Bacteriological assessment of some vegetables and ready-to-eat salads in Alexandria, Egypt

Original Article

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ABSTRACT

Background: Fresh vegetables and ready-to-eat salads (RTES) are essential components of human diet. Despite their benefits, they remain a major public health concern, because they have been implicated in foodborne illness outbreaks in numerous countries.

Aim: The present study aimed to assess the bacteriological quality of some fresh vegetables and RTES in Alexandria, Egypt.

Materials and Methods: This cross-sectional study included 121 samples of vegetables and RTES that were randomly purchased from different markets, restaurants, and street vendors in three districts in Alexandria. All samples were subjected to heterotrophic plate count using pour plate method; detection and enumeration of total coliforms, fecal coliforms and *Escherichia coli* by multiple tube dilution method; and isolation and detection of *Salmonella* spp. using standard microbiological methods.

Results: The aerobic colony count (ACC) for the 71 tested fresh vegetable samples ranged from 2.0 to 10.4 log CFU/g. Green pepper had the highest ACC mean value (8.4 log CFU/g), whereas lettuce showed the lowest ACC mean (5.1 log CFU/g). Fecal coliforms were detected in 90.1% of tested vegetable samples and 66% of the examined RTES samples. Of the 22 street-vended RTES samples, 18.2% were significantly unsatisfactory regarding *E. coli. Salmonella* spp. was not detected in any of the examined samples.

Conclusion: All examined samples were contaminated and yielded growth of aerobic mesophilic bacteria with varying densities. According to the Public Health Laboratories guidelines, only street-vended RTES samples yielded unsatisfactory levels of *E. coli*, which indicates the need for close supervision and regular inspection of hygienic practices and preparation methods of street-vended salads.

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Key Words: Aerobic colony count, aerobic mesophilic bacteria, fecal coliforms, microbiological quality, pathogenic organisms, ready-to-eat salads, total coliforms, vegetables

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INTRODUCTION

Vegetables are important components of a healthy and balanced diet. They are an extraordinary dietary source of nutrients, micronutrients, vitamins, and fiber for humans and are thus vital for health and well-being[1]. Within the past decade and because of intensified public awareness of the benefits of healthy nutrition, attention on vegetables as a vital dietary component has significantly increased their consumption. Moreover, health agencies in many countries as World Health Organization (WHO), European Food Safety Authority (EFSA), Food and Agriculture Organization (FAO), and French Agency for Food Safety (AFSSA) encourage their consumption to protect against a range of illnesses such as cancers and cardiovascular diseases[2].

Vegetables are composed of different range of plant parts (leaves, roots, tubers, fruits, and flowers). A vegetable is the tender plant part that is not sweet and may be flavored or spiced with condiments before consumption. These plants or plant parts may be eaten raw as salad or added to some cooked foods like rice[3].

'Ready to eat' (RTE) is defined as the status of the food being ready for immediate consumption at the point of sale. Ready-to-eat salads (RTES) are low-calorie convenience foods of good dietary value. RTES technology implies procurement of raw vegetables from vertically coordinated farms, cutting, sorting, washing, drying, packaging in permeable plastics, and retailing in cold chain regime. The increased consumption of RTE fresh vegetables, in particular leafy greens, which are used in salad mixtures that are consumed raw, has resulted in increase in foodborne illnesses associated with these products in different regions of the world. They are increasingly being recognized as

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important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin[4].

Foodborne illness is a major public health concern worldwide in terms of numbers of persons affected and economic cost. An estimated 600 million – almost one in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years[5].

Many foodborne illness outbreaks in numerous countries have been associated with consumption of contaminated fresh vegetables. Hence, many consumers question the quality and safety of these foods. Problems linked with pathogens in fresh produce, including the associated public health and trade implications, have been previously reported in a number of countries worldwide[6]. The quality of RTE leafy greens has been surveyed in the United Kingdome (UK)[7,8] and Brazil[9]. Most of the reported foodborne outbreaks originates from Europe, North America, Australia, and New Zealand, as these locations have well-developed epidemiological surveillance systems. In developing countries, paucity of data on food safety together with inaccessibility to safe water, lack of agricultural infrastructures, and limitations to implementing good agricultural practices are persistent challenges[10]. Foodborne disease outbreaks originating in prepared raw green vegetable salads were more likely to occur on commercial food service premises than outbreaks from other sources, with restaurants and hotels accounting for almost 75% of outbreaks. Several outbreaks have been associated with consumption of the products from salad bars[11].

Coliforms are usually indicators of intestinal contaminants from man and animals. This may not be too surprising, as most often the source of watering the gardens is usually sewage from domestic sources and runoff water, which is mostly used for irrigation purposes in some communities. If the counts are high, then they pose dangers to consumers[3]. On the other hand, human pathogens such as *Escherichia coli* (*E. coli*) and *Salmonella* spp. are among the greatest concerns during food-related outbreaks. Several cases of typhoid fever outbreaks have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage[12].

Most people will experience a foodborne disease at some point in their lives. This highlights the importance of making sure the food we eat is not contaminated with potentially harmful microorganisms, toxins, or chemicals. Food can become contaminated at any point during production, distribution, and preparation. Everyone along the production chain, from producer to consumer, has a role to play to ensure the food we eat does not cause diseases[12].

This study was performed to assess the bacteriological quality of some fresh vegetables and ready-to-eat (RTE) vegetable salads collected from local markets, restaurants, and street vendors in Alexandria, Egypt.

MATERIALS AND METHODS

The present cross-sectional study was carried out during a 4-month period from January to April 2017. It included 121 samples of fresh vegetables (71) and RTE vegetable salads (50) that were randomly purchased from different markets, restaurants, and street vendors in three Alexandria districts, Egypt.

The 71 vegetable samples were distributed as follows: arugula 10 (14.1%), coriander 10 (14.1%), parsley 10 (14.1%), celery nine (12.7%), dill eight (11.3%), lettuce seven (9.9%), cucumber six (8.5%), green pepper four (5.6%), tomato four (5.6%), and mint three (4.2%) (Table 1). On the other hand, the 50 RTES samples comprised 28 samples from restaurants and 22 samples from street vendors.

Sample size

Using PASS 2000 Software (Stuart (Bibby Scientific US) Colony Counter SC6PLUSCole-Parmer Ltd. Beacon Road, Stone, Staffordshire, ST15 0SA, United Kingdom Tel: +44 (0)1785 810475 Email: cpservice@coleparmer.com Web: www.stuart-equipment.com), a sample size of 100 samples was required to detect a contamination rate of 22%[13] in vegetables and RTES with unsatisfactory level of aerobic colony count (ACC), with a precision of 8% at a 95% confidence level.

Data collection

Each sample was accompanied by a sheet including all the relevant information such as type and source of sample, date of sampling, site and time of sample collection, place of storage, duration of storage, and temperature of storage.

Every collected sample was properly labeled and transported to the Microbiology Laboratory at the High Institute of Public Health, in an ice box within 1–2 h of collection, where they were processed for bacteriological examination.

Samples processing[14]

Twenty-five grams of each sample was mixed with 225 ml of sterile buffered peptone water in a sterile stomacher plastic bag and were blended thoroughly in the stomacher for 1–2 min for complete homogenization. This constituted 1 : 10 dilution of the original sample.

All collected samples were subjected to the following: 1- Determination of heterotrophic plate count using pour plate method[14].

Using separate sterile pipettes, decimal dilutions of

10^{-2}, 10^{-3}, and 10^{-4} were prepared of food homogenate by transferring 10 ml of previous dilution to 90 ml of diluents, while avoiding sampling foam. All dilutions were shaken 25 times. One ml of each dilution was pipetted into sterile, separate, appropriately marked, and duplicate Petri dishes. Approximately 12–15 ml of plate count agar (cooled to 45±1ºC) was added to each plate within 15 min of original dilution. Agar was left to solidify. Then solidified Petri dishes were inverted and incubated promptly for 48±2 h at 35°C. Following the appropriate duration of incubation, colonies were counted using a Quebec digital colony counter. Plates having counts between 25 and 250 colonies were chosen. The average number of colonies/plate was multiplied by the dilution factor and recorded as colony forming unit (CFU)/g. Bacterial counts were calculated as CFU/g and were also converted into $\log_{10}$ values.

2- Detection and enumeration of total coliforms, fecal coliforms, and E. coli using multiple tube dilution method[14]

Presumptive test: Using at least three consecutive dilutions, 1-ml aliquots from each dilution were inoculated into three lauryl sulfate tryptose (LST) broths with Durham tubes for a three-tube most probable number (MPN) analysis. Tubes were incubated aerobically at 37ºC. Tubes were examined, and reactions were recorded at 24 h for turbidity and gas production. Gas-negative tubes were reincubated for an additional 24 h, and reactions were examined and recorded again at 48 h. Confirmation test on all presumptive positive (gas) tubes was performed.

Confirmed test for total coliforms: From each positive LST broth tube, a loopful of suspension was transferred to a tube of brilliant green lactose bile broth. Brilliant green lactose bile broth tubes were incubated at 35±0.5ºC and examined for turbidity and gas production at 48 h. MPN of coliforms was calculated.

Confirmed test for fecal coliforms and *E. coli*: From each positive presumptive LST broth tube, a loopful of suspension was transferred to a tube of *E. coli* broth. Inoculated *E. coli* tubes were incubated at 44.5°C and examined for turbidity and gas production at 24 h. Gas-negative tubes were reincubated for an additional 24 h, and reactions were examined and recorded again at 48 h. MPN of thermotolerant coliforms [fecal coliforms (FC)] was calculated.

Completed test for *E. coli:* A loopful of each agitated positive *E. coli* broth was removed and streaked on a Levine's eosin methylene blue agar plate and incubated aerobically for 24 h at 35±0.5ºC. Plates were examined for suspicious *E. coli* colonies, that is, dark centered and flat, with or without metallic sheen. Up to five suspicious colonies from each Levine's eosin methylene blue plate were transferred to plate count agar slants and incubated for 24 h at 35±0.5ºC and used for further testing. They were Gram-stained and tested for indole, methyl red, Voges–Proskauer, and citrate (IMViC) reactions. Isolates that fermented lactose with gas production within 48 h at 35°C, appeared as Gram-negative non-spore-forming rods, and were positive for indole and methyl red tests and negative for Voges–Proskauer and citrate tests (biotype 1) or gave IMViC patterns of -+-- (biotype 2) were considered to be *E. coli*.

These isolates were confirmed and further identified using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) test, which was performed in the Microbiology Department at the Faculty of Medicine, Alexandria University.

Isolation and detection of Salmonella spp.[14]

A 0.1-ml mixture of homogenized sample was transferred to 10-ml Rappaport-Vassiliadis medium and another one ml mixture to 10-ml tetrathionate broth and vortexed. Inoculated selective enrichment media were incubated for 24 h at 42°C in a circulating, thermostatically controlled, water bath. About 3-mm loopful (10 µl) of mixed tubes was streaked onto bismuth sulfite agar and xylose lysine deoxycholate agar, and incubated for 24 h at 35°C. Pink colonies with or without black centers on xylose lysine deoxycholate and brown, gray, or black colonies with sometimes metallic sheen on bismuth sulfite plates suspicious of being Salmonella spp. were tested using conventional biochemical tests such as triple sugar iron agar, IMViC, urease, arginine, lysine, and ornithine amino acid decarboxylation tests. These isolates were further confirmed and identified using MALDI TOF MS test, which was performed in the Microbiology Department at the Faculty of Medicine, Alexandria University.

Statistical analysis[15]

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp, Armonk, New York, USA). The Kolmogorov–Smirnov, Shapiro, and D'agstino tests were used to verify the normality of distribution of variables. Comparisons between groups for categorical variables were assessed using $\chi 2$-test. Kruskal–Wallis test was used to compare different groups for abnormally distributed quantitative variables and followed by post-hoc test (Dunn's multiple comparisons test) for pairwise comparison. Significance of the obtained results was judged at the 5% level.

RESULTS

In the present study, the 121 collected samples included 71 (58.6%) fresh vegetables purchased from different markets located in the three Alexandria districts, together with 50 RTE vegetable salads bought from restaurants and street vendors [28 (23.1%) and 22 (18.2%), respectively]. All examined samples (100%) were contaminated and yielded aerobic mesophilic bacteria of varying densities. The ACC for the 71 examined fresh vegetable samples ranged from 2.0 to 10.4 log CFU/g, with a median of 7.08 and a mean value of 6.76± 1.94. Regarding the 28 RTES samples collected from restaurants, ACC ranged from

3.8 to 9.4 log CFU/g, with a median of 7.7 and a mean of 7.5±1.4, whereas the 22 RTES samples purchased from street vendors had an ACC range of 2.7–5.9 log CFU/g, a median of 3.8 and a mean of 4.1±1.0 (Table 1).

Green pepper had the highest ACC mean value (8.4 ±2.0 log CFU/g), followed by parsley (7.9±1.0 log CFU/g), arugula (7.7±1.9 log CFU/g), mint (7.7±1.0log CFU/g), and coriander (6.5±2.0 log CFU/g). The lowest ACC mean value was recorded for lettuce samples (5.1±1.4 log CFU/g) (Table 1).

Table 1 also displays that the 71 tested fresh vegetables had a mean value for total coliforms (TC) of 4.15±0.0 log MPN/100 g. On the other hand, FC mean values ranged between 3.0 and 4.1 log MPN/100 g, where arugula, cucumber, and mint showed the highest FC mean values (4.1 log MPN/100 g each), and tomato samples had the lowest mean (3.0 log MPN/100 g). There was no statistical significant difference between any of these figures.

Table 1: Distribution of 71 examined fresh vegetables and their ACC, TC[#], and FC mean and median values

Vegetables (n=71)	n (%)	Aerobic colony count (ACC) (log CFU/g)	Aerobic colony count (ACC) (CFU/g)	Fecal coliforms (FC) (log MPN/100 g)	Fecal coliforms (FC) (MPN/100 g)
Arugula					
Mean ±SD	10 (14.1)	7.7a±1.9	$3x10^9$a±$3x10^5$	4.1a±0.3	$1x10^4$a±$3x10^3$
Median (minimum–maximum)		7.6 (3.8–10.2)	$4x10^7$ ($6x10^3$-$2x10^{10}$)	4.1 (3.3–4.1)	$14x10^3$ ($2x10^3$-$14x10^3$)
Coriander					
Mean ±SD	10 (14.1)	6.5a±2.0	$2x10^8$a±$4x10^8$	3.9a±0.4	$1x10^3$a,b±$5x10^3$
Median (minimum–maximum)		6.9 (3.8–9.1)	$2x10^7$ ($6x10^3$-$11x10^{8)}$	4.1 (3–4.1)	$14x10^3$ (950-$14x10^3$)
Parsley					
Mean ±SD	10 (14.1)	7.9a±1.0	$5x10^8$a±$1x10^9$	3.6a±1.2	$9x10^3$ab±$5x10^3$
Median (minimum–maximum)		7.9 (6.5–9.6)	$7x10^7$($3x10^6$-$4x10^9$)	4.1 (3–4.1)	$14x10^3$(2-$14x10^3$)
Celery					
Mean ±SD	9 (12.7)	6.2a±2.3	$2x10^8$a±$4x10^8$	3.6a±0.7	$7x10^3$a,b±$6x10^3$
Median (minimum–maximum)		6.8 (3.8–9.0)	$7x10^6$ ($6x10^3$-$11x10^8$)	4.0 (2.5–4.1)	$11x10^3$ (300-$14x10^3$)
Dill					
Mean ±SD	8 (11.3%)	5.8a±1.6	$2x10^7$a±$6x10^7$	3.8a±0.6	$10x10^3$a,b±$6x10^3$
Median (minimum–maximum)		6.1 (3.8–8.2)	$2x10^6$ ($6x10^3$-$2x10^8$)	4.1 (2.6–4.1)	$14x10^3$ (400-$14x10^3$)
Lettuce					
Mean ±SD	7 (9.9%)	5.1a± 1.4	$7x10^6$a±$2x10^7$	4.0a±0.2	$8x10^3$a,c±$6x10^3$
Median (minimum–maximum)		4.6 (3.8–7.7)	$4x10^4$($6x10^3$-sx10^7)	4.1 (3.7–4.1)	$11x10^3$ (0-$14x10^3$)
Cucumber					
Mean ±SD	6 (8.5%)	5.9a±2.1	$1x10^7$a±$1x10^7$	4.1a±0.1	$8x10^3$a,c±$6x10^3$
Median (minimum–maximum)		6.4 (2.0–7.5)	$1x10^7$ (103-$3x10^7$)	4.1 (4–4.1)	$11x10^3$ (0-$14x10^3$)
Green pepper					
Mean ±SD	4 (5.6)	8.4a±2.0	$7x10^9$a±$1x10^{10}$	3.6a± 0.8	$6x10^3$b,c±$7x10^3$
Median (minimum–maximum)		8.6 (5.8–10.4)	$1x10^9$ ($6x10^5$-$83x10^{10}$)	4.1 (2.7–4.1)	$5x10^3$ (0-$14x10^3$)
Tomato[##]					
Mean ±SD	4 (5.6)	7.6a± 0.9	$1x10^8$a±$2x10^8$	3.0a±0.6	850c±$1x10^3$
Median (minimum–maximum)		7.5 (6.8–8.6)	$9x10^7$ ($7x10^6$-$4x10^8$)	3.0 (2.6–3.5)	200 (0–3000)
Mint					
Mean ±SD	3 (4.2)	7.7a± 1.0	$2x10^8$a±$4x10^8$	4.1a± 0.1	$13x10^3$a,b±$1x10^3$
Median (minimum–maximum)		7.3 (7.0–8.8)	$2x10^7$ ($9x10^6$-$7x10^8$)	4.1 (4–4.1)	$14x10^3$($11x10^3$-$14x10^3$)
P		0.053	0.053	0.368	0.042

Qualitative data were described using number and percent, whereas abnormally distributed data were expressed in median (minimum–maximum) and mean ±SD ; ACC, aerobic colony count; CFU, colony forming unit; FC, fecal coliforms; MPN, most probable number; TC, total coliforms ; Means with common letters are not significant, whereas means with different letters are significant ; [#]All 71 tested vegetables had total coliforms mean values of 4.15±0.01 log MPN/100 g ; [##]As the botanical term vegetable is defined as the edible part(s) of a plant, such as fruits; tomato is considered a vegetable, and it is an essential ingredient in the Egyptian green salad.

Table 2 illustrates that the ACC mean value of the 71 tested vegetables was 6.76±1.94 log CFU/g, whereas the corresponding figures for RTES from restaurants and street vendors were 7.5±1.4 and 4.1±1.0 log CFU/g, respectively. There was a statistical significant difference between vegetables and RTES from street vendors ($P_2<0.001$) and between RTES from restaurants and street vendors ($P_3<0.001$).

The mean value of TC for the examined vegetables and RTES from restaurants was 4.15 ±0.0 log MPN/100 g each, whereas a lower value was recorded for RTES from street vendors (4.04±0.31 log MPN/100 g). The difference between vegetables and RTES from street vendors was found to be statistically significant ($P_2=0.002$). Moreover, the difference between RTES from restaurants and street vendors was also found to be statistically significant ($P_3=0.046$) (Table 2).

FC were detected in 64 (90.1%) of 71 tested vegetable samples. On the other hand, of the 50 examined RTES samples, 33 (66%) were positive for FC. They were distributed as 15 (30.0%) samples from restaurants and 18 (36.0%) samples from street vendors.

FC mean values for vegetables, RTES from restaurants, and street vendors were 3.81±0.67, 4.44± 1.64 and 2.85± 1.02 log MPN/100 g, respectively. There was a statistical significant difference between vegetables and RTES from street vendors (P2<0.001) and between RTES from restaurants and street vendors (P3=0.010) (Table 2).

Table 2: Comparison between 121 examined fresh vegetables and RTES regarding ACC, TC and FC mean and median values

		Ready to eat salads (RTES) (n=50)		
	Vegetables (n=71)	Restaurants (n=28)	Street vendors (n=22)	*P*
Aerobic colony count (log CFU/g)				
Mean ±SD	6.76± 1.94	7.5± 1.4	4.1± 1.0	<0.001*
Median (minimum–maximum)	7.08 (2.0–10.4)	7.7 (3.8–9.4)	3.8 (2.7–5.9)	
Significance between groups		P_1=0.092, P_2<0.001*, P_3<0.001*		
Total coliforms (log MPN/100 g)				
Mean ±SD	4.15±0.0	4.15±0.0	4.04± 0.31	0.001*
Median (minimum–maximum)	4.15 (4.15–4.15)	4.15 (4.15–4.15)	4.15 (2.98–4.15)	
Significance between groups		P_1=1.000, P_2=0.002*, P_3=0.046*		
Fecal coliforms (log MPN/100 g)	n=64	n=15	n=18	
Mean ±SD	3.81±0.6	4.44± 1.64	2.85± 1.02	0.001*
Median (minimum–maximum)	4.15 (0.30–4.15)	5.04 (1.60–6.15)	2.53 (1.60–4.15)	
Significance between groups		P_1=0.265, P_2<0.001*, P_3=0.010*		

Abnormally distributed data were expressed in median (minimum–maximum)
P_1: *P* value for comparing between vegetables and RTES from restaurants.
P_2: *P* value for comparing between vegetables and RTES from street vendors.
P_3: *P* value for comparing between RTES from restaurants and street vendors.
ACC, aerobic colony count; CFU, colony forming unit; FC, fecal coliforms; MPN, most probable number; RTES, ready-to-eat salads; TC, total coliforms.
*Statistically significant at $P\leq0.05$

Table 3 shows that all the 22 (100%) RTES samples obtained from street vendors were satisfactory regarding ACC, while of the 28 RTES samples collected from restaurants, 13 (46.4%) were acceptable with ACC ranging between 10^6 and less than 10^7 CFU/g; 10 (35.7%) were unsatisfactory, with an ACC equal to or more than 10^7 CFU/g; and only five (17.9%) were satisfactory, with an ACC of less than 10^6 CFU/g. The difference between these figures was found to be statistically significant (*P<0.001*). All 71 fresh vegetable samples together with all RTES (28) samples from restaurants were satisfactory regarding *E. coli* (<20 CFU/g). On the other hand, 18/22 (81.8%) RTES samples from street vendors were satisfactory and only four (18.2%) were unsatisfactory (>100 CFU/g). The difference between these figures was found to be statistically significant (P_2*=0.003,* P_3*=0.032*) (Table 3).

In this piece of work, all the 121 studied samples were satisfactory regarding *Salmonella* spp. as it was not detected in 25 g of any of these samples (Table 3).

Table 3: Microbiological quality of the 121 studied fresh vegetables and ready-to-eat vegetable salads according to the Public Health Laboratories guidelines[17]

		Ready-to-eat salads (RTES) (n=50)			
	Vegetables (n=71)	Restaurants (n=28)	Street vendors (n=22)	RTES (n=50) total	*P*
Aerobic colony count					
Satisfactory (<10^6 CFU/g)	NA	5 (17.9)	22 (100.0)	27 (54.0)	<0.001*
Acceptable (10^6 to <10^7 CFU/g)	NA	13 (46.4)	0 (0.0)	13 (26)	
Unsatisfactory (≥10^7 CFU/g)	NA	10 (35.7)	0 (0.0)	10 (20.0)	
E. coli					
Satisfactory (<20 CFU/g)	71 (100)	28 (100.0)	18 (81.8)	-	0.001*
Unsatisfactory (>100 CFU/g)	0 (0)	0 (0)	4 (18.2)	-	
Significance between groups		P_1=NA, P_2*=0.003*,* P_3*=0.032**			
Salmonella spp.					
Satisfactory (not detected in 25 g)	71 (100)	28 (100.0)	22 (100.0)	-	-
Unacceptable/potentially hazardous (detected in 25 g)	0 (0)	0 (0)	0 (0)	-	

Satisfactory=test results indicating good microbiological quality.
Acceptable=an index reflecting a borderline limit of microbiological quality.
Unsatisfactory=test results indicating that further sampling may be necessary and that environmental health officers may wish to undertake a further inspection of the premises concerned to determine whether hygiene practices for food production or handling are adequate or not.
Unacceptable/potentially hazardous=test results indicating that urgent attention is needed to locate the source of the problem; a detailed risk assessment is recommended.
Qualitative data were described using n (%).
P1: P value for comparing between vegetables and RES from restaurants.
P2: P value for comparing between vegetables and RES from street vendors.
P3: P value for comparing between RES from restaurants and street vendors.
CFU, colony forming unit; NA, not applicable; RES, ready to eat; RTES, ready-to-eat salads.
*Statistically significant at P≤0.05

DISCUSSION

Global production and consumption of fresh vegetables has been increasing for the past three decades. This is because of the increased demand for healthy food that ensures sufficient intake of minerals, fibers, and vitamins with antimicrobial properties, and it also decreases the risk of cardiovascular diseases, cancer, and stroke[6].

In addition, it has become well documented that there is an increased outdoor consumption of RTES as a result of a fast-paced lifestyle, awareness on their nutritional benefits, and enhanced processing technology. However, when they are not carefully prepared, they can be subjected to microbial contamination and become hazardous to health, particularly when eaten raw[10].

Microbial safety of vegetables and RTES is currently a global concern. The present study aimed to evaluate the bacteriological quality of some fresh vegetables and RTES in Alexandria, Egypt; as fresh vegetables and mixed vegetable salads constitute an essential and indispensable component of most of the Egyptians' daily diet.

This work included 121 samples distributed as 71 (58.6%) fresh vegetables and 50 (41.3%) RTE mixed vegetable salads. All these samples yielded growth of aerobic mesophilic bacteria with varying densities. It has been documented that aerobic organisms reflect the exposure of samples to any contamination and generally the existence of favorable conditions for microorganisms' multiplication.

The acceptable ACC limit of fresh vegetables by some countries for export purposes should not exceed 6.69 log CFU/g[17]. The ACC of the 71 examined fresh vegetable samples in this study showed a wide range of microbial load from 2.0 to 10.4 log CFU/g, with a median of 7.08 and a mean of 6.76±1.94 log CFU/g. This was in line with the findings of Faour-Klingbeil *et al.*[10] in Lebanon who reported that the mean ACC levels ranged from 2.90 to 7.38 log CFU/g, with counts above 10^7 CFU/g recorded for 17% of their samples. Furthermore, in accordance with the present results, Khalil and Gomaa[18] in Egypt recorded a wide range of aerobic mesophilic count (AMC) for conventional vegetable samples (3.63–7.17 log CFU/g). On the other hand, Weldezgina and Muleto in Ethiopia[19] and Nyenje *et al.*[20] in South Africa observed narrower ranges of high AMC from 6.94 to 8.06 and from 6.3 to 6.8 log CFU/g, respectively). A much lower AMC range of 2.95–3.75 log CFU/g was reported by Buyukunal *et al.*[21] in Turkey. The difference in the ACC mean values may be attributed to the different areas of vegetables cultivation and different irrigation sources. It has been noted that plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality, and most foods are regarded as harmful when they have large populations of these microorganisms, even if the organisms are not known to be pathogens[22].

In this study, green pepper had the highest ACC mean value (8.4±2.0 log CFU/g), followed by parsley (7.9±1.0 log CFU/g), arugula (7.7±1.9 log CFU/g), mint (7.7±1.0 log CFU/g), and coriander (6.5±2.0 CFU/g). A higher count of parsley was recorded in Lebanon by Halablab *et al.*[23], where the total aerobic plate count of parsley samples collected from Barelias ranged from 8.3 to 9.85 log10 CFU/g. However, Faour-Klingbeil *et al.*[10] found that of the 118 examined fresh-cut vegetable samples, coriander had the highest mean count of 7.38±0.00 log CFU/g, followed by lettuce (5.50±1.55 log CFU/g), parsley (5.42±1.32 log CFU/g), radish (5.09±2.2 log CFU/g), cucumber (4.60±2.01 log CFU/g), and arugula (3.99±2.44 log CFU/g). In contrast to the present results, Khalil and Gomaa[16] reported that the maximum AMC mean value was obtained from radish (7.17±2.8 log CFU/g), followed by basil and mint (6.88±0.23 and 6.77±0.53 log CFU/g, respectively).

Furthermore, in the current work, the lowest ACC mean value was found in lettuce samples at 5.1±1.4 log CFU/g. This was in agreement with Khalil and Gomaa[18] who found that lettuce had the lowest mean AMC (3.15±0.46 log CFU/g). On the contrary, Buyukunal *et al.*[21] in Turkey reported that green leaf lettuce, iceberg lettuce, cos lettuce, and spinach had significantly higher microbial loads than other tested commodities. Moreover, Halablab *et al.*[23] in Lebanon documented that all lettuce samples collected from different locations in Bekaa Valley had higher incidence of aerobic organisms than any other vegetable samples collected from the same locations. In Barelias, for instance, the aerobic bacterial count on lettuce ranged from 8.0 to 10.4 log10 CFU/g compared with Rawda (El Establ), Jib-Janine, and Kaaroun, where the aerobic counts were 8.0–9.27, 7.17, and 6.75 log10 CFU/g, respectively.

Indicator bacteria may be associated with an increased likelihood of the presence of pathogens. They are useful in the assessment of food product safety because they tend to be present in higher numbers than most pathogens and are relatively quick and easy to identify[24]. In the present study, all 71 tested vegetables had TC mean values of 4.15±0.0 log MPN/100 g. This agreed with the findings of Nguz *et al.*[25], who showed that mixed vegetables were still found to harbor high levels of TC (5.9 log CFU/g). High loads of coliforms in RTE vegetables at retail levels can be directly influenced by intense use of untreated manure during preharvest and extensive handling during postharvest[22].

Results of FC in this study exhibited the presence of fecal contamination in 90.1% of the examined fresh vegetable samples. FC mean values ranged between 850 and $1x10^4$ MPN/100 g, where arugula, cucumber, and mint showed the highest FC mean values (1×10^4, 8×10^3, and 13×10^3 MPN/100 g, respectively), whereas tomato samples had the lowest mean 850 MPN/100 g. In accordance with the current results, heavy contamination with FCs between 4.0×10^3 and 9.3×10^8 MPN/g was also observed in a survey that was carried out in Ghana on some vegetables cultivated with poor-quality irrigation water[26]. In Ethiopia, Weldezgina and Muleta[19] stated that MPN of total and FC and their overall mean in vegetables ranged from 865.3 to 1036.0 and 524.0 to 716.0 MPN/100 ml, respectively. In line with the present findings, in an early study conducted by Ashenafi[27] in Ethiopia, raw consumed food like tomato had TC and FC counts of 1.5×10^3 and 3.7×10^2 MPN/10 g, respectively.

Raw vegetables are widely consumed in the form of salads in most countries. They are considered to be suitable and convenient meals for today's lifestyles. In Egypt, RTE vegetable salad (known as green salad) is one

of the most popular and widely consumed dishes in the Egyptians' daily food. RTESs are considered as a high-risk food because they do not require heating, and may not be cleaned or washed properly before consumption[13].

Contamination of RTE foods sold in restaurant premises and by street vendors rendering them unacceptable for human consumption has become a global health problem. In this piece of work, 50 RTES samples were examined (28 from restaurants and 22 from street vendors).

The ACC mean value for the 22 street-vended RTESs was 4.1±1.0 log CFU/g. This was nearly similar to the results recorded by Amiko *et al.*[28] in Ghana, where raw mixed vegetable salads obtained from five randomly selected vendors had a mean aerobic count of $\log_{10}$ 4.70 CFU/g (5×10^4 CFU/g). A higher count was reported earlier by Kubheka *et al.*[29] in South Africa, as the 55 salads collected from street vendors had an ACC mean value of 5.9 (±0.6) log CFU/g and the count ranged between 2.7 and 8.9 log CFU/g. Another higher ACC mean value was reported in a study done in India by Mritunjay and Kumar[30], where a total of 480 samples of eight different raw salad vegetables from local markets had a mean AMC of 6.1 log CFU/g, ranging from 2.0 to 9.6 log CFU/g.

On the contrary, in this study, the ACC mean value for the 28 RTES samples purchased from restaurants was 7.5±1.4 log CFU/g, ranging from 3.8 to 9.4 log CFU/g. In concordance with the results in the present study, Pamuk *et al.*[31] in Turkey reported that 55.1% of the RTES samples from different private restaurants, cafes, and shopping centers were found to have total viable counts of more than 6 log CFU/g, and Jeddi *et al.*[32] in Iran reported that the count of these bacteria in salads ranged from 5.5 to 7.4 log CFU/g.

In the present study, the ACC mean value of RTES from restaurants was significantly higher than that of street vendors. This may be the result of the greater number of people that can possibly be involved in the handling and preparation of this type of food in restaurants. In addition, street-vended foods are prepared and sold in the streets for immediate consumption, as there is usually a lack of storage facilities, whereas in restaurants, the salads are stored, and leftovers may be used for several days. The leftovers could serve as a good source for contaminating freshly prepared salads[32]. According to Frank-Peterside and Waribor[33], bacterial load on vegetables increase with time during storage. It is advisable to separate the leftovers from freshly prepared salads to prevent cross contamination. The leftovers could be kept for sale the next day, only if they are kept under cold storage and then reheated above 70°C before being sold. However, as salads are generally not heated, it is advisable to discard any leftovers[34]. In agreement with the current findings, Khater *et al.*[35] in Egypt documented that salad samples obtained from restaurants had a higher ACC value than the street-vended ones (5.70 and 4.92 log CFU/g, respectively). In contrast to these results, in Togo, Soncy *et al.*[36] reported that the microbial loads of salad samples from the street vendors in Lomé were higher than that of the studied Domino restaurant.

The present study showed that FC were detected in 90.1% of tested vegetable samples. On the contrary, 66% of the examined RTES samples were positive for FC (distributed as 30.0% RTES samples from restaurants and 36.0% RTES samples from street vendors). This was in accordance with the results of Gomez-Aldapa *et al.*[11] in Mexico, who found that 95.5% of the 220 analyzed samples were contaminated with FC, and those of Cerna-Cortes *et al.*[37], where FC were identified in 32% of 100 tested RTES samples (50 samples from different supermarkets and 50 from street-vendor stalls). Moreover, in Brazil, Froder *et al.*[9] detected FC concentrations higher than 10^2 CFU/g (Brazilian standard) in 73% of the samples, and Castro-Rosas et al. [4] reported that 99% of the 130 samples collected from restaurants in Mexico were contaminated with FC.

Over the past 10 years, there has been an increasing demand for RTE meals as people changed their eating habits because of healthier lifestyle interest. Nevertheless, RTE meals may be recognized as a source of food poisoning outbreaks in developed countries. Street foods are enjoying increasing patronage owing to industrialization which is forcing many city dwellers to eat their major daily meals out of home, as street food vendors provide cheap and enjoyable food to millions of consumers. In developing countries, street food vending is a common feature of most cities and towns[34].

According to the Public Health Laboratories guidelines (PHLS)[16] for the microbiological quality of some RTE foods, of the 50 examined RTES samples in this study, 54.0% in this study were satisfactory regarding ACC, 26% were acceptable, and 20.0% were unsatisfactory. Nearly similar results were reported by Hannan *et al.*[13] in Pakistan, where 58% of the 50 tested salads from different vendors and restaurants showed satisfactory levels of ACC, 20% were on the borderline, whereas 22% had unsatisfactory levels.

Examination for the presence of pathogens in RTE food products contributes to food safety. *Salmonella* spp. is a common foodborne pathogen that causes food contamination, which has resulted in higher economic losses and poses a significant threat to public health. *Salmonella* spp. has been implicated in disease outbreaks associated with consumption of fresh and RTE vegetables[24]. In this piece of work, all the 121 studied samples were satisfactory regarding *Salmonella* spp., as it was not detected in 25 g of any of the samples. The current findings were in concordance with the results of many researchers from different countries, where *Salmonella* spp.

were not detected in their samples: Soncy *et al.*[36] in Togo, Allen *et al.*[38] in Canada, Caponigro *et al.*[39] in Italy and earlier, Sagoo *et al.*[8] in the UK. It has been documented that negative results may suggest that levels of occurrence of these pathogens on the sampled population of RTES may be below the sensitivity of the used detection method and could be minimally affected by season and other considered factors[39]. In contrast to the present findings, *Salmonella* was detected in the samples examined by Weldezgina and Muleta[19] in Ethiopia, Gomez-Aldapa in Mexico[11], and Toe *et al.*[40] in the Ivory Coast with isolation percentages of 20.7%, 6.8% and 2.6%, respectively.

As specified by PHLS guidelines[16], RTE food products should contain less than 20 *E. coli* CFU/g to be satisfactory. Accordingly, the current study showed that all the 71 fresh vegetable samples, together with all RTES (28) samples from restaurants, were satisfactory regarding *E. coli* (<20 CFU/g), whereas 81.8% RTES samples from street vendors were found to be satisfactory. In line with the findings in this study, Sagoo *et al.*[8] revealed that the vast majority of their examined vegetable salads (99.3%) were of satisfactory or acceptable microbiological quality based on the PHLS guidelines for some RTE foods sampled at the point of sale, whereas only 0.5% of samples were of unsatisfactory microbiological quality owing to *E. coli.* Moreover, earlier, Sagoo *et al.*[7] stated that 98.5% of 3200 RTE organic vegetables were satisfactory, 1% acceptable, and 0.5% were of unsatisfactory microbiological quality according to the same guidelines. *E. coli* was detected in 1.5% (48/3200) of RTE organic vegetable samples, and was present at 10^2 CFU/g or more in 0.3% samples. The presence of *E. coli* in food indicates recent direct or indirect fecal contamination and possible presence of other enteric pathogens known to be causative agents of foodborne gastroenteritis and bacterial diarrheal diseases[24].

It is worth mentioning that in this study, 18.2% of street-vended RTES samples were significantly unsatisfactory regarding *E. coli* levels (>100 CFU/g) compared with RTES from restaurants and fresh vegetables from markets. Unsanitary handling of street foods by some of the vendors has been commonly found to be the source of contamination. This could be attributed to the fact that most of the street vendors do not take the needed precautions to avoid contamination of the raw salads during preparation and sale, as they are usually unaware of food contamination causes. In addition, in many developing countries, street food vending activities are not usually protected or regulated by the governments. Furthermore, it has been previously noted that stands used by street vendors are usually of inefficient construction, running water is not easily accessible, and hand and dish washing are performed in the same bucket, sometimes without soap. Wastewater is usually discarded right there in the streets, and garbage is likewise conveniently discarded right next to the stands, providing attraction, food, and harborage for insects and rodents. In many cases, toilets are not available, thus forcing the vendors to eliminate their body wastes also in areas close by and to return to their vending sites without washing their hands. Such conditions and practices are likely to lead to cross contamination of street food, thus adequate measures for treatment and cleaning of raw materials, environment and utensils together with hygienic practices of vendors must be strictly implemented to ensure good quality of fresh vegetables and RTES and significantly reduce their contamination[34].

LIMITATIONS OF STUDY

One of the limitations of this study was not testing for other foodborne pathogens such as *Listeria monocytogenes*. This would have added to the results in the present study, but unfortunately it was not feasible during the study period.

CONCLUSION

All examined samples were contaminated and yielded the growth of aerobic mesophilic bacteria with varying densities, where RTES samples purchased from restaurants had the highest ACC mean values compared with fresh vegetables from local markets and RTES from street vendors. Most fresh vegetables were contaminated with FC. According to the PHLS guidelines for the microbiological quality of some RTE foods, only street-vended RTES samples yielded unsatisfactory levels of *E. coli*.

The present findings highlight the importance of training restaurant staff regarding sanitary methods of salad preparation, together with avoiding long storage duration and usage of leftovers. Moreover, they emphasize the particular attention that should be paid to hygienic handling of raw vegetables to ensure that microbiological standards for managing vegetables are effectively followed. They also indicate the need for regular inspection and close supervision of handling practices and preparation methods of street-vended salads.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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