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# Microbiological evaluation and possible origins of the microbial contamination of vegetables in Ouagadougou (Burkina Faso)

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**This study aimed to contribute to the health safety of widely consumed vegetables in Ouagadougou by investigating the potential contamination of onion, tomato, cucumber and lettuce with pathogenic microorganisms. A survey was conducted, involving 102 producers in fields, 102 vendors in markets, and 205 consumers. The most commonly consumed vegetables were identified as onion, tomato, cucumber and lettuce. A total of 264 samples, comprising 80 from fields and 184 from markets, were subjected to microbiological analysis, focusing on the isolation and identification of *Escherichia coli* and *Salmonella spp*. In market samples, onions exhibited the highest contamination with *E. coli*, registering a value of 316.2 × 103 CFU/g, while tomatoes showed the least contamination with *E. coli* at a load of 6.7 × $10^3$ CFU/g. Lettuce had the highest prevalence of Salmonella at 20.31%, while onions had the lowest prevalence at 2.38%. For field samples, cucumbers demonstrated the highest contamination with *E. coli* at 10 × 103 CFU/g, whereas onions had the least contamination at 2.4 × $10^3$ CFU/g. *Salmonella* was only detected in lettuces, with a prevalence of 4.76%.**

**Key words**: Convenience vegetables, *Escherichia coli*, evaluation, microbial contamination, *Salmonella* spp.

## INTRODUCTION

Fruit and vegetables constitute a crucial component of a healthy diet, serving as vital sources of nutrients, vitamins, and fiber. They play a significant role in promoting human health and well-being, with a particular emphasis on preventing vitamin C and A deficiencies (Gomes and Reynolds, 2021; Kumar et al., 2013). According to the Food and Agriculture Organization/World Health Organization (FAO/WHO), a daily intake of at least 400 g of fruits and vegetables is recommended to prevent chronic diseases such as heart disease, cancer, diabetes, and obesity. This intake is also effective in preventing or alleviating various micronutrient deficiencies, especially in developing countries. Despite the well-established health benefits

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associated with fruit and vegetable consumption, an unfortunate correlation exists between increased consumption of raw vegetables and a rise in the frequency of diseases linked to raw fruits and vegetables (Adjrah et al., 2011).

Vegetables commonly consumed in the sub-region include lettuce, cucumber, carrot, tomato, onion, parsley, and garlic (Toe et al., 2017). However, these vegetables are also recognized as a significant source of infection by various pathogens, including fungi, bacteria, viruses, and parasites (FAO/WHO, 2004). Incidents of food poisoning associated with the ingestion of contaminated vegetables have been documented worldwide (Panisset et al., 2003; Alegbeleye et al., 2018). Concerns about the safety of fresh fruits and vegetables have escalated as food-borne illnesses linked to these products continue to rise (EFSA, 2013).

Bacterial pathogens are identified as the most common agents causing foodborne illnesses, with viruses following closely (Phoeurk et al., 2019). Many of these enteric bacteria are implicated in an increasing number of collective foodborne infections (Martínez-Vaz et al., 2014). The consumption of raw vegetables heightens the risk of transmitting infectious diseases such as cholera, typhoid fever, and gastroenteritis, particularly if consumers neglect to observe proper hygiene rules (FAO/WHO, 2004).

Contamination of vegetables by *Salmonella and Escherichia coli* poses a global public health concern, particularly in developing countries (FAO/WHO, 2007). However, key participants in the vegetable value chain, including producers, processors, traders, and consumers, often prioritize the health benefits of vegetables over considerations of quality and hygiene (Adjrah et al., 2011; Antwi-Agyei et al., 2015).

Despite the nutritional and health advantages of vegetables, there has been a rise in human infections linked to the consumption of prepared fresh fruits and vegetables in recent years (Anin et al., 2016). Diseases associated with the consumption of contaminated fruit and vegetables are prevalent in various regions of developing countries, yet they are often underestimated due to a lack of reliable survey and surveillance data (Traoré et al., 2015). In Burkina Faso, there is a dearth of data on the hygienic quality of mass-market vegetables.

The objective of this study is to evaluate the microbiological quality and identify the source of contamination in mass-market vegetables in Ouagadougou.

## MATERIALS AND METHODS

### Study site and sampling

The study was conducted in Ouagadougou, as shown in Figure 1. Microbiological analyses were performed at the Laboratory of Molecular Biology, Epidemiology and Surveillance of Food-Transmissible Bacteria and Viruses (LaBESTA) at the Joseph KI-Zerbo University. Survey sites were chosen based on literature data, which helped identify key areas for vegetable production and sales, whether wholesale or retail, in Ouagadougou.

The selected production areas included Boulmiougou, Tanghin, Loumbila, Basky, and Ouaga 2000. Sales sites encompassed markets in Zone 1, Rayongo, Cité An II, El Nour, Gounghin, Zogona, Dassasgho, and Nabi Yaar. Actors present at these sales sites were interviewed as part of the survey. To ensure a representative sample of consumers, individuals were randomly selected from the streets of various neighborhoods for inclusion in the survey.

### Survey carried out

A comprehensive survey was conducted in Ouagadougou, encompassing fields (102 growers), markets (102 market women), and consumers (205 individuals). The primary objectives were to identify widely consumed vegetables in the city and assess practices posing a risk of biocontamination in both cultivation fields and public markets. Three distinct types of cards were employed for consumers, producers, and vendors to gather information relevant to each group.

The field survey specifically focused on pinpointing cultivation practices that could contribute to vegetable biocontamination. The investigation utilized the five key principles for growing safe fruits and vegetables, as outlined by the World Health Organization (FAO/WHO, 2007). These key areas included the quality of water used for irrigation, the use of manure as fertilizer, maintenance and hygiene of tools and storage areas, personal hygiene practices of producers, and control over animal access to cultivation fields.

At the market level, the survey aimed to elucidate the behavior and practices of women vendors, as well as the hygiene conditions within markets that might impact the microbiological quality of vegetables. Information was sought regarding the conditions under which vegetables are sold, the personal hygiene practices of female vendors, the overall hygiene of sales premises and the market environment, and the storage conditions for unsold vegetables at the end of the day.

The consumer survey gathered information on vegetable consumption patterns, including details about where, when, and how often vegetables are consumed. Additionally, consumers' perceptions of the hygiene practices of female vendors and the potential association of vegetables with instances of illness were documented.

### Sampling

Fresh vegetable samples were systematically obtained from the surveyed production areas and markets. Each sample, comprising 59 tomato samples, 49 cucumber samples, 106 lettuce samples, and 50 onion samples, was carefully collected in sterile bags and meticulously labeled with a unique identification number. To maintain sample integrity, they were promptly transported to the laboratory in a cooler equipped with ice.

The sampling strategy encompassed firm tomatoes without visible damage or cracks, damaged tomatoes, whole cucumbers, onions with their outer shells, and bunch of lettuce. Sampling occurred in both the fields for tomatoes, cucumbers, and lettuce, and in the markets for onions. Due to the limited availability of data on the prevalences of Salmonella and E. coli strains specific to the tested vegetables, namely tomatoes, cucumbers, onions, and lettuce, the sample size required for representativeness was determined using the formula provided by the World Health Organization (WHO, 1991) for probability sampling (Formula 1).

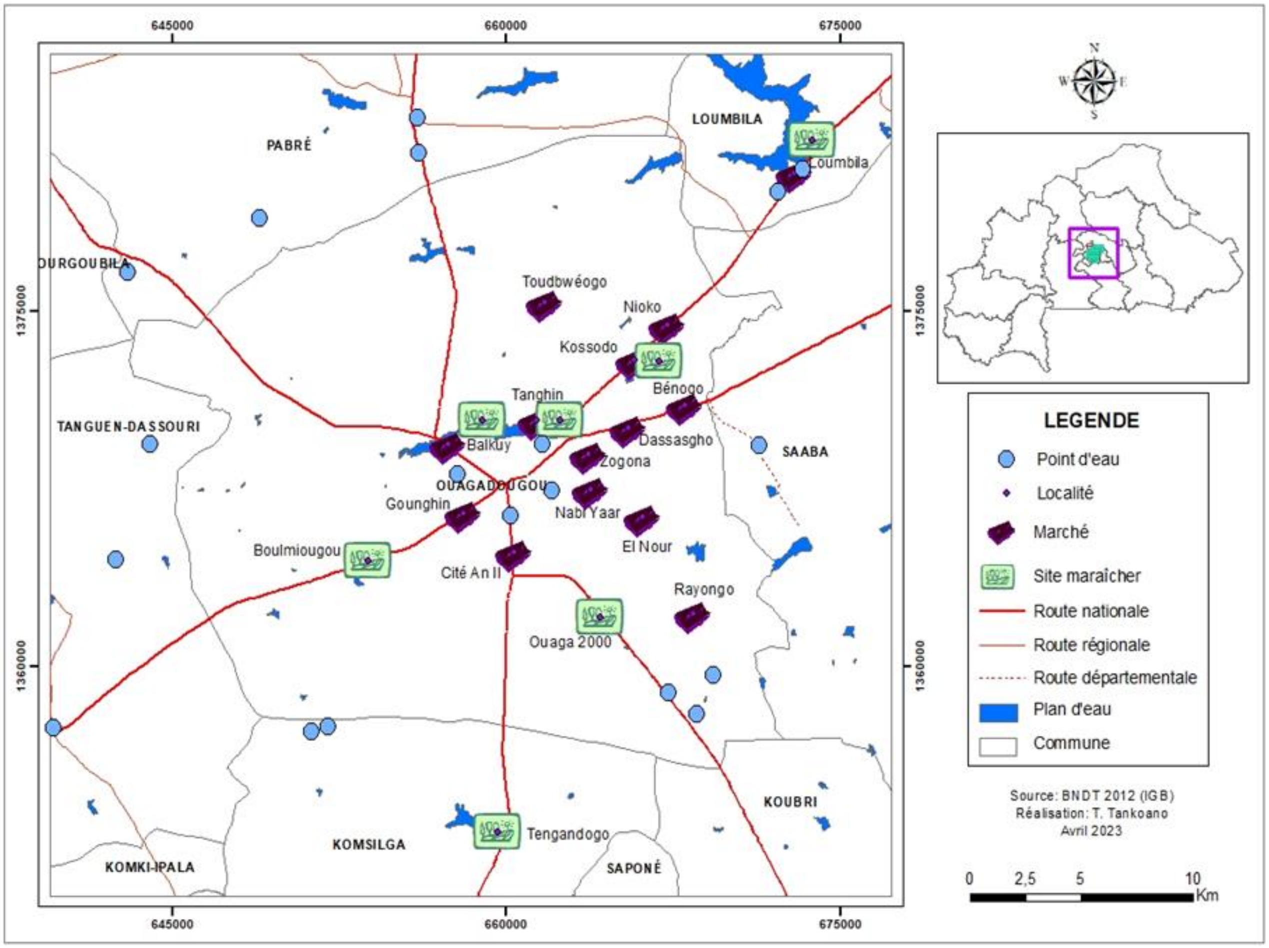


**Figure 1.** Map of the city of Ouagadougou with surveyed and sampled sites

N = PQ/(E/L) 2

Where N is the minimum sample size, P is the estimate of the expected proportion (prevalence rate), Q is the value of (1-P), E is the tolerated margin of error (statistical risk in %) and L is the smallest deviation for the accepted statistical risk (1.96 for the 5% risk). The above relationship for p equals 0.8 gives a minimum of 246 samples.

A lettuce sample consisted of two lettuce plants taken at random from different parts of the same field or from the same vendor's lots. For tomatoes, a sample consisted of 10 balls. For cucumbers, a sample was made up of 1 to 2 depending on size, and an onion sample was made up of 5 whole bulbs. These vegetables were taken from different parts of the same field, or from different lots held by the same vendor. All the onions were collected only in the markets, with the exception of one batch of 8 samples taken in the Boulmiougou market garden. In the fields, only market-ready vegetables were taken into account. Samples were taken in both fields and markets in a single pass (Figure 2).

## Microbiological analysis

*Salmonella* detection was conducted using a method adapted from NF EN ISO 6579:2002. Initially, a predetermined quantity of Eau Peptonnée Tamponnée (EPT) was added to 225 ml vials, and the contents of the sachets were mixed. Following this, 25 g of each sample, contained in sterile sachets, was added to 225 ml of Buffered Peptone Water and homogenized using a stomacher. The mixture underwent incubation at 37ºC for 18 to 24 h, constituting the pre-enrichment stage. Subsequently, 0.01 ml of the pre-enriched suspension was added to 10 ml of Rappaport Vassiliadis Soja (RVS) broth and incubated at 42ºC for 24 h. After incubation, 10 ul of Rappaport Vassiliadis Soja (RVS) was streaked onto XLD agar, followed by incubation at 37ºC for 24 h. *Salmonella* strains on XLD agar exhibited black colonies with a red background, consistent with the color of the XLD medium. Three characteristic colonies from each XLD medium were transferred to XLD agar for colony purification and then incubated at 37ºC for 24 h. Subsequently, three characteristic colonies from the XLD medium were transferred to Muller Hilton (MH) agar and incubated at 37ºC for 24 h for phenotypic identification. Phenotypic identification involved determining biochemical characteristics such as glucose fermentation, lactose oxidation, gas and hydrogen sulfide ($H_2S$) production on Kligler-Hajna agar, urease and indole production, and citrate utilization as the sole carbon source on Simmons citrate agar.

For *E. coli* enumeration, 25 g of each vegetable sample was added to a vial containing 225 ml of peptone-buffered water and subjected to stomaching for 5 min to prepare the stock solution. This stock solution was used to prepare cascade dilutions in tubes containing 9 ml of buffered peptone water. For plating, 100 ul of the 10 to 2 and 10 to 3 dilutions were spread directly onto


**Figure 2.** Vegetables.

Methylene Blue Eosin agar (EMB) and incubated at 44ºC for 24 h. Characteristic *E. coli* colonies on EMB (dark purple with or without metallic sheen and dark center) were counted. These colonies were then transferred to Mueller-Hinton (MH) agar and incubated at 37ºC for 24 h. Suspected *E. coli* colonies underwent identification through biochemical tests, including glucose fermentation, lactose oxidation, gas and hydrogen sulfide (H2S) production on Kligler-Hajna agar, and urease, indole, and citrate production as the sole carbon source on Simmons citrate agar. Results were expressed as colony-forming units (CFU) per plate.

## RESULTS

### Microbial loads in field vegetables

Bacteriological analysis revealed varying *E. coli* loads in different vegetables, with successive high levels observed in cucumbers, tomatoes, lettuces, and onions, ranging from $2.4 \times 10^3$ to $10 \times 10^3$ CFU/g. Cucumbers exhibited the highest contamination with *E. coli*, while onions showed the least contamination. Notably, onions, tomatoes, and cucumbers did not show contamination with *Salmonella*. Conversely, two lettuce samples were found to be contaminated with Salmonella, resulting in a prevalence of 4.76% (Table 1).

The microbiological analysis of samples collected from markets revealed varying *E. coli* loads in different vegetables, with successive high levels observed in onions, cucumbers, lettuces, and tomatoes, ranging from $6.7 \times 10^3$ to $316.2 \times 10^3$ CFU/g. Onions exhibited the highest contamination with *E. coli*, while tomatoes showed the least. Notably, onions, tomatoes, lettuces, and cucumbers were found to be contaminated with *Salmonella*. Lettuces demonstrated a notably high presence of Salmonella with a prevalence of 20.31%, followed by tomatoes at 11.36%, cucumbers at 2.94%, and onions at 2.38% (Table 2).

### Vegetables producers' practices

Analysis of our survey data revealed that: 100% of fields visited had no sanitary facilities. In the event of injuries or lesions to market gardeners, protective measures

**Table 1.** Average *E. coli* load (CFU/g) and *Salmonella spp* prevalence in vegetables collected from field.

| Microorganism | Tomato (n=15) | Onion (n=8) | Cucumber (n=15) | Lettuce (n=42) |
|---|---|---|---|---|
| *E. coli* | $6.6 \times 10^3$ | $2.4 \times 10^3$ | $10 \times 10^3$ | $3.5 \times 10^3$ |
| *Salmonella* spp. | Absent (0%) | Absent (0%) | Absent (0%) | 2 (4.76%) |

**Table 2.** Average load of *E. coli (*CFU/g) and presence of *Salmonella* in staple vegetables sampled in Ouagadougou markets.

| Microorganism | Tomato (n= 44) | Onion (n= 42) | Cucumber (n=34) | Lettuce (n=64) |
|---|---|---|---|---|
| *E. coli* | $6.7 \times 10^3$ | $316.2 \times 10^3$ | $34.3 \times 10^3$ | $13.8 \times 10^3$ |
| *Salmonella* spp. | 5 (11.36%) | 1(2.38%) | 1(2.94%) | 13 (20.31%) |

were not applied (39%). Most of the fields were not fenced (72%), and there were piles of garbage (33%), domestic animals (14%) or livestock close to the fields (51%). Most farmers (94%) use animal manure to improve the soil, and 45% admit that they do not treat it before use. Water from wells and dams is used mainly (75%) for watering vegetables, and (100%) of this water was untreated. When harvested, vegetables are placed in contact with the soil (21.6%), packed in plastic bags (56%) and transported to markets by motorcycle (80%).

### Vegetables vending conditions in markets

Vegetables are mostly washed at reception with dirty water (83%). Vendors say they use public toilets for their needs (78.4%) and wash their hands afterwards with soap and water (85%). Vegetables are mostly sold in open-air markets (65%) and in unsanitary conditions (35%). The rest of the vegetables not sold during the day are kept at room temperature (100%).

### Knowledge about vegetable-related illnesses

More than half (75%) of the consumers questioned said that vegetables can carry diseases. Consumer perceptions differed significantly according to the level of education. The main reasons given by consumers for the involvement of vegetables in cases of disease transmission were the absence or inadequate cleaning of vegetables (44.3%), lack of hygiene during preparation (26.9%) and poor storage conditions (19.9%).

### Knowledge of the main place of vegetable consumption

Vegetables are eaten exclusively in restaurants in 28% of cases, at home in 48% and in other places in 24% of cases. Vegetables are mainly eaten for dinner (61%) and lunch (32%), with 77% consuming them at least once a day.

## DISCUSSION

The surveys conducted revealed that the production of staple vegetables in Ouagadougou is exclusively carried out by men. These findings align with previous studies by Alio Sanda et al. (2017) and Toe et al. (2017), which demonstrated that in Niger and Abidjan, respectively, market gardening is predominantly practiced by men (100 and 73.3%). In Ouagadougou, growers are primarily aged between 30 and 45 (69.5%) and operate medium-sized fields (63.7%). The absence of female representation and the average size of farms may be attributed to cultural practices in the country and the demanding nature of irrigation work.

The study suggests that the presence of *enterobacteria*, such as *Salmonella and E. coli*, in vegetables is likely due to the precarious hygienic conditions under which they are cultivated and sold, including equipment contact and the immediate environment of the products. The high microbial load observed in vegetables from both markets and fields could be linked to inadequate storage facilities, poor personal hygiene among vendors and growers, insufficient waste disposal and sanitation facilities, the use of water for plant irrigation, animal manures for soil amendment, and a lack of sanitary facilities.

In onion samples from both fields and markets, the microbial load of Escherichia coli was successively observed (2.4 × 103 CFU/g and $316.2 \times 10^3$ CFU/g). In contrast, Anin et al. (2016) found no Escherichia coli in their onion samples from Abidjan (0 CFU/g). The Escherichia coli microbial load ($9.2 \times 10^2$ CFU/g) reported by Anin et al. (2016) in tomato purée in Abidjan is higher than the levels found in the present study for tomato samples ($6.6 \times 10^3$ CFU/g for fields and 6.7 × 103 CFU/g for markets).

*Salmonella* prevalences of 0% in onions, tomatoes, and field cucumbers align with the guidelines set by the WHO, which recommend no presence of Salmonella in any raw vegetable intended for consumption (WHO, 2012). However, the prevalences of tomatoes, onions, cucumbers, and lettuces from markets did not meet this recommendation (Prevalence over 0%). The prevalence of Salmonella in lettuce samples from markets (20.31%) and fields (4.76%) is lower than the 50% reported by Traoré et al. (2015) in lettuce from fields in Burkina Faso. Additionally, the prevalence of Salmonella in tomato samples (5.3%) reported by Toé et al. (2017) in Abidjan is lower than the levels found in tomato samples collected from markets in this study (11.36%).

The disparity in *Salmonella* prevalence between field and market lettuces could be attributed to the practice in the field where vegetables remain unwashed, coming into contact with potentially contaminated water and manure used as fertilizer. Some authors in Burkina Faso have noted that fertilizers utilized by farmers are often untreated and may contain pathogenic bacteria detected in animals' feces (Bako et al., 2018; Kagambèga et al., 2013). However, the Salmonella prevalence in vegetables from fields reported in this study is lower than the levels reported by Toe et al. (2017) in lettuce (16.1%) and cucumbers (4.8%), but similar in onions (0%).

The prevalence of *Salmonella* in onions found in our study from the fields (0%) aligns with the findings by Anin et al. (2016). Conversely, we observed a prevalence of Salmonella in market tomato samples (11.36%), while Anin et al. (2016) found no Salmonella in their tomato purées. Alio Sanda et al. (2017) in Niger reported higher *Salmonella* prevalence (36.94%) than that found in lettuce from fields (4.76%) and markets (20.31%) in our study. In Malaysia, Saw et al. (2020) found a *Salmonella* prevalence of 0% in tomato samples, which is consistent with the present results for tomatoes from fields but lower than that found in tomatoes from markets (11.36%). The cucumber prevalence reported by Saw et al. (2020) at 10% was higher than that in our market and field samples, respectively (2.94% and 0%).

Additionally, Saw et al. (2020) found a prevalence of 0% in their lettuce samples, which is lower than the levels observed in our field and market lettuces (4.76 and 20.31%, respectively). These differences in *Salmonella* prevalence in vegetables across different countries could be explained by variations in climate, production practices, and the level of hygiene control in each country.

## Conclusion

A significant portion of our diet includes raw foods, and depending on their nature and production methods, these raw foods can carry germs, some of which may be pathogenic for humans. These germs are typically susceptible to cooking. However, in the absence of cooking, the risks associated with consuming raw food must be addressed by controlling the microbiological quality of raw materials. The contamination of staple vegetables in Ouagadougou is attributed to factors such as ignorance, inadequate, or poorly applied good hygiene practices. Notably, the vegetables consumed in Ouagadougou are more commonly contaminated by *E. coli* than *Salmonella*, posing a risk of food poisoning. Raising awareness among various stakeholders in this sector, including producers, vendors, and consumers, has the potential to significantly reduce contamination issues associated with these products, which are an important source of nutrients.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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