



Bacteriological Analysis of Contamination Level of Selected Vegetables Sold in Some Markets in Niamey, Niger

Almou Abdoulaye Alio, Alio Fody Mahamadou, Chaibou Yaou, Alio Sanda Abdel-Kader, Rabiou Mamane Moustapha, Hamidou Djelifa, Soumana Sourounda Idé, Mamadou Lewamy, Haoua Sabo Seini, Sadou Hassimi

► To cite this version:

Almou Abdoulaye Alio, Alio Fody Mahamadou, Chaibou Yaou, Alio Sanda Abdel-Kader, Rabiou Mamane Moustapha, et al.. Bacteriological Analysis of Contamination Level of Selected Vegetables Sold in Some Markets in Niamey, Niger. European Journal of Nutrition and Food Safety, 2024, 16, pp.70 - 84. <10.9734/ejnfs/2024/v16i41409>. <hal-04542668>

HAL Id: hal-04542668

<https://hal.science/hal-04542668v1>

Submitted on 11 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Bacteriological Analysis of Contamination Level of Selected Vegetables Sold in Some Markets in Niamey, Niger

**Almou Abdoulaye Alio ^{a*}, Alio Fody Mahamadou ^b,
Chaibou Yaou ^a, Alio Sanda Abdel-Kader ^c,
Rabiou Mamane Moustapha ^d, Hamidou Djelifa ^a,
Soumana Sourounda Idé ^a, Mamadou Lewamy ^a,
Haoua Sabo Seini ^a and Sadou Hassimi ^a**

^a Department of Chemistry, Faculty of Science and Technology, Laboratory of Nutrition and Valorization of Agro-Resources, Abdou Moumouni University, Niamey, Niger.

^b Department of Life and Earth, Ecole Normale Supérieure, Abdou Moumouni University, Niamey, Niger.

^c Department of Biology, Faculty of Science and Technology, Laboratory of Management and Valorization of Biodiversity in the Sahel, Abdou Moumouni University, Niamey, Niger.

^d Department of Biology, Faculty of Science and Technology, Dan Dicko Dankoulodo University, Maradi, Niger.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2024/v16i41409

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/114442>

Original Research Article

Received: 15/01/2024
Accepted: 19/03/2024
Published: 05/04/2024

*Corresponding author: Email: almouabdoulayea@gmail.com;

ABSTRACT

Vegetables are an important food source of nutrients, vitamins and dietary fiber, and therefore play a vital role in human health and well-being. Unfortunately, they are perishable products that could be vectors for the transmission of infectious diseases. The aim of this study was to analyse the level of bacteriological contamination of the samples of tomatoes (29), carrots (12), lettuces (47) and onions (12) sold in five (5) markets of community urban of Niamey. To this end, contamination indicator germs such as Total Aerobic Mesophilic Flora (TAMF), Total Coliforms (TC), Faecal Coliforms (FC), Enterobacteria (Ent), Faecal Streptococci (FS), *Clostridium perfringens* (CP) and *Escherichia coli* (*E. coli*) were enumerated, using methods specific to each germ. Analysis of the results showed tomatoes to be highly contaminated with FS, CP and *E. coli* ($9.75 \pm 1.81 \cdot 10^5$; $1.33 \pm 2.30 \cdot 10^5$ and $2.25 \pm 2.48 \cdot 10^5$ CFU/g respectively), carrots with TAMF, FC, Ent ($1.37 \pm 1.24 \cdot 10^7$; $4.48 \pm 0.34 \cdot 10^6$; $7.44 \pm 0.34 \cdot 10^6$ CFU/g respectively) and onions with TC ($4.45 \pm 0.84 \cdot 10^6$ CFU/g). The sanitary quality of these vegetables is low. Hence the need for strict compliance with good hygiene practices in markets to ensure a healthy vegetable.

Keywords: Vegetable; contamination; bacteriological quality; market; Niamey.

1. INTRODUCTION

Africa's urban and peri-urban areas are favorable to fruit and vegetable cultivation, contributing to the food security of populations and creating jobs for many low-income households [1,2]. The consumption of fruit and vegetables is recommended in several countries for protection against various diet-related chronic non-communicable diseases such as certain forms of cancer, obesity and cardiovascular disease, and the benefits of their dietary fiber are also recognized in the smooth functioning of intestinal transit [3,4,5]. Despite the benefits associated with fruit and vegetable consumption, the safety of those eaten fresh remains a major concern, as these foods are considered vectors for the transmission of infectious diseases [6]. Thus, their consumption constitutes a potential risk factor for infection by enteropathogenic bacteria such as *Salmonella* and *Escherichia coli* 0157 [7]. Cases of food poisoning linked to the ingestion of contaminated vegetables have been identified all over the world [4,7,8,9]. Indeed, plant contamination can occur at any point in the food chain, from farms to consumer plates, via transport, distribution and markets, with the presence or absence of pathogens [6,10]. In recent years, the quality of foodstuffs on the market has tended to be disregarded by consumers in underdeveloped countries [11,12,13]. Many risk factors are considered to be the main contributors to contamination, such as poor post-harvest handling, particularly in markets, and unhygienic food handling practices [6,14,15,16]. The present study aimed to analyze the level of bacteriological contamination of four

types of high-consumption vegetable commonly sold on Niamey markets.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the urban community of Niamey, the capital of the Republic of Niger. The urban community of Niamey is located in the south-western part of Niger between 13°24' and 13°35'N latitude and 2°00' and 2°15'E longitude, with an altitude of between 160 and 250 m. Its administrative boundaries extend over 552.27km², including around 297.46km² of urbanized area [17]. Niamey's population is estimated at around 1,407,635. The urban community of Niamey is subdivided into 5 communal districts, with the following population breakdown by communal district: Niamey I: 287,902 inhabitants; Niamey II: 338,455 inhabitants; Niamey III: 223,685 inhabitants; Niamey IV: 376,271 inhabitants; Niamey V: 181,321 inhabitants [18]. Five (5) Niamey vegetable markets were studied namely Petit marche, Dar Es Sallam, Dole, Wadata and harobanda markets (market 1,2,3,4, and 5 respectively).

2.2 Study Vegetables

2.2.1 Carrot (*Daucus carota*)

The carrot is a vegetable of the *Apiaceae* family, widely cultivated for its fleshy, edible taproot, generally orange in color, eaten as a vegetable. The root is rich in carotene [19]. Carrots are used

for food and this is their best-known use (consumption of the root). Carrot essential oil, distilled from the seeds, is used in perfumery and aromatherapy [20]. Carrots are grown throughout Niger in all three seasons: cool dry season (November-February), hot dry season (March-June) and winter season (July-September). The main production period is between September and April [19].

2.2.2 Lettuce (*Lactuca sativa*)

Lettuce (*Lactuca sativa*) is an annual vegetable of the Compositae family, cultivated for its leaves, which are eaten as a vegetable in the form of salad in combination with other vegetables. Lettuce plays an important role in vegetable gardens, is fairly simple to grow and is a gardener's pride and joy. It is grown throughout Niger in all three seasons: cool dry season, hot dry season and winter season. The main production occurs during the cool dry season [21].

2.2.3 Onion (*Allium cepa* L.)

The onion (*Allium cepa* L.) is a species of the Liliaceae family, native to Asia [22]. It is used as a condiment in many types of dishes, either cooked as a ragout, fried or as a thickener [23]. However, it is also eaten raw, particularly the sweet-tasting sweet onion [24]. The onion is also used for its virtues against the risk of cardiovascular disease [23,25]. Onions are grown in all regions of Niger where soil and climatic conditions allow, with areas and production varying from one region to another [26]. This makes onions Niger's leading vegetable crop [27]. The onion production cycle comprises four (4) essential stages: seed production, nursery setting, transplanting and cultivation. It should also be noted that the production cycle depends on seasonality (dry or rainy season) and soil type (clay or sandy loam) [28].

2.2.4 Tomato (*Solanum lycopersicum* L.)

The tomato (*Solanum lycopersicum* L.) is a fruit in the botanical sense, but is eaten as a vegetable. Raw, it can be eaten plain, in a salted snack, in a vegetable salad or as a puree. Cooked, tomatoes are most often eaten in sauce. In Niger, tomatoes can be found in several off-season locations. Production is highest around the big cities. Urban and peri-urban areas are major tomato producers [29]. Tomatoes are mainly

produced for a maximum of 6 months (January to June). Niger exports it during the dry season and imports it during the rainy season [30].

2.3 Sampling

Carrot, Lettuce, tomato and onion samples were taken from five (5) markets in the urban community of Niamey. Samples were taken under sterile conditions, using single-use sterile gloves and alcohol to avoid external contamination. These samples concerned lettuce without roots, whole tomatoes showing no apparent damage or visible cracking, whole carrots and onions. A lettuce sample corresponds to three (3) bunches, tomato samples correspond to three (3) fruits, carrots samples to three carrots and onion samples corresponds to three onion bulb weighing approximately 150 g, taken at random from same batch of seller. An information sheet was attached to each sample.

2.4 Transport Conditions

Each sample collected was well packaged in a polyethylene bag, then carefully labeled. The samples were then transferred to the microbiology laboratory of the Faculty of Science and Technology (FAST), after being packaged and placed in a cooler containing carboglass to keep the temperature down to around 4°C.

2.5 Bacteriological Analysis of Samples

Preparation of stock solutions: The stock solution was prepared by grinding the vegetable sample (150 g) in a sterile polyethylene bag around the flame of a Bunsen burner. Then, 25 grams of the crushed material were taken and introduced directly into 225 mL of previously prepared and sterilized buffered peptone water. Next, 1 mL of each stock solution was taken and introduced into a test tube containing 9 mL of buffered peptone water to perform the various decimal dilutions in accordance with ISO 6887-V08-010-6 (2013) [31].

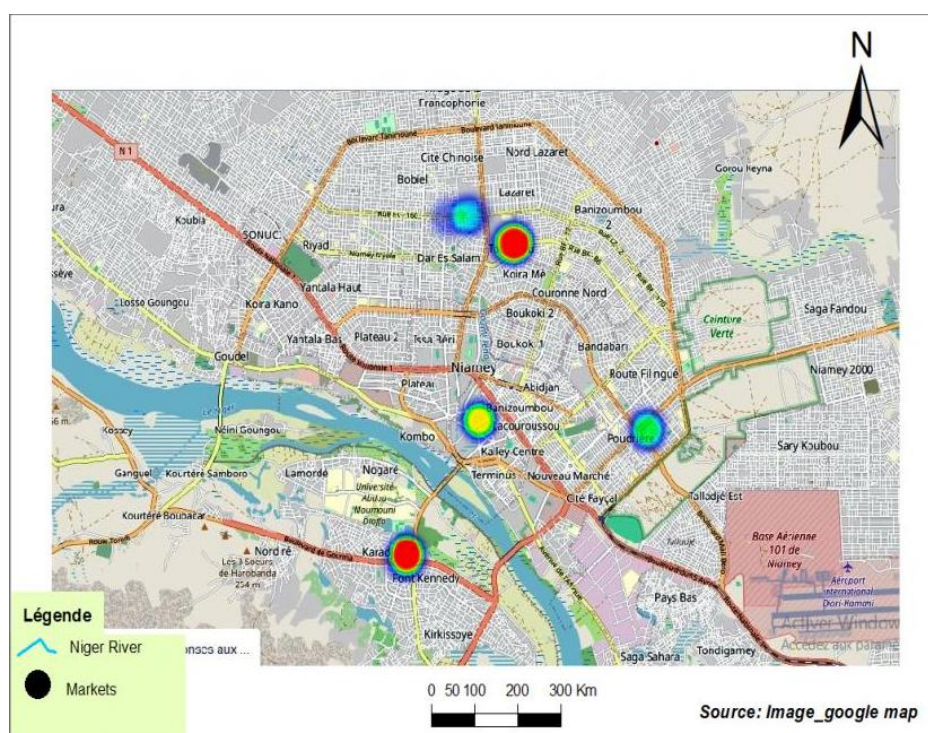
Bacterial culture:

- Total Aerobic Mesophilic Flora (TAMF) was enumerated in accordance with ISO V08-051(1992) / ISO 4833. This flora was counted on PCA (Plat Count Agar). Incubation was carried out at 37° C for 24 hours.
- Coliforms were tested on VRBL (Violet Red Bile Lactose) medium by incubation at

hours, characteristic *E. coli* colonies (green with a metallic sheen) were counted.

2.6 Interpretation

$$N = \frac{\sum c}{(n_1 + 0.1x n_2) n d} \quad [31]$$





A. Street seller of vegetable at market 1



B. Retailer seller of vegetable at market 3



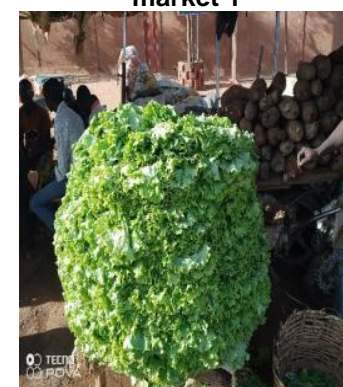
C. Street seller of carrot at market 1



D. Street seller of carrot at market 2



E. Seller of lettuce at market 5



F. Seller of lettuce at market 2

Plates A – F. Indicate the type of fruit or vegetable in the plates

2.7 Determination of Fecal Contamination Origin

Table 1. Criteria for determination of fecal contamination origin

Ratio CF/SF (R)	Origin of contamination
$R < 0,7$	Strictly animal
$0,7 < R < 1$	Mixed predominantly animal
$1 < R < 2$	Uncertain
$2 < R < 4$	Mixed predominantly human
$R > 4$	Strictly human

(Borrego et Romero, 1982; Wognin, 2014)

2.8 Statistical Analysis

The data were analyzing using SPSS 23.0.0.0. The results were expressed as means \pm standard deviation for bacterial loads and percentage for frequency. The statistical differences among the means of data calculating using one-way analysis of variance and Duncan's test (DMRT). Differences were considered significant for values of $P < .05$. The document was drafted using Microsoft Word. Arc

Gis software was used to design the geographical map of the study area.

3. RESULTS

3.1 Contamination Level of Lettuce Samples (*Lactuca sativa*)

Table 2 shows the contamination levels of lettuce samples by market. Contamination levels of lettuce samples vary from one market to another.

The lowest level of contamination was found for *Clostridium perfringens* ($7.0 \pm 14.70.10^2$ CFU/g) at market 1, and the highest for TAMF ($1.10 \pm 0.32.10^7$ CFU/g) at market 4. Fecal Streptococci, *Clostridium perfringens* and *Escherichia coli* were found least frequently. Samples from markets 3 and 2 showed no *Clostridium perfringens* load.

3.2 Contamination Level of Tomato (*Solanum lycopersicum* L.) Samples in the Selected Markets

The contamination levels of tomato samples are shown in Table 3. Average loads range from $1.25 \pm 2.31.10^2$ CFU/g for *Escherichia coli* (market 5) to $1.15 \pm 0.49.10^7$ CFU/g for TAMF (market 4). TAMF is the highest contamination indicator in all markets. Of all the contamination indicators tested, *Clostridium perfringens* was absent from samples from markets 2, 3 and 5. In fact, market 4 samples are higher in TAMF, total coliforms, faecal coliforms and enterobacteria ($1.15 \pm 0.00.10^7$; $8.50 \pm 2.12.10^5$; $5.50 \pm 3.54.10^5$ and $2.05 \pm 0.29.10^6$ CFU/g respectively). Thus, samples from market 1 are more loaded with fecal Streptococci ($9.75 \pm 1.81.10^5$ CFU/g) and *Clostridium perfringens* ($1.33 \pm 2.30.10^5$ CFU/g) and those from market 5 with *Escherichia coli* ($2.25 \pm 2.48.10^5$ CFU/g).

3.3 Contamination Level of Carrot (*Daucus carota*) Samples in the Selected Markets

Table 4 shows the average loads of the various contamination indicators tested in carrot samples. Average loads range from $5.00 \pm 7.00.10^2$ CFU/g for fecal *Streptococcus* (market 2) to $1.37 \pm 1.24.10^7$ CFU/g for TAMF (market 5). For all markets, TAMF is the highest indicator. Market 1 showed high loads of total coliforms ($3.96 \pm 0.28.10^6$ CFU/g), fecal coliforms ($4.48 \pm 0.34.10^6$ CFU/g), Enterobacteria ($7.44 \pm 0.34.10^6$ CFU/g) and fecal Streptococci ($3.32 \pm 1.70.10^5$ CFU/g). Market 4 had the highest *Escherichia coli* load ($8.75 \pm 0.99.10^4$ CFU/g). However, all the carrot samples analyzed were free from *Clostridium perfringens*.

3.4 Onion (*Allium cepa* L.) Sample Contamination Levels in the Selected Markets

Table 5 shows the contamination levels of onion samples by market. Market 4 records the highest level of contamination at around $3.05 \pm 0.21.10^7$ CFU/g (TAMF), while the lowest level of

contamination is observed at market 2 at around $5.00 \pm 7.00.10^2$ CFU/g (faecal Streptococci). *Clostridium perfringens* were absent from all onion samples, while *Escherichia coli* were absent from samples from markets 4, 3 and 2. The samples from market 3 were higher in total coliforms ($4.45 \pm 0.84.10^6$ CFU/g) and Enterobacteria ($4.68 \pm 6.01.10^6$ CFU/g); those from market 4 in faecal coliforms ($2.40 \pm 1.84.10^6$ CFU/g) and faecal Streptococci ($8.27 \pm 0.04.10^5$ CFU/g). Finally, samples from market 1 have a higher *Escherichia coli* content ($1.98 \pm 0.20.10^5$ CFU/g).

3.5 Overall Prevalence of Contamination Indicators by Fruit and Vegetable Type

The overall prevalence of contamination indicators by type of vegetable is shown in Fig. 2, and varies from one type of vegetable to another. The highest prevalence is observed for TAMF, For the fruit and vegetable. This ranged from 43.13% for tomatoes to 70.22% for lettuce. Differences are significant between vegetables (p -value = .015). Next come enterobacteria, which vary from 14.57% for carrots to 21.07% for tomatoes. The difference between vegetables is significant (p -value = .000). Coliform prevalences ranged from 2.74% (fecal coliforms) in lettuce samples to 10.94% (total coliforms) in tomato samples. Differences were significant between coliforms and vegetable type (p -value = .000). *Clostridium perfringens* and *Escherichia coli* were the lowest indicators for all vegetable types. Only tomato samples showed high prevalences in these indicators (1.39% for *Clostridium perfringens* and 1.79% for *Escherichia coli*). Differences were not significant between vegetables (p -value >0).

3.6 Correlation by Contamination Indicator

Table 6 shows the Person correlations coefficients calculated between indicators. Strong positive and highly significant correlations are observed between fecal streptococci and *Clostridium perfringens* ($r = 0.800$; $p = .000$), total coliforms and fecal coliforms ($r = 0.734$; $p = .000$), total coliforms, fecal coliforms and enterobacteria ($r = 0.682$; $p = .000$ and $r = 0.569$; $p = .000$ respectively). Weak positive correlations were observed between total coliforms, enterobacteria and TAMF ($r = 0.398$; $p = .000$ and $r = 0.355$; $p = .000$ respectively). However, weak negative correlations were observed between

Table 2. Level of contamination of lettuce (*Lactuca sativa*) samples in the selected markets

Markets	Average load \pm standard deviation of lettuce sample (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
1	1,56 \pm 2,30.10 ^{6a}	1,41 \pm 1,40.10 ^{5a}	1,30 \pm 1,82.10 ^{5a}	5,00 \pm 4,72.10 ^{5a}	1,40 \pm 1,80.10 ^{3a}	7,0 \pm 14,70.10 ^{2a}	1,20 \pm 2,04.10 ^{3a}
2	5,21 \pm 9,11.10 ^{6ab}	7,37 \pm 2,63.10 ^{5ab}	2,33 \pm 2,88.10 ^{5a}	2,08 \pm 0,32.10 ^{6bc}	2,22 \pm 1,38.10 ^{4ab}	0 ^a	2,90 \pm 3,98.10 ^{4ab}
3	6,48 \pm 1,20.10 ^{6ab}	1,37 \pm 0,85.10 ^{6b}	2,40 \pm 4,44.10 ^{5a}	2,19 \pm 1,64.10 ^{6bc}	3,66 \pm 3,46.10 ^{4b}	0 ^a	1,13 \pm 1,79.10 ^{4ab}
4	1,10 \pm 0,32.10 ^{7b}	6,65 \pm 1,21.10 ^{5ab}	2,50 \pm 2,89.10 ^{4a}	1,26 \pm 0,35.10 ^{6ab}	9,85 \pm 3,18.10 ^{4c}	1,00 \pm 1,15.10 ^{3a}	4,25 \pm 2,37.10 ^{4b}
5	1,44 \pm 1,77.10 ^{6a}	4,17 \pm 6,88.10 ^{5a}	4,94 \pm 1,04.10 ^{5a}	3,22 \pm 1,56.10 ^{6c}	9,57 \pm 22,28.10 ^{3a}	7,00 \pm 1,78.10 ^{3a}	3,87 \pm 3,92.10 ^{4ab}

Values with the same letter in the same column are not significantly different ($P > .05$); market 1: Petit marché ; market 2 : Dar es salam ; market 3 : Dolé ; market 4 : Wadata ; Market 5 : Harobanda ; TAMF : Total Aerobic mesophilic Flora ; TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; FS : Faecal Streptococci ; CP : Clostridium perfringens ; E. coli : Escherichia coli.

Table 3. Tomato (*Solanum lycopersicum* L.) sample contamination levels by market

Markets	Average load \pm standard deviation of tomato sample (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
1	2,50 \pm 4,66.10 ^{4a}	1,28 \pm 1,92.10 ^{5a}	8,90 \pm 1,46.10 ^{4a}	2,24 \pm 3,39.10 ^{5a}	9,75 \pm 1,81.10 ^{5a}	1,33 \pm 2,30.10 ^{5a}	1,25 \pm 2,31.10 ^{2a}
2	2,86 \pm 4,91.10 ^{5a}	1,47 \pm 2,42.10 ^{5ab}	1,87 \pm 3,01.10 ^{5ab}	3,41 \pm 6,40.10 ^{5a}	2,41 \pm 3,20.10 ^{4a}	0 ^a	3,44 \pm 3,44.10 ^{4a}
3	4,75 \pm 8,81.10 ^{5a}	2,96 \pm 3,14.10 ^{5ab}	5,50 \pm 9,64.10 ^{4a}	6,71 \pm 8,21.10 ^{5a}	1,35 \pm 2,50.10 ^{5a}	0 ^a	2,04 \pm 2,22.10 ^{4a}
4	1,15 \pm 0,49.10 ^{7b}	8,50 \pm 2,12.10 ^{5b}	5,50 \pm 3,54.10 ^{5b}	2,05 \pm 0,35.10 ^{6b}	1,14 \pm 0,05.10 ^{5a}	1,50 \pm 0,70.10 ^{3a}	8,50 \pm 4,95.10 ^{3a}
5	9,93 \pm 4,70.10 ^{5a}	5,70 \pm 1,01.10 ^{5ab}	3,75 \pm 5,10.10 ^{5ab}	6,20 \pm 1,11.10 ^{5a}	5,63 \pm 7,01.10 ^{4a}	0 ^a	2,25 \pm 2,48.10 ^{5b}

Values with the same letter in the same column are not significantly different ($P > .05$); market 1: Petit marché ; market 2 : Dar es salam ; market 3 : Dolé ; market 4 : Wadata ; Market 5 : Harobanda ; TAMF : Total Aerobic mesophilic Flora ; TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; FS : Faecal Streptococci ; CP : Clostridium perfringens ; E. coli : Escherichia coli

Table 4. loads of carrot (*Daucus carota*) samples in the selected markets

Markets	Load \pm standard deviation of carrot sample (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
1	5,04 \pm 5,66.10 ^{6b}	3,96 \pm 0,28.10 ^{6b}	4,48 \pm 0,34.10 ^{6c}	7,44 \pm 0,34.10 ^{6c}	3,32 \pm 1,70.10 ^{5b}	0	8,70 \pm 0,99.10 ^{4b}
2	9,50 \pm 0,71.10 ^{5a}	4,80 \pm 1,13.10 ^{5a}	2,75 \pm 0,64.10 ^{5a}	1,00 \pm 0,06.10 ^{6ab}	5,00 \pm 7,00.10 ^{2a}	0	3,60 \pm 0,99.10 ^{4ab}
3	6,30 \pm 0,71.10 ^{6a}	7,90 \pm 0,14.10 ^{5a}	6,60 \pm 1,41.10 ^{5ab}	2,27 \pm 0,13.10 ^{6ab}	1,50 \pm 0,70.10 ^{3a}	0	2,60 \pm 0,57.10 ^{4a}
4	7,50 \pm 0,71.10 ^{6a}	2,00 \pm 1,41.10 ^{5a}	3,00 \pm 2,83.10 ^{5a}	5,00 \pm 4,24.10 ^{5a}	1,86 \pm 0,48.10 ^{5b}	0	8,75 \pm 2,90.10 ^{4b}
5	1,37 \pm 1,24.10 ^{7a}	2,60 \pm 1,79.10 ^{6ab}	2,72 \pm 1,46.10 ^{6bc}	4,21 \pm 2,36.10 ^{6bc}	5,00 \pm 5,77.10 ^{3a}	0	2,73 \pm 3,21.10 ^{4a}

Values with the same letter in the same column are not significantly different ($P > .05$); market 1: Petit marché ; market 2 : Dar es salam ; market 3 : Dolé ; market 4 : Wadata ; Market 5 : Harobanda ; TAMF : Total Aerobic mesophilic Flora ; TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; FS : Faecal Streptococci ; CP : Clostridium perfringens ; E. coli : Escherichia coli

Table 5. Contamination level of onion (*Allium cepa* L.) samples by market

Markets	Load \pm standard deviation of onion sample (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
1	7,70 \pm 0,99.10 ^{6b}	1,90 \pm 0,28.10 ^{5a}	2,05 \pm 0,07.10 ^{5a}	3,40 \pm 0,14.10 ^{5a}	5,50 \pm 6,36.10 ^{3a}	0	1,98 \pm 0,20.10 ^{5b}
2	1,60 \pm 1,41.10 ^{6a}	8,20 \pm 3,11.10 ^{5a}	7,90 \pm 0,42.10 ^{5ab}	2,42 \pm 0,14.10 ^{5ab}	5,00 \pm 7,00.10 ^{2a}	0	0 ^a
3	1,32 \pm 0,11.10 ^{7c}	4,45 \pm 0,84.10 ^{6b}	1,41 \pm 0,13.10 ^{6ab}	4,68 \pm 6,01.10 ^{6b}	8,27 \pm 0,04.10 ^{5b}	0	0 ^a
4	3,05 \pm 0,21.10 ^{7d}	1,45 \pm 0,21.10 ^{6a}	2,40 \pm 1,84.10 ^{6b}	2,85 \pm 0,92.10 ^{6ab}	1,55 \pm 1,77.10 ^{5a}	0	0 ^a
5	5,60 \pm 5,62.10 ^{5a}	8,78 \pm 9,99.10 ^{5a}	2,45 \pm 2,47.10 ^{5a}	8,73 \pm 9,94.10 ^{5a}	3,73 \pm 2,69.10 ^{4a}	0	2,93 \pm 1,73.10 ^{4a}

Values with the same letter in the same column are not significantly different ($P>.05$); market 1: Petit marché ; market 2 : Dar es salam ; market 3 : Dolé ; market 4 : Wadata ; Market 5 : Harobanda ; TAMF : Total Aerobic mesophilic Flora ; TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; FS : Faecal Streptococci ; CP : Clostridium perfringens ; *E. coli* : Escherichia coli

Table 6. Person's correlation coefficients between contamination indicators

	FAMT	CT	CF	Ent	SF	CP	<i>E. coli</i>
FAMT	1						
CT	0,398**	1					
CF	0,525**	0,734**	1				
Ent	0,355**	0,682**	0,569**	1			
SF	0,005	0,091	0,044	0,000	1		
CP	-0,061	-0,053	-0,025	-0,054	0,800**	1	
<i>E. coli</i>	-0,043	-0,099	-0,042	-0,024	-0,106	-0,069	1

** Significant Correlation ($p<0,01$)

Table 7. Fecal contamination origin of vegetables by markets

Markets		Ratio faecal coliforms / faecal streptococci			
		Carrot	Lettuce	Onion	Tomato
1	Ratio	13,49	92,86	37,27	0,09
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OA
2	Ratio	550	10,53	1580	7,75
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OH
3	Ratio	440	6,55	1,7	0,41
	Origin	Strictly OH	Strictly OH	Uncertain	Strictly OA
4	Ratio	1,61	0,26	15,48	0,48
	Origin	uncertain	Strictly OA	Strictly OH	Strictly OA
5	Ratio	544	51,64	6,58	6,67
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OH
Total	Ratio	21,05	11,87	4,98	0,44
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OA

Market 1 : Petit marché ; market 2 : Dar es salam ; market 3 : Dolé ; market 4 : Wadata ; Market 5 : Harobanda ; OH : Human Origin ; OA : Animal Origin

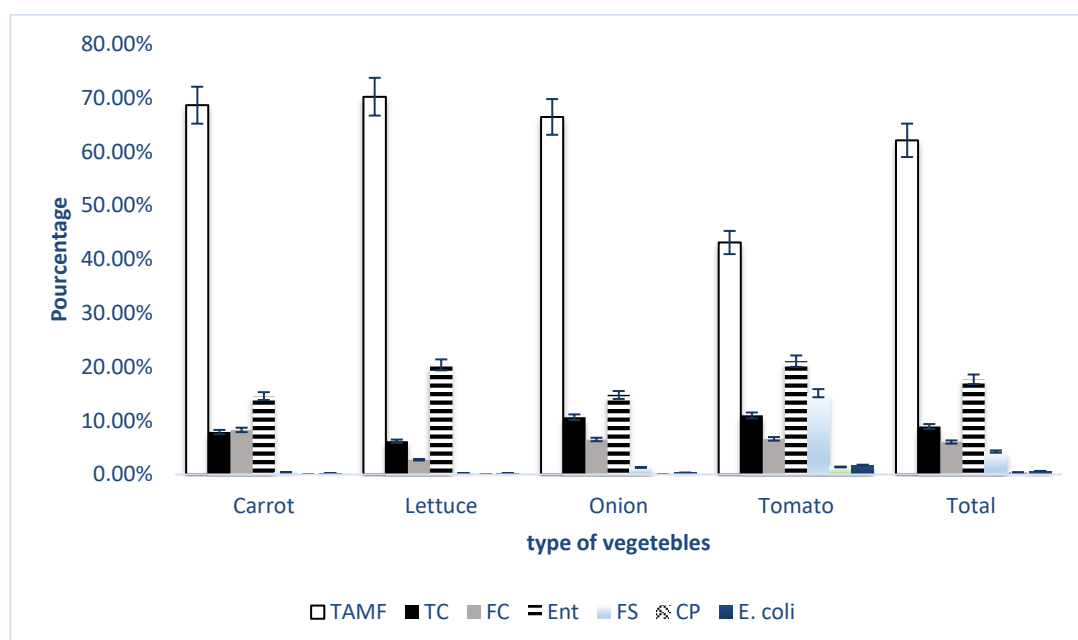


Fig. 2. Overall prevalence of contamination indicators by vegetable type

E. coli and all other indicators. *Clostridium perfringens* was also negatively correlated with all other indicators except faecal streptococci. All negative correlations are not significant.

3.7 Origin of Fecal Contamination of Vegetables

Fecal coliform/streptococcus ratios are presented in Table 7. Three (3) origins of contamination were observed: strictly human, strictly animal and of uncertain origin. The origin of contamination in markets 1 and 2 is strictly human (CF/SF > 4). Strictly origin animal contamination is observed in markets 2 and 4 for tomato, and in market 3 for lettuce and tomato (CF/SF ratio < 0.7). However, uncertain origin is observed at markets 3 and 4 for carrot and onion respectively ($1 < \text{CF/SF} < 2$).

4. DISCUSSION

The bacteriological quality of vegetable samples taken from five (5) markets in urban community of Niamey was assessed in this study. Four (4) types of vegetables were studied. The TAMF gives an indication of the degree of overall contamination of the food, and its acceptability for consumption. It is also known as food spoilage flora [32]. The study highlighted the presence of TAMF in the vegetable samples analyzed. The TAMF loads obtained were all above the bacteriological quality standards

defined by French Standardization Association AFNOR concerning vegetables. A similar finding was made in Nigeria by Abdullahi et al. [33] and Eni et al. [34]. Expenses vary significantly from one market to another. Variations in costs are not significant from one type of vegetable to another. Indeed, the variability of TAMF contamination from one vendor to another in the same locality could depend on the density of street traffic, which influences environmental hygiene and therefore product pollution [32]. Several authors have reported high contamination of foods other than vegetables by TAMF. Like Kasse et al. [32], who reported significant contamination of mango slices sold in Dakar by TAMF. For example, Anoman et al. [35] recorded high loads of TAMF in samples of "Garba", a street food from Côte d'Ivoire.

Total and faecal coliforms give an idea of the hygienic conditions under which the product was manufactured and stored. They are indicators of process and environmental hygiene. A high level of coliform contamination of vegetables was observed in all samples analyzed. Coliform loads tended to be high for carrots. These results are similar to those obtained in Cameroon by Maiwore et al. [2]. In Côte d'Ivoire, Anin et al. [36] recorded high loads of faecal Coliforms in fruit, onion and tomato purée, with values ranging from 9.1×10^2 to 1.3×10^4 CFU/g. Barour et al. [37] also found very high levels of faecal coliform contamination at most water sampling sites

during both study periods in the far east of Algeria. The strong presence of these germs is justified by their ubiquitous nature. These bacteria, which are widespread in the environment and saprophytic to humans and warm-blooded animals, are found in flours during processing [38].

The enterobacteria to which coliforms belong also contain pathogenic strains, dangerous to consumers. They are indicators of food safety (absence of danger) [32]. The results show very high enterobacteria loads for all the vegetables analyzed. A similar observation was made by Wognin [38]. These are indicators of fecal contamination that provide a more complete picture of potentially pathogenic germs [39,40]. The presence of bacteria of enteric origin in lettuces suggests a lack of good hygiene practices and fecal contamination that could be due to the inappropriate processing undergone by these raw edible vegetables [41].

It is also important to note the high level of fecal streptococcal contamination of vegetables. A similar finding was made by Agbossou et al. [42]. However, these results corroborate those found by Kheira Ghazi and A. Niar [43] in Algeria in milk samples. They also corroborate those of N'goran-Aw et al. [38] in samples of corn flour sold in markets in the city of Abidjan. They reported a fecal *Streptococcus* load ranging from 10 to 8.1×10^5 CFU/g for white flour and from 1×10^5 to 1.9×10^8 CFU/g for yellow flour with potash. Fecal streptococci are widespread in the animal's environment, but are little or non-pathogenic [44,45,46]. They are also excellent indicators of faecal pollution; their determination is very important as they are more resistant to disinfectants than *E. coli* [38,47].

Escherichia coli is a coliform that testifies to human fecal contamination and therefore to the processor's hygiene. It also provides an indication of the presence of possible enteropathogenic strains [32]. Some samples are free of *Escherichia coli*. But the average *Escherichia coli* loads of the different types of vegetables tested are high and above the standards defined for raw vegetables. There was a non-significant difference between these loads and vegetable types (P -value = .339). Our results corroborate those obtained by Toe et al. [6] in vegetable samples sold in Abidjan markets. Secondly, many authors have made similar findings on other types of food: Kasse et al. [32], in mango slices sold in Dakar, Anin et al. [37], in

4th range products sold in Abidjan markets, Anoman et al. [35], in Garba samples, Almou [48], in kilichi samples (Niger).

However, slightly high *Clostridium perfringens* loads were recorded on lettuce ($7.0 \pm 14.70.10^2$ CFU/g) and no sprouts on carrots and onions. Tomatoes, on the other hand, were highly contaminated ($1.33 \pm 2.30.10^5$ CFU/g). There were significant differences between these germs, the different markets and the type of vegetable (p -value=.033 and 0.022). The dust generated by traffic could therefore be a major vector for the transmission of sulfite-reducing anaerobes (*Clostridium*) [49,50]. The presence of these bacteria could be due to the fact that these germs are highly resistant in the environment thanks to their ability to sporulate and can persist for a long time [51]. These germs are used as indicators of long-term faecal contamination [52].

In addition, vegetable contamination levels in markets are high. These high levels of contamination in markets could be explained by the poor practices observed. Vegetables are rinsed with water generally taken from taps installed in public toilets. They are also sold mainly on the ground covered with bags at the roadside of markets and in an unsanitary environment characterized by the presence of anarchic garbage dumps, gutters and drains that would also be niches for pathogenic bacteria [15, 6]. Thus, the microbiological quality of food could also be directly linked to the quality of the available water used by vendors to prepare food. Access to a safe water supply leads the way in promoting food safety, while the environments where street foods are prepared and sold significantly affect their safety [36,53]. Wholesalers and resellers pack lettuces in inappropriate net bags, sometimes in trays without covers when transporting these vegetables. These poor practices are thought to contribute to increased bacterial loads on raw edible vegetables [39]. The presence of people in the market is a factor in the introduction of microorganisms and increases the level of contamination of raw edible vegetables [54,55]. Furthermore, Alvaro et al. [52] and Ameko et al. [56], have indicated that numerous actions, notably rinsing operations and failure to protect vegetables during sale, create favorable environments and opportunities for pathogenic micro-organisms to multiply. The origin of vegetable contamination is mainly human (the faecal coliform/streptococcus ratio is well over 4). It should be noted that human faecal flora

contains more faecal coliforms than streptococci [57]. The results are similar to those obtained by Hassoune et al. [58]; Barour et al. [37].

5. CONCLUSION

This study assessed the level of bacteriological contamination of vegetables sold in Niamey markets. The results show that the loads of contamination indicators are well above the standards recommended by the French Standardization Association (AFNOR), which vegetables must meet. These results suggest that the consumption of these vegetables without any precautions could represent a health risk for consumers. In view of this non-compliant quality of vegetables, it would be necessary to teach (train and raise awareness) vegetable sellers about good hygiene practices at the point of sale. To gain a better understanding of this contamination, it will be important to characterize phenotypically and genotypically the species of pathogenic enterobacteria in these vegetables sold on Niamey markets.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Koffi-Nevry R, Assi-Clair BJ, Assemmand EF, Wognin AS, Koussemon M. Origine des témoins de contamination fécale de l'eau d'arrosage de la laitue (*Lactuca sativa*) cultivée dans la zone périurbaine d'Abidjan. *Journal of Applied Biosciences*. 2012;52:3669–3675.
- Maïwore J, Adamou M, Djaouda M, Baane MP, Amale Y, Youssouf D, Ngoune LT. Influence de quelques sources bactériologiques de contamination sur la qualité de la laitue consommée à Maroua (Cameroun), identification des entérobactéries. *Journal of Applied Biosciences*. 2020;154:15926–15939.
- Idogun ES, Famodu AA, Olasunkanmi LA, Osilesi O, Adebawo OO. Effects of fruits and vegetables on electrolytes and blood pressure of hypertensive patients seen in Nigeria. *AJFAND*. 2008;8(3):349–357.
- Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P, Frankel G. Minireview: Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ. Microbiol*. 2010; 12: 2385–2397.
- Wognin AS, Ouffoue SK, Assemmand EF, Tano K, Koffi-Nevry R. Perception des risques sanitaires dans le maraîchage à Abidjan, Côte d'Ivoire. *International Journal of Biological and Chemical Science*. 2013;7(5):1829–1837.
- Toe E, Dadié A, Dako E, Loukou G, Djé K. Bacteriological quality and risk factors for contamination of raw mixed vegetables salads served in collective catering in Abidjan (Ivory Coast). *Advances in Microbiology*. 2017;7:405–419.
- Petterson SR, Ashbolt NJ, Sharma A. Microbial risks from wastewater irrigation of salad crops: A screening-level risk assessment. *J Food Sci*. 2010;75(5):283–290.
- Ackers ML, Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS, William FB, Daniel HR, Timothy JB, Patricia MG, Laurence S. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J Infect Dis*. 1998;177:1588–1593.
- Wendel AM, Johnson DH, Sharapov U, Grant J, Archer JR, Monson T, Koschmann C, Jeffrey PD. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August–September 2006: the wisconsin investigation. *Clin Infect Dis*. 2009;48:1079–1086.
- Adjrah Y, Soncy K, Anani K, Blewussi K, Karou DS, Ameyapoh Y, De Souza C, Gbeassor M. Socio-Economic Profile of Street Food Vendors and Microbiological Quality of Ready-to-Eat Salads in Iomé. *International Food Research Journal*. 2013;20:65–70.
- Sanny MS. Etude des bio-contaminants et migration des agents toxiques dans les cultures maraîchères: Cas du périmètre maraîcher de Houéyiho à Cotonou. Mémoire D.I.T EPAC, Université d'Abomey Calavi, Bénin. 2002;102p.
- Gnandli K, Tozo K, Edorh AP, Agbeko AK, Amouzouvi K, Baba G, Tchabbedji G, Killi K. La bioaccumulation du plomb et autres métaux lourds dans les produits maraîchers cultivés sur les sols urbains le long de l'autoroute Lomé- Aného, Sud Togo. *Journal of African Earth Sciences*. 2006;37:1–2.
- Agueh V, Degbey CC, Sossa-Jerome C, Adomahou D, Paraiso MN, Vissoh S,

- Makoutode M, Fayomi B. Niveau de contamination des produits maraîchers par les substances toxiques sur le site de Houéyiho au Bénin. *Int. J. Biol. Chem. Sci.* 2015;9(1):542-551.
14. Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A. Street foods in accra, Ghana: How safe are they? *Bulletin of the World Health Organization.* 2002;80:546-554.
15. Koffi-Nevry R, Assi-Clair BJ, Koussemon M, Wognin AS, Coulibaly N. Potential Enterobacteria risk factors associated with contamination of lettuce (*Lactuca sativa*) grown in the peri-urban area of Abidjan (Côte d'Ivoire). *International Journal of Biology and Chemical Sciences.* 2011; 5(1):279–290.
16. Amoah DS. Microbial risk assessment of mixed vegetable salads from selected canteens in the kumasi metropolis kwame nkrumah university of science and technology, Kumasi, Ghana. (PhD thesis.) University of science and technology, Kumasi, Ghana. 2014;93.
17. INS. Niamey regional statistical yearbook 2013 – 2017. 2018;93p.
18. INS. General information on the region: Administrative division of the Niamey region, projection based on RGP/H_2012 data; 2022.
19. Prom AP (Programme de la promotion de l'agriculture productive). Techniques de production des cultures irriguées (laitue), ministère de l'agriculture, Niger. 2012;25p.
20. Lecomte M. Analyse des mécanismes de défense de la carotte (*Daucus carota*) face au champignon pathogène *altrenaria dauci*, responsable de l'alternariose ou brûlure foliaire. Thèse de doctorat en biochimie et biologie moléculaire, Université d'Angers. 2013;183p.
21. RECA. Fiche technique pour la culture de la laitue et pratiques des producteurs, Niger ; 2023.
22. Rabiou MM, Moussa I, Mella T, Sadou H. Panorama of onion production in tillabéri, A region of the far west of Niger. *European Scientific Journal.* 2018a;14:175-196.
23. Foury C, Schweisguth B. L'oignon in amelioration des espèces végétales cultivées, Gallais A, Bannerot H, édés, Paris : INRA. 1992;406-419.
24. Doré C, Varoquaux F. Histoire et amélioration de cinquante plantes cultivées. Paris : INRA, pp. 481-493. Editions Quae; 2006.
25. Craig WJ. Health-promoting properties of common herbs. *Am. J. Clin. Nutr.* 1999; 70 (3): 491-499.
26. Laouali A, Aichatou A, Mansour Am. Analyse de la chaîne de valeurs d'oignon (*Allium cepa* L.) blanc de soucoucoutane au Niger. *European Scientific Journal ESJ.* 2019;15:99–117.
27. Boukary H, Roumba A, Adam T, Barage M, Saadou M. Interactions entre la variabilité des écotypes de l'oignon (*Allium cepa* L.) et les facteurs agro-climatiques au Niger, *Tropicultura.* 2012;30(4):209 – 215.
28. Boukar MAK. Analyse comparée des systèmes de production des écotypes locaux d'oignon (*Allium cepa* L.) dans la vallée de la Korama au Sud-Est du Niger. *Afrique Science.* 2022;20(5): 23-35.
29. PPEAP. Étude sur la facilitation du commerce couvrant les filières agricoles; 1999.
30. RECA. Des tomates en toutes saisons; 2016.
31. Guiraud JP, Bimbenet J-J, Duquenoy A. et Trystram G. Microbiologie alimentaire. Paris : Dunod. 2003;576p. ISBN 2 10 007259 5
32. Kasse M, Cisse M, Toure A, Ducamp-Collin A, Guisse A, Qualité microbiologique des tranches de mangues (*Mangifera indica* L.) vendues à Dakar (Sénégal). *Int. J. Biol. Chem. Sci.* 2014;8(4): 1611-1619.
33. Abdullahi IO, Abdulkareem S. Bacteriological quality of some ready to eat vegetables as retailed and consumed in sabon-gari, Zaria, Nigeria. *Bayero Journal of Pure and Applied Sciences.* 2011; 3(1): 173–175.
34. Eni AO, Oluwawemitan IA., Solomon OU. Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *African Journal of Food Science.* 2010;4(5):291-296.
35. Anoman AT, Koussemon M, Kouassi KI, Ake-Assi Y. Qualité microbiologique du garba, un aliment de rue de Côte d'Ivoire. *Int. J. Biol. Chem. Sci.* 2018;12(5):2258-2265.
36. Anin LA, Patrice DYA, Monnet YM, Yapi MAY, Soro CL, Kouadio KAK. Evaluation microbiologique et origines de la contamination des produits de 4ème gamme vendus sur les marchés d'abidjan, Cote D'ivoire. *European Scientific Journal.* 2016;12(36):1857–7881.
37. Barour AA, Benslama M, Chefrour A, Barour C. Contribution à l'étude microbiologique des eaux de l'oued

- Medjerda dans l'extrême Est Algérien: Souk Ahras. Rev. Sci. Technol. 2012;25: 88- 96.
38. N'goran-Aw EBZ, Coulibaly Jk, Assidjo EN, N'gatta C. Qualité microbiologique des farines de maïs commercialisées sur les marchés de la ville d'Abidjan (2018). Rev. Mar. Sci. Agron. Vét. 2018;6(4):476-482.
 39. Wognin AS. Facteurs de risques de contamination et gènes de virulences associés à *Escherichia coli* dans l'environnement maraîcher: Cas de la laitue (*Lactuca sativa*) en zone péri-urbaine d'Abidjan. Thèse de doctorat de l'université Nangui Abrogoua: Microbiologie et Biologie Moléculaire. 2014;122.
 40. Anonyme 2. Dénombrement des entérobactéries dans les aliments et les échantillons environnementaux au moyen des plaques de numération des entérobactéries petrifilm^{mc} 3mc. Direction générale des produits de santé et des aliments Ottawa (Canada) MFLP-09. 2007;5p.
 41. Wognin As, Ouattara Mb, Assi-Clair Bj, Koffi-Nevry R. Evaluation des niveaux de contamination bactériologique de la laitue selon les sites de production et de vente dans les sites de maraîchage d'Abidjan et zone périurbaine. Int. J. Biol. Chem. Sci. 2022;16(4):1580-1592.
 42. Agbossou KE, Sanny S, Zopodo B, Ahamide B, Guedegbe HJ. Evaluation qualitative de quelques légumes sur le périmètre maraîcher de Houéyiho, à Cotonou au Sud-Benin. Bulletin de la recherche agronomique du Benin. 2003;42:1-12.
 43. Kheira G, Niar A. Qualité hygiénique du lait cru de vache dans les différents élevages de la Wilaya de Tiaret (Algérie). Tropicultura. 2011;29(4):193-196.
 44. Baazize D. Qualité hygiénique et sanitaire du lait cru de vache. Mémoire de Magistère en hygiène et qualité du lait, Université Saad Dahleb de Blida (Algérie) ; 2005.
 45. Stine SW, Song I, Choi CY, Gerba C. Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce and bell peppers. Journal of food protection. 2005;68(7):1352-1358.
 46. Kouamé-Sina SM, Bassa A, Dadié A, Makita A, Grace A, Dje M, Bonfoh B. Analyse des risques microbiens du lait cru local à Abidjan (Côte d'Ivoire). Revue Africaine de Santé et de Productions Animales. 2010;8:35-42.
 47. Ami L, Jean F, St-Ursanne; Vania A. Contamination bactérienne des eaux de boisson: Recherche de vorigine (humaine ou animale) par l'identification des streptocoques fécaux. Québec (CDN). 1992;895-900.
 48. Almou AA, Alio Sanda A, Yaou C, Mahamane Idi Issa A, Sabo Seini H, Sadou H. Evaluation of the microbiological quality of three varieties of kilichi produced in Niger. International Journal of Food Science; 2023.
 49. Al Saif N, Brazier JS. The distribution of clostridium diffi cile in the environment of South Wales. J Med Microbiol. 1996;45: 133–137.
 50. Keessen EC, Lipman LJA. Clostridium difficile transmission risks for humans through diseased animals and symptomless but colonized animals. Wiener Tierärztliche Monatsschrift – Veterinary Medicine Austria (WTRN). 2012;99:286-297.
 51. Karou GT, Ouattara H, Bakayoko S. Prevalence of *Salmonella* and distribution of serovars isolated from retail raw chicken gizzards in Abidjan, Côte D'ivoire. Octa Journal of Biosciences. 2010;1(2):115– 121.
 52. Alvaro JE, Moreno S, Dianez F, Santos M, Carrasco G, Urrestarazu M. Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. Journal. of Food Engin. 2009;95:11-15.
 53. Vanselow BA, Krause DO, McSweeney CS. The shiga toxin – producing *Escherichia coli*, their ruminant hosts, and potential on-farm interventions: A review. Australian Journal of Agricultural Research. 2005;56(3):219–244.
 54. Ndiaye ML, Dieng Y, Niang S, Pfeifer HR, Tonolla M, Peduzzi R. Effect of irrigation water on the incidence of *Salmonella spp.* on lettuces produced by urban agriculture and sold on the markets in Dakar, Senegal. African Journal of Microbiology Research. 2011;5(19):2885-2890.
 55. Adjrah Y, Karou DS, Djéri B, Anani K, Soucy K, Ameyapoh Y, Souza C, Gbeassor M. Hygienic quality of commonly consumed vegetables, and perception about disinfecting agents in Lomé. International. Food Research. Journal. 2011;8(4):1499-1503.

56. Ameko E, Achio S, Alhassan S, Kassim A. Microbial safety of raw mixed- vegetable salad sold as an accompaniment to street vended cooked rice in Accra, Ghana. African. Journal of Biotechnonology. 2012; 11(50):11078-11085.
57. Geldreich EE, Kenner BA. Bacterial population and indicator concepts in feces swage, storm water and solid wastes. In: Indicators of virus in water and food, G. Berg. Ann Arbor Science Publishers (Eds), Michigan, USA. 1978;51-97.
58. Hassoune E, El kettani S, Koulali Y, Bouzidi A. Contamination bactériologique des eaux souterraines par les eaux usées de la ville de Settlat. Microbiol. Ind. San. and Environn. 2010;4(1):1-21.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<https://www.sdiarticle5.com/review-history/114442>