host-Microorganisms and Evolutions Interactions (LIHME) of University Jean Lorougnon Guede.

2.3.2 Methods of Analysis

Several methods were used in this study. Buffered Peptone Water (BPW) broth was used in the preparation of 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 h 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. For the search, isolation and enumeration of *Salmonella* sp, media Buffered Peptone Water (BPW), broth Rappaport of Vassiliadis Soya Broth and Hecktoen Enteric Agar were used as described in the reference standard NF/ISO 6579:2002 Amd 1: 2007. Baird-Parker Agar with Telluride Egg Yolk and 0.2% Sulphamethazine served the identification of *Staphylococcus aureus* at 37 °C for 48 h according to the French standard NF/ISO 6888: 2004. Rapid'E coli 2 agar served the isolation and enumeration of *Escherichia coli* at 44 °C for 24 to 48 h as recommended in standard NF/ISO 16140: 2013. Yeasts and molds were counted with Sabouraud agar chloramphenicol 25 °C for 5 days according to the NF/ISO 16212: 2011 standard. The different culture media used were prepared according to the manufacturers' instructions.

2.3.2.1 Preparation of the Stock Solution and Decimal Dilutions

Twenty-five grams (25 g) of lettuce's sample was aseptically transferred to an Erlenmeyer flask containing 225 mL of sterile (BPW) medium to prepare the stock solution. Everything is carefully mixed, taking care to soften the leaves with the fingers 2 to 3 minutes. After a 1 hour rest on the bench at room temperature, the stock solution was decimally diluted in sterile Tryptone Salt medium up to 10⁶.

2.3.2.2 Inoculations and Incubations

Research of mesophilic aerobic germs, yeasts and molds, fecal coliforms, enterobacteria and *Escherichia coli*

According to the prescriptions of the standards adopted, the pour plate method was applied. Thus, one milliliter (1 mL) of the diluted lettuce sample to be analyzed is aseptically transferred to a sterile Petri dish and mixed with 20 mL of the respective agar. After solidification, the dishes are inverted and incubated at temperatures as given in the respective standard. Three Petri dishes were inoculated per dilution. The characteristic colonies according to the different media are then counted taking into account the calculation standard (NF/ISO 7218: 2007).

Research of *Staphylococcus aureus*

The surface spreading method was used for the detection of *S. aureus*. It consisted of taking 0.1 mL of the stock solution or a dilution of the lettuce sample to be analyzed, using a sterile pipette, and transferring to a Petri dish containing the Baird Parker agar medium already poured and solidified. The dilution is spread on the agar using a spreader rake. These manipulations are all performed under aseptic conditions near the Bunsen burner flame. Petri dishes are then inverted and incubated at 37 °C for 48 h. Two plates of Petri were seeded by dilution. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) are counted (15-150 characteristic colonies) taking into account the dilution.

Highlighting *Salmonella* sp

It is done in three stages. Pre-enrichment is performed by incubating the stock solution at 37 °C for 24 h. The enrichment consisted of taking 0.1 mL of the stock solution (pre-enriched) and transferred to a tube containing

10 mL of Vassiliadis Rappaport previously prepared and sterilized. After homogenization, the tube is incubated at 42 °C for 24 h. Finally the isolation was carried out from the enrichment medium incubated on a solid selective medium: Hecktoen agar. A drop is taken using a Pasteur pipette and then seeded by streaks on the surface of the Hecktoen agar. The dish is incubated at 37 °C for 24 h, and sometimes even for 48 h, in the absence of characteristic colonies after the first incubation. On Hecktoen agar, the typical *Salmonella* colonies observed are green or blue with a black center.

Enumeration

The number of Colony Forming Units per milliliter of sample (CFU/g) from the number of colonies obtained in the Petri dishes is carried out according to standard NF/ISO 7218: 2007.

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

$\sum Ci$: Sum of characteristic colonies counted on all retained Petri dishes;

N_1 : Number of Petri dishes retained at the first dilution;

N_2 : 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).

Standards for assessing the microbiological quality of lettuce

The microbiological quality assessment standards for lettuce are 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).

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.

3. Results

3.1 Characteristics of Urban Producers of Lettuce and Diagnosis of Their Production Process

The profile of the urban lettuce producers of the different sites investigated is summarized in **Table 1**. Lettuce is produced by both genera. It is dominated by the female gender (58.1 %) against 41.9 % for the male gender. The age of the producers varies between 30 and 60 years. They are mostly Ivorian (58.1 %) and have a low level of education.

Table 1. Profile of urban producers and characteristics of lettuce production

	Frequency	Percentage (%)
Age (years)	< 30 years	22,6
	30-60 years	45,2
	> 60	32,3
Gender	Male	41,9
	Female	58,1
Nationality	Ivorian	58,1
	Burkinabe	25,8
	Malian	16,1
Level of study	Illiterate	80,6
	Primary	9,7
	Secondary	9,7
Agricultural inputs	Surface water	100
	Poultry manure + chemical fertilizers	48,4
	Poultry manure + cow dung + chemical fertilizers	51,6
		15

3.2 Technical Process of Urban Lettuce Production

The technical process of producing lettuce from the different study sites investigated is summarized in the diagram below (Figure 2). According to the survey, the quantities of chemical or natural fertilizer used are not quantified, so the earth planks for the crops are made by punching.

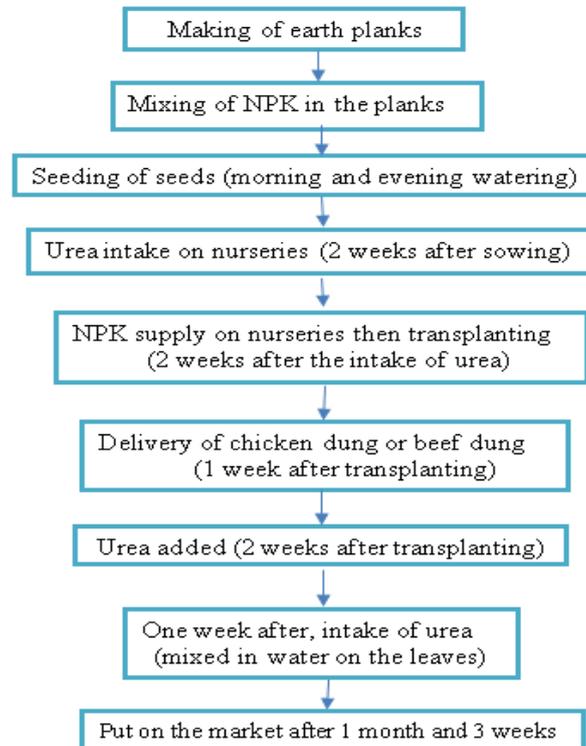


Figure 2. Urban production process of lettuce (*Lactuca sativa* L.)

3.3 Microbiological Characteristics of the Lettuce Produced

3.3.1 Presence of Microflora in Lettuce

The microbiological study assessed the level of microbiological contamination of lettuce produced in urban areas of Daloa. Microbiological analyzes made it possible to count microorganisms; major alteration microfloras and/or floras suggestive of a deficit in good production and hygiene practices of lettuce produced in urban areas in Daloa. These are fungal flora (yeasts/molds), mesophilic aerobic germs, fecal coliforms and enterobacteria. All samples from the study site were heavily contaminated by these different microfloras. In addition, all charges (CFU/g) of the flora were all well above the expected microbiological quality standards. The CFU/g load for yeasts and molds ranged from 3.4×10^5 to 9.3×10^5 while the standard predicts 10^4 (Figure 3). The loads of the fungal flora of the different study blocks were unequally distributed and therefore statistically different ($p > 0.05$) from one block to another. For mesophilic aerobic germs, the CFU/g load ranged from 1.5×10^7 to 6.3×10^7 while the standard indicated $3 \cdot 10^6$ (Figure 4). As for fecal coliforms and enterobacteria, their loads ranged between 1.2×10^5 to 2.8×10^5 CFU/g for the first and 7.2×10^5 to 1.1×10^6 CFU/g for the second while the standards are 10^2 CFU/g and 10^4 CFU/g respectively (Figures 5 and 6). These microfloras varied from one block to another and therefore statistically different ($p > 0.05$). The B12 block samples were more contaminated by the mesophilic aerobic germs (6.3×10^7 CFU/g), those of the B5 block contained the highest yeast and mold load (9.3×10^5 CFU/g); the B8 block samples contained both the largest fecal coliforms and enterobacteria load (2.8×10^5 CFU/g) for the first and (10^6 CFU/g) for the second.

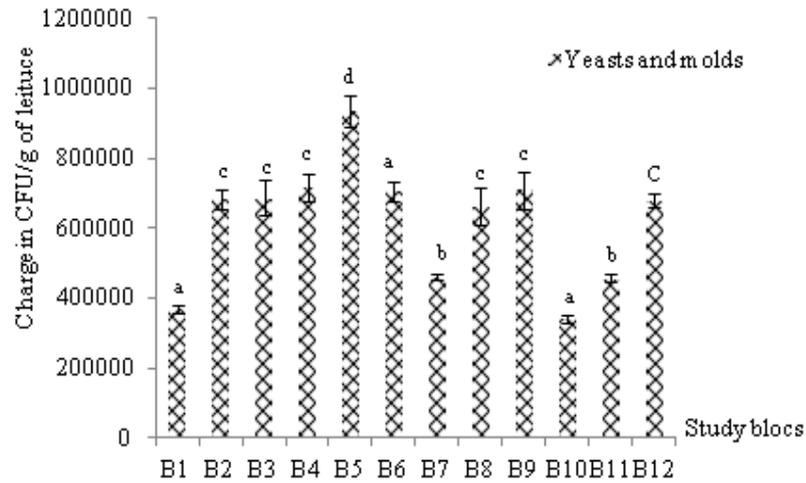


Figure 3. Numbers in CFU/g of yeasts and molds in lettuce according to the study blocks. Values with the same letters are not significantly different ($P>0.05$)

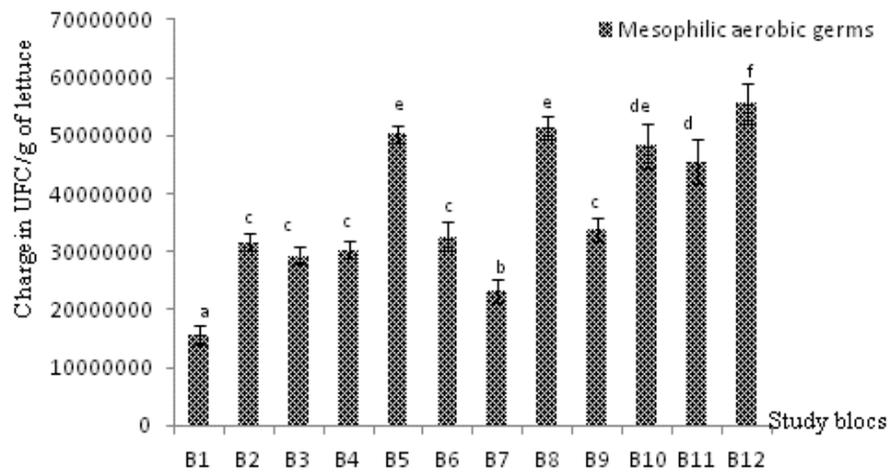


Figure 4. Numbers in CFU/g of mesophilic aerobic germs in lettuce according to the study blocks. Values with the same letters are not significantly different ($P>0.05$)

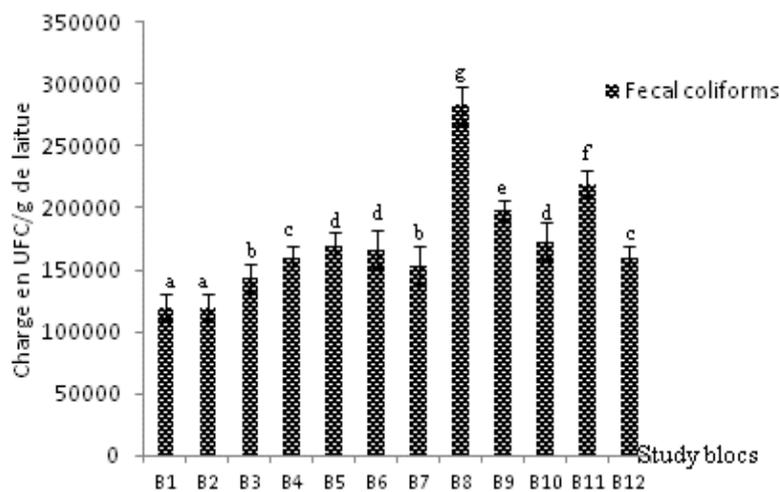


Figure 5. Numbers in CFU/g of fecal coliforms in lettuce according to the study blocks. Values with the same letters are not significantly different ($P>0.05$).

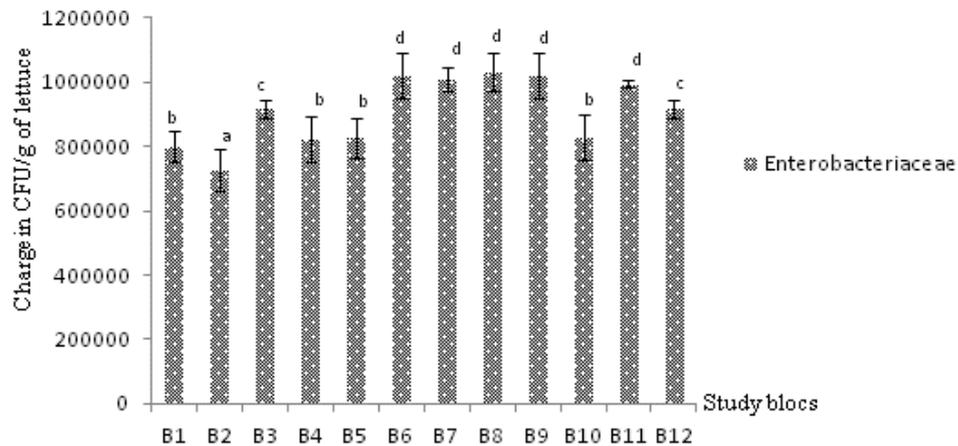


Figure 6. Numbers in CFU/g of enterobacteriaceae in lettuce according to the study blocks. Values with the same letters are not significantly different ($P>0.05$)

3.3.2 Presence of Pathogenic Species

Lettuce from the investigated site was contaminated with pathogenic bacterial species including *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* coagulase positive. Worse, all the samples from the twelve study blocks 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 9.4×10^4 to 1.8×10^5 , whereas the standard predicts 10 to 10^2 (Figure 7). For *S. aureus* coagulase +, the charges in CFU/g ranged from 5.9×10^4 to 1.4×10^5 while the standard indicated 10^2 (Figure 7). Where all the standards in food microbiology on *Salmonella* sp are a total absence in 25 g of food, there were loads of 6.1×10^4 to 8.2×10^4 CFU/g for the lettuce samples of the different study blocks (Figure 7). The charges for *E. coli*, *Salmonella* sp and *S. aureus* coagulase positive from the different study blocks were unevenly distributed from one block to another. These loads were therefore statistically different ($p > 0.05$) in the different blocks.

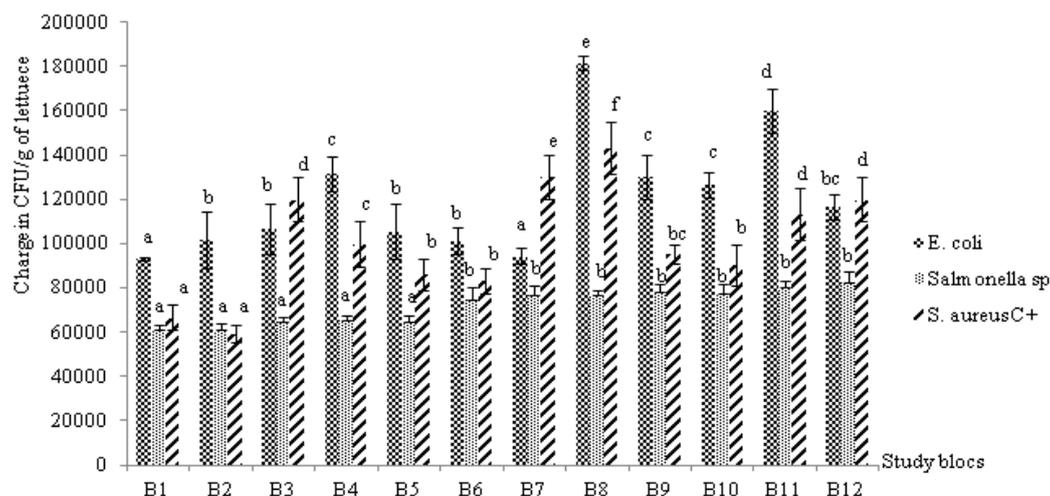


Figure 7. Numbers in CFU/g of pathogenic bacterial species in lettuce according to the study blocks. Values with the same letters are not significantly different ($P>0.05$)

4. Discussion

The production of lettuce for the consumption of the people of the city of Daloa is urban. This activity was practiced by adults aged between 30 and 60, then dominated by the female gender (58.1 %) against (41.9 %) for men. These data differ from other data provided in other similar studies. Indeed, the works of Ackerson & Awuah (2010) and those Kenmongue *et al.* (2010) carried out in Ghana and Cameroon respectively, urban and periurban market gardening was dominated by men and the majority of producers were between 24 and 68 years old. Urban and periurban production of lettuce farmers in Abidjan was also dominated by men (83 %) according to

Koffi *et al.* (2012). If the age of producers between 30 and 60 years could be explained by the arduous nature of market gardening, the sudden dominant appearance of women could have a purely local explanation. In fact, in agricultural areas dominated by cash crops (coffee or cocoa); men's main activity, food crops such as vegetable crops are left to women. Urban lettuce producers in Daloa were predominantly Ivorians (58.1 %), unlike those in Abidjan who were dominated by non-nationals (88.7 %) in a similar study (Koffi *et al.*, 2012). The preponderance of nationals in this study is explained by the fact that agriculture is still the main activity in the deep country (Daloa) unlike the big capitals like Abidjan where the activities are much diversified. The high rate of illiterate producers (80.6 %) results from the high rate of illiteracy among the populations in the study area (61.9 %) according to (RGPH, 2014). In addition, a study on market gardening in Bouake (central Côte d'Ivoire) revealed that this activity was practiced by people with no education (Fondio *et al.*, 2011). The main inputs for production were either poultry manure or a mix of dung, cow dung and chemical fertilizer. In addition, all producers (100 %) used surface or runoff water for watering lettuce. These same risky practices have been noted in other studies. In a study of the diversity and dynamics of *Salmonella* isolated from lettuce in Niger, soil amendments were mainly cow dung and chemical fertilizer (Alio *et al.*, 2017). Exclusive use of surface water or gutters was also reported. In studies on perceptions of risk of contamination of urban and periurban crops, in Accra (Ghana), (Keraita *et al.*, 2008) as in Belgium (Holvoet *et al.*, 2014a), the majority of producers also used surface water or sewage for watering vegetables. The technical process of lettuce production practiced by urban producers was therefore empirical and unconventional or unknown. Some crucial provisions on the boards for a good production (width, length and spacing between boards) were royally ignored by these producers. In addition, no initial disinfection of the plants was carried out to avoid feathers, due among other things to fungi, insects and nematodes. In addition, soil amendments (fed with poultry and cow dung) were not processed and were used without precautions. All these facts are due to the low level of education of producers. The survey revealed that only about 20% were educated. Amponsah-Doku *et al.* (2010), Soendjojo (2012) and Woldetsadik *et al.* (2017) also reported empirical or artisanal production techniques in their studies of urban and periurban market gardeners. The informal nature of this agriculture, the high level of illiteracy and the lack of training programs on good practices in urban farming could justify the behavior of producers who were not aware of the risks of contamination from gardening practices. These farming practices would make lettuce susceptible to contamination, including microbial contamination.

Microbiological analyzes have shown the contamination of lettuce produced at the study site. The lettuces of the different blocks were heavily contaminated by different flora including mesophilic aerobic germs, total coliforms, enterobacteria and yeasts and molds. The same flora had also been identified in urban production lettuce in other works (Mohammad *et al.*, 2013). Similarly, Koffi *et al.* (2011), Mngoli & Ng'ong'ola-Manani, (2014) and Akusu *et al.* (2016) isolated enterobacteria in lettuce in similar studies in Côte d'Ivoire, Malawi and Nigeria, respectively. The strong presence of these microfloras would translate into a marked deficit of good production and hygiene practices in the study site. High loads of these microfloras were also reported in other studies. Fecal coliforms were found in lettuce at production sites with loads ranging from 10^3 to 10^5 CFU/g and 10^3 to 10^4 CFU/g respectively, in Ghana in the works of Cobbina *et al.* (2013) and Ethiopia those Woldetsadik *et al.* (2017). Mesophilic aerobic germs (3×10^5 to 1.1×10^6 CFU/g) and fungal flora (2×10^3 to 2×10^4) were reported in lettuce grown in Romania (Soendjojo, 2012). The site of this study, located in downtown Daloa and in shallow water, regularly receives wastewater, sewage and gutter water on a continuous basis without any treatment. In addition, these waters are either directly used for watering or they communicate directly in the surface waters of the lowlands that were used for this purpose. These waters, once contaminated would therefore be the source of contamination of lettuce produced. Several studies have reported this form of contamination. For Koffi *et al.* (2011) in Côte d'Ivoire, Abbou *et al.* (2014) in Morocco and Holvoet *et al.* (2014b) in Belgium, irrigation water from urban vegetable crops was the main source of microbial contamination of production. Coagulase positive *Staphylococcus aureus* has been reported in work in lettuce in Brazil (César *et al.*, 2015). In the present study, the presence of enterobacteriaceae could be due to the precarious hygienic conditions in which these leafy vegetables are grown as already stated by other authors Amoah *et al.* (2007) and Koffi *et al.* (2012) after work on vegetables produced in urban areas or periurban. The urban lettuce producers of the investigated site used large quantities of poultry droppings (sometimes fresh), and beef dung as fertilizer for soil fertilization. This agricultural practice would favor permanent fecal contamination, hence the strong presence of fecal germs such as *E. coli*, *Salmonella* sp. The presence of these species has already been reported in several similar studies (Pettersson *et al.*, 2010; Schikora *et al.*, 2011; Koffi *et al.*, 2012; Jensen *et al.*, 2015; Traoré *et al.*, 2015). In a similar study in Ghana, the load of *E. coli* counted in lettuce on an urban site ranged from 10^3 to 10^4 CFU/g (Cobbina *et al.*, 2013). In New Zealand, *S. aureus* was counted in lettuce from various production systems with feeds ranging from 10^2 to 10^3 CFU/g (Wadamori *et al.*, 2016). *Salmonella* sp (0 to 10^4) loads have been found

recently in urban lettuce production in Ghana (Abakari *et al.*, 2018). The high contamination of lettuce is also due to soil that has been heavily contaminated by the presence of dumpsites in the area, surroundings in addition to wastewater. Indeed, human excrement, farm animals such as oxen, sheep, other animals (lizards, migratory birds, dogs and cats) are constantly straying there. Their excreta are carried by rain runoff to soils, wells and other water sources used for watering. The urban lettuce of the investigated site would be a source of microbiological hazards, which would cause multiple infectious diseases such as diarrhea, gastroenteritis, typhoid and paratyphoid fevers. Its consumption constitutes a real risk of infection or a source of food poisoning which can lead to a public health problem.

5. Conclusion

Urban agriculture is a major source of lettuce (*Lactuca sativa* L.) for urban populations, as is the case in Daloa. The assessment of the risk of microbial contamination revealed that consumption of lettuce (*Lactuca sativa* L.) from urban production in the city of Daloa would pose a risk to the health of consumers. High loads of microflora reflecting a deficit of good production and hygiene practices such as fecal coliforms, enterobacteria, mesophilic aerobic germs, yeasts and molds have been discovered. Pathogenic bacterial species such as *Escherichia coli*, coagulase positive *Staphylococcus aureus* and *Salmonella* sp were detected in all samples from the 12 blocks of the investigated site. The consumption of this lettuce would present a real danger to the health of consumers. The profile of producers, the upstream empirical production route and the difficult production conditions would increase the risk of microbial contamination and even other types of contamination. Thus, the competent authorities must raise awareness and raise awareness of the health risks to consumers. Establishing regulations for urban agriculture could limit the risk of contamination. An organization of urban agriculture with good training in agricultural production routes would contribute to the food security of urban populations and create jobs for urban vulnerable groups. Health education would be needed to prevent the health risks of consuming urban lettuce and to prevent possible foodborne infections. Good communication about these identified hazards would help the well being of consumers.

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