

Analysis of bacteriological contamination level of vegetables sold on the Niamey markets (Niger)

Abstract:

Fruits and 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 is to analyse the level of bacteriological contamination of tomatoes, carrots, lettuces and onions sold in Niamey markets. 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, microbiology, 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* O157 [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 aims to analyse 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

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Comment [AA2]: Fruits ??? Your abstract included fruits and vegetables

Comment [AA3]: The title of the article can be rephrased. The Niger in bracket, is it the name of a country ?

Comment [AA4]: Was to analyse the level of....

Comment [AA5]: Quantity of the fruits and vegetables, how many markets ?

Comment [AA6]: Be more specific on the methods used.

Comment [AA7]: Is it only vegetable ? What about the fruits ? Rephrase the conclusion

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(Market 1: petit marché; market 2: Dar Es Sallam market; market 3:Dolé market; market 4:Wadata market et market 5:Harobanda market) (figure 1).

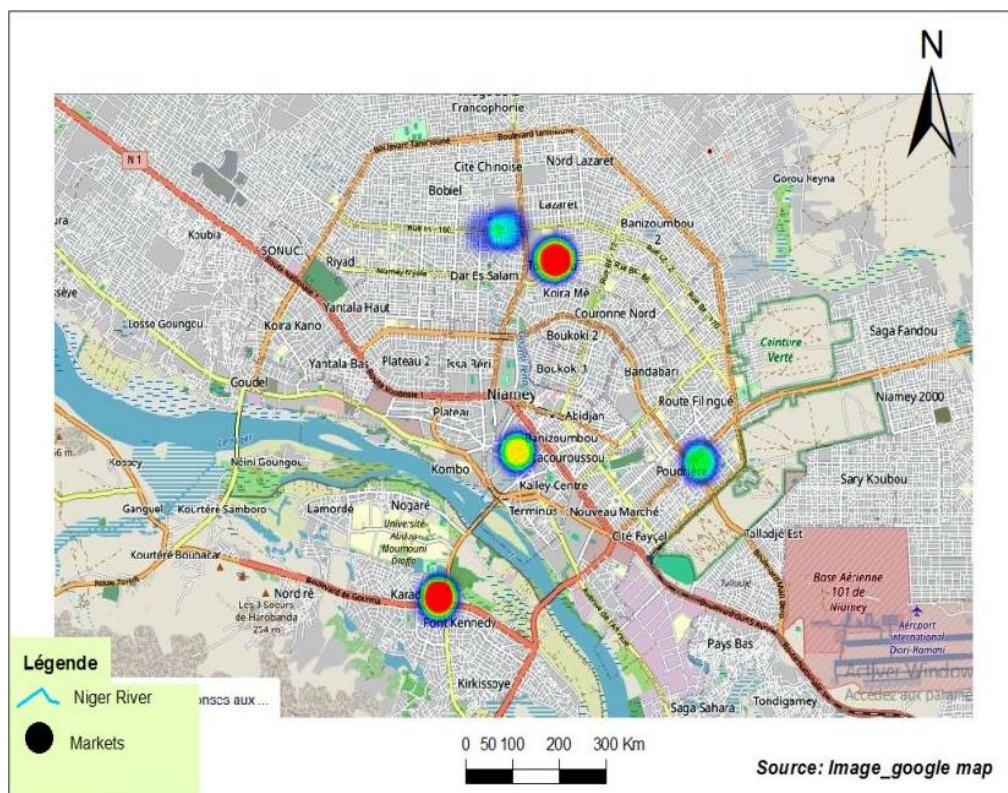


Figure 1 : Geological map of the urban community of Niamey presenting the study markets



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

Figure 2 (A, B, C, E et F): some vegetables sellers at their site of sale (market)

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.)

Comment [AA14]: The above are not figures but plates. So label each as Plate 1 – 5 or Plate A – F. Indicate the type of fruit or vegetable in the plates, don't just write vegetable

Comment [AA15]: Brief information on the fruit and vegetable should be included in the introduction section not in materials and method section. Information on the quantity of samples and where they are obtained from is needed in this section.

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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].

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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].

Comment [AA19]: Since it's a fruit you refer to it as such. Tomatoes not a vegetable

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) bunch, tomato samples corresponds to three (3) fruits, carrots samples to three carrot and onion samples corresponds to three onion weighing approximately 150g, taken at random from same batch of seller. An information sheet was attached to each sample.

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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. Microbiological analysis of samples

Preparation of stock solutions: The stock solution was prepared by grinding the vegetable sample 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].

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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 medium by incubation at 37°C for 24 hours for total and 44°C for fecal. All characteristic colonies were counted in accordance with ISO 4832 (February, 2006). Coliforms showed purplish colonies equal to or greater than 0.5 mm in diameter after 24 hours' incubation. Plates containing more than 15 characteristic colonies and less than 300 total colonies were retained.

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- Fecal *Streptococcus* enumeration was carried out on Kanamycin-Asculin-Azid-Agar (KAA) agar in accordance with the AFNOR NF 190-0411 standard (1989). Inoculation was carried out using the surface spreading method. Incubation was at 37°C for 24 hours. After 24 hours, faecal streptococci appeared as small translucent colonies surrounded by black halos.
- *Clostridium perfringens* enumeration was carried out on TSN agar according to the ISO 7937 standard. A first reading was taken after 24 hours to prevent total blackening of the tube, followed by a second reading after 48 hours when the large colonies visible in the tube were counted.
- *E. coli* were counted on EMB agar (Bio-rad, France) in accordance with ISO 18140. After incubation at 37°C for 24 hours, characteristic *E. coli* colonies (green with a metallic sheen) were counted.
- Enterobacteria were counted on Mac Conkey agar, in accordance with ISO Standard 21528-1. Inoculation was carried out by spreading 0.1 mL of each dilution onto Mac Conkey agar, which had previously been poured into Petri dishes. The plates were then incubated at 37°C for 24 hours. Red colonies characteristic of Enterobacteria were counted.

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2.6. Interpretation

Bacterial load was calculated in accordance with ISO 7218 (2007) using the following formula:

$$N = \frac{\sum c}{(n_1 + 0,1x n_2)v.d} [31]$$

- $\sum c$ = Total number of colonies counted in plates with colony counts between 15 and 300.
- n_1 = number of plates counted in the first dilution;
- n_2 = number of plates counted in the second dilution;
- d = dilution factor at which 1st counts were made;
- v = inoculum volume.

2.7. Statistical analysis

The data were analyzed using SPSS 23.0.0.0. The results were expressed as means ± 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 $P < 0.05$. 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 1) by market

Table 1 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* ($7.0 \pm 14.70 \cdot 10^2$ CFU/g) at market 1, and the highest for TAMF ($1.10 \pm 0.32 \cdot 10^7$ 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.

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Table 1: Level of contamination of lettuce (*Lactuca sativa*) samples by market

Comment [AA41]: In the selected markets

	Average load ± standard deviation of lettuce sample (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
Marché 1	1,56±2,30.10 ^{5a}	1,41±1,40.10 ^{5a}	1,30±1,82.10 ^{5a}	5,00±4,72.10 ^{5a}	1,40±1,80.10 ^{5a}	7,0±14,70.10 ^{2a}	1,20±2,04.10 ^{3a}
Marché 2	5,21±9,11.10 ^{6ab}	7,37±2,63.10 ^{5ab}	2,33±2,88.10 ^{5a}	2,08±0,32.10 ^{6bc}	2,22±1,38.10 ^{4ab}	0 ^a	2,90±3,98.10 ^{4a}

Comment [AA42]: There should be an heading on top, 'Markets' Then below just write 1, 2, 3, 4, and 5. You dont need to be repeating markets several times.

Marché 3	6,48±1,20.10 ^{6ab}	1,37±0,85.10 ^{6b}	2,40±4,44.10 ^{5a}	2,19±1,64.10 ^{6bc}	3,66±3,46.10 ^{4b}	0 ^a	1,13±1,79.10 ^{4ab}
Marché 4	1,10±0,32.10 ^{7b}	6,65±1,21.10 ^{5ab}	2,50±2,89.10 ^{4a}	1,26±0,35.10 ^{6ab}	9,85±3,18.10 ^{4c}	1,00±1,15.10 ^{3a}	4,25±2,37.10 ^{4b}
Marché 5	1,44±1,77.10 ^{6a}	4,17±6,88.10 ^{5a}	4,94±1,04.10 ^{5a}	3,22±1,56.10 ^{6c}	9,57±22,28.10 ^{3a}	7,00±1,78.10 ^{3a}	3,87±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.

3.2. Contamination level of tomato (*Solanum lycopersicum* L., Table 2) samples by market

Comment [AA43]: Check table 1

The contamination levels of tomato samples are shown in Table 2. Average loads range from $1.25±2.31.10^2$ CFU/g for *Escherichia coli* (market 5) to $1.15±0.49.10^7$ 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 ($1.15±0.00.10^7$; $8.50±2.12.10^5$; $5.50±3.54.10^5$ and $2.05±0.29.10^6$ CFU/g respectively). Thus, samples from market 1 are more loaded with fecal Streptococci ($9.75±1.81.10^5$ CFU/g) and *Clostridium perfringens* ($1.33±2.30.10^5$ CFU/g) and those from market 5 with *Escherichia coli* ($2.25±2.48.10^5$ CFU/g).

Table 2: Tomato (*Solanum lycopersicum* L.) sample contamination levels by market

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

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3.3. Contamination level of carrot (*Daucus carota*, table 3) samples by market

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Table 3 shows the average loads of the various contamination indicators tested in carrot samples. Average loads range from $5.00±7.00.10^2$ CFU/g for fecal *Streptococcus* (market 2) to $1.37±1.24.10^7$ CFU/g for TAMF (market 5). For all markets, TAMF is the highest indicator. Market 1 showed high loads of total coliforms ($3.96±0.28.10^6$ CFU/g), fecal coliforms ($4.48±0.34.10^6$ CFU/g), Enterobacteria ($7.44±0.34.10^6$ CFU/g) and fecal Streptococci ($3.32±1.70.10^5$ CFU/g). Market 4 had the highest *Escherichia coli* load ($8.75±0.99.10^4$ CFU/g). However, all the carrot samples analyzed were free from *Clostridium perfringens*.

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Table 3: average loads of carrot (*Daucus carota*) samples by market

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

3.4. Onion (*Allium cepa L.*, table 4) sample contamination levels by market

Table 4 shows the contamination levels of onion samples by market. Market 4 records the highest level of contamination at around $3.05 \pm 0.21 \cdot 10^7$ CFU/g (TAMF), while the lowest level of contamination is observed at market 2 at around $5.00 \pm 7.00 \cdot 10^2$ 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 ($4.45 \pm 0.84 \cdot 10^6$ CFU/g) and Enterobacteria ($4.68 \pm 6.01 \cdot 10^6$ CFU/g); those from market 4 in faecal coliforms ($2.40 \pm 1.84 \cdot 10^6$ CFU/g) and faecal Streptococci ($8.27 \pm 0.04 \cdot 10^5$ CFU/g). Finally, samples from market 1 have a higher *Escherichia coli* content ($1.98 \pm 0.20 \cdot 10^5$ CFU/g).

Table 4: contamination level of onion (*Allium cepa L.*) samples by market

	Charges moyennes ± écart type des échantillons d'oignon (UFC/g)						
	TAMF	TC	FC	Ent	FS	CP	<i>E. coli</i>
Marché 1	7,70±0,99.10 ^{6b}	1,90±0,28.10 ^{5a}	2,05±0,07.10 ^{5a}	3,40±0,14.10 ^{5a}	5,50±6,36.10 ^{3a}	0	1,98±0,20.10 ^{5b}
Marché 2	1,60±1,41.10 ^{6a}	8,20±3,11.10 ^{5a}	7,90±0,42.10 ^{5ab}	2,42±0,14.10 ^{5ab}	5,00±7,00.10 ^{2a}	0	0 ^a
Marché 3	1,32±0,11.10 ^{7c}	4,45±0,84.10 ^{6b}	1,41±0,13.10 ^{6ab}	4,68±6,01.10 ^{6b}	8,27±0,04.10 ^{5b}	0	0 ^a
Marché 4	3,05±0,21.10 ^{7d}	1,45±0,21.10 ^{6a}	2,40±1,84.10 ^{6b}	2,85±0,92.10 ^{6ab}	1,55±1,77.10 ^{5a}	0	0 ^a
Marché 5	5,60±5,62.10 ^{5a}	8,78±9,99.10 ^{5a}	2,45±2,47.10 ^{5a}	8,73±9,94.10 ^{5a}	3,73±2,69.10 ^{4a}	0	2,93±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 ;

Comment [AA47]: you dont need to write average

Comment [AA48]: Reconstruct all the table titles. What you are investigating is the bacterial load or counts obtained from the different samples

Comment [AA49]: average load of what?

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Comment [AA51]: Use English language

Comment [AA52]: Check above

TAMF : Total Aerobic mesophilic Flora ; TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; FS : Faecal Streptococci ; CP : Clostridium perfringens ; E. coli : Escherichia coli

3.5. Overall prevalence of contamination indicators by vegetable type

The overall prevalence of contamination indicators by type of vegetable is shown in **Figure 2**, and varies from one type of vegetable to another. The highest prevalence is observed for TAMF, whatever the 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).

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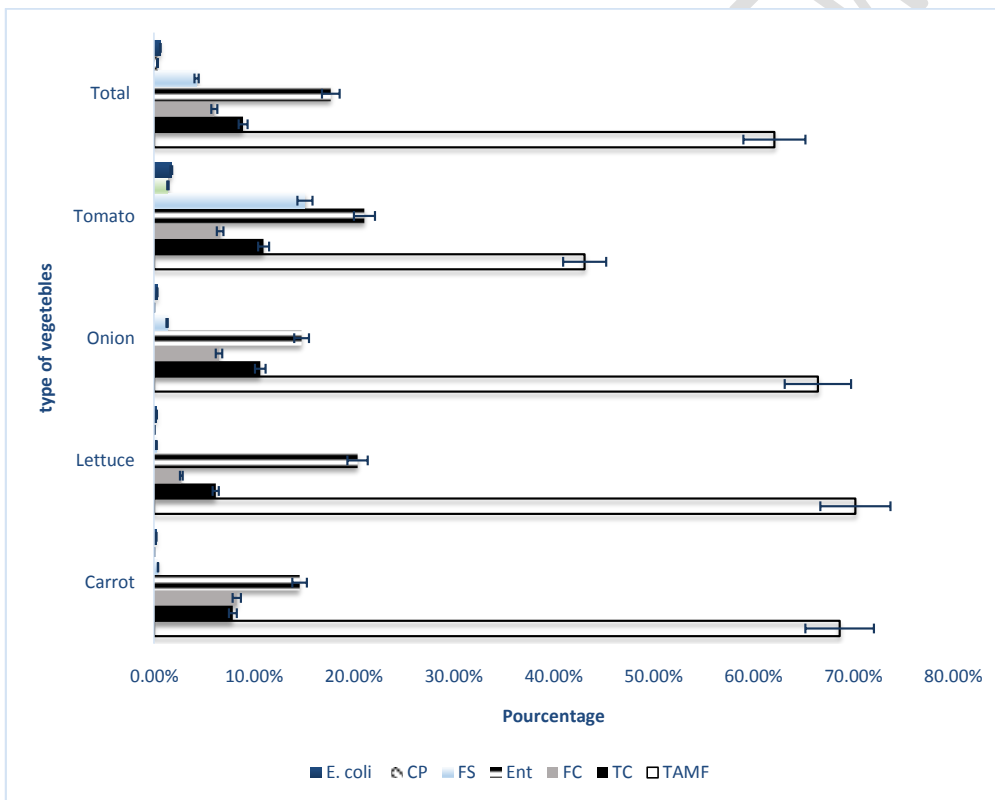


Figure 3: Overall prevalence of contamination indicators by vegetable type

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3.6. Correlation by contamination indicator

Table 5 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 *E. coli*

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

Table 5: 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 ($p < 0,01$)

3.7. Origin of fecal contamination of vegetables

Fecal coliform/streptococcus ratios are presented in **Table 6**. 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 ($1 < CF/SF < 2$).

Table 6: fecal contamination origin of vegetables by markets

Markets		Ratio faecal coliforms / faecal streptococci			
		Carrot	Lettuce	Onion	Tomato
Marché1	Ratio	13,49	92,86	37,27	0,09
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OA
Marché2	Ratio	550	10,53	1580	7,75
	Origin	Strictly OH	Strictly OH	Strictly OH	Strictly OH
Marché3	Ratio	440	6,55	1,7	0,41
	Origin	Strictly OH	Strictly OH	Uncertain	Strictly OA
Marché4	Ratio	1,61	0,26	15,48	0,48
	Origin	uncertain	Strictly OA	Strictly OH	Strictly OA
Marché5	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

4. Discussion

The microbiological 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. These were lettuce, tomato, carrot and onion. This analysis was carried out by researching and enumerating contamination indicators such as Total Aerobic Mesophilic Flora (TAMF), Total and Faecal Coliforms (TC and FC), Faecal Streptococci (FS), *Clostridium perfringens* (CP), Enterobacteria and *Escherichia coli*.

Comment [AA56]: Is this a result or is just data

Comment [AA57]: Fecal

Comment [AA58]: Which method did you use to obtain this result. It was not mentioned in the materials and method section

Comment [AA59]: What you did was bacteriological, not microbiological because you didn't determine fungal counts.

Comment [AA60]: This is discussion section you don't mention materials again

Comment [AA61]: This is not an extension of materials and methods section.

TMAF 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 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 (p -value = .007). Variations in costs are not significant from one type of vegetable to another (p -value = .074). Indeed, the variability of TMAF 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.

Comment [AA62]: Write in full

Comment [AA63]: You need to include the standard in the table

Comment [AA64]: You dont need to mention p value here

Total and faecal coliforms give an idea of the hygienic conditions under which the product is 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 (TC = $3.96 \pm 0.28 \cdot 10^6$ and FC = $4.48 \pm 0.34 \cdot 10^6$ CFU/g). These results are similar to those obtained in Cameroon by Maïworee *et al.* [2]. In Côte d'Ivoire, Aninet *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. Barouret *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].

Comment [AA65]: was

Comment [AA66]: you dont need to mention values here. This is not result section. Just discuss the result, give reasons for having such results and compare with other researchers findings

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. These loads ranged from $2.24 \pm 3.39 \cdot 10^5$ UFC/g (Table 2) for tomato to $7.44 \pm 0.34 \cdot 10^6$ UFC/g (Table 3) for carrot. 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].

Comment [AA67]: dont repeat results

It is also important to note the high level of fecal streptococcal contamination of vegetables. The average faecal streptococcus loads of the various vegetables analyzed ranged from $5.00 \pm 7.00 \cdot 10^2$ CFU/g (carrot) to $9.75 \pm 1.81 \cdot 10^5$ CFU/g (tomato). A similar finding was made by Agbossouet *al.*[42]. However, these results corroborate those found by Kheira Ghasi 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].

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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. These loads range from $1.25 \pm 2.31 \cdot 10^2$ UFC/g to $2.25 \pm 2.48 \cdot 10^5$ UFC/g for tomatoes. 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, Aninet *al.*[37], in 4th range products sold in Abidjan markets, Anoman *et al.*[35], in Garba samples, Almou[48], in kilichi samples (Niger).

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However, slightly high *Clostridium perfringens* loads were recorded on lettuce ($7.0 \pm 14.70 \cdot 10^2$ CFU/g) and no sprouts on carrots and onions. Tomatoes, on the other hand, were highly contaminated ($1.33 \pm 2.30 \cdot 10^5$ CFU/g). There were significant differences between these germs, the different markets and the type of vegetable (p -value=0.033 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].

Comment [AA70]: result values should not be mentioned again in discussion section, instead refer to the table where these results were represented.

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]; Barouret *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.

Comment [AA71]: There was no place this standard was presented

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Comment [AA72]: Journal names should be written in full

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Comment [AA73]: Be consistent with the author's guidelines for referencing