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Bacteriological Quality of Fresh Vegetables Salad Sold in Schools Canteens and Restaurants in Gaza Strip-Palestine

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Abstract

This research aimed to investigate the bacteriological quality of fresh vegetables salad sold in the local school canteens and restaurants in Gaza strip, Palestine. Samples examined included different types of fresh vegetables salad. A total of 200 random samples were collected from school canteens (100 samples) and different restaurants (100 samples) in Mid Zone, Khan Younis and Rafah governorates during the period from April to June 2013. All samples were examined for Total Plate Count, Total Coliform bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria* spp., *L. monocytogenes*, Salmonella spp., *Shigella* spp., and *E. coli* O157:H7. Eighty eight percentage of vegetables salad samples failed to comply with Guidelines for the microbiological examination of ready-to-eat foods, Food Standards Australia New Zealand. The percentage of failure is distributed as follows; 79.5% with Total Plate Count, 60.5% with Total Coliform bacteria, 53.5% with *E. coli*, 21.5% with *S. aureus*, 14% *B. cereus*, 7.5% *Listeria* spp., 2% *L. monocytogenes*, 5% *Salmonella* spp., 1% *Shigella* spp., and 1% *E. coli* O157:H7. In this study the mixed vegetables salad samples were showed the highest non-compliant. Moreover, several potential pathogens were isolated; *Cronobacter sakazakii* (12.5%; 25), *Pasteurella* spp. (3%; 6) and *Aeromonas hydrophila* (0.5%; 1). The results indicated that the tested vegetables salads had poor microbiological quality, and could act as a vehicle for food-borne pathogens such as *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7 and *L. monocytogenes*, which justifies the necessity for the urgent actions to promote awareness about the possible health hazards.

Keywords:
Vegetables salad,
Bacteriological quality,
Foodborne pathogens,
Indicator bacteria,
Gaza strip.

1. Introduction:

Demand for fresh-cut and mixed ready to eat vegetables has been increased in recent years, due to the nutritional value as well as health benefits (Mohammad *et al.*, 2012). Different international organizations (WHO, FAO, USDA, EFSA) recommend increasing fruits and vegetables consumption to decrease the risk of some diseases such as cardiovascular, cancer (Allende *et al.*, 2006; Mohammad *et al.*, 2012), stroke, and reduced mortality (Venneria *et al.*, 2012).

Fresh fruits and vegetables are increasingly recognized as a source of food-borne outbreaks in many parts of the world (Falomir *et al*., 2010; Lynch *et al*., 2009; Sivapalasingam *et al*., 2004). Fresh vegetables can be a vehicle for transmission of bacterial, parasitic and viral pathogens capable of causing human illness and a number of reports refer to raw vegetables, harboring potential food borne pathogens as *Escherichia coli*, *L. monocytogenes* and *Salmonella* (Abadias *et al*., 2008).

Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables have occurred with increased frequency during the past decade (Beuchat, 1996, 2002; Johnston *et al*., 2005). Raw vegetables are widely consumed in the form of salads in most countries. Consumption of raw or slightly cooked vegetables can increase the risk of food-borne disease (Puspanadan *et al*., 2012), and increasingly recognized as vehicles for transmission of human enteric pathogens (Gu *et al*., 2011). The major health problems can arise from consumption of contaminated prepared vegetable salads if hygiene practices breakdown (Little and Gillespie, 2008). Among the more common pathogenic microorganisms that can be transmitted to humans by fresh vegetables are *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* (Fröder *et al*., 2007). Tambekar and Mundhada (2006) analyzed fresh vegetables salad and isolated 86 bacterial pathogens, among them *E. coli*, *Enterobacter aerogenes*, *Pseudomonas* spp., *S. aureus*, *Salmonella* spp. and *Shigella* spp.

Vegetables can be contaminated with pathogenic microorganisms; pre-harvest (Puspanadan *et al*., 2012), during growing in the field through contact with soil, dust, irrigation water (Beuchat, 2006; Halablab *et al*., 2011), and manure of human and animal feces (Aycicek *et al*., 2006; Puspanadan *et al*., 2012) or during harvesting, post harvesting, handling, processing, distribution and marketing, or in the home kitchen (Aycicek *et al*., 2006; Amoah *et al*., 2007; Goja *et al*., 2013; Puspanadan *et al*., 2012; Tambekar and Mundhada, 2006). Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host (Halablab *et al*., 2011). Application of untreated manure may represent a risk of contamination of the vegetables; as such, manure may harbor human pathogenic bacteria as *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7 and other verotoxin-producing bacteria that may contaminate soil, irrigation water and the plants (Johannessen *et al*., 2004).

This study intended to evaluate the microbiological quality of fresh vegetable salads, which is very popular food in Gaza strip, Palestine.

2. Material and Methods:

Food Samples

Two hundred samples of different types of fresh vegetable salads were collected from school canteens (100 samples) and different restaurants (100 samples) in Mid Zone, Khan Younis and Rafah governorates during the period from April to June 2013. The distribution of salad types according to sample sources is shown in Table 1.

Table 1 *Distribution of salad type in samples collected from school canteens and restaurants*

Salad type	Source of samples					
	School canteens		Restaurants		Total	
	N	%	N	%	N	%
Single vegetable	6	6	5	5	11	5.5
Mixed vegetables	92	92	75	75	167	83.5
Vegetables with additives*	2	2	20	20	22	11
Total	**100**	**100**	**100**	**100**	**200**	**100**

** Vegetables with additives (with lemon, oil and/or vinegar)*

Sample collection

Each sample (300-400 g) was properly identified and labeled, placed separately in a sterile plastic bag and transported to the laboratory in an ice box within 2-4 hours of collection, where they were processed for bacteriological examination. Sample type, source and other relevant data were recorded for each sample. All tests were carried out at the Public Health Laboratory for Food and Water, Gaza-Palestine.

Bacteriological analysis

At the laboratory, 25 grams of vegetables salad were weighed and 225 ml peptone water (0.1%) was added to a 500 ml- capacity blender jar and was mixed for 2 minutes. Ten-fold dilutions were prepared under aseptic conditions from each sample using 0.1% peptone water as diluents (BAM, 2001).

The following tests were performed for all samples; Total Plate Count, enumeration of Coliform bacteria and *E. coli,* enumeration of *Staphylococcus aureus* and *Bacillus cereus* according to the procedures indicated in Bacteriological Analytical Manual (BAM, 2001).

Isolation of Salmonella and Shigella

Twenty-five grams sample were suspended in 225 ml of sterile peptone water and blended for 2 min. Then incubated at 37ºC for 24 ± 2 h. One ml pre-enrichment mixture was transferred to 10 ml Selenite Cystine (SC) broth and Gram Negative broth (GN) incubated for 24 ± 2 h at 35ºC. Enrichment samples were then streaked on *Salmonella-Shigella* agar (SS), Hektoen Enteric agar (HE) and Brilliant Green Agar (BG) (HiMedia) and incubated at 37ºC for 24 ± 2 h. *Salmonella* and *Shigella* suspected colonies were examined using API 20 E system and agglutinated with polyvalent antisera (BAM, 2001).

Detection of *Listeria monocytogenes*

Twenty-five grams of the vegetable sample were inoculated into Buffered *Listeria* Enrichment Broth (BLEB) (HiMedia), with supplement (Acriflavin, nalidixic acid and cycloheximide). Blended in a stomacher for one minute at high speed and incubated at 37°C for 48 h. After incubation period streak on *Listeria* agar with supplement and incubated at 30°C for 24-48 h. Suspected colonies were examined by *Listeria* API system (BAM, 2001).

Statistical analysis

Data were tabulated using Microsoft Excel and then uploaded to SPSS v. 13 (Statistical Package for Social Sciences). Chi square test was used for assessing the statistical significance of the data, and P-values of ≤ 0.05 were considered significant.

3. Results:

The compliance percentage of salad samples:

The results revealed that 88% (176/200) of vegetables salads samples did not comply (failed) the standards (at least failed to comply with one parameter) according to the microbiological parameters used in this study "Guidelines for the microbiological examination of ready-to-eat foods, Food Standards Australia New Zealand (2001)" (FSANZ, 2001).

The compliance according to governorates, sample source and salad type:

The results in this study showed that the percentage of non-compliance according to governorates as follows; Khan Younis samples 92.2% (71/77) Mid Zone 91.3% (63/69) and Rafah 77.8% (42/54) (*P*= 0.025). The non-compliance in restaurants samples 90% (90/100) and in school canteens 86% (86/100), single salad was 90.9% (10/11 samples) and higher than the mixed vegetables salad 89.9% (150/167 samples) and salad with additives 72.7% (16/22 samples), as shown in Table 2.

Table 2 *The compliance percentage of salad samples according to governorates, sample source and salad type*

Compliance		Governorates			Sample source		Salad type			Total
		Rafah	Khan Younis	Mid Zone	School	Resta-urants	Single vegetable	Mixed vegetables	W/. additives	
Pass	**N**	12	6	6	14	10	1	17	6	**24**
	%	22.2	7.8	8.7	14	10	9.1	10.2	27.3	**12%**
Fail	**N**	42	71	63	86	90	10	150	16	**176**
	%	77.8	92.2	91.3	86	90	90.9	89.9	72.7	**88%**
Total	**N**	**54**	**77**	**69**	**100**	**100**	**11**	**167**	**22**	**200**
	%	**27**	**38.5**	**34.5**	**50**	**50**	**5.5**	**83.5**	**11**	**100%**
P value			**0.025***		**0.384**			**0.065**		

* *Statistically significant*

The percentage of compliance of salad samples based on various microbiological parameters:

There is a statistically significant correlation between salad type and Total Plate Count (*P*= 0.001) and *Shigella* (*P*= 0.021). The highest non-compliance was because of exceeding the limits in Total Plate Count (79.5%) as shown in Table 3.

Table 3 *The percentage of compliance of salad samples based on various microbiological parameters*

Parameter	C	Salad type			Total	*P value*
		Single	Mixed	W/. additives		
TPC	**P**	2 (18.2%)	28 (16.8%)	11(50%)	41 (20.5%)	0.001*
	F	9 (81.8%)	139 (83.2%)	11 (50%)	159 (79.5%)	
TC	**P**	6 (54.5%)	60 (35.9%)	13 (59.1%)	79 (39.5%)	0.065
	F	5 (45.5%)	107 (64.1%)	9 (40.9%)	121 (60.5%)	
E. coli	**P**	7 (63.6%)	73 (43.7%)	13 (59.1%)	93 (46.5%)	0.200
	F	4 (36.4%)	94 (56.3%)	9 (40.9%)	107 (53.5%)	
S. aureus	**P**	8 (72.2%)	129 (77.2%)	20 (90.9%)	157 (78.5%)	0.304
	F	3 (27.3%)	38 (22.8%)	2 (9.1%)	43 (21.5%)	
B. cereus	**P**	9 (81.8%)	141 (84.4%)	22(100%)	172 (86.0%)	0.130
	F	2 (18.2%)	26 (15.6%)	0 (0 %)	28 (14%)	
***Salmonella* spp.**	**P**	10 (90.9%)	159 (95.2%)	21(95.5%)	190 (95%)	0.814
	F	1 (9.1%)	8 (4.8%)	1 (4.5 %)	10 (5 %)	
***Shigella* spp.**	**P**	10 (90.9%)	166 (99.4%)	22(100%)	198 (99 %)	0.021*
	F	1 (9.1%)	1 (0.6%)	0 (0 %)	2 (1%)	
***Listeria* spp.**	**P**	9 (81.8 %)	155 (92.8%)	21(95.5%)	185 (92.5%)	0.348
	F	2 (18.2%)	12 (7.2%)	1 (4.5%)	15 (7.5 %)	
***E. coli* O157:H7**	**P**	11 (100%)	165 (98.8%)	22 (100%)	198 (99 %)	0.819
	F	0 (0%)	2 (1.2%)	0 (0 %)	2 (1 %)	
Total		**11(5.5%)**	**167 (83.5%)**	**22 (11%)**	**200 (100%)**	

TPC; *Total Plate count,* TC; *Total coliforms,* C; *Compliance,* P; *Pass,* F; *Fail,*
* *Statistically significant*

Distribution of microbiological parameters compliance according to samples source:

The source of samples showed statistically significant correlation with Total Coliform (P=0.002) and *E. coli* ($P \leq 0.001$) while a non-significant correlation with other parameters was detected Table 4.

Table 4 *Distribution of samples source according to microbiological parameters compliance*

Parameter	C	Source of samples		*P value*
		School canteens	Restaurants	
TPC	**P**	20 (20 %)	21 (21 %)	0.861
	F	80 (80 %)	79 (79 %)	
TC	**P**	50 (50 %)	29 (29%)	0.002*
	F	50 (50 %)	71 (71%)	
E. coli	**P**	60 (60 %)	33 (33 %)	0.000*
	F	40 (40 %)	67 (67 %)	
S. aureus	**P**	84 (84 %)	73 (73%)	0.058
	F	16 (16 %)	27 (27 %)	
B. cereus	**P**	89 (89 %)	83 (83 %)	0.221
	F	11 (11 %)	17 (17 %)	
***Salmonella* spp.**	**P**	97 (97%)	93 (93 %)	0.194
	F	3 (3%)	7 (70%)	
***Shigella* spp.**	**P**	100 (100%)	98 (98 %)	0.155
	F	0 (0%)	2 (2%)	
***Listeria* spp.**	**P**	89 (89 %)	96 (96 %)	0.060
	F	11 (11%)	4 (4 %)	
***E. coli* O157:H7**	**P**	99 (99%)	99 (99 %)	1.000
	F	1 (1%)	1 (1 %)	
Total		**100 (100%)**	**100 (100 %)**	

TPC; *Total Plate count,* TC; *Total coliforms,* C; *Compliance,* P; *Pass,* F; *Fail,*
* *Statistically significant*

Distribution of *Listeria* species according to governorates, sample source and salad type:

Listeria spp. was found in higher frequency in Khan Younis governorate (7, 3.5%), followed by Mid Zone (5, 2.5%), and Rafah (3, 1.5%). (P= 0.108). The ratio of *Listeria* spp. in school samples (11, 5.5%) was higher than in the restaurants (4, 2%). *L. monocytogenes* and *L. welshimeri* were detected only in school canteens salad samples. *Listeria* spp. was found in mixed vegetables (12, 6.6%) higher than other type of salads as seen in Table 5.

Table 5 *Distribution of Listeria species with governorates an sample source*

Listeria spp.	Governorates			Sample source	
	Rafah	Khan Younis	Mid Zone	Schools	Restaurants
L. monocytogenes	1 0.5%	1 0.5%	2 1%	4 2%	0 0%
L. ivanovii	0 0%	5 2.5%	0 0%	4 2%	1 0.5%
L. welshimeri	1 0.5%	1 0.5%	0 0%	2 1%	0 0%
L. grayi	1 0.5%	0 0%	3 1.5%	1 0.5%	3 1.5%
Total	**3** **1.5%**	**7** **3.5%**	**5** **2.5%**	**11** **5.5%**	**4** **2%**
P value		**0.108**		**0.059**	

This study showed the presence of *Listeria* spp. in 15 (7.5%) vegetables salad samples, was found in higher frequency in mixed vegetables (12, 6.6%) higher than other type of salads, (*P*= 0.071) as seen in Figure 1.

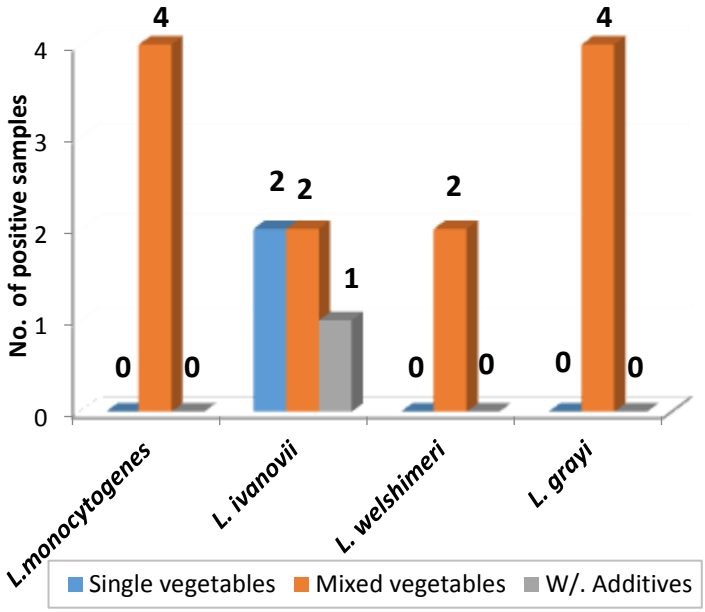

Figure 1 *Distribution of L. monocytogenes and other species according to salad type*

Distribution of *Salmonella* species according to governorates, sample source and salad type:

Salmonella spp. was found in higher frequency in Mid Zone governorate (8, 11.6%). The statistical significance were shown in *Salmonella* spp. with governorates (*P*= 0.008), was isolated from restaurants samples (7, 7%) and school canteens samples (3, 3%), (*P*= 0.194). *Salmonella* spp. was detected in higher frequency in mixed vegetables salad (8, 5%), (*P*= 0.814) as seen in Figure 2.

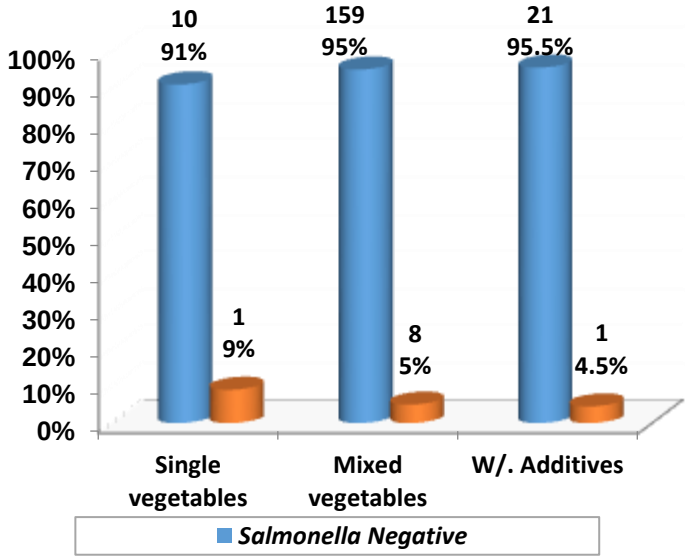

Figure 2 *Distribution of Salmonella spp. with salad type*

A statistically significant correlation was found in bacteria with sample source (*P*= 0.022). In this study the higher ratio of *Cronobacter sakazakii* (formerly known as *Enterobacter sakazakii*) was found in Khan Younis governorate (17; 8.5%), followed by Mid Zone (6; 3%), and Rafah (2; 1%). The ratio of *C. sakazakii* detected in school samples (18; 9%) was higher than that detected in restaurants samples (7; 3.5%). Contamination with *C. sakazakii* in mixed vegetables was (21; 10.5%) higher than other type of salads as seen in Table 6.

Table 6 *Distribution of isolated bacterial species according to governorates, sample source and salad type*

Other Isolates	Governorates			Sample source		Salad type			Total
	Rafah	Khan Younis	Mid Zone	Schools	Restaurants	Single vegetables	Mixed vegetables	W/. additives	
C. sakazakii	2 1%	17 8.5%	6 3%	18 9%	7 3.5%	2 1%	21 10.5%	2 1%	25 12.5%
Pasteurella spp.	1 0.5%	3 1.5%	2 1%	1 0.5%	5 2.5%	1 0.5%	5 2.5%	0 0%	6 3%
Aeromonas hydrophila	0 0%	1 0.5%	0 0%	0 0%	1 0.5%	0 0%	1 0.5%	0 0%	1 0.5%
Total	**3 1.5%**	**21 10.5%**	**8 4%**	**19 9.5%**	**13 6.5%**	**3 1.5%**	**27 13.5%**	**2 1%**	**32 11.5%**
P value		**0.855**		**0.022***			**0.898**		

* *Statistically significant*

Distribution of indicators bacteria according to salad type:

Indicator bacteria were distributed according to salad type as shown in table (7). A statistically significant correlation was shown in Total Plate Count ($P< 0.05$).

Table 7 *Distribution of indicators bacterial count according to salad type*

Bacterial count (CFU/g)	Salad type	TPC	TC	*E. coli*	*S. aureus*	*B. cereus*
0 - <10²	**S**	0 (0%)	1 (9.1%)	7 (63.6%)	7 (63.6%)	8 (72.7%)
	M	1(0.6%)	13 (7.8%)	73 (43.7%)	99 (59.3%)	128 (76.6%)
	W	1 (4.5%)	4 (18.2%)	13 (59.1%)	18 (81.8%)	18 (81.8%)
10² - <10³	**S**	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	1 (9.1%)
	M	0 (0%)	8 (4.8%)	11 (6.6%)	30 (%18)	13 (7.8%)
	W	0 (0%)	3 (13.6%)	1 (4.5%)	2 (9.1%)	4 (18.2%)
10³ - <10⁴	**S**	0 (0%)	5 (45.5%)	3 (27.3%)	2 (18.2%)	2 (18.2%)
	M	6 (3.6%)	39 (23.4%)	49 (29.3%)	32 (19.2%)	20 (12%)
	W	2 (9.1%)	6 (27.3%)	6 (27.3%)	1(4.5%)	0 (0%)
10⁴ - <10⁵	**S**	2 (18.2%)	2 (18.2%)	1 (9.1%)	1 (9.1%)	0 (0%)
	M	21 (12.6%)	57 (34.1%)	26 (15.6%)	5 (3%)	6 (3.6%)
	W	8 (36.4%)	6 (27.3%)	1 (4.5%)	1 (4.5%)	0 (0%)
10⁵ - <10⁶	**S**	6 (54.5%)	3 (27.3%)	0 (0%)	0 (0%)	0 (0%)
	M	105(62.9%)	49 (29.3%)	8 (4.8%)	1 (0.6%)	0 (0%)
	W	10 (45.5%)	3 (13.6%)	1 (4.5%)	0 (0%)	0 (0%)
≥ 10⁶	**S**	3 (27.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	M	34 (20.4%)	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)
	W	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

S; *Single vegetables salad,* M; *Mixed vegetables salad,* W; *Vegetables with additives,*
TPC; *Total Plate Count,* TC; *Total Coliform*

The results in this study revealed that there were several statistically significant correlation ($P < 0.05$) between; The compliance and non-compliance samples in different governorates. Moreover, compliance samples of school canteens and governorates. Furthermore, salad type showed that statistically significant with samples source, Total Plate Count (TPC) and *Shigella*. Also, the source of samples showed statistically significant correlation with Total Coliform (TC), *E. coli* and *S. aureus*. Moreover, governorates with TPC and *Salmonella*. Also, Total Plate Count with *S. aureus*, *E. coli* and TC. Furthermore, TC with *S. aureus*,

E. coli, TPC and *B. cereus*. The isolated bacteria *Cronobacter sakazakii*, *Pasteurella* spp. and *Aeromonas hydrophila* showed statistically significant correlation with samples source.

4. Discussion:

The bacteriological quality of fresh vegetables salad sold in restaurants and school canteens in Gaza-strip, Palestine was investigated in the present work. However, due to the absence of published local data, the results obtained in the present study could not be compared with any previous local data.

In this study, it was found that the percentage of samples that failed to comply with standards was 88% and this is very much higher than those found in UK (5.1%) (Meldrum *et al*., 2009) and lower than those found in Iran (98%) (Avazpour *et al*., 2013), the difference in the percentage compliance may be due to one or more of several reasons (e.g., the number of samples and type of samples used, the level of hygiene practiced in the study area and due to the use of different standards for evaluation).

The high levels of Total Coliform and *E. coli* in the tested samples and the detection of *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7 among other pathogens, pose a potential risk of food-borne illnesses to consumers especially children. In this study, the mixed vegetables salad samples exhibited the highest non-compliance with Total Plate Count, Total Coliform, *E. coli, S. aureus*, *Listeria* spp., *Salmonella* spp. and *E. coli* O157:H7, respectively.

These differences were probably due to the fact that mixed vegetables salad undergo a great deal of preparation processes such as peeling, washing with water, slicing, cutting and mixing of more one type of vegetable may causing cross contamination. In addition, salad samples with additives showed the least failure percentage, this may be because of the low pH of the food, which may inhibit or slow down the growth of bacterial contaminants. The action of acetic acid (vinegar) is based essentially on lowering the pH value of the product to be preserved (Sun-Young, 2004).

Total Plate Count in food is one of the microbiological indicators for food quality. This reflects the exposure of the sample to any contamination **(**Halablab *et al*., 2011). In this study, results demonstrated that percentage of salad vegetables samples that failed to comply with standards of Total Plate Count was 79.5% (159/200) (≥ 10^5 CFU/g).

In this study, the level of Total Plate Count in all vegetables salad samples ranged from 10^3 to 10^6 CFU/g. Similar findings had been reported in other studies; in Turkey, 3.3-7.4 $\log_{10}$ CFU/g (Aycicek *et al*., 2006) and in Egypt, reported TPC of raw salad vegetables as <10^6 CFU/g (Aycicek *et al*., 2006; Elmacioglu *et al*., 2010; Halablab *et al*., 2011). Furthermore, in Sudan it was found that TPC range from 1.2×10^5 to 2.8×10^7 CFU/g (Goja *et al*., 2013) and in Turkey reported a range from 10^2 to 10^5 CFU/g in 76 (84.4%) of salads samples (Elmacioglu *et al*., 2010). Meanwhile other studies reported higher range; in Turkey reported TPC range from 2.2×10^6 to 4.3×10^7 CFU/g (Erkan and Vural, 2008). In Nigeria two studies reported a range 3.0×10^5 to 5.7×10^8 CFU/g in ready to eat leafy vegetables (Abdullahi and Abdulkareem, 2010) and another reported bacterial loads ranging from 1.6×10^6 to 2.9×10^8 CFU/g (Itohan *et al*., 2011). In another study from Iran, it was found that TPC ranged from 4.1 to 8.3 $\log_{10}$ CFU/g in mixed fresh-cut salads and from 4.3 to 8.3 $\log_{10}$ CFU/g in mixed green leaves vegetables (Mohammad *et al*., 2012). While in Spain, a study reported that TPC ranged from 2.7 to 8.0 $\log_{10}$ CFU/g in whole fresh vegetables, from 4.3 to 8.9 $\log_{10}$ CFU/g in fresh-cut vegetables (Abadias *et al*., 2008). Total Plate Count results showed direct correlation with other bacterial indicators and these findings emphasize its role as superior indicator.

Total Coliform Counts (TC) are commonly used as bacterial indicator of sanitary quality of foods and water and can be considered as a hygiene indicator, especially for fecal contamination. Their presence indicates that pathogens might be present due to fecal contamination by human and animal or irrigation water (Goja *et al*., 2013; Osamwonyi *et al*., 2013; Halablab *et al*., 2011; Tambekar and Mundhada, 2006). The high prevalence of Coliform in vegetables could be due to water used to irrigate vegetable crops and the manure used as fertilizer are reported to contain Coliform and other enteric bacteria contaminated water used to clean equipment and cutting/slicing machines leading to cross-contamination, handlers not practicing proper sanitation (Hosein *et al*., 2008).

In this study, results demonstrated that percentage of salad vegetables samples that failed to comply with standards of Total Coliform was 60.5% (121/200) at ≥ 10^4 CFU/g as shown in table (3). There is a statistical significance correlation ($P < 0.05$) were shown in Total Coliform with *S. aureus*, *E. coli*, TPC and *B. cereus*. These findings emphasize its role as indicator for hygiene and

sanitary quality. The mixed vegetables salad showed the highest non-compliance with Total Coliform as 64.1% (107/167) (count ≥10^4 CFU/g), followed by single vegetables salad were 45.5% (5/11) and 40.9% (9/22) of vegetables with additives as shown in table (3).

In this study it was found that Total Coliform ranged from 2×10^2 to 3×10^6 CFU/g. Similar findings had been reported in two studies from Turkey; TC ranged from 3.0 to 6.9 $\log_{10}$ CFU/g (Halablab *et al*., 2011; Aycicek *et al*., 2006) and from 1.6×10^3 to 8.2×10^5 CFU/g (Erkan and Vural, 2008). Furthermore, in a study from Nigeria, it was found that TC range from 8.0 x 10^5 to 1.3 x 10^9 CFU/g in ready to eat leafy vegetables (Abdullahi and Abdulkareem, 2010).

E. coli is regarded as primary indicator for microbiological quality of water and food (Afolabi and Oloyede, 2010). The presence of *E. coli* in foods is an indicator of direct or indirect fecal contamination (Goja *et al*., 2013; Nma and Oruese, 2013; Osamwonyi *et al*., 2013; Khiyami *et al*., 2011). Presence of *E. coli* indicates recent contamination by fecal matter and possible presence of other enteric pathogens known to be causative agents of food-borne gastroenteritis and bacterial diarrhea disease (Nma and Oruese, 2013).

This results demonstrated that percentage of salad vegetables samples that failed to comply with standards of *E. coli* was 53.5% of samples. It was higher than those found in a study from UK which reported *E. coli* in 1.5% of samples, and it was at 10^2 CFU/g or more in 0.3% (11) samples (Sagoo *et al*., 2001). Another study in UK, reported that 57 (4.7%) of salad vegetable samples were found to be of unsatisfactory microbiological quality due to *E. coli* and/or *S. aureus* levels at ≥10^2 CFU/g (Meldrum *et al*., 2009). Moreover, in a study from India, it was reported in (38.3%) of different salad vegetables (Avazpour *et al*., 2013; Tambekar and Mundhada, 2006) and in Nigeria, it was reported in (6.7%) in salad vegetables (Itohan *et al*., 2011).

The statistical significance ($P < 0.05$) were shown in *E. coli* with TC, Total TPC and *B. cereus*. *E. coli* count results also showed direct correlation with other bacterial indicators and these findings emphasize its role as hygienic indicators.

In this study, the level of *E. coli* in all vegetables salad samples ranged from 2×10^2 to 3×10^5 CFU/g. Similar findings had been reported in Turkey, it was found that *E. coli* at range from 4.0×10^2 to1.9×10^5 CFU/g (Erkan and Vural, 2008). Moreover, two other studies in Turkey, *E. coli* was detected at level 10^2-10^4 CFU/g in 9 (10.0%) (Elmacioglu *et al*., 2010) and from 1.0 to 3.8 $\log_{10}$ CFU/g (Aycicek *et al*., 2006) of salad samples tested. Furthermore, in a study from USA, it was reported that *E. coli* level for all green leafy vegetable and herbs of less than 2 to 3 $\log_{10}$ CFU/g (Johnston *et al*., 2005). While higher levels was reported in Lebanon, it was that level of *E. coli* in all fresh vegetable samples ranged from 1.0 to 8.77 $\log_{10}$ CFU/g (Halablab *et al*., 2011). Moreover, in a study from Bangladesh had a range of *E. coli* in salad vegetables from 1.0×10^4 to 5.0×10^8 CFU/g (Rahman and Noor, 2012).

In the present study, *E. coli* was detected at count ≥ 10^2 CFU/g in 36.4% (4/11 samples) of single vegetables salad, 56.3% (94/167 samples) of mixed vegetables salad and 40.9% (9/22 samples) of vegetables salad with additives. It was lower than the percentage reported in a study from Iran, which detected *E. coli* in 69% of the fresh salad vegetables samples at counts ≥10^2 CFU/g (Avazpour *et al*., 2013) and in another study from Iran, it was detected that *E. coli* in 19.1% of mixed fresh-cut salads and 27.8% of mixed green leaves vegetables. 6.3% of all mixed green leaves vegetables and mixed fresh-cut salads (five samples), had *E. coli* counts ≥ 2 log CFU/g (Mohammad *et al*., 2012). Moreover, in Spain, a study reported that *E. coli* in 7.1% (2/28) of whole vegetable samples and in 11.4% (27/236) of fresh-cut vegetable and only two of them (0.8%) had *E. coli* counts >10^2 CFU/g (Abadias *et al*., 2008).

E. coli O157:H7 was isolated from two salad samples. In other studies, it was not detected in any salad sample in a study from Spain (Abadias *et al*., 2008), USA (Johnston *et al*., 2005) and UK (Sagoo *et al*., 2001). While in a study from Iran, it was found that *E. coli* O157:H7 in 6.5% of mixed fresh-cut salads and 11.4% of mixed green leaves vegetables (Mohammad *et al*., 2012).

In this study the high percentage of incidence Coliform counts, *E. coli* and *E. coli* O157:H7 presence may be due to used animal manure in fertilization vegetables crops or irrigation with contaminated or untreated sewage water and poor hygienic standard in the handling of these salad vegetables or it could be also be from contamination during harvest. In Gaza strip farms animal manures is used directly without any treatment thus carries huge numbers of pathogenic and indicator bacteria for long periods.

The percentage of salad vegetables samples that failed to comply with standards of *S. aureus* in this study was 21.5% (43/200) (≥ 10^3 CFU/g). This is higher than those reported in a study from UK, (2.7%) (Meldrum *et al*., 2009) and in a study from India, (15.1%) (Tambekar and Mundhada, 2006). Other studies showed high levels, in Bangladesh, had been a range of *S. aureus* (2.0×10^5 - 5.95×10^7 CFU/g) (Rahman and Noor, 2012) and in Lebanon, a study reported that level of *S. aureus* ranged from 1.47 to 8.77 $\log_{10}$ CFU/g (Halablab *et al*., 2011). Moreover, in Nigeria, a study reported that *S. aureus* in 46.7% of salad samples (Itohan *et al*., 2011) and in Iran, a study found *S. aureus* in 94.9% of all samples (Mohammad *et al*., 2012).

Contamination with *S. aureus* has been linked to carriage in nasal passages of food handlers or by infected workers (Itohan *et al*., 2011; Tambekar and Mundhada, 2006). Food handlers are usually the main source of food contamination in *S. aureus* food poisoning, but equipment and environmental surfaces can also be sources of contamination with *S. aureus* (Meldrum *et al*., 2009). The presence of *S. aureus* in ready-to-eat salad vegetables is an indication of poor hygiene practices (Abdullahi and Abdulkareem, 2010; Meldrum *et al*., 2009; Harris *et al*., 2003).

The results of study demonstrated that percentage of vegetables salad samples that failed to comply with standards of *B. cereus* is 14% (28/200) (≥ 10^3 CFU/gIt was detected in 23% (46) of vegetables salad samples at count from 2×10^2 to 4×10^4 CFU/g. Similar findings had been reported in Turkey where it was detected at range of 3.3×10^2-2.7×10^4 CFU/g (Erkan and Vural, 2008). The presence of *Bacillus* species in vegetables may be due to environmental factor (Abdullahi and Abdulkareem, 2010; Nma and Oruese, 2013).

This study showed the presence of *Listeria* spp. in 15 (7.5%) vegetables salad samples, 11 samples of them were in school canteens. Four species of *Listeria* were detected; *L. monocytogenes* (2%; 4 samples), *L. grayi* (2%; 4), *L. ivanovii* (2.5%; 5), and *L. welshimeri* (1%; 2).

A lower prevalence of *Listeria* spp. was reported in a study from UK, that found *Listeria* spp. in only 0.2% (6 samples) vegetables salad (not including *L. monocytogenes*) (Sagoo *et al*., 2001), and in Spain, a study reported *L. monocytogenes* in 0.7% (2) samples (Abadias *et al*., 2008). Moreover, in a study from Brazil, it detected *L. monocytogenes* only in one sample out of 181 (0.6%) (Fröder *et al*., 2007). A slightly lower detection rate was reported by a study from Chile (5.9%) (Cordano and Jacquet, 2009). While in another study from USA, *L. monocytogenes* not detected in any salad samples (Johnston *et al*., 2005).

L. monocytogenes is widely diffused in the environment and this fact can cause the contamination of vegetables during growing, harvesting, post-harvesting, handling or distribution. Salads have an additional risk of contamination through preparation, distribution and storage (Cordano and Jacquet, 2009; Sant'Ana *et al*., 2012). Cattle feces used as fertilizer are source for contamination grown vegetables with *L. monocytogenes* (Loncarevic *et al*., 2005).

This study showed the presence of *Salmonella* spp. in (5%; 10/200) of vegetables salad samples, three of them were found in school canteens samples. Other studies showed similar findings (5.8%) in India (Avazpour *et al*., 2013; Tambekar and Mundhada, 2006) and in Nigeria reported in (6.7%) of vegetables salad samples (Itohan *et al*., 2011). Furthermore, in Spain, it had been detected in four (1.3%) of the samples analyzed (Abadias *et al*., 2008), in USA, isolated 0.7% (3 of 398) of samples (Johnston *et al*., 2005), in Iran, two studies detected *Salmonella* spp. in 5.6% and 9.4% of mixed fresh-cut salads and mixed green leaves vegetables, respectively (Mohammad *et al*., 2012). While other studies reported no *salmonella* in any samples; as from Iran (Avazpour *et al*., 2013), Turkey (Erkan and Vural, 2008**)** and UK (Sagoo *et al*., 2001).

The presence of *E. coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. on vegetables irrigated with polluted stream water and well water is an indication of faecal contamination (Afolabi and Oloyede, 2010). The presence of *Salmonella* in salad vegetables has been linked to a number of recent outbreaks in the UK and elsewhere (Meldrum *et al*., 2009). Its presence in food is of serious concern to safety. According to the WHO (2002), effect of *Salmonella* on food safety is now a major public health concern worldwide (Itohan *et al*., 2011).

Contamination with this bacteria could arise from washing vegetables with contaminated water or handling of vegetables by carrier or infected workers, contaminated irrigated water and organic manures widely is used by Gaza farmers for soil fertilization. Contaminated manure compost and irrigation water can play an important role in contaminating soil and root vegetables with *Salmonellae* for several months (Islam *et al*., 2004).

In this study, *Shigella* spp. was found in 1% (2 samples) of vegetables salad samples whereas, in a study from India, *Shigella* spp. was found in (3.4%) of different vegetables salad (Avazpour *et al*., 2013; Tambekar and Mundhada, 2006) and in a study from Nigeria, *Shigella* spp. was reported in (6.7%) vegetables salad (Itohan *et al*., 2011).

Shigella spp. has been frequently found in salads. It is a principal agent of bacterial dysentery (Itohan *et al*., 2011). Laboratory studies demonstrated the rapid growth of *Shigella* on shredded lettuce stored at temperatures that were too warm (Berger *et al*., 2010). The possible sources of contamination of *Salmonella* spp. and *Shigella* spp. in vegetables may be due to washing of vegetables with contaminated water, handling of vegetables by infected workers, vendors and consumers in the market place, which helps to spread pathogenic microorganisms (Tambekar and Mundhada, 2006).

Bacterial contamination of salad vegetables was linked to the fact that they are usually consumed without any heat treatment. Contamination of vegetables with different pathogens such as enteric bacteria can occur directly or indirectly *via* animals or insects, soil, dust, use of untreated wastewater and water supplies contaminated with sewage for irrigation, processing equipment, transportation, and human handling (Avazpour *et al*., 2013; Goja *et al*., 2013; Harris *et al*., 2003; Itohan *et al*., 2011).

5. Conclusions

In conclusion, the percentage of samples that failed to comply with standards was 88% of all vegetable salad samples. The results elucidated the poor microbiological quality of salads and the possibility to act as a vehicle for pathogens such as *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7 and *L. monocytogenes*. It is therefore, recommended that good hygiene practices and high standards of cleanliness should be maintained at all times to avoid microbiological contamination.

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الجودة البكتيرية لسلطة الخضار الطازجة في مقاصف المدارس والمطاعم في قطاع غزة

كلمات مفتاحية:
سلطات الخضار،
الجودة البكتيرية،
الممرضات التي تنتقل عبر الطعام،
مؤشرات التلوث،
قطاع غزة.

هدفت هذه الدراسة إلى التحقق من الجودة البكتيرية لسلطات الخضروات في مقاصف المدارس والمطاعم المحلية في قطاع غزة، فلسطين. العينات المفحوصة شملت أنواع مختلفة من سلطات الخضار.

تم جمع ما مجموعه 200 عينة عشوائية من مقاصف المدارس (100 عينة) ومن مطاعم متنوعة (100 عينة) من محافظات الوسطى وخانيونس ورفح في الفترة ما بين إبريل إلى مايو 2013. جميع العينات أخضعت لفحص العد الكلي للبكتيريا، عد بكتيريا الكوليفورم الكلي، الايشريشيا القولونية، المكورات العنقودية الذهبية، الباسيليوس سيريس، والليستيريا. هذا بالإضافة إلى الكشف عن وجود اليستيريا مونوسيتوجينس، السلمونيلا، الشيجلا والايشريشيا القولونية O157:H7.

ثمانية وثمانين بالمائة من عينات سلطات الخضار فشلت في مطابقة المواصفة. وتوزعت نسب الفشل كما يلي: 79.5% للعد الكلي للبكتيريا، 60.5% لبكتيريا الكوليفورم الكلي، 53.5% للايشريشيا القولونية، 21.5% للمكورات العنقودية الذهبية، 14% للباسيليوس سيريس، 7.5% لعدد الليستيريا، 2% بسبب وجود اليستيريا مونوسيتوجينس، 5% بسبب السلمونيلا، 1% لكل من الشيجلا والايشريشيا القولونية O157:H7. في هذه الدراسة وجد أن السلطات التي تحتوي أكثر من نوع من الخضروات هي الأكثر فشلاً للمطابقة مع المواصفة. هذا بالإضافة إلى عزل عديد من الممرضات المحتملة مثل كرونوباكتر ساكازاكي (12.5%: 25)، الباستوريلا (3%: 6) وبكتيريا الايروموناس هيدروفيلا (0.5%: 1).

أظهرت النتائج بان سلطات الخضروات المفحوصة كانت رديئة من الناحية الميكروبيولوجية وربما تشكل وسيلة نقل للأمراض المنقولة عبر الطعام مثل السالمونيلا، الشيجلا، الايشريشيا القولونية O157:H7 واليستيريا مونوسيتوجينس وهذا يستلزم إجراءات عاجلة وزيادة الوعى بالمخاطر الصحية المحتملة.