Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Microbiology

Prevalence of Pathogenic Enterobacteria in Market Garden Produce Grown in the Peri-Urban Area of Abidjan, Ivory Coast

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DOI: 10.36347/sajb.2023.v11i10.004 | **Received:** 27.08.2023 | **Accepted:** 03.10.2023 | **Published:** 09.10.2023

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Abstract **Original Research Article**

This study, which is a contribution to national health surveillance of pathogens, was conducted to determine the prevalence of pathogenic Enterobacteriaceae in the market garden environment of Abidjan. To this end, samples of lettuce, irrigation water, manure and soil were taken from three (03) major market gardening sites in Abidjan. Microbiological analyses of these samples were carried out. The enterobacteria obtained after isolation were identified. The results revealed ten (10) bacterial genera belonging to the Enterobacteriaceae family, including 15 bacterial species in the samples of lettuce, irrigation water, manure and soil analysed. These included Salmonella enterica with serotypes (*Salmonella Gallinarum*, *Salmonella Arizonae*, *Salmonella Choleraesus*), Salmonella spp, *Escherichia coli*, *Shigella boydii*, *Shigella sonnei*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter gergoviae*, *Enterobacter agglomerans*, *Serratia marcesens*, *Serratia phymutica*, *Afnia alvei*, *Citrobacter freuidii Citrobacter diversus*, *Providencia alcalifasciens*, *Proteus vulgaris* and Yersinia spp. The presence of these pathogenic Enterobacteriaceae indicates a potential risk associated with the consumption of lettuce produced in Abidjan. The Ivorian authorities should take preventive measures, such as educating market gardeners about the harmful effects of contaminated water used to irrigate vegetables, and the use of animal droppings as fertiliser to fertilise the soil, to avoid the future proliferation of infectious diseases such as typhoid fever and bacillary dysentery among the population of Abidjan.
Keywords: Prevalence, pathogenic Enterobacteriaceae, market gardening, Abidjan.

1-INTRODUCTION

Urban and peri-urban agriculture is a widespread practice throughout the world, and is of particular interest in African cities (Koffi *et al.*, 2012). In West Africa, it is a source of fresh fruit and vegetables and has become increasingly important over the last 30 years (Maundu *et al.*, 2009; Chepkoech *et al.*, 2018). Following the example of many African countries, Côte d'Ivoire has seen prodigious growth in urban and peri-urban agriculture, particularly in Abidjan. Vegetable production there has risen by 30% in ten years, reaching 700,000 tonnes in 2001 (CNRA, 2004). In Abidjan, leafy vegetables have become an important part of market garden production, helping to supply fresh produce to the markets of 10 communes. Among these leafy vegetables, lettuce (*Lactuca sativa*), commonly known as "salad" is much appreciated for its soft, tender and crunchy leaves (Wognin *et al.*, 2013).

The benefits of regular consumption of these vegetables have been clearly demonstrated in terms of protection against chronic degenerative diseases such as cancer, cardiovascular disease and diabetes, and prevention of vitamin A and C deficiencies (Berger *et al.*, 2009; Ramos *et al.*, 2013), There is some evidence that raw, edible vegetables are a source of infection (Koffi-Nevry *et al.*, 2011; Olaimat *et al.*, 2012). Vegetables can harbour human pathogens without showing any signs of deterioration, which raises concerns about food safety. Throughout the production chain (cultivation and harvesting), vegetables can be exposed to various routes of contamination by enteric pathogens (Lenzi *et al.*, 2021).

Contamination of market garden produce is often linked to several factors, including the quality of irrigation water and animal droppings used as fertiliser for the soil (Alio *et al*., 2017) Cases of food poisoning linked to the ingestion of vegetables produced under these conditions have been identified throughout the world (Tanouti, 2016; Maiwore *et al*., 2017). Barro *et al.*, (2002) revealed that vegetables sold in the streets of Ouagadougou and Bobo-dioulasso in Burkina Faso were the cause of 65% of cases of diarrhoea and digestive disorders. Consumption of fruit and vegetables is therefore a potential risk factor for infection by enteropathogenic bacteria. To date, few studies in Côte d'Ivoire have revealed the prevalence of pathogenic enterobacteria in the market garden environment. The general objective of this study is to determine the prevalence of pathogenic enterobacteria in market gardening in Abidjan.

2-MATERIALS AND METHODS

Study materials

The study was carried out on lettuce (Lactuca sativa), irrigation water, manure and vegetable growing soil from three major market gardening sites, namely site 1 (Port Bouet ASECNA), site 2 (Port Bouet Adjahui) and site 3 (Cocody M'Pouto).

Culture media and reagents

Buffered peptone water was used to dilute the samples and prepare the stock suspensions. VRBG agar (Bile Glucose Agar with Crystal Violet and Neutral Red, AES CHEMUNEX - Combourg - France) was used for enumeration of enterobacteria. The API 20E gallery (Biomérieux, Paris, France) was used to identify strains of Enterobacteriaceae isolated using API Web software.

Methods

Description and selection criteria for the study areas

Description of the study areas

A survey of the various production sites in Abidjan identified three main market garden production sites for this study. Three (03) sampling sites were selected:

- Site 1, located in the commune of Port-Bouet, alongside the road to the Félix Houphouet Boigny airport (ASECNA), is one of the largest market garden sites in the city of Abidjan. It covers an area of around 30 hectares and is dominated by lettuce;
- Site 2, located in the same commune, near the village of Adjahui, covers an area of 35 hectares;
- Site 3, located in the commune of Cocody (Pouto) on the edge of the Ebrié lagoon, with an area of 23 hectares, is also dominated by lettuce cultivation (Figure 2).

Study area selection criteria

Lettuce-growing sites were identified on the basis of a number of factors, including the nature of the vegetable production, the age of the farmers, the length of time they had been farming and the area farmed.

Inclusion factors

On the production sites, the choice of respondents was guided by the size of their farms, which were at least one (01) hectare in size and mainly produced lettuce. Market gardeners using manual, watering, with at least two (02) years of experience in market gardening and working on well-defined sites were selected. Their main activity is market gardening, and they do this all year round.

Exclusion factors

The people who were not included in this survey are those who grow vegetables in isolation, i.e. in a domestic setting and on small plots. People who do not grow lettuce were not included.

Sampling

Sampling was carried out at the three production sites selected (Port-Bouët ASECNA, Port Bouet Adjahui and Cocody M'Pouto). At the market garden production sites, samples were taken from plots set up by gardeners. At each study site, a plot measuring approximately 35 m x 20 m, i.e. 700 m2, was marked out and used as a sampling point for lettuce, irrigation water, manure and crop soil. One sample of lettuce corresponded to five plants pulled directly from the ground and placed in sterile stomachers. Also, a sample of irrigation water corresponds to 500 mL of water taken by dipping each of the 500 mL sterile bottles directly into the wells. As for manure, one sample corresponds to 100 g obtained directly from the gardeners' stocks and then placed in sterile stomacher bags. Finally, a sample of cultivated soil corresponds to approximately 100 g of soil taken directly from the place where the lettuces are harvested and also placed in sterile stomacher bags. Single-use sterile gangs were used to take the various samples. Four samples of each matrix studied (lettuce, water, irrigation, manure and soil) were taken each month, depending on the season and the study site. Sampling took place over a twelve (12) month period from April 2013 to March 2014.

A total of 130 lettuce samples were taken from the production sites, 128 irrigation water samples, 136 manure samples and 92 soil samples were taken from the various market garden production sites. A total of 486 samples were taken from all the sites studied.

The collected and labelled samples were immediately placed in a cooler containing dry ice and transported to the microbiology laboratory of the Centre Ivoirien Anti-pollution (CIAPOL) for analysis.

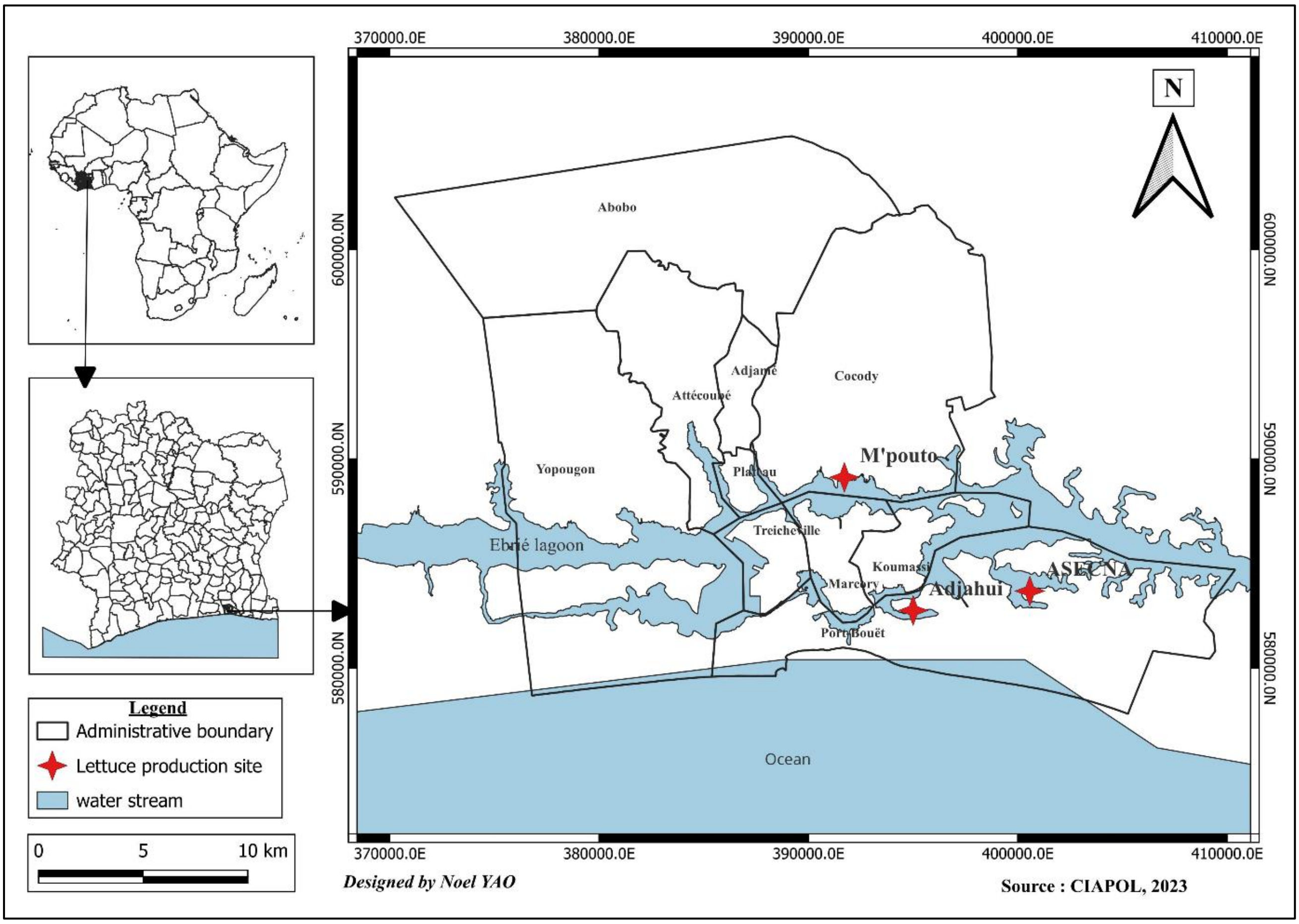

Figure 1: Map of Abidjan showing production sites

Bacteriological analyses

Enumeration of enterobacteria (Standard ISO 21528-1)

Enterobacteria were counted on VRBG agar (Crystal Violet, Neutral Red, Bile, Glucose). Inoculation was carried out by spreading 0.1 mL of each dilution on VRBG agar previously poured into Petri dishes. The plates were then incubated at 37°C for 24 hours. The red colonies characteristic of Enterobacteriaceae were counted. Plates containing between 15 and 150 colonies were selected for counting.

Expression of Results

Methods for calculating average germ loads

Bacterial loads in lettuce samples were determined according to germ category. The number N of germs, expressed in colony-forming units (CFU) per gram of sample, was determined using the following equation:

$$N\ (CFU)/g\ ou\ mL) = \frac{\sum C}{V.\ (n1+0{,}1\ n2).d}$$

C: Number of colonies in two successive dilutions

V: Volume of inoculum

n1: Number of Petri dishes inoculated at 1st dilution

n2: Number of Petri dishes inoculated at 2nd dilution

d: 1st dilution used.

The formula for calculating the frequency of isolation of Enterobacteriaceae is as follows:

$$F = \frac{n}{N_T} \times 100$$

With F: Frequency of isolation of the germ in (%),

n: Number of each bacterial species identified,

NT: Total number of species.

Search for Enterobacteriaceae

Determination of morphological characteristics

Morphological characteristics were determined using Gram staining. Observations were made using an objective x 100. The slide was gently dried with water. Gram-negative bacilli selected for further identification appear in pink.

Determination of family characteristics

Family characteristics were determined by testing for catalase and oxidase.

Identification of Enterobacteriaceae using the API 20E gallery

The API 20E gallery is a standardised system comprising 20 microtubes containing dehydrated substrates (AFNOR, 1996). The microtubes are inoculated with enterobacteria suspensions. The reactions produced during the incubation period resulted in spontaneous colour changes or were revealed by the addition of reagents. These reactions were read using the reading table and identification was obtained using the identification software (API WEB).

Statistical analysis

A one-factor analysis of variance (ANOVA) was performed using Graph pad version 5.0 software to study the degree of difference between the means of the survey data and the bacteriological data.

3- RESULTS

Prevalence of pathogenic Enterobacteriaceae in peri-urban market gardening in Abidjan

Prevalence of pathogenic Enterobacteriaceae in lettuce

The prevalence of pathogenic Enterobacteriaceae at production and sales sites is presented in Table 1. The species of the Enterobacteriaceae family identified were almost the same regardless of the sampling site. In total, fourteen (14) species of Enterobacteriaceae belonging to eight (8) genera were identified. *Escherichia coli* was isolated from sites 1 and 3 with frequencies of 3.74% and 6.54% respectively. Three *Salmonella enterica* serotypes were identified at site 3: *Salmonella Gallinarum*, *Salmonella Arizonae* and *Salmonella Choleraesus*. *Shigella boydii* was identified in samples from site 3 with a frequency of 6.5%. *Providencia alcalifasciens* was isolated only from samples from site 1 and *Salmonella Choleraesus* only from those from site 2.

Table 1: **Percentage (%) of occurrence of Enterobacteriaceae in lettuce from production sites**

	Sampling sites		
Enterobacteriaceae identified	Site 1	Site 2	Site 3
Salmonella Gallinarum	-	$4,67^{b}$	$5,61^{b}$
Salmonella Arizonae	-	$0,93^{d}$	$1,87^{c}$
Salmonella spp.	-	-	$2,8^{c}$
Salmonella Choleraesus	-	$0,93^{d}$	-
Escherichia coli	$3,74^{b}$	$2,8^{c}$	$6,54^{a}$
Shigella boydii	-	-	$3,74^{b}$
Shigella sonnei	-	$0,93^{d}$	$0,93^{d}$
Enterobacter cloacae	-	-	-
Enterobacter aerogenes	-	$1,87^{c}$	$2,8^{c}$
Enterobacter gergoviae	-	$0,93^{d}$	-
Enterobacter agglomerans	-	$0,93^{d}$	-
Serratia marcesens	$2,8^{c}$	$0,93^{d}$	$7,48^{a}$
Serratia phymutica	$0,93^{d}$	$1,87^{c}$	$4,67^{b}$
Afnia alvei	-	-	$1,87^{c}$
Citrobacter freuidii	-	-	-
Providencia alcalifasciens	$1,87^{c}$	-	-
Proteus vulgaris	-	-	-

Not identified; in rows and columns, values with the same letters are equal according to the Neumann-Keuls test (α= 0.05).

Prevalence of Enterobacteriaceae in irrigation water

Table 2 shows the Enterobacteriaceae identified in the irrigation water of the vegetable production sites. The highest isolation frequencies, i.e. 11.76%, were observed at site 2 with Salmonella spp. and *Escherichia coli*. At site 3, *Salmonella arizonae* and *Escherichia coli* were isolated with frequencies of 10.29% and 7.35% respectively. The lowest frequency of isolation, 1.47%, was obtained at site 3 with *Salmonella Gallinarum*, *Salmonella choleraesus*, *Citrobcater diversus* and *Proteus vulgaris*. There was a significant difference (P<0.05) between the frequency of isolation of strains at site 2 and site 3.

Table 2: **Percentage (%) of isolation of Enterobacteriaceae in lettuce irrigation water from market garden production sites**

	Sampling sites		
Enterobacteriaceae identified	Site 1	Site 2	Site 3
Salmonella Gallinarum	-	8,82^{a}	1,47^{c}
Salmonella Arizonae	-	-	10,29^{a}
Salmonella spp.	-	11,76^{a}	-
Salmonella Choleraesuis	-	-	1,47^{c}
Escherichia coli	5,88^{b}	11,76^{a}	7,35^{a}
Enterobacter aerogenes	2,94^{bc}	-	4,41^{b}
Serratia marcesens	-	-	5,88^{b}
Serratia phymutica	-	5,88^{b}	2,94^{bc}
Afnia alvei	-	2,94^{bc}	-
Citrobacter freuidii	-	-	2,94^{bc}
Citrobacter diversus	-	4,41^{b}	1,47^{c}
Proteus vulgaris	4,41^{b}	-	1,47^{c}

Not identified; in rows and columns, values with the same letters are equal according to the Neumann-Keuls test (α= 0.05).

Prevalence of Enterobacteriaceae in manure

The prevalence of Enterobacteriaceae in manure from the three (3) production sites selected is given in Table 3. The prevalence varied from site to site for all the samples studied. For site 1, the highest frequency of isolation was observed with *Serratia phymutica* species (5.19%), followed by *Escherichia coli* (3.9%). *Salmonella Gallinarum* was isolated with a frequency of 10.39% in samples from site 2, unlike site 3, where the same prevalence of 10.39% was obtained simultaneously with *Escherichia coli* and *Enterobacter gergoviae* species.

Among the strains isolated, the prevalence of *Salmonella Gallinarum* in site 2, *Enterobacter gergoviae* and *Escherichia coli* in site 3 samples was significantly (P<0.05) higher than that of the other enterobacteria found in the manure. In addition, the genus Yersinia was only found in samples from site 3 at 2.6%.

Table 3: **Frequency of isolation (%) of Enterobacteriaceae isolated from manure from vegetable**

	Sampling sites		
Enterobacteriaceae identified	Site 1	Site 2	Site 3
Salmonella Gallinarum	-	10,39^{a}	5,19^{b}
Salmonella Arizonae	-	-	3,9^{bc}
Salmonella spp.	-	-	6,49^{b}
Salmonella Choleraesuis	-	-	2,6^{c}
Escherichia coli	3,9^{bc}	6,49^{b}	10,39^{a}
Shigella boydii	-	-	2,6^{c}
Enterobacter aerogenes	-	5,19^{b}	-
Enterobacter gergoviae	-	-	10,39^{a}
Enterobacter agglomerans	-	3,9^{bc}	-
Serratia marcesens	2,6^{c}	1,3^{c}	2,6^{c}
Serratia phymutica	5,19^{b}	3,9^{bc}	1,3^{c}
Afnia alvei	2,6^{c}	-	-
Citrobacter freuidii	-	-	2,6^{c}
Citrobacter diversus	-	2,6^{c}	-
Providencia alcalifasciens	1,3^{c}	-	-
Yersinia spp	-	-	2,6^{c}

Not identified; in rows and columns, values with the same letters are equal according to the Neumann-Keuls test (α= 0.05).

Prevalence of Enterobacteriaceae in cultivated soil

Table 4 shows the Enterobacteriaceae isolated from the market garden soil. *Escherichia coli* species were isolated from site 1 with a prevalence of 9.1%. Prevalences of 5.45% were obtained in samples from site 2 with *Salmonella Gallinarum*, *Salmonella Arizonae* and *Escherichia coli*. For site 3, the highest prevalences were observed with *Salmonella Gallinarum* and *Escherichia coli,* at 23.64% and 10.91% respectively. *Salmonella Choleraesus, Shigella boydii, Enterobacter aerogenes, Enterobacter gergoviae, Enterobacter agglomerans, Proteus vulgaris, Erwinia amylovera* and Yersinia spp. Were not found in the soil samples analysed from the 3 sites.

Table 4: Frequency of isolation (%) of Enterobacteriaceae from the growing soils of the vegetable production sites

	Sampling sites		
Enterobacteriaceae identified	Site 1	Site 2	Site 3
Salmonella Gallinarum	-	5,45[b]	23,64[a]
Salmonella Arizonae	1,81[c]	5,45[b]	-
Salmonella spp.	-	-	3,64[bc]
Escherichia coli	9,1[b]	5,45[b]	10,91[b]
Shigella sonnei	-	-	1,81[c]
Enterobacter cloacae	1,81[c]	-	1,81[c]
Serratia marcesens	1,81[c]	1,81[c]	7,27[b]
Serratia phymutica	1,81[c]	-	-
Afnia alvei	1,81[c]	1,81[c]	-
Citrobacter freuidii	1,81[c]	-	1,81[c]
Citrobacter diversus	-	3,64[bc]	-
Providencia alcalifasciens	3,64[bc]	1,81[c]	-

Not identified; in rows and columns, values with the same letters are equal according to the Neumann-Keuls test (α= 0.05).

4-DISCUSSION

The presence of Enterobacteriaceae in lettuce samples from production sites could be due to untreated irrigation water and manure, but also to the market garden soil. Numerous studies have implicated untreated irrigation water as a factor in plant surface contamination (Petterson *et al*., 2010; Nikaido *et al*., 2010). Direct and indirect contamination of plants by raw animal droppings, sewage or contaminated soil, wild birds and poor post-harvest practices are well documented (Barak and Liang, 2009; Mitra *et al*., 2009).

Three serotypes of *Salmonella enterica* have been identified: *Salmonella Gallinarum, Salmonella Arizonae and Salmonella Choleraesuis*. These serotypes are pathogens responsible for human infections. Many microorganisms colonise plant roots and leaf surfaces. The use of poultry manure as fertiliser could explain the presence of *Salmonella Gallinarum* on cultivation sites. Indeed, the ecological reservoir of this serotype is the digestive tract of poultry (Kokosharov and Phetisova, 2002). This result is in line with that of Beuchat and Ryu (1997), who indicated that manure composed mainly of poultry droppings makes a major contribution to the contamination of lettuce by *Salmonella Gallinarum*. The presence of *Salmonella Choleraesuis* suggests that pig faeces are also used as organic fertiliser for soil fertilisation, since the digestive tract of pigs is the main reservoir of this serotype. The ecological niche of *Salmonella arizonae*, a telluric serovar, unlike the other two serotypes, is the intestine of cold-blooded animals such as lizards, geckos and frogs (Hardouin, 2008). The presence of this bacterium in the soil could be linked to the presence of these animals in large numbers on the three production sites, due to the high level of insalubrity. The presence of *Shigella sonnei* can be explained by the presence of human waste. Production sites are sometimes used as defecation sites by neighbours. The unsanitary hands of growers who have no knowledge of hygiene practices could also contribute to the contamination of lettuce by *Shigella sonnei* (Koffi-Nevry *et al*., 2011).

Manure and irrigation water contribute significantly to the spread of human pathogens in fields and the crops grown there (Natvig *et al*., 2002; Okonko *et al*., 2008). Furthermore, the use of untreated manure to fertilise crop soils led to the isolation of Salmonella species, *Shigella flexneri* and *Escherichia coli* from lettuce and tomatoes in 2001 in Accra, Ghana (Mensah *et al*., 2001). Similar observations were also reported in the annual report of the Ghana Health Service in 2007, where school children who were fed rice, stew and salad, experienced gastroenteritis which was attributed to contaminated greens (Felgo and Sakyi, 2012). In this study, lettuce samples were contaminated with *Salmonella Gallinarum*, *Salmonella Arizonae*,

Salmonella spp, *Salmonella Choleraesus*, *Escherichia coli*, *Shigella boydii*, *Shigella sonnei*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Serratia marcesens*, *Serratia phymutica*, *Afnia alvea*, *Citrobacter freundii*, *Providencia alcalifasciens and Porteus vulgaris*. These Enterobacteriaceae were found with different prevalences in the samples analysed. Poor sanitary conditions in the areas where lettuce is grown in Abidjan could explain the presence of these enterobacteria species. According to some authors, such as Mañas *et al*., (2009) and Tham *et al*., (2010), enteric pathogens are the main contaminants of vegetables as a result of poor hygiene practices.

5- CONCLUSION

This study of enterobacteria isolated 10 bacterial genera belonging to the enterobacteria family, including 14 bacterial species in the samples of lettuce, irrigation water, manure and soil analysed. The presence of these pathogenic germs indicates a potential risk associated with the consumption of lettuce produced and sold in Abidjan. Awareness campaigns aimed at market gardeners on the risks associated with using contaminated water for irrigation and animal dung as fertiliser for fertilising the soil of vegetables such as lettuce should be carried out by the relevant authorities, in order to avoid possible contamination from food sources.

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