

# Evaluation of fresh vegetable microbiological contamination in fast-food restaurants

Contamination  
in fast-food  
restaurants

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## Abstract

**Purpose** – Fresh vegetables contain advantageous phytochemical components, making them one of the most significant sources of nutrition. The threat of harmful bacteria still exists because these vegetables are not heated in restaurants before being consumed. Therefore, this study aimed to evaluate the microbial quality of fresh vegetables in restaurants of different levels.

**Design/methodology/approach** – A total of 499 fresh vegetable samples (from sandwiches and fresh-cut vegetable salads) were collected from 3 different types of food service establishment: 201 from international restaurants (IRs), 210 from national restaurants (NRs), and 88 from cafeterias (CAs). The samples were prepared and inoculated on specific growth media. The aerobic mesophilic bacteria (AMB) *Campylobacter* spp., *Staphylococcus aureus* (*S. aureus*), *Enterobacteriaceae*, *Escherichia coli* (*E. coli*) and yeast and molds were counted, and *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157 were detected using specialized medium.

**Findings** – High counts of *S. aureus*, above 3 log cfu/g, suggested that 71.5% of samples collected from NRs and 77.3% from CA were not accepted, whereas 81.6% of samples collected from IRs were accepted. The low population of *E. coli*, less than 2 log cfu/g, suggested that 99.0, 97 and 92.0 % of samples collected from IRs, NRs and CA, respectively, were accepted. *Listeria monocytogenes* and *Escherichia coli* O157 were absent from every sample. One sample was positive for *Salmonella* spp. in each of the NR and CA sample groups.

**Originality/value** – RIs adhere to health and hygiene standards better than NRs and CAs, according to the findings of vegetable contamination tests.

**Keywords** Food contamination, Vegetables, Fast-food restaurants, Pathogens

**Paper type** Research paper

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**Conflicts of interest:** The authors declare that there is no conflict of interest related to this study.

**Corrigendum:** It has come to the attention of the publisher that the article: Saleh, F.A. and Al-Otaibi, M.M. (2023), "Evaluation of fresh vegetable microbiological contamination in fast-food restaurants", *Arab Gulf Journal of Scientific Research*, Vol. ahead-of-print No. ahead-of-print. <https://doi.org/10.1108/AGJSR-02-2023-0066> incorrectly listed the funding details in the acknowledgments as No: 22774.

Our guidelines make it clear that funding information is to be checked at proof stage.

The error has been corrected in the online version of the article to include the correct funding information.



## 1. Introduction

The spread and growth of fast-food restaurants are steadily increasing in all parts of the world. According to public sector figures for 2020, Saudi Arabia now has 34,357 catering outlets, or a 70.8% rate, the majority of which are service-oriented restaurants. The market for restaurants was worth 22.4bnUS\$ in 2020, and it is anticipated that it will expand at a 12.2% annual pace from 2020 to 2025 (Anonymous, 2020). A crucial subset of ingredients for fast-food restaurants includes fresh vegetables like tomatoes, cucumbers, lettuce, watercress, parsley, carrots, rocca, broccoli and green peppers. Because they include vital substances including vitamins, minerals, plant sterols, natural antioxidants and dietary fibers, these fresh vegetables are regarded as one of the most significant food groups for human health.

As knowledge of obesity and cardiovascular disease patterns in the Gulf Cooperation Council (GCC) region grows, people are being encouraged to adopt healthy eating practices. The demand for high-quality fruits and vegetables is rising as a result of this healthy eating pattern (Mordor Intelligence, 2023).

Given the importance of eating vegetables, the World Health Organization (WHO) has recommended that a person eat 400 grams per day of fresh vegetables or fruits (WHO and FAO, 2003). Although vegetables are a source of important nutrient compounds, they can also be a source of microbial contamination and pathogens. It has been proven in a lot of research that fast-food restaurants are frequently linked to foodborne diseases that affect human health (Hazra, Collison, & Davis, 2022; U.S. Food & Drug Administration, 2023). In recent times, complaints about increases in foodborne diseases, especially those that are carried through vegetables and fruits, have increased in different regions of the world (Esmael *et al.*, 2023). Oliveira *et al.* (2010) and (Zhang *et al.*, 2018) reported an increase in the number of foodborne illnesses linked to vegetables and fruits in the United States of America (USA) and European Union (EU) countries. Leafy vegetables are the second-largest vector of foodborne bacteria in the USA (Kyle, Parker, Goudeau, & Brandl, 2010). A multi-state *Listeria monocytogenes* outbreak in the USA in 2022 was linked to bagged leafy greens from Dole. There have been reports of an epidemic of *Listeria monocytogenes* in 18 individuals from 13 different states (U.S. Food & Drug Administration, 2023). Despite the fact that everyone can contract a foodborne illness, sometimes known as food poisoning, some populations are more susceptible to it and to a serious sickness. These groups comprise people over the age of 65, children under the age of 5, individuals with compromised immune systems and pregnant individuals (Hazra *et al.*, 2022). Vegetables are a common way for many infections to spread (Rahman *et al.*, 2022), causing 2,191 disease cases and 11 deaths as a result of eating food loaded with foodborne bacteria in 2018 in Saudi Arabia (Ministry of Health, 2019). There is a close correlation between the type of foodborne bacteria and the type of food; fresh lettuce leaves and also a green salad containing lettuce carry many pathogenic bacteria, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Shigella sonnei*, *Campylobacter jejuni* and *Salmonella newport*. Fresh green parsley may be a carrier of bacteria such as *Shigella sonnei*, while green basil leaves may be carriers of microbes such as *Salmonella senftenberg* and *Cyclospora cayetanensis* (Mercanoglu Taban & Halkman, 2011).

The sources of contamination of fresh vegetables in fast-food restaurants have been studied by previous researchers (Soriano *et al.*, 2001a, b; Sospedra, Rubert, Soriano, & Mañes, 2013; Darwish, 2018), who have found that many different sources can cause contamination of vegetables in restaurants, including a lack of hygienic practices. A study by Sospedra *et al.* (2013) analyzed 555 samples of vegetables taken from restaurants and found that 13% of them were unsatisfactory and contained high levels of mesophilic aerobic bacteria. The samples most contaminated with *Enterobacteriaceae* were tomatoes and lettuce. Pathogenic bacteria have also been evaluated in fast-food establishments, which were found to have *Staphylococcus aureus* in 97.3% of cases, followed by *Salmonella* spp. in 86.7% of cases (Usman, Oladimeji, & Oluwasogbo, 2018). Soriano *et al.*, (2001a) also studied the microbial

contamination of fresh green salad and fresh lettuce, finding contamination with *Staphylococcus aureus*. [Shahbaz \(2022\)](#) found that out of 82 samples of fresh vegetables, 7% were found to be unsatisfactory or exceeded acceptable limits. The researchers attributed this contamination to unclean practices for dealing with fresh vegetables or to insufficient health standards in restaurants. [Soriano et al., \(2001b\)](#) also isolated different genera of *Listeria* from ready-to-eat salad. Moreover, cutting boards and knives may be considered important sources of vegetable contamination. [Bukhari et al. \(2021\)](#) found food contact surface materials contaminated with nine food-borne bacteria in some restaurants in Makkah city. Even the water used for irrigation can be a source of contamination for fresh vegetables before they reach restaurants ([Weldezigina & Muleta, 2016](#); [Saab et al., 2022](#)).

In the Al-Ahsa region of Saudi Arabia, all sorts of fast-food restaurants use fresh vegetables; however, no research on the detection of microbial contamination of these vegetables has been found after analyzing the documentation of previous studies. The present work was a survey study to determine the extent of contamination of fresh vegetables in fast-food restaurants in the Al-Ahsa region of Saudi Arabia. We identified the types of microbes transported through the fresh vegetables, documented useful information for consumers to maintain their health and take care of necessary meals and informed the regulatory authorities and bodies concerned with food safety and human health about the actual reality of the extent of contamination of those meals. This information is for those restaurants to encourage them to follow health conditions to provide a clean and healthy product.

## 2. Materials and methods

### 2.1 Samples collection

Three different types of fast-food restaurants (food service establishments) provided a total of 499 samples (sandwiches and fresh-cut vegetable salads): 201 from international restaurants (IRs), 210 from national restaurants (NRs) and 88 from cafeterias (CAs) in the Al-Ahsa area of Saudi Arabia. The samples were taken at random from several locations in Saudi Arabia's Al-Ahsa Governorate, including Hofuf, Al-Mubarraz, Al-Uyoun, Al-Tarf, Al-Omran and Al-Jafr. The number of samples, the kind of samples and the vegetable ingredients are illustrated in [Table 1](#). IRs: It is a regular entity that prepares and provides meals for its customers to be eaten inside the dining hall attached and prepared for this purpose or outside (it has multiple branches in different parts of the world). NRs: It is like IRs, but there are no other branches outside the country, and it also serves traditional meals. CA: It is a regular entity whose customers serve themselves from a counter, in which they prepare sandwiches and light meals and serve hot and cold drinks, and payment is made before eating. The samples were collected in May and June 2018, June 2019 and August 2020. The samples were kept in an icebox until they reached the lab. Three to five restaurant samples were collected daily, and samples were analyzed on the same day.

### 2.2 Microbiological analyses

For all samples, 25 g of the vegetable part of a sandwich or fresh-cut vegetable salad was mixed with 225 mL of 0.1% buffered peptone water (BPW) into a sterile stomacher bag (Difco, Becton Dickinson). The samples were blended in a stomacher blender (Lab Blender 400UK) for 5 minutes. The serial dilution was made up to  $10^{-5}$  in 0.1% BPW. Samples of 1.0-0.1 mL of each dilution were plated in duplicate on the appropriate media. Plate count agar medium (CM0107, Oxoid) was used to determine the aerobic mesophilic bacteria (AMB) according to [Atlas \(2004\)](#). The plates were incubated at 30 °C for 72 h. For the enumeration of *Campylobacter* species, a blood-free selective agar base plate medium (CM0739, Oxoid) was used. The plates were incubated under microaerophilic conditions at 37 °C for 48 h using a gas generating kit (Oxoid, BR0060) in conjunction with an anaerobic jar. *Staphylococcus* medium

| Food service establishments | Number of samples                                     | Kind of samples              | Fresh vegetable ingredients  |   |
|-----------------------------|---|------------------------------|------------------------------|---|
| IRs                         | 31  | Beef burger                  | Tomato, cucumber and lettuce |   |
|                             | 43  | Chicken sandwich             | Lettuce                      |   |
|                             | 32  | Chicken burger               | Tomato and lettuce           |   |
|                             | 95  | Beef burger                  | Tomato and lettuce           |   |
|                             | NRs   | 15                           | Chicken sandwich             | Lettuce                                       |
|                             |   | 19                           | Chicken sandwich             | Tomato and lettuce                            |
|                             |   | 91                           | Fresh-cut vegetable salad    | Tomato, cucumber, lettuce and carrot          |
|                             |   | 29                           | Fresh-cut vegetable salad    | Tomato, cucumber and lettuce                  |
|                             |   | 13                           | Fresh-cut vegetable salad    | Cucumber, cabbage and carrot                  |
|                             |   | 17                           | Fresh-cut vegetable salad    | Tomato, cucumber, lettuce, cabbage and carrot |
|                             |   | 11                           | Fresh-cut vegetable salad    | Tomato, cucumber and lemon slices             |
|                             |   | 15                           | Fresh-cut vegetable salad    | Tomato, cucumber, lettuce and lemon slices    |
|                             |   | CA                           | 13                           | Sandwich of falafel* and French-fried potato  |
| 18                          |   |                              | Falafel sandwich             | Tomato, lettuce, onion and rocca              |
| 11                          | Sandwich of falafel and French-fried potato           |                              | Tomato and lettuce           |   |
| 27                          | Beef burger   |                              | Lettuce                      |   |
| 19                          | Sandwich of falafel, French-fried potato and eggplant |                              | Tomato and cabbage           |   |
| 18                          | Falafel sandwich                                      |                              | Tomato, cucumber and lettuce |   |
| 17                          | Sandwich of falafel and French-fried potato           |                              | Tomato, cucumber and lettuce |   |
| 12                          | Sandwich of falafel, French-fried potato and eggplant | Tomato, cucumber and cabbage |                              |   |

**Table 1.**

Descriptions of fresh vegetable samples collected from IRs, NRs and CAs

**Note(s):** 1. \*Falafel is a deep-fried patty or ball prepared from ground chickpeas or fava beans with garlic, leek and coriander

**Source(s):** Table by authors

no. 110 (CM0145, Oxoid) was used for the enumeration of *Staphylococcus aureus*. The plates were incubated at 37 °C for 24-48 h (producing yellow-orange pigment colonies and clearing zones around them) and identified using the staphylase test (DR0595, Oxoid). The *Enterobacteriaceae* were counted using a violet-red bile glucose agar (VRBGA) medium (CM1082, Oxoid). The plates were incubated at 37 °C for 24 h (Atlas, 2004). The *Escherichia coli* were enumerated in RAPID *E. coli* 2 agar medium (3564024, BIO-RAD). The plates were incubated for 24 h at 44 °C, and colonies colored violet to pink were considered to be *E. coli*. Among the *E. coli* detected on RAPID *E. coli* 2 agar medium, the presence of *E. coli* O157 was tested using the *Escherichia coli* O157 Latex Test (DR0620, Oxoid) (Goncuoglu, Erol, Ayaz, Ormanci, & Kaspar, 2010). For enumeration of yeasts and molds, potato dextrose agar medium (CM0139, Oxoid) was used and the plates were incubated at 30 °C for 72 h. The counts of tested microorganisms were expressed as logarithm colony-forming units (log CFU) per gram (Atlas, 2004). The method of Rhodes and Quesnel (1986) was used for isolation and identification of *Salmonella* spp., 0.1 and 1 mL measures of BPW were placed into the Rappaport–Vassiliadis broth (RV), (CM0669B, Oxoid) and Muller–Kauffmann tetrathionate broth (MTTn), (CM0343, Oxoid), respectively. The enrichment broths were incubated at 37°C (for MTTn broth) and 42 °C (for RV broth) for 24 h. Confirmation of the positive cultures was performed using the API (analytical profile index)20E identification system (BioMerex,

Marcy l'Etoile, France). After incubation, the streaked xylose–lysine–desoxycholate (XLD) agar plates (CM0469 Oxoid) were incubated at 37 °C for 24 h. For isolation and identification of *Listeria monocytogenes*, 1 mL quantities of BPW were inoculated into a Fraser broth (CM0895 Oxoid). The enrichment broth was incubated for 48 h at 37 °C. The turbid tubes were streaked onto Palcam agar (CM0877 Oxoid) and incubated at 37 °C for 24 h. The gram-positive, motile, catalase-positive and oxidase-negative colonies were identified by API Listeria (BioMérieux, Mancy l'Etoile, France).

### 2.3 Statistical analysis

The minimum, maximum, mean and standard deviation values of the microbial counts and simple mathematical operations such as the percentage of samples contaminated with numbers of microorganisms were calculated by one-way analysis of variance (ANOVA) using Minitab 17 software.

## 3. Results and discussion

### 3.1 Contaminated microorganisms

**3.1.1 Aerobic mesophilic bacteria (AMB).** As shown in Table 2, the AMB count ranged from  $<10^2$  to  $10^3$  cfu/g in IR samples, from  $<10^2$  to  $>10^5$  cfu/g in NR samples and from  $<10^2$  to  $10^5$  cfu/g in CA samples. The highest sample percentages (91%, 92.9% and 77.3% from IRs, NRs and CA, respectively) were found in 2, between 3 and  $>5$  and in 5 log cfu/g for IR, NRs and CA, respectively. In IRs, 91% of the samples had an AMB count  $>2$  log cfu/g, while only 4.7% and 9.12% of NRs and CA had the same count, respectively. According to the technical guidelines of hazard analysis critical control point and total quality management (HACCP-TQM) (Anonymous, 1998), if the count of AMB (at 21.1°C) in raw foods  $< 4$  log cfu/g considered “good”, 4-6.7 log cfu/g “average”, 6.7-7.7 log cfu/g “poor” and  $>7.7$  log cfu/g “spoiled”. In our results, no samples from either IRs or CA contained AMB above 3 and 5 log cfu/g. However,

| Microorganisms               | Food service establishments | Percentage of samples in the indicated interval |                 |                 |                 |         |
|------------------------------|-----------------------------|---|-----------------|-----------------|-----------------|---------|
|                              |                             | $<10^{2a}$                                      | $10^2$ - $10^3$ | $10^3$ - $10^4$ | $10^4$ - $10^5$ | $>10^5$ |
| Aerobic mesophilic bacteria  | IRs                         | 91.0  | 9.0             | 0               | 0               | 0       |
|                              | NRs                         | 4.7   | 2.4             | 28.6            | 42.9            | 21.4    |
|                              | CA                          | 9.12  | 2.3             | 11.4            | 77.3            | 0       |
| <i>Campylobacter</i> spp.    | IRs                         | 0   | 0               | 0               | 0               | 0       |
|                              | NRs                         | 0   | 0               | 0               | 0               | 0       |
|                              | CA                          | 0   | 0               | 0               | 0               | 0       |
| <i>Staphylococcus aureus</i> | IRs                         | 9.0   | 72.6            | 18.4            | 0               | 0       |
|                              | NRs                         | 21.4  | 7.1             | 57.1            | 14.3            | 0       |
|                              | CA                          | 17.0  | 5.7             | 33.0            | 44.3            | 0       |
| Enterobacteriaceae           | IRs                         | 27.4  | 54.2            | 18.4            | 0               | 0       |
|                              | NRs                         | 14.3  | 7.1             | 35.2            | 35.7            | 7.6     |
|                              | CA                          | 22.7  | 21.6            | 55.7            | 0               | 0       |
| <i>Escherichia coli</i>      | IRs                         | 99.0  | 1.0             | 0               | 0               | 0       |
|                              | NRs                         | 97.1  | 1.9             | 1.0             | 0               | 0       |
|                              | CA                          | 92.0  | 5.7             | 2.3             | 0               | 0       |
| Yeast and molds              | IRs                         | 45.3  | 36.3            | 18.4            | 0               | 0       |
|                              | NRs                         | 43.3  | 42.4            | 14.3            | 0               | 0       |
|                              | CA                          | 44.3  | 33.0            | 22.7            | 0               | 0       |

Note(s): <sup>a</sup>in cfu/g

Source(s): Table by authors

**Table 2.** Contaminated microorganism counts in fresh vegetable samples collected from IRs, NRs and CA in each interval

21.4% of NR samples had a population above 5 log cfu/g. The mean counts of AMB were 0.25, 4.16 and 3.82 log cfu/g for IR, NR and CA samples, respectively, with the lowest count in IR samples (Figure 1).

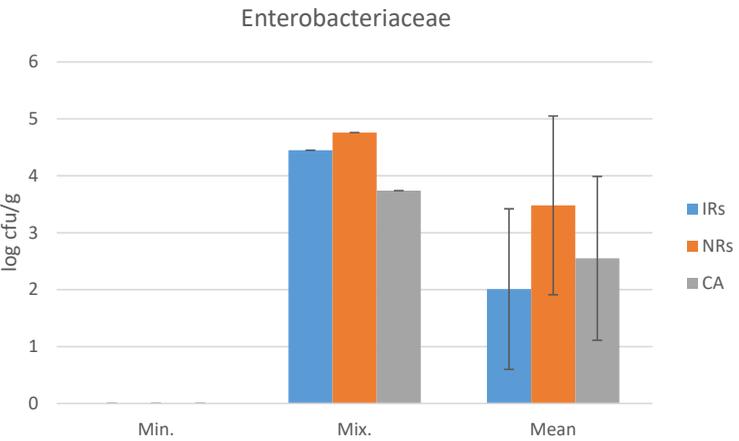
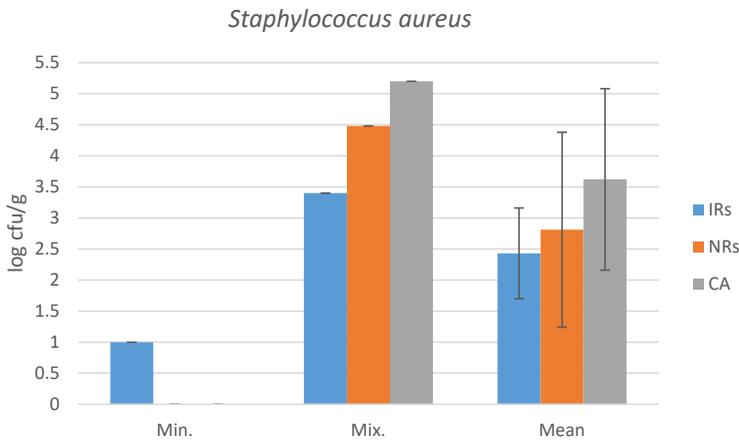
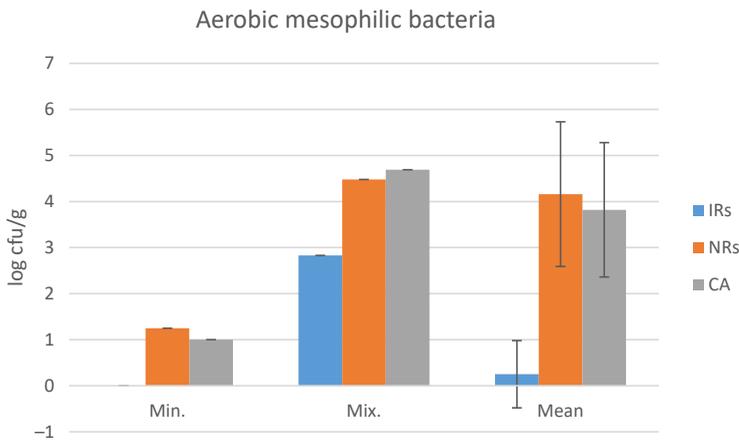
Many results from the literature confirmed that fresh vegetables are the samples most contaminated with AMB (Oliveira *et al.*, 2010; Sospedra *et al.*, 2013; Shahbaz, 2022). Sospedra *et al.* (2013) and Shahbaz (2022) found that aerobic bacteria counts in 13% and 22% of samples were not satisfactory, respectively. Moreover, Oliveira *et al.* (2010) reported that the AMB count in conventional and organic lettuce ranged from 2.85 to 7.04 and 5.19 to 7.81 log cfu/g, respectively. Additionally, in ready-to-eat salad samples, Ssemanda *et al.* (2017) found that the mean count of aerobic plate count bacteria was 4.9 log cfu/g. Faour-Klingbeil, Todd and Kuri (2016) explained that the mean AMB count in 17% of ready-to-eat fresh vegetable samples provided in restaurants was above  $10^7$  cfu/g. The population of AMB was considered evidence for microbial quality of food and the shelf-life duration but was not related to food safety hazards (Pianetti *et al.*, 2008).

**3.1.2 Campylobacter spp.** *Campylobacter* spp. was not detected in any sample from IRs, NRs or CAs (Table 2). These results are in agreement with the results obtained by Santarelli *et al.* (2018) and Zhang, Yamamoto, Murphy, and Locas (2020), who confirmed that the *Campylobacter* spp. was not detected in ready-to-eat fresh and raw vegetables. *Campylobacter* spp. is commonly found in many types of food animals other than chicken (Humphrey, O'Brien, & Madsen, 2007). Some fresh vegetable samples were adjacent to animal source foods, such as beef and chicken (Table 1); nevertheless, the samples were free from *Campylobacter* spp. *Campylobacter* spp. is known to be the leading cause of bacterial foodborne diarrhea worldwide (Silva *et al.*, 2011).

**3.1.3 Staphylococcus aureus (S. aureus).** *S. aureus* is widespread and is one of the main pathogens that cause infection in humans and animals (Sergelidis & Angelidis, 2017). The counts of *S. aureus* ranged from  $<10^2$  to  $10^4$ , from  $<10^2$  to  $10^5$  and from  $<10^2$  to  $10^5$  cfu/g in IRs, NRs and CA, respectively (Table 2). The highest sample percentages (72.6%, 57.1% and 77.3% from IRs, NRs and CA, respectively) were found in 3, 4 and between 3 and 5 log cfu/g for IRs, NRs and CA, respectively. The IR and CA samples (14.3 and 44.3%) had *S. aureus* counts above 4 log cfu/g. No IR samples contained *S. aureus* above 4 log cfu/g. The average counts of *S. aureus* in IR, NR and CA, samples were 2.43, 2.81 and 3.03 log cfu/g, respectively, with the lowest count in IR samples (Figure 1). According to GCC standardization organization GSO (2015), the count of *S. aureus* should not be more than  $10^3$  cfu/g. As a result, our findings show that the IRs, NRs and CA samples, respectively, 81.6, 28.5 and 22.7%, are regarded as acceptable because they were below the maximum allowed (Table 2). *S. aureus* is a common commensal of the skin and mucosal membranes of humans (Kluytmans & Wertheim, 2005). Thus, food handlers carrying *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. Santarelli *et al.* (2018) found that 0.33% of tested precut or uncut vegetable samples contained *S. aureus*. Ssemanda *et al.* (2017) found that the mean coagulase-positive *S. aureus* count was 3 log cfu/g in ready-to-eat salad samples. Faour-Klingbeil *et al.* (2016) and Shahbaz (2022) stated that more than 41.5% and 48% of the ready-to-eat fresh vegetable and salad samples provided in restaurants were found to contain *S. aureus*, respectively.

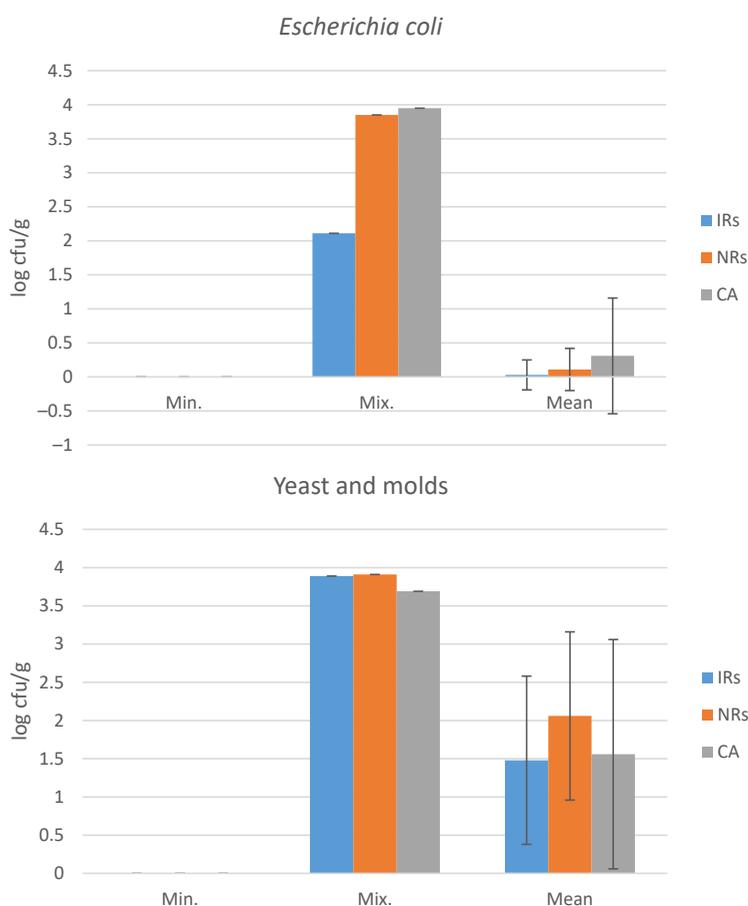
**3.1.4 Enterobacteriaceae.** *Enterobacteriaceae* microorganism counts were similar to those of *S. aureus*, with ranges between  $<10^2$  to  $10^4$  cfu/g in both IR and CA samples and from  $<10^2$  to  $>10^5$  cfu/g in NR samples (Table 2). The highest sample percentages (81.6, 70.9 and 100% from IRs, NRs and CA, respectively) were found between 2 and 4, 3 and 5 and  $<2$  and 4 log cfu/g for IRs, NRs and CA, respectively. The NR samples (35.7%) had *Enterobacteriaceae* counts above 5 log cfu/g. There were no samples that contained more than 4 log cfu/g in IR or CA samples. The average counts of *Enterobacteriaceae* in IR, NR and CA, samples were 2.01, 3.48 and 2.55 log cfu/g, respectively, with the lowest count in IR samples (Figure 1). Oliveira *et al.*

Contamination  
in fast-food  
restaurants



**Figure 1.**  
Counts of  
microorganisms  
contaminating fresh  
vegetables collected  
from IRs, NRs and CA

(continued)



Source(s): Figure by authors

Figure 1.

(2010) found that the average count of *Enterobacteriaceae* in conventional lettuce and organic lettuce was 5.61 and 3.80 log cfu/g, respectively. Sospedra *et al.* (2013) found that 23% of samples collected from restaurants had unsatisfactory levels of *Enterobacteriaceae*. They pointed out that lettuce and tomato are the most contaminated vegetables. *Enterobacteriaceae* are generally intestinal bacteria that live in animal guts and do not cause disease. However, there are genera/species of *Enterobacteriaceae* that cause a range of diseases in humans and animals (Octavia & Lan, 2014). The presence of *Enterobacteriaceae* in fresh vegetables is considered normal, but these numbers must be reduced or absent after washing and before serving vegetables for consumption.

**3.1.5 *Escherichia coli* (*E. coli*).** *E. coli* is a member of the fecal coliform group and is a more specific indicator of fecal contamination than other fecal coliforms (Odonkor & Ampofo, 2013). Therefore, its presence is considered evidence of inadequate hygiene conditions in food service establishments. *E. coli* was present in low numbers compared to other bacteria, with averages between  $<10^2$  to  $10^3$  cfu/g in IR samples and from  $<10^2$  to  $>10^4$  cfu/g in the samples of NRs and CA (Table 2). The highest sample percentages (99.0, 97.1 and 92% from IR, NR and CA samples,

respectively) were found in 2 log cfu/g for all food service establishments. There were no samples that contained >3 log cfu/g (only 1% of samples had 2 log cfu/g) in IRs. Some NR and CA samples (only 1% and 2.3%) had counts of *E. coli* above 3 log cfu/g. The *E. coli* average counts in IR, NR and CA samples were 0.03, 0.11, and 0.31 log cfu/g, respectively, with the lowest counts in IR samples (Figure 1). This means that food service establishments follow hygiene requirements, especially IRs. GSO (2015) reported that the number of *E. coli* should be not more than  $10^2$  cfu/g. Accordingly, 99.0, 97 and 92.0 % from samples collected from IRs, NRs and CA, were accepted, respectively, (Table 2). Some previous microbiological studies that were conducted on fresh vegetables served in restaurants found that 6.6% of lettuce samples and 27% of salad samples were contaminated with *E. coli* (Sospedra *et al.*, 2013; Shahbaz, 2022, respectively). *E. coli* was isolated from 31.3% ready-to-eat salad samples in a previous study, with bacterial counts between 1.00 and 7.15 log cfu/g (Sospedra *et al.*, 2013).

**3.1.6 Yeast and molds.** The presence of molds in vegetables can cause potential health problems, as some of them may produce dangerous mycotoxins, and it is known that some types produce large numbers of conidia, causing an allergic reaction (Tournas, 2005; Tournas & Katsoudas, 2005). Food spoilage from mold can also be a food safety issue due to the production of mycotoxins by these molds (Marchetti, Casadei, & Guerzoni, 1992). Yeasts and molds were present in small numbers compared to bacteria, except for *E. coli*, with ranges between  $<10^2$  and  $10^4$  cfu/g in all of the samples of studied food service establishments (Table 2). The highest sample percentage (81.6, 85.7 and 77.3% from IRs, NRs and CA, respectively) was found between <2 and 3 in all food service establishments. The largest percentage of samples that contained  $10^4$  cfu/g was found in the CA samples (22.7%). The average count of yeast and molds in IR, NR and CA samples were 1.48, 2.06 and 1.56 log cfu/g, respectively (Figure 1). Tournas (2005) reported that the count of fungi in ready-to-eat salads ranged between  $3.8 \times 10^4$  and  $1.1 \times 10^5$  cfu/g. Oliveira *et al.* (2010) found the count of yeast and molds was 4.21 log cfu/g in conventional lettuce and 4.74 log cfu/g in organic lettuce. Marchetti *et al.* (1992) and Liao and Fett (2001) also found a high concentration of yeast counts in salads, peeled carrots and Romaine lettuce.

**3.1.7 Positive samples for detection of pathogenic bacteria.** The detection of pathogenic bacteria in food service establishment samples is tabulated in Table 3. *L. monocytogenes* and *E. coli* O157 were not identified in any tested food service establishment samples. The GSO (2015) requires that vegetables and fruits must be free of *L. monocytogenes*, *E. coli* O157 and *Salmonella* spp. *Salmonella* spp. was detected in only one sample collected from each of NRs and CA (sample percentage of 0.5 and 1.1%, respectively). Therefore, all the samples collected from IRs were accepted, whereas 99.5 and 98.9% samples from NRs and CA were accepted, respectively (Table 3).

The spread of pathogenic bacteria, inclusive of *Salmonella* spp., *L. monocytogenes* and *Escherichia coli* O157, was limited or absent in fresh vegetables related to the results of previous research. Zhang *et al.* (2020) reported that *E. coli* O157:H7 and *Salmonella* spp. were unidentified in 5,379 fresh-cut vegetable samples, while 13 samples were contaminated by *Listeria monocytogenes*. Moreover, Santarelli *et al.* (2018) noted that 0.33% of precut and uncut vegetables were contaminated by *Salmonella* spp., while *L. monocytogenes* was unidentified in the same samples. In a study of vegetable dishes from food service

| Pathogenic bacteria           | IRs (n = 201) | NRs (n = 210) | CA (n = 88) |
|-------------------------------|---------------|---------------|-------------|
| <i>Escherichia coli</i> O157  | nd            | nd            | nd          |
| <i>Salmonella</i> spp.        | nd            | 0.5%          | 1.1%        |
| <i>Listeria monocytogenes</i> | nd            | nd            | nd          |

**Note(s):** nd: not detected

**Source(s):** Table by authors

**Table 3.** Percentages of samples contaminated with pathogenic bacteria of fresh vegetable collected from IRs, NRs and CA

establishments, [Sospedra et al. \(2013\)](#) found that only 0.7% of samples were contaminated by *Salmonella* spp. and *E. coli*, while *L. monocytogenes* was not detected. It is easy for *L. monocytogenes* to spread in vegetables because environmental microorganisms normally live in soil and water. The presence of *Salmonella* spp. in some samples may be due to the transition from the surfaces of knives and cutting boards to fresh vegetables. [Faour-Klingbeil et al. \(2016\)](#) isolated groups of bacteria from the surfaces of knives and cutting boards used to chop fresh vegetables. In general, contamination of vegetables with microbes can be caused by many reasons, such as irrigation with untreated water, organic fertilization or during harvesting, transportation, handling and other practices ([Liao & Fett, 2001](#); [Mercanoglu Taban & Halkman, 2011](#); [Oliveira et al., 2010](#)).

#### 4. Conclusions

Our results showed that the IR samples showed the smallest counts of tested microorganisms (AMB, *S. aureus*, Enterobacteriaceae, *E. coli*, yeasts and molds). The samples most contaminated by AMB, yeasts and molds were obtained from NRs, while the samples obtained from the CA retained the highest counts of *S. aureus* and *E. coli*. All vegetable samples collected from all food service establishments were free of *L. monocytogenes* and *E. coli* O157, while 0.5 and 1.1% from the samples collected from NRs and CA were contaminated with *Salmonella* spp., respectively. In general, especially samples taken from IRs, the majority of vegetable samples taken from three food service outlets were below the upper limit for contamination. According to the guidelines of [GSO \(2015\)](#), the accepted samples collected from IRs, NRs and CA were 81.6, 28.5 and 22.7% from the total for *S. aureus*, 99.0, 97 and 92.0 % for *E. coli* and 100, 99.5 and 98.9% for *Salmonella* spp., respectively. These results reflect the fact that IRs are more compliant with health and hygiene requirements compared to NRs and CAs.

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### Further reading

Annual Report on Foodborne Disease Outbreaks-2018 (2019).

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