



# **Microbial Contamination Potential and Antibiotic Resistance Profile of *Staphylococcus aureus* and *Escherichia coli* Isolated from *Ipomoea batatas* Leaf**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. Authors PYA, CKK and HS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PYA, CKK and MW managed the analyses of the study. Authors PYA and LBM managed the literature searches. All authors read and approved the final manuscript.

## **Article Information**

DOI: 10.9734/IJPR/2020/v5i430147

Editor(s):

(1) Dr. Olufemi, Frederick Oyewole, Federal University of Agriculture, Nigeria.

Reviewers:

(1) Daira Radouane, University of Skikda, Algeria.

(2) Cherry C. Favor, Southern Luzon State University, Philippines.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63450>

**Original Research Article**

**Received 02 October 2020**  
**Accepted 09 December 2020**  
**Published 26 December 2020**

## **ABSTRACT**

Traditional leafy vegetables are recommended because there are important sources of nutrients and they protect against certain non-communicable diseases. This study aimed at assessing the microbiological quality of *Ipomoea batatas* leaves and evaluating the susceptibility to antibiotics of isolated *S. aureus* and *E. coli* strains.

Heighten samples of *I. batatas* leaves collected were analyzed for research / enumeration of mesophilic total aerobic flora, enterobacteriaceae, yeasts, molds, fecal coliforms, fecal

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streptococci, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* strains and *Escherichia coli*, *Staphylococcus aureus*. The microorganisms were identified using appropriate medium and classical biochemical tests. The susceptibility to eleven conventional antibiotics was investigated by the disk diffusion method.

The *I. batatas* leaves showed high contamination by various flora, in particular flora indicative of general pollution (aerobic mesophilic germs, yeasts and molds, enterobacteria) and flora of fecal origin (fecal coliforms and fecal streptococcus). In addition, the presence of pathogenic species such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. was noted in the samples analyzed. Globally, the highest resistance proportion has been observed with penicillin (~65%) and none of the *S. aureus* strains was resistant to vancomycin.

From a microbiological point of view, *Ipomoea batatas* leaf intended for consumption is not suitable for human consumption. It is therefore important to educate producers to improve their production route in order to have better microbiological quality *I. batatas* leaves.

**Keywords:** Microbial contamination; *Ipomoea batatas* leaf; pathogenic bacteria; Ivory Coast.

## 1. INTRODUCTION

Urban agriculture has developed due to the growth of the population and the evolution of the eating habits of city dwellers. This agriculture is also supported by the employment crisis and the impoverishment of city dwellers. This situation contributes to increasing the number of market gardeners and to increasing the cultivated areas in the cities. The direct advantage of this kind agriculture is the rapid supply of fresh produce to urban populations [1]. In addition, it produces economic and social functions for the city; through the direct and indirect jobs, it provides [2-3]. Several types of crops are thus produced, notably the leafy vegetables and especially traditional leafy vegetables.

Traditional leafy vegetables, also called native leafy vegetables, denote cultivated or wild plant species whose leaves are used in food [4]. The consumption of traditional leafy vegetables in general is highly recommended for protection against certain diseases such as cancer, obesity, cardiovascular diseases and diseases of the intestinal transit [5-6]. They are important sources of nutrients such as vitamins, proteins, minerals, and soluble fiber, among others [7-8]. However, despite all the nutritional and economic advantages of growing and consuming traditional leafy vegetables, the health risks associated with production conditions are numerous [9].

In most developing countries marked by the lack of financial support for drinking water and fertilizers for soil fertilization, market gardeners are forced to use wastewater for irrigation and animal excrement as soil fertilizer [10-12]. In addition, the flow of rainwater to these crops also

carries other types of pathogenic microorganisms of fecal origin, largely originating from the defecation of domestic or wild animals [13]. These practices could favor a strong contamination of vegetables by microorganisms, some of which can be dangerous for the consumer. According to Petterson et al. [11], the consumption of such vegetables is a potential risk factor for infection with enteropathogenic bacteria such as *Salmonella* and *Escherichia coli* O157. Thus, many cases of food poisoning linked to the ingestion of contaminated vegetables have been identified around the world [14-16]. Annual global estimates are in the region of tens millions cases of vegetable-borne diseases, often with deaths, especially in Africa. It is therefore imperative to minimize the risks of contamination of urban crops, especially that of traditional leafy vegetables.

In Ivory Coast, urban and peri-urban agriculture is often practiced on marshy sites, in cities or around cities. It is growing rapidly in large cities with strong urbanization such as Abidjan, Yamoussoukro, Bouaké or Daloa where food needs are growing and diversified [12]. According to Atchibri et al. [17], Ivoirians consume several traditional leafy vegetables such as vegetable coretes (*Corchorus olitorius*), amaranth (*Amaranthus hybridus*), sorrel (*Hibiscus sabdarifa*), black nightshade (*Solanum nigrum*), potato leaves (*Ipomoea batatas*), celosia (*Celosia argentea*), spinach (*Spinach oleracea*) and African cabbage (*Cleome gynandra*). Production from the cultivation of these leaves is highly subject to the risk of microbial contamination. This study aims to assess the microbiological quality of *Ipomoea batatas* leaves collected in the city of Daloa and, in the other hand, evaluate the susceptibility to

antibiotics of isolated *S. aureus* and *E. coli* strains.

## 2. MATERIALS AND METHODS

### 2.1 Sampling and Sample Collection

The sample of *Ipomoea batatas* leaves were collected directly on the market garden products (Fig. 1). Thus, three planks of *I. batatas* leave constitute a study block. From each block, three mature *I. batatas* plants were randomly selected. For this study, 18 samples were collected in sterile stomacher bags, then transported to laboratory for microbial analysis in icebox (4-8°C).

### 2.2 Microbial Analysis

For the microbiological analysis, 25 g of each *I. batatas* leave sample was mixed with 225 ml of sterile buffered peptone water and then was incubated at 37°C for 1 to 3 h the enrichment. From the mixed solution, a serial decimal dilution was made with peptone water. Dilutions  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  were used for inoculation in appropriate media.

### 2.2.1 Research of mesophilic total aerobic flora

The enumeration of mesophilic total aerobic flora was performed, in duplicates, on Plate Count Agar (PCA). For each above-mentioned dilutions, 1 ml was spread into sterile Petri dishes and 20 ml of PCA agar precooled at 50°C [18] were added. After homogenization and solidification, the samples were incubated at 30°C for 72 h. Only whitish colonies were counted. The retained boxes contain between were those containing between 30 and 300 colonies.

### 2.2.2 Research of enterobacteriaceae

The enumeration of mesophilic total aerobic flora was performed, in duplicates, on VRBG Agar. The each selected dilutions; 1 mL was transferred to sterile Petri dish and 20 mL of cooled to 50°C VRBG Agar [19] were added. After homogenization and solidification, each sample were incubated at 37°C for 24 h. Only red or pink colonies were counted and boxes containing between 15 and 150 colonies were considered.

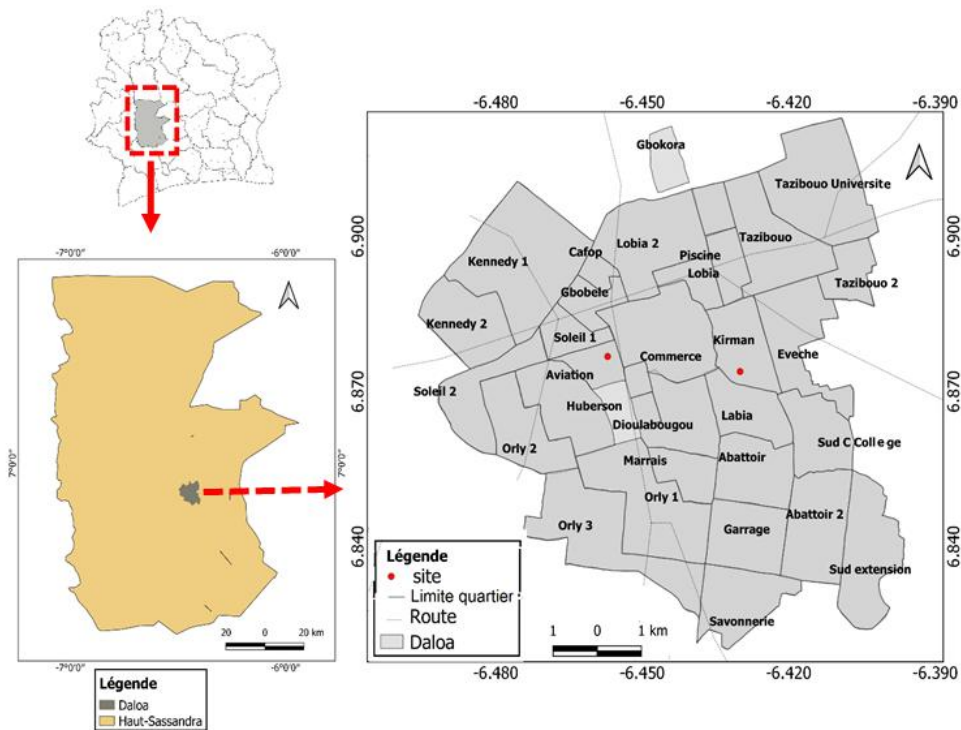


Fig. 1. Geographic location of the area and sampling site

### 2.2.3 Research of yeasts and molds

The enumeration of yeasts and molds was performed, in duplicates, on Sabouraud Chloramphenicol Agar. Then, 1 ml of each selected dilution was transferred to sterile Petri dish and about 20 mL of the medium Sabouraud Chloramphenicol precooled at 50°C were added [20]. After solidification, the dishes were incubated at 25°C for 5 days. Yeast colonies (white, creamy, transparent) and mold (downy, rough) were counted and dishes containing between 15 and 150 colonies were considered.

### 2.2.4 Research of fecal coliforms

Fecal coliforms were enumerated on VRBG agar in duplicates. One ml of each dilution was transferred to a sterile Petri dish and about 20 mL of VRBG agar were added [19]. After homogenization dishes were incubation of 44°C for 24 h. Characteristic colonies were red. Dishes considered for enumeration were those containing between 15 and 150 characteristic colonies.

### 2.2.5 Research of fecal streptococci

Fecal streptococci were enumerated on BEA agar medium, in duplicates. One ml of each selected dilution was transferred to a sterile Petri dish and about 20 ml of BEA agar medium, precooled to 50°C, was added [21]. After homogenization dishes were incubation at 37°C for 24-48 h. Characteristic fecal streptococci colonies appear black. Dishes considered for enumeration were those containing between 15 and 150 characteristic colonies.

### 2.2.6 Research of *Staphylococcus aureus*

*Staphylococcus aureus* were identified and enumerated, in duplicates, on Baird Parker + egg yolk + potassium tellurite + 0.2% sulphamethazine. Thus, 0.1 ml of each selected dilution was spread to a sterile Petri dish containing Baird Parker + egg yolk + potassium tellurite + 0.2% sulphamethazine [22]. Dishes were incubated at 37°C for 48 h. Characteristic *Staphylococcus aureus* colonies appear Black, shiny, convex surrounded by a clear zone. Dishes considered for enumeration were those containing between 15 and 150 characteristic colonies.

### 2.2.7 Research of *Escherichia coli*

*Escherichia coli* were identified and enumerated, in duplicates, on Rapid *E. coli* medium. Thus, 0.1

ml of each selected dilution was spread to a sterile Petri dish containing Rapid *E. coli* medium [23]. Dishes were incubated at 44°C for 24-48 h. Dishes considered for enumeration were those containing between 15 and 150 characteristic colonies.

### 2.2.8 Search of *Salmonella* strains

*Salmonella* strains were isolated following three steps: pre-enrichment on buffered peptone water (at 37°C for 24 h), enrichment on Rappaport Vassiliadis (at 44°C for 24 hours) and isolation on Hecktoen agar (at 37°C for 24-48 h) [24]. The typical colonies observed were green or blue with a black center.

## 2.3 Enumeration

Number was evaluated of microorganisms per milliliter of sample (CFU/mL) from the number of colonies obtained in the chosen Petri dishes was carried using the equation previously described [25]. The standards for assessing the microbiological quality of potato leaf were taken from the Microbiological Criteria for Foodstuffs - Guidelines for Interpretation of Luxembourg, supplemented by the reference normative of microbiological criteria of human foods [26]. The determination of the origin of fecal contamination was based on the criteria defined by authors of reference [27]. According to these authors, the contamination was of animal origin if the fecal coliform / fecal streptococci ratio indicates 0.7 and of human origin if this ratio was greater than 4.

## 2.4 Susceptibility of *Staphylococcus aureus* and *Escherichia coli* Isolates to Antibiotics

The susceptibility of isolated *E. coli* and *Staphylococcus aureus* strains to even conventional antibiotics (Bio Rad, England) was performed using the recommendations and interpretation of the French Society of Antibioqram [28]. The tested antibiotics were ceftriaxone (CEF 30 µg), penicillin (PEN 10 µg), amoxicilline (AMX 25 µg), kanamycine (K 30µg), Gentamycine (G 10µg), tobramycine (TM 10 µg), levofloxacin (LVX 5 µg), norfloxacin (NOR 10 µg), ofloxacin (OFX 5 µg), Vancomycine (VAN 30µg), Erythromycine (ERY 15 µg), Imipenem (IMP 10 µg).

## 2.5 Data Analysis

The raw data was collected on benchtop sheets and analyze with Microsoft Office Excel 2016

spreadsheet. The statistical texts and graphs were produced using the GraphPad Prism 7. The threshold of statistical significance was set at  $p < 0.05$ .

### 3. RESULTS

#### 3.1 Contamination Flora and Fecal Contamination Flora of *Ipomoea batatas* Leaves

The microbiological analysis shows the level of microbiological contamination of the samples of *I. batatas* leaf. Thus, microorganisms count reveals the main spoilage and good hygiene level. Those microorganisms including fungal flora (yeasts / molds), mesophilic aerobic germs and enterobacteria were observed in all samples. In addition, all the CFU / g loads were all well above the expected microbiological quality standards. The load in CFU / g for aerobic mesophilic germs varied from  $2.5 \times 10^7$  to  $3.7 \times 10^7$  CFU / g while the standard only provides for  $10^6$  CFU / g. For the fungal flora, the charges varied from  $1.3 \times 10^7$  to  $1.9 \times 10^7$  CFU / g while the standard provides for the value of  $10^4$  CFU / g. As for enterobacteriaceae, the load oscillated between  $1.3 \times 10^6$  and  $2.4 \times 10^6$  CFU/g while the standard only provides  $10^4$  CFU / g (Table 1).

The results of the microbiological analyzes showed that all the samples were highly contaminated with fecal coliforms and fecal streptococci. All of the loads exceeded the expected microbiological quality standards. The load in CFU/g for fecal coliforms (FC) varied from  $7.7 \cdot 10^4$  to  $3.36 \cdot 10^5$  CFU/g, while the standard

only provides for  $10^2$  CFU/g. That of fecal streptococci (FS) ranged between  $2.03 \cdot 10^5$  and  $5.3 \cdot 10^5$  CFU/g (Fig. 2). The FC/FS ratio gives 0.61. This result shows that the origin of fecal contamination of the potato leaf is strictly animal (FC/FS  $< 0.7$ )

#### 3.2 Pathogenic Flora of *Ipomoea batatas*

The samples of *Ipomoea batatas* leaf shows high contamination loads of *Escherichia coli*, Coagulase-positive *Staphylococcus aureus* and *Salmonella*. In addition, the samples analyzed all showed the presence of both *E. coli*, *S. aureus* and *Salmonella* with loads higher than the standards. The load of *E. coli* ranged from  $9 \cdot 10^4$  to  $2.52 \cdot 10^5$  CFU / g while the standard provides for  $10$  to  $10^2$  CFU / g. that of coagulase-positive *S. aureus* ranged from  $5 \cdot 10^3$  to  $1.63 \cdot 10^5$  CFU / g while the standard provided for only  $10^2$  CFU / g (Fig. 3). The standard on *Salmonella* indicates a total absence in 25 g of food, while all the analyzed samples presented high loads of *Salmonella*.

#### 3.3 Susceptibility of *Staphylococcus aureus* and *Escherichia coli* Isolates to Antibiotics

The susceptibility of the isolated *E. coli* and *S. aureus* varies according to the tested antibiotics (Fig. 4). Thus, independently to the strains, the highest resistance proportions (about 65%) were recorded with penicillin. Vancomycin is efficient (0% of resistance) on *Escherichia coli* strains while imipenem *Staphylococcus aureus*.

**Table 1. Contamination flora (CFU/g) of *Ipomoea batatas* leaf samples collected in Abidjan**

Samples	Aerobic mesophilic germs	Enterobacteria	Yeast and mold
E1	$3 \cdot 10^6 \pm 2 \cdot 10^5$	$1.5 \cdot 10^6 \pm 4.1 \cdot 10^5$	$1.6 \cdot 10^7 \pm 1.6 \cdot 10^5$
E2	$3.5 \cdot 10^7 \pm 1.6 \cdot 10^6$	$1.8 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.8 \cdot 10^7 \pm 8.1 \cdot 10^4$
E3	$3.6 \cdot 10^7 \pm 2.5 \cdot 10^6$	$1.8 \cdot 10^6 \pm 1.6 \cdot 10^5$	$1.9 \cdot 10^7 \pm 1.7 \cdot 10^5$
E4	$3.1 \cdot 10^7 \pm 2.5 \cdot 10^6$	$1.3 \cdot 10^6 \pm 1.6 \cdot 10^5$	$1.6 \cdot 10^7 \pm 5.7 \cdot 10^4$
E5	$2.5 \cdot 10^7 \pm 3.3 \cdot 10^6$	$2 \cdot 10^6 \pm 2.1 \cdot 10^5$	$1.3 \cdot 10^7 \pm 1.6 \cdot 10^5$
E6	$3.1 \cdot 10^7 \pm 1.6 \cdot 10^6$	$1.7 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.6 \cdot 10^7 \pm 1.6 \cdot 10^5$
E7	$3.6 \cdot 10^7 \pm 4.1 \cdot 10^6$	$2 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.9 \cdot 10^7 \pm 8.2 \cdot 10^4$
E8	$3.2 \cdot 10^7 \pm 2 \cdot 10^6$	$1.7 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.7 \cdot 10^7 \pm 1.6 \cdot 10^5$
E9	$2.5 \cdot 10^7 \pm 4 \cdot 10^6$	$2.4 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.4 \cdot 10^7 \pm 1.6 \cdot 10^5$
E10	$3.1 \cdot 10^7 \pm 4 \cdot 10^6$	$1.8 \cdot 10^6 \pm 1.6 \cdot 10^5$	$1.6 \cdot 10^7 \pm 8.2 \cdot 10^4$
E11	$2.7 \cdot 10^7 \pm 4 \cdot 10^6$	$2.3 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.5 \cdot 10^7 \pm 6.5 \cdot 10^4$
E12	$2.8 \cdot 10^7 \pm 4 \cdot 10^6$	$2.4 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.5 \cdot 10^7 \pm 5 \cdot 10^4$
E13	$3.7 \cdot 10^7 \pm 4 \cdot 10^6$	$1.6 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.9 \cdot 10^8 \pm 1.6 \cdot 10^5$
E14	$3.7 \cdot 10^7 \pm 4 \cdot 10^6$	$2 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.9 \cdot 10^7 \pm 4.9 \cdot 10^4$
E15	$2.9 \cdot 10^7 \pm 2.9 \cdot 10^6$	$2 \cdot 10^6 \pm 3.3 \cdot 10^5$	$1.5 \cdot 10^8 \pm 2.4 \cdot 10^5$
E16	$3.6 \cdot 10^7 \pm 4 \cdot 10^6$	$2.4 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.9 \cdot 10^7 \pm 2.4 \cdot 10^5$
E17	$3.3 \cdot 10^7 \pm 4 \cdot 10^6$	$1.8 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.7 \cdot 10^7 \pm 1.2 \cdot 10^5$
E18	$3.4 \cdot 10^7 \pm 2.4 \cdot 10^6$	$1.8 \cdot 10^6 \pm 2.4 \cdot 10^5$	$1.8 \cdot 10^7 \pm 2.4 \cdot 10^5$

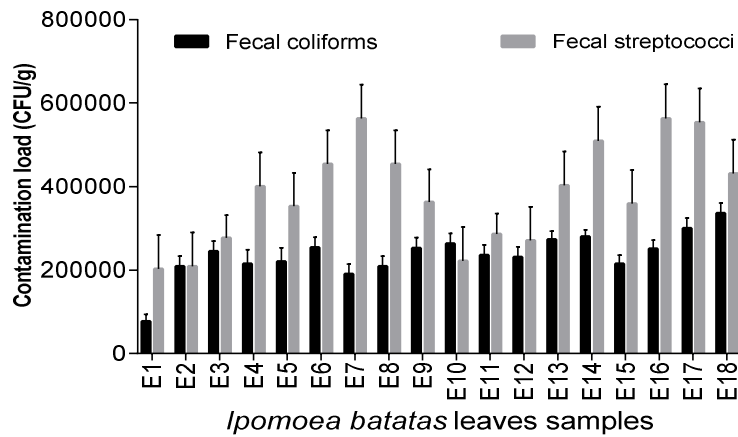


Fig. 2. Contamination load of *I. batatas* leaves samples by fecal coliforms and streptococci

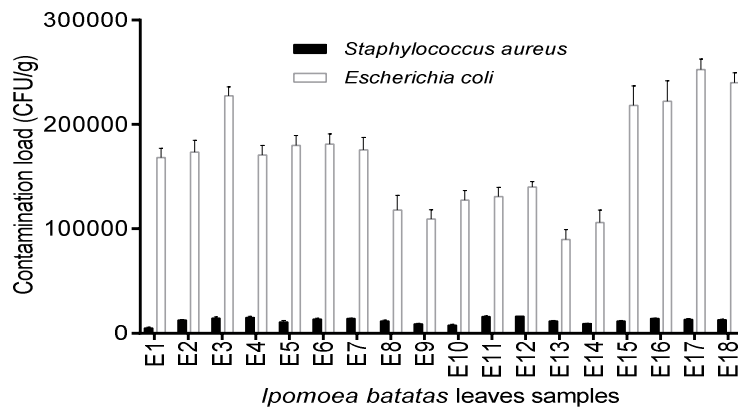


Fig. 3. Average number of contaminations by *S. aureus* and *E. coli* load of *I. batatas* leaf samples

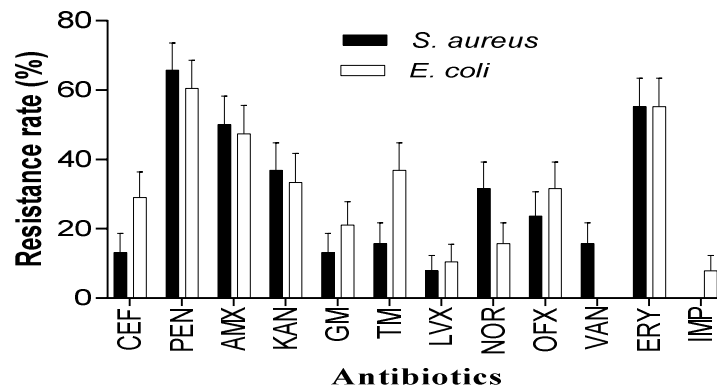


Fig. 4. Susceptibility of the isolated *S. aureus* and *E. coli* strains to the twelve tested antibiotics

CEF: ceftriaxone, PEN: penicillin; AMX: amoxicilline, KAN: kanamycine, GM: Gentamycine, TM: tobramycine, LVX: levofloxacin, NOR: norfloxacin, OFX: ofloxacin, VAN: Vancomycine, ERY: Erythromycine, IMP: Imipenem

#### 4. DISCUSSION

Microbiological analyzes have shown a high contamination by aerobic mesophilic germs (AMG), enterobacteria, yeasts, molds, faecal coliforms and streptococci. Worse, this vegetable was contaminated by both *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp, potential pathogens with variable loads. The contaminant load was well above the threshold. Levels of microbial contamination of potato leaf samples at market gardening sites varied differently. The AMG loads of the potato leaf varied from  $2.47 \times 10^7$  to  $3.7 \times 10^7$  CFU/g. The load of the fungal flora varied from  $1.33 \times 10^7$  to  $1.94 \times 10^7$  CFU/g while that of the enterobacteria oscillated between  $1.29 \times 10^6$  and  $2.45 \times 10^6$  CFU/g. Concerning faecal coliforms and streptococci, their loads varied respectively from  $7.7 \times 10^4$  to  $3.36 \times 10^5$  CFU/g and from  $2.03 \times 10^5$  to  $5.3 \times 10^5$  CFU/g. These heavy loads could be explained by the lack of hygiene in the production environment on the sites. The production site for this leaf vegetable is located in the city center, which is the city's main container for wastewater, sewage and household waste. Loads lower than Uzeh et al. [29] in Lagos, Nigeria observed those obtained during our study. Indeed, these authors have shown that the highest load of aerobic mesophilic germs in samples of other leafy vegetables was  $5.9 \times 10^6$  CFU/g. This difference could be due to the environment and the techniques of growing vegetables that differ from a country to another. On vegetable production sites, the contamination of leafy vegetables by enterobacteria could be induced by the combination of factors such as continuous watering with water from ceanes, the use of poultry droppings and the cow purse for soils enrichment. This is in agreement with the observations made by Matthys et al. [30] during their work on the social network of market gardeners in Abidjan. Similar observations indicating the involvement of wastewater in the contamination of vegetables produced on market garden production sites have been reported in Ghana [10,31], Togo [32], Senegal [33] and Ivory Coast [34].

It has been noted that the use of poultry manure and poorly composted manure as fertilizer to fertilize the soil promotes permanent fecal contamination of vegetables. In fact, enterobacteria are normal hosts of the digestive tract of humans and animals. It is able to proliferate in abundance in the environment and participate in the major cycles of organic matter

degradation [35]. These indicators of fecal contamination provide a more picture of potentially pathogenic germs. In addition, the high loads of faecal coliforms in the leafy vegetables of the two sites could be explained by the use of the beef purse and poultry manure associated with the presence of faeces of human origin. Uzeh et al. [29] made similar observations during their study on the contamination of lettuce by *E. coli*. In addition, the presence of animals such as reptiles, birds, domestic animals can contribute to fecal contamination of production sites. The excreta of the above-mentioned animals are transported by runoff during the rainy season to the sources of irrigation water for vegetables [32,36], which could explain the presence of these sprouts in vegetables. The high rate of faecal streptococci would also be justified by the presence of animals such as dogs on the sites, the use of the purse, droppings as fertilizer and the presence of faeces of human origin. *E. coli* are potentially pathogens (diarrhea) and are often reported in environments where health conditions are precarious [37-38]. Consequently, the consumption of these vegetables without any precaution could be associated with diarrheal diseases [39]. In addition, the presence of pathogens indicates that good hygienic practices are not observed during production [40].

The Ratio faecal coliforms / faecal streptococci (FC / FS) is 0.61 (R < 0.7), which indicates animal contamination. Our results corroborate those of several authors who indicated that the contributions of wild bird and domestic animal excrement, human excrement and household waste that end up in unprotected shallow wells on vegetable farms are causing faecal contamination of vegetables [36,41]. The contamination with *Staphylococcus aureus* could be explained by a lack of hygiene of the market gardeners. Indeed, *S. aureus* is an indicator of handling, which can result from human pathways, skin and superficial wounds [42]. Our results confirm those of several authors who indicated that excessive handling of vegetables contributes to increasing the risks of contamination by *S. aureus* [43-44]. The presence of this bacterium in vegetable samples reflects poor hygiene practice in the production chain and poses a risk to the health of consumers. Note that *S. aureus* is a pathogenic bacterium responsible for foodborne illness [45]. According to some authors, enteric pathogens are the main contaminant of vegetables following poor hygiene practice [46-47]. To end, all the

analyzed samples were contaminated with *Salmonella* sp. This species was found with different proportions in the samples analyzed. The use of poultry droppings, the presence of domestic animals such as pigs and cold-blooded wild animals such as frogs and lizards, poor sanitary conditions at the vegetable production sites could explain the presence of this species.

## 5. CONCLUSION

The lack of sanitation in the various markets, accompanied by a total lack of disinfection of vegetables by the vendors would predispose to all forms of contamination if they were not contaminated at the production sites. Microbiological analyzes have shown that the potato leaf produced was highly contaminated with various flora, in particular flora indicative of general pollution (aerobic mesophilic germs, yeasts and molds, enterobacteria) and a flora of fecal origin (fecal coliforms and streptococcus). In addition, there is a presence of potentially pathogenic bacterial species such as *S. aureus*, *E. coli* and *Salmonella* sp. The presence of these germs would reflect a deficit in good production practices and hygiene, which would represent a danger for consumers. It is thus recommended to avoid the consumption of the crude *Ipomoea batatas* leaves without cleaning with appropriate detergents.

## DATA AVAILABILITY

The data used in this manuscript are available from the corresponding author upon request.

## ACKNOWLEDGEMENTS

We address our sincere thanks to the gardeners and all those who assisted us in carrying out this work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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