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Bacterial Community in Fresh Fruits and Vegetables Sold in Streets and Open-Air Markets of Dakar, Senegal

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Abstract: This study aimed to assess the microbiological quality of set of fruits and vegetables sold on stalls in the streets and open markets of Dakar, the capital city of Senegal. Samples purchased in seven sites were analyzed to isolate *E. coli*, *Salmonella spp.* and *Vibrio spp*. Some primary samples with a positive microbiological culture were subjected to DNA extraction and subsequent metagenomic analysis. A total of 240 fresh fruit and vegetable samples were collected including lettuce (n=40), tomatoes (n=40), mango slices (n=40), onion slices (n=40), mint leaves (n=40), strawberries and grapes (respectively n=20). Of these samples, 50.83% (122/240) and 60.41% (145/240) was contaminated with *Escherichia coli* and *Vibrio spp.* respectively, while *Salmonella* was not isolated in any product. Levels of contamination with both *E. coli* (1.3 10^3 to 6 10^6 CFU/g) and *Vibrio spp.* (4.55 10^2 to 8.73 10^6 CFU/g) were significantly above the thresholds acceptable for human consumption. The most contaminated samples were lettuce with a prevalence of 98% (39/40) for *E. coli* and for *Vibrio spp.* followed by mint leaves with 100% (40/40) and 93% (37/40) of the samples containing for *E. coli* and *Vibrio spp.* respectively. Out of 46 samples sequenced, metagenomic analysis revealed high contamination rates for *E. coli*, *Vibrio spp.* and *Salmonella spp.* with 100%, 67.39% and 93.47% of prevalence respectively. On the other hand, the alpha diversity analysis shows a high bacterial diversity in lettuce and mint leaf samples while beta diversity analysis highlighted the presence of two major clusters. Our results stress the need of a surveillance system that extends this investigation to a national scale while increasing the number of sampling sites and products analyzed.

Keywords: food safety; fresh fruits and vegetables; *E. coli*; *Vibrio spp.*; *Salmonella spp.*; metagenomic

1. Introduction

As primary sources of essential nutrients, fruits and vegetables are a vital part of our daily diet [1,2]. Phytonutrients they contain, prevent cardiovascular diseases as well as certain cancers [3]. These valuable constituents (fatty acids, vitamins, minerals and fibers) are intact in freshly harvested fruits. Low consumption of fruits and vegetables may potentially leads to nutritional deficiencies and favor diseases such are malnutrition in children, overweight or obesity in adults [4,5]. Consequently, regular intake of fruit and vegetable is an important part of a healthy diet [6,7] and nutritionists recommend to ideally consume at least five portions per day (5/d) [3,8]. The sale of fruits and vegetables in the streets and open-air markets is a widespread practice in low-and-middle income countries (LMIC) and represent an important part of daily food consumption in urban and peri-urban areas [9–12]. This activity constitutes a business opportunity and has a significant socio-economic impact, especially with regard to the value chain of horticultural products. Indeed, in LMIC, sale of fruits and vegetables in batch or even in single of portion of a piece provide a regular source of income

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for millions of people. In addition, this activity contributes to the economic empowerment of smallholder farmers, local food processors, wholesalers and different retailers [11,13].

According to the 2018 *Codex alimentarius* report, despite many education and training initiatives, and efforts to frame the horticulture sector, many actors involved in food processing still have limited food safety skills [14]. This raises concerns on the quality, safety [7,15], management and proper preservation of fruits and vegetables in LMIC. For example, post-harvest losses of mangoes are estimated at around 60% [12,16]. This is one of the main reasons that promote the sale of these food matrices on the streets as fourth-range products [17].

Fresh vegetables are rich in carbohydrates, low in proteins and have a pH ranging from neutrality to a slight acidity. They constitute a favorable ecosystem for the development of microorganisms that can cause their putrefaction making these products highly perishable. Additionally, when these products are frequently marketed without proper guidelines [18], they can act as vehicles for infection by bacteria, parasites, viruses, fungi, especially when eaten uncooked [1,2,17,19]. In LMIC where poor hygiene conditions are frequent, fruits and vegetables can be contaminated with pathogens through fecal transmission or from the environment (irrigation water or contaminated soil) [2,15]. These contaminants cause a food safety concern and therefore food poisonings which pose a persistent threat to public health [2,15]. Indeed, most raw-eaten foods have been recognized as sources of transmission of infectious diseases [1–3].

Although the majority of food poisoning events are due to the consumption of animal source foods [15], the number of cases associated with fresh fruits and vegetables continues to increase [20]. Thus, a wide range of contaminated fresh fruits and vegetables have recently caused major outbreaks of microbial infections [21]. Moreover, several studies showed that unsafe fresh fruits and vegetables can be vectors for various human pathogens [1,18,19,22].

In Senegal as in many LMIC, food safety remains one of the major concerns with multiple challenges. Street foods have developed strongly over the past thirty years [11,15,23–25]. This phenomenon is linked to the combined effect of changing eating habits [19], rural exodus, population growth in cities and also due to the high annual production of horticultural products [21]. In a context of food transition, the agri-food sector has experienced a considerable change since the beginning of the 2000s. In addition, compliance with health standards in the fruit and vegetable value chain remains a controversial subject given the prevalence of foodborne diseases in resource-limited countries [26].

This study aimed to assess the microbiological quality of set of fruit and vegetable sold on stalls in the streets and open-air markets in Dakar.

2. Materials and Methods

2.1. Collection and sampling sites

Samples were purchased in seven sites including five in the district of Dakar city (Tilène, Kermel, Castor markets, Dakar – down town and Cheikh Anta Diop Avenue) and two in its suburbs (Gueule Tapée, Parcelles Assainies and Keur Massar markets) (Figure 1). In order to obtaining representative samples, study sites were chosen according to the high density of the surrounding populations reflecting level of frequentation. For each type of sample and regardless of the collection site, a quantity of 250 g was purchased from different sellers. We have arbitrarily set to 3 and 5 for a minimum and maximum of samples purchased per seller. It is noteworthy that one of the sites i.e "Kermel market" is located in Dakar city center, a neighborhood where high-income individuals reside. Samples collection was carried out over the period from June to December 2021 on all sites.

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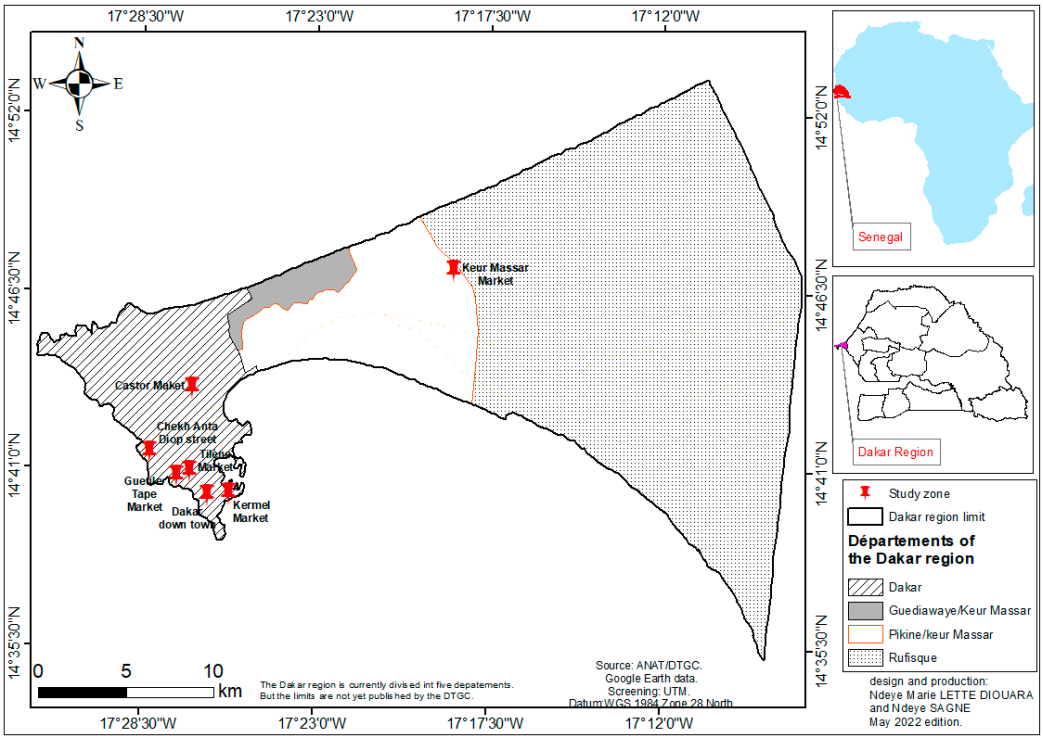

Figure 1. Sampling sites.

The collected samples were individually packaged in sterile plastic bags and hermetically sealed. Each sample was clearly identified by a code with a permanent marker. Samples were transported to the laboratory in a cooler box bag containing ice packs maintained at 4°C, within 2 hours following collection. Each sample was split in two with 150 g stored in a biobank at - 80°C for molecular tests and the remaining immediately submitted to rub-shake-rub to dislodge microbial populations from the surface.

2.2. Bacterial culture

For each sample, 25g were mixed with 225mL of Buffered Peptone Water in a sterile filter bag stomacher. This matrix was subjected to rub-shake-rub to dislodge microbial populations from the surface. Part of the mixture obtained was incubated at 37°C during 24 hours for detection of *Salmonella spp*. For detection of *E. coli* and *Vibrio spp.*, a serial dilution (up to 10^{-4}) was carried out from this pre-enrichment solution, all according to the matrix for inoculating the microbial suspension at different concentrations. Then we used specific culture media for *E. coli*, *Vibrio spp*. and *Salmonella spp*. suspicion i.e Tryptone Bile Glucuronate (TBX), Thiosulfate Citrate Sucrose (TCBS) and Rappaport, respectively. For *E. coli* investigation, a deep inoculation is carried out by pouring the TBX culture medium into a Petri dish containing 1mL of microbial suspension. Dishes are then incubated at 42°C for 48 hours. The determination of *Vibrio spp*. was carried out by seeding on the surface by spreading 100 µL of microbial suspension on the surface of the TCBS culture medium. The dishes are incubated at 37°C for 24 hours. For the suspicion of *Salmonella*, 100 µL of the pre-incubated pre-enrichment are inoculated into 10 mL of Rappaport. The tubes are then incubated at 42°C for 24 hours. All microbial analyses were carried out in duplicate. Characteristic blue colonies of *E. coli* were counted in TBX, a chromogenic medium. For *Vibrio spp*., yellow colonies around 2 mm diameter were counted. Counting is performed in accordance with the ISO 16649 2001 standard. For *Salmonella spp*., suspicion would result in a change in the Rappaport medium which becomes colorless whereas initially blue. After counting characteristic colonies of each germ, bacterial contamination was recorded and expressed as the number of colonies forming units per gram of sample (CFU g^{-1}) according to the following formula ISO 7218 [27] : N=(∑Colonies)/V(n1+0,1n2)D.

ΣColonies: sum of the numbers of bacterial colonies in dishes considered;

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N: CFU number per gram of initial product;
V: Volume (in mL) of the inoculated suspension;
n1 & n2: number of interpretable dishes chosen at the 1st and at the 2nd dilution considered;
D: dilution factor of the 1st dilution considered.

The interpretation of the microbiological results was carried out according to European Commission Regulation (EC) No. 2073/2005 based on two and three class plans for *Salmonella spp.* and *Vibrio spp.* on one side and for the *E. coli* respectively. The presence of *Salmonella spp.* and *Vibrio spp.* in fresh fruits and vegetables indicates fecal contamination which declares the product corrupted and therefore not suitable for human consumption. The following parameters were considered: m, M, Sat and Cor.

m: minimal number of CFU of bacteria per gram of sample tolerated in the product
M: maximal number of CFU/g tolerated in the product; M = 10m
Sat: degree of satisfaction with the established standards; Sat = 3m
Cor: degree of corruption of product; this means the number of CFU beyond which the product is declared not suitable for human consumption which is set as follow Cor = 1000m. Below this threshold, the product may be contaminated and does not meet the standards established by *Codex Alimentarius* but suitable for human consumption. In case of the product is declared unsatisfactory but fit for consumption because inferior to Cor level; this situation would indicate a margin of tolerance for consumption of contaminated product without health risk.

For each product, according to standards established by *Codex Alimentarius* [14], the different values of these parameters were summarized in Table 1.

Table 1. Summarize the standards for the presence of *E. coli* in foodstuffs according to the type of matrix.

Matrix	m	M	Sat	Cor
Strawberries	100 CFU/g	1000 CFU/g	300 CFU/g	100,000 CFU/g
Grapes				
Lettuce				
Tomatoes				
Mango slices				
Onion slices	10 CFU/g	100 CFU/g	30 CFU/g	10,000 CFU/g
Mint leaves				

2.3. Nucleic Acids isolation and Sequencing

Nucleic acids were extracted using the Quick-DNA/RNA™ Miniprep Plus kit (Zymo Research Quick- DNA/RNA™ Miniprep Plus Catalog D7003; lot 207477; 50 preps) according to manufacturer's instructions. The quantity and integrity of the DNA was examined using Qubit 4 fluorometer (Thermo Scientific) and Qubit ™ 1X dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits.

The Rapid Barcoding kit 96 (SQK-RBK110.96) was used for sequencing according to the manufacturer's recommendations. An amount of 50 ng was used for library preparation. This step included sample barcoding, purification and washes with AMPure XP beads and ethanol 80% respectively, elution with 15µL of Elution Buffer (EB), and addition of Rapid Adapter (RAP-F) to barcoded fragments. The library was loaded into a R9.4.1 (FLO-MIN 106) flow cell according to the kit manufacturer's instructions.

2.4. Data analysis

Statistical analysis for microbiological results was performed using Microsoft excel program. For metagenomic analysis, sequences obtained after 3 hours of run were retrieved and analyzed using the EPI2ME Agent Desktop version 3.6.2 (**https://epi2me.nanoporetech.com**) of Oxford Nanopore Technologies (ONT) for real-time sequence analysis. The What's In My Pot for taxonomic

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classification workflow was used with microbial quantification from metagenomic samples according to sequences available in NCBI RefSeq database that is linked to EPI2ME [28,29]. This pipeline utilizes "centrifuge" kmer-based read identification through FastQ files outpout generated after basecalling. The abundance of species obtained after this analysis was used for the subsequent steps, which consisted in describing the microbial community of each sample using Microsoft Excel program. We labeled « OTHERS » all species that have relative a abundance less than 5%.

For intra sample diversity [30], we used the following metrics: Observed OTUs (for different taxa observed in a sample at taxonomic level), Chao1 (for the estimation of diversity through abundances), Simpson (based on the probability that two species taken from the sample at random are different) and Shannon index (estimator for both species richness and evenness, but with weight on the richness) [31]. Theses metrics were calculated with the vegan and phyloseq packages in Rstudio. Wilcox-Test were performed to find out the significance between observed differences. All plots were carried out with ggplot2 package in R.

For the inter samples diversity (beta), non-phylogenetic based metrics were used for the dissimilarity analysis: Bray-Curtis Dissimilarity (examines the abundances of microbes that are shared between two samples, and the number of microbes found in each; ranged from 0 to 1) and Jaccard distances (abundances are not taken into account; just the presence of microbes in one or both samples) [32,33]. These parameters were calculated in R with ecodist package. Dissimilarities were plotted with the Principal Coordinates Analysis (PCoA) approach using ggplot2's R package

3. Results

In this study, we analyzed 240 fruit and vegetable samples collected at different supply sites (streets and open-air markets) in Dakar, Senegal. The samples included lettuce (n = 40), tomatoes (n = 40), mango slices (n = 40), onion slices (n = 40), mint leaves (n = 40), strawberries (n = 20) and grapes (n = 20) (**Table 2**).

Table 2. Shows the distribution of samples by matrix and by collection site.

SITES	Grapes	Strawberries	Lettuce	Mango slices	Tomatoes	Onion slices	Mint leaves	Total
Tilene	17	0	0	0	0	30	8	**55**
Dakar- down town	0	20	0	0	0	0	0	**20**
Cheikh Anta Diop (CAD) street	0	0	0	40	0	0	0	**40**
Castor	0	0	10	0	15	0	0	**25**
Gueule tapée	0	0	0	0	10	0	0	**10**
Keur Massar	3	0	10	0	0	10	32	**55**
Kermel	0	0	20	0	15	0	0	**35**
TOTAL	**20**	**20**	**40**	**40**	**40**	**40**	**40**	**240**

3.1. Fruit and vegetables contamination by Escherichia coli and Vibrio spp

Microbiological analysis revealed the presence of *E. coli* and *Vibrio spp.* in most of the samples (Table 3). *E. coli* was isolated from all mint samples (100%) and from 97.5% (39/40) of the lettuce. In addition, most of the onion samples (85%, 34/40) were contaminated by *E. coli.* Other samples including strawberries (40%, 8/20), mango slices (22.5%, 9/40) and tomatoes (2.5%, 1/40) were less contaminated by *E. coli* (Table 3). Contamination of *Vibrio spp* was recorded in most of the mint leaves (92.5%, 37/40), lettuce (97.5%, 39/40) and onion samples (90%, 36/40). *Vibrio spp*. was also significantly present in mango slices (47.5%, 19/40), tomatoes (27.5%, 11/40) and strawberries (15%, 3/20) (Table 3). Interestingly, no sample was positive for *Salmonella*. It is important to note that no positive culture was obtained from grape samples.

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Table 3. Shows the contamination rates with *E. coli*, *Vibrio spp.* and *Salmonella spp*. according to the matrix.

Samples	*E. coli* +	*Vibrio spp* +	*Salmonella spp* +
Grapes	0% (n=0)	0% (n=0)	0% (n=0)
Strawberries	40% (n=8)	15% (n=3)	0% (n=0)
Lettuce	97.5% (n=39)	97.5% (n=39)	0% (n=0)
Tomatoes	2.5% (n=1)	27.5% (n=11)	0% (n=0)
Mangoes slices	22.5% (n=9)	47.5% (n=19)	0% (n=0)
Mint leaves	100% (n=40)	92.5% (n=37)	0% (n=0)
Onion slices	85% (n=34)	90% (n=36)	0% (n=0)

When analyzing product contamination according to the supply sites (Figure 1), we found 90.91% (50/55) and 87.27% (48/55) of the samples from Keur Massar market were contaminated by *E. coli* and *Vibrio spp* respectively (Table 4). Tilene and Castor markets showed a comparable level of contamination by *E. coli* and *Vibrio spp.* (Table 4). Contrary to what was found in the Dakar downtown, *Vibrio spp.* was more frequently found than *E. coli* in samples from the Kermel market and Cheikh Anta Diop street (Table 4).

Table 4. Contamination rate of samples by collection site.

Samples	*E. coli* +	*Vibrio spp.* +	*Salmonella spp.* +
Tilene	61.82% (n=34)	61.82% (n=34)	0% (n=0)
Keur Massar	90.91% (n=50)	87.27% (n=48)	0% (n=0)
CAD street	22.5% (n=9)	47.5% (n=19)	0% (n=0)
Dakar-down town	40% (n=8)	15% (n=3)	0% (n=0)
Kermel	54.28% (n=19)	80% (n=28)	0% (n=0)
Castor	44% (n=11)	52% (n=13)	0% (n=0)
Gueule tapée	0% (n=0)	0% (n=0)	0% (n=0)

The high level of contamination of samples from Kermel market was somehow unexpected since this market is located within downtown area and is mainly used by high-income households, contrary to the other supply sites that are located in densely populated suburbs with mostly low-income and deprived inhabitants. In this regard, it is interesting to note that none of the samples collected at Gueule-Tapée market contained any of the analyzed bacteria. These results suggest that the location of the supply sites has a little contribution to the contamination of the products.

3.2. Samples contamination and standard criteria for human consumption

We determined the level of contamination by calculating the bacterial densities in positive fruit and vegetables samples. For each product, the proportions of samples contaminated by *E. coli* were shown in Figure 2. The most contaminated samples are lettuce and mint leaves.

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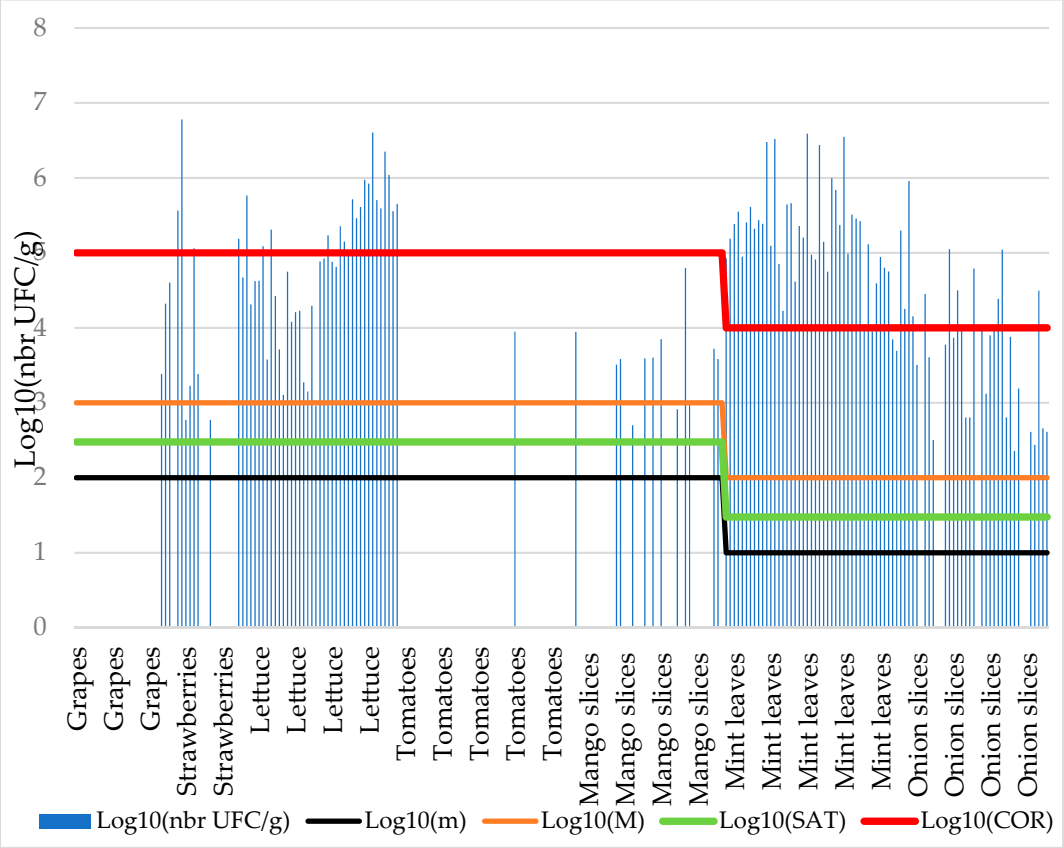

Figure 2. *E. coli* contamination level in different products.

As shown in Table 5, contamination levels ranged from 1,300 to 6,000,000 CFU g^{-1} for *E. coli* and thus, most of the samples were significantly above the acceptable thresholds for human consumption.

Table 5. Bacterial densities (nbr CFU/g) per matrix.

Samples	***E. coli***	***Vibrio spp.***	***Salmonella spp.***
Grapes	0	0	0
Strawberries	1700 to 6000000	1820 to 45500	0
Lettuce	1300 to 4000000	455 to 8730000	0
Tomatoes	8,9E+03	1820 to 81800	0
Mangoes slices	3200 to 62000	4090 to 2020000	0
Mint leaves	8900 to 3500000	6360 to 1950000	0
Onion slices	230 to 900000	1360 to 1710000	0

With regard to the acceptable satisfaction threshold (Sat) and corruption threshold (Cor) criteria for *E. coli*, 50.83% (n=122) of the 240 samples contained the maximal number (M) of CFU g^{-1} tolerated and 57.08% (n=137) were above the satisfaction threshold (Sat). Of these, 53.28% (73/137) were declared corrupted for *E. coli* (contamination level higher than Cor); i.e. 30.42% (73/240) if considering all the samples.

Vibrio spp. contamination reveals that 60.41% (n=145) of the samples were corrupted and the contamination level ranged from 455 to 8,730,000 CFU g^{-1} (Table 5). Thus, the bacterial loads in these samples were above the acceptable thresholds (i.e absence in the sample) (Figure 3).

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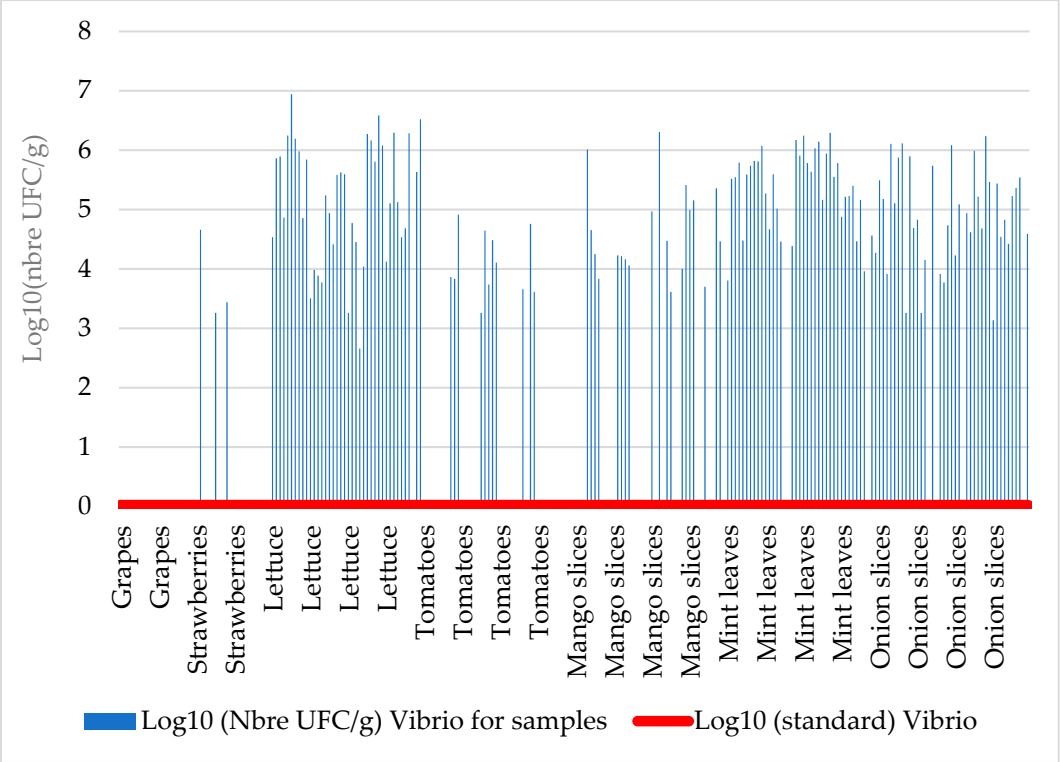

Figure 3. Vibrio spp. contamination level in different products.

Table 5 shows details of density intervals for each targeted bacterium in each analyzed matrix. It also gives an idea of the levels of high contamination of these products in comparison with the maximum standard threshold (M).

Analysis of the standard for consumption according to the sample collection sites showed that *E. coli* and *Vibrio spp.* germs were predominant in samples collected at Keur Massar market (90.91% and 87.27% respectively; n=55). Kermel market was ranked in second place with proportions of corrupted samples of 54.28% for *E. coli* against 80% for *Vibrio spp.* (n=35) following by Tilène market (61.82% for both *E. coli* and *Vibrio spp.* n=55), Castor market (44% and 52% for *E. coli* and *Vibrio spp.* respectively; n=25), Cheikh Anta Diop street (22.5% and 47.5% for *E. coli* and *Vibrio spp.* respectively ; n=40) and Dakar Down-Town (40% and 15% for *E. coli* and *Vibrio spp.* respectively ; n=20) (table 4). In contrast, none sample collected to Gueule Tapée's market were contaminated with targeted germs (n=10).

3.3. Metagenomic analysis

3.3.1. Bacterial communities

Given the the high contamination level of many products, we selected 46 samples for metagenomic analysis. These samples included mango slices (n=2), onion slices (n=7), lettuce (n=21) and mint leaves (n=16). The bacterial diversity for each sample using the relative abundance of each species were depicted in Figure 4.

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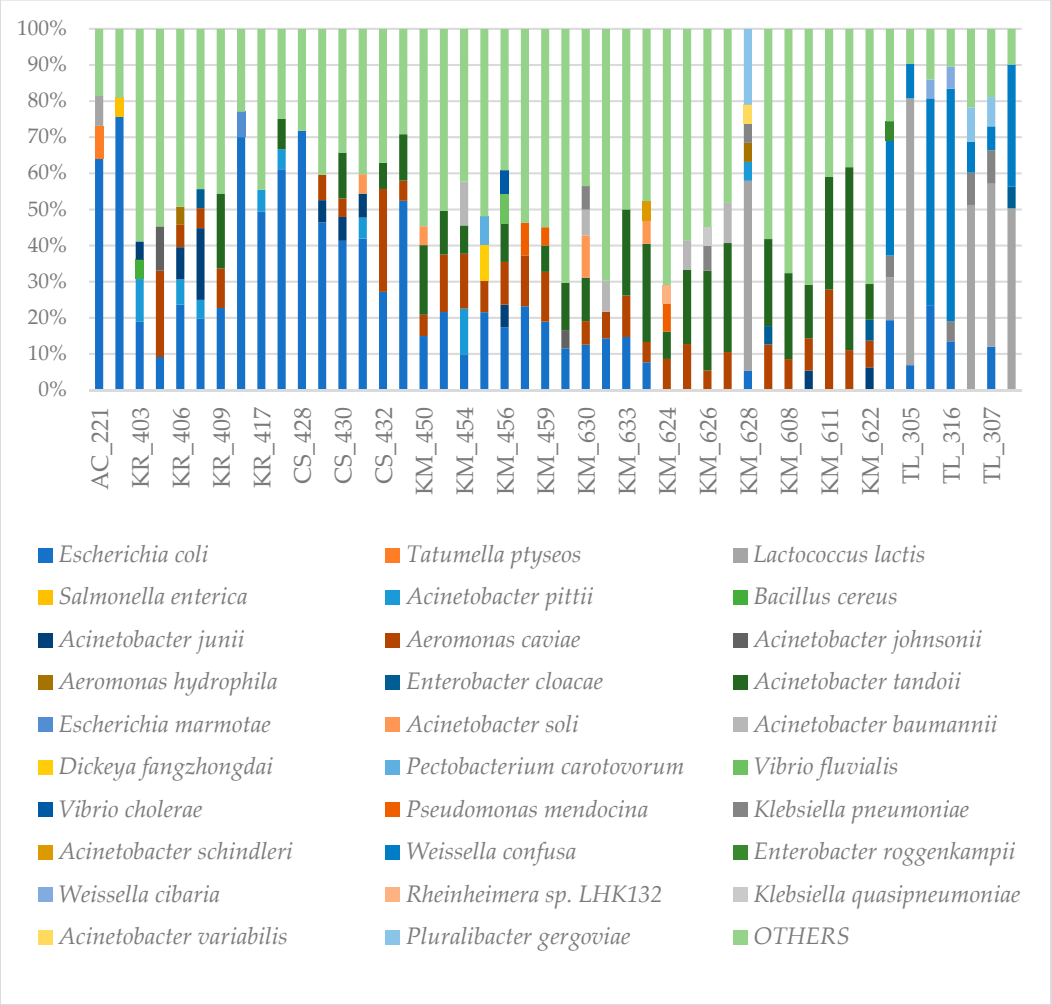

Figure 4. Relative abundance of species for each sample.

Details of metagenomic analysis showed a predominance of *E. coli* in 34 samples in which the relative abundance was greater than 5% (for all mangoes and lettuce samples; n=6 and n=5 for mint leaves and onion slices respectively) with the highest abundance noted in a mango slice sample (75.6%). Interestingly, pathogenic serotype *E. coli O157:H7* were found in 2/46 samples (4.35%), albeit in low abundance. The microbial community in onion slice samples was dominated by *Lactococcus lactis* found in 71.43% (n=5) of the samples, with the highest abundance being 73.8%. As for the mint leaf samples, the bacterial community was diverse and dominated by species classified as « OTHERS ».

Salmonella spp. was found in 43/46 (93.47%) samples with a maximum of 79 reads obtained. For *Salmonella enterica subsp. enterica*, the following serovars were found: *Senftenberg*, *Kentucky*, *Stanleyville*, *Enteritidis*, *Saintpaul*, *Thyphimurium*, *Cubana* among others. *Vibrio spp*. was found in 31/46 (67.39%) samples. The highest abundance (560 reads) was found in lettuce sample.

3.3.2. Alpha diversity

Figure 5 shows a heterogeneous alpha diversity from one matrix to another, with the same profiles for all the diversity parameters studied (ranged from mint leaves, lettuce, onions to mango slices, respectively the highest to the lowest alpha diversity).

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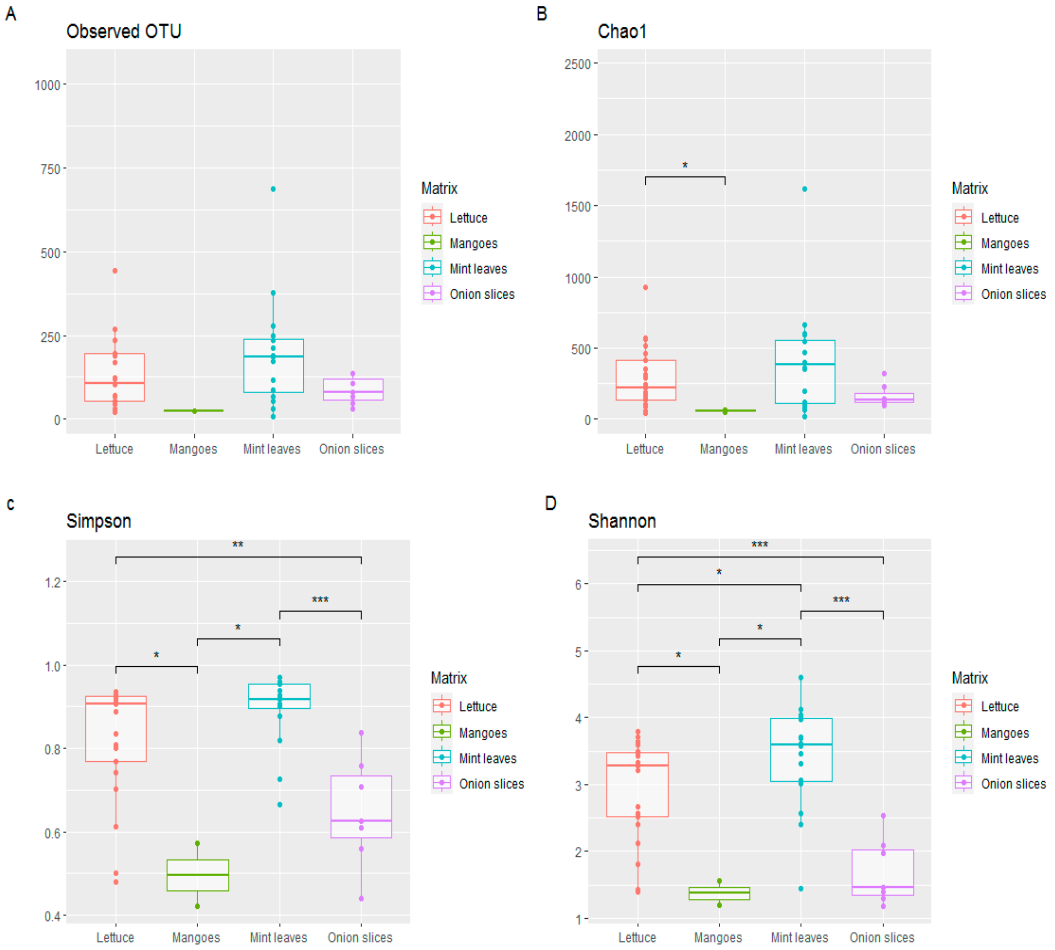

Figure 5. Alpha diversity metrics according to type of matrix. Significance of difference with Wilcox-Test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

High diversity was noted in the mint leaves and lettuce samples, particularly for the Shannon and Simpson indexes. The Wilcox test carried out on the alpha diversity metrics showed significant differences from one matrix to another (p-value=0.05). For the other diversity metrics (Observed OTUs and Chao1 index) the difference was not significant, so diversity is almost the same for all matrices combined, with the exception of that observed with the Chao1 index between lettuce and mango slices, showing a significant difference between the microbial communities.

3.3.3. Beta diversity

Dissimilarity was noted between the samples. Metrics such as the Bray-Curtis dissimilarity and the Jaccard distance indicate the presence of three clusters according to the community observed between the matrices when visualized using Principal Coordinates Analysis (Figure 6).

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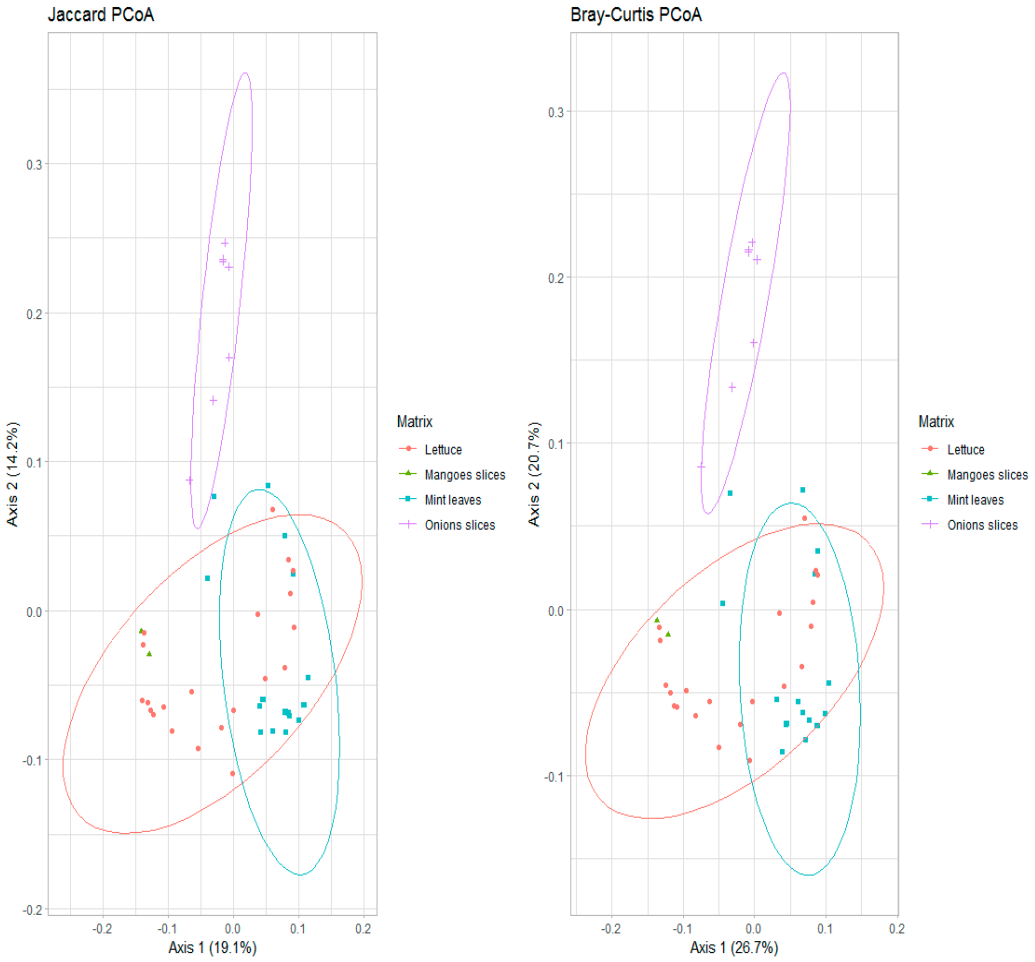

Figure 6. Beta diversity depending to the type of matrix.

The samples from mango slices matrix were included in the cluster formed by the lettuce samples. Clearly, we can distinguish two different large clusters: the first grouping together the mango slice, mint leaves and lettuce matrices, and the second entirely comprising onion slice samples. This observation shows that the microbial communities of samples in the first cluster are closer compared to samples in the second cluster.

4. Discussion

This study aimed to assess *E. coli*, *Salmonella spp*. and *Vibrio spp*. contamination level in fresh fruits and vegetables sold on stalls in streets and open-air markets of Dakar, the capital city of Senegal. Our findings show a heterogeneity in microbiological quality status of the analyzed samples. The proportions of corrupted samples varied depending on the bacterial species and the type of sample. The levels of contamination observed were above the critical threshold with more than 6.0 10^6 and 8.73 10^6 CFU g^{-1} for *E. coli* and *Vibrio spp*. respectively. A previous study related to microbiological quality of mango slices sold in Dakar (Senegal) also reports high level of *E. coli* contamination **[12]**. Similar results have been reported by other authors, particularly in the fourth-line products **[17]**, fruits and vegetables **[12]**. In this study, we did not detect *Salmonella spp*. in any of the samples. Similar findings was reported in ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market **[34]**.

The high prevalence of *E. coli* and *Vibrio spp*. could indicate fecal contamination of the products. The most unsatisfactory microbiological quality samples were mint leaves and lettuce. This could be caused by the use of untreated wastewater to grow these products **[14,35–43]**. Additionally, some farmers directly use raw fecal matter as organic fertilizers for horticultural production **[1,14,44]**,

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which could be a potential source of contamination of fruits and vegetables. Indeed, several studies reported that the type of watering plays an important role in the level of contamination when the used water directly contact the edible part of the plant instead of being poured at the he roots**[1,14,42]**.

All of the sources of contamination mentioned above are associated with the primary production phases. Moreover, although less contaminated than mint leaves and lettuce, other products such as onion slices and mangoes slices could also be corrupted by fecal contamination associated with poor hand hygiene during post-harvest handling. In particular, this concerns equipment for common use, such as multipurpose knives, rinsing and humidification water, contact surfaces of worktops where products are placed during the minimal processing for sale **[3,14,17,19]**. In addition, the high contamination of product may be due to the environmental hygiene of the open-air markets and streets of Dakar. These include the high attendance of visitors and traffic density **[12]**.

The relatively low prevalence of *E. coli* and *Vibrio spp*. in tomatoes can be explained by the difference in texture between tomato and the other products investigated. The smooth skin of tomatoes, compared to the folds of lettuce and mint leaves, could limit bacterial adhesion and proliferation. The absence of contamination observed in the grape samples can be explained by the smooth texture of the fruit and by the fact that the fruit is not in contact with the soil during cultivation and harvesting. Most of the time, it is imported and is delivered in primary packaging, which limits the potential handled contaminants.

Our metagenomic analysis further depicts the microbial community colonizing the analyzed fruits and vegetables. The high bacterial diversity noted in the different matrices analyzed could underline the impact of agricultural inputs, farming practices and handling of products sold in the streets and markets. Members of *Enterobacteriaceae* were widely present and included a wide range of important enteric foodborne pathogens, which represent a strong threat to public health and food safety **[45–47]**. Moreover, the application of poultry manure and other incomplete compost to the crops can also result in contamination with enteric bacteria in feces **[21]**.

The predominance of certain enteric species in the metagenomic analysis, in this case *E. coli* (in 100% of the samples sequenced), suggests a contamination of fecal origin and confirms the results obtained in bacterial culture **[48]**. The high prevalence of *Salmonella spp*. in the metagenomic analysis (93.47%), besides supporting fecal contamination, poses limits concerning the microbiological approach in which no sample was positive to *Salmonella spp*. This confirms the sensitivity of sequencing, which have the capacity to detect bacteria present in quantity below the threshold for positive culture **[49]**. Beata Kowalska et *al*. in their meta-analysis review found that the average prevalence of *Salmonella spp*. in lettuce was 4.1%, with values ranging from 0.1% for Japan to 50% for Burkina Faso **[50]**, figures that are below the results obtained in this study. These prevalences are lowest comparing to those found in this study.

Regarding the sources of contamination, data from the literature show that some pathogens including *Escherichia coli O157: H7*, *Listeria monocytogenes*, and *Salmonella spp*. are commonly isolated from animal feces including poultry and cattle, which are mostly used as fertilizers for horticultural products **[51–53]**. It was shown a few years ago that *E. coli O157: H7* can be transmitted to lettuce through the soil and irrigation water, and can persist throughout the life cycle of the plant and be transmitted to consumers **[51–53]**. The plant morphology (for example mint leaves) may influence the contamination rate due to close contact with the soil.

The high prevalence of *E. coli*, *Vibrio spp*. and *Salmonella spp*. observed in this study indicate a significant health risk associated with the consumption of these products analyzed. Given that most of these products are eaten raw, the possibility of foodborne epidemics is a reality, especially for children and immunocompromised people, who are the most vulnerable population group **[55–57]**.

5. Conclusion

The main objective of this study was to assess the level of contamination by *E. coli*, *Salmonella spp*. and *Vibrio spp*. of lettuce, tomatoes, mango and onion slices, mint leaves, strawberries and grapes sold on stalls in the streets and open-air markets of Dakar. Our findings show a high prevalence of the three bacterial species suggesting a fecal contamination that might originate from the use of

contaminated water for growing these products or from poor hand hygiene during post-harvest handling.

This study reports a very high rate of contamination of fruit and vegetables by the targeted germs, as well as the first use of the NGS approach in Senegal to describe the bacterial community in these products. Our results stress the need to raise awareness among the actors involved in the value chain of horticultural products, for good agricultural and hygiene practices in order to better integrate health safety and hygiene into their practice. Regarding the diversity of potential origins of contamination, these results represent a call to extend the investigation we conducted to a national scale while increasing the number of sites and of products analyzed.

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