

## Evaluation of Bacteriological Profile of the Fresh Produce in Beheira Governorate, Egypt

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The consumption of fresh produce has increased significantly in the recent decades. So far, no data are available on the bacteriological burden and the prevalence of foodborne pathogens in fresh produce of Beheira Governorate. The aim of the present study was to evaluate some food-borne pathogens namely: *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, in Beheira fresh produce. A total of 200 fresh produce samples were collected during a period of 11 month originating from different 21 agricultural fields, 13 marketplaces and 19 grocery stores in Beheira Governorate (Damanhour, El Mahmodya, Abo Homous and Kafr El Dawar) during April 2016 through March 2017. Out of 200 samples, 62 (31%) were contaminated with coliform. The highest coliform contaminated sources were that collected from grocery followed by market place then by agricultural field. The total mean log CFU/g  $\pm$  SD for arugula, spinach and radish were the highest coliforms contaminated fresh produce among all tested fresh produce samples while cucumber was the lowest coliform contaminated fresh produce among all tested fresh produce samples. In the present study, 18 *Salmonella*, 4 *L. monocytogenes* and 104 fecal *E. coli* were detected (representing 9% , 2% and 52%, respectively). *E. coli* O157:H7 was not detected in all collected fresh produce samples. Proteomic identification of the isolated bacteria was made using MALDI TOF spectrometry. It was revealed that *Salmonella* isolates were considered as multidrug-resistant organisms. They showed resistance against some members of more than 3 antibiotic classes, cephalosporins (Cephradine, CE), tetracycline (Oxytetracycline, OT) and amphenicols (Chloramphenicol, C). ESBL was detected in all *Salmonella* isolates of the present work. *L. monocytogenes* isolates showed to be sensitive to all tested antibiotics.

**Keywords:** Fresh produce, food-borne pathogens, MALDI TOF spectrometry, Beheira.

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Fresh produce is important components of a fit and balanced diet. They are a major source of fiber and micronutrients such as iron, manganese, and copper. Compounds like

carotenoids, polyphenolics, and glucosinolates, present in fruits also have nutritional value. They are a good source of vitamins<sup>1</sup>. The consumption of fresh produce has increased in the United States by more than 30% in the past few years.

The fresh-cut vegetables and fruit sector have experienced sustained market growth since the early 1990s<sup>3</sup>. According to James &

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Ngarmsak<sup>4</sup> Salad vegetables are good sources of pathogenic microorganisms. Pathogenic bacteria can contaminate vegetables at any stage from planting to consumption. The use of untreated wastewater and water supplies contaminated with sewage used for irrigation, post-harvest handling, and preparation in unhygienic environments in food services and home settings are among the commonly reported sources of vegetable contamination<sup>5</sup>. In September 2006, there was an outbreak of food-borne illness caused by *Escherichia coli* found in uncooked spinach in 26 U.S. states<sup>6</sup>. Thirty-one person suffered from a type of kidney failure called hemolytic uremic syndrome after eating spinach contaminated with the *E. coli* O157:H7 in the United States<sup>7</sup>. The present study was aimed to investigate the contamination of Beheira Governorate fresh produce with food-borne pathogens, detect the occurrence of multi-drug resistant and Shiga toxin-producing *E. coli* (STEC) and *E. coli* O157:H7 in fresh produce.

## MATERIALS AND METHODS

### Sample collection

A total of 200 samples namely cucumber, green Pepper, lettuce, spinach, parsley, tomato, arugula, and radish were collected from the grocery, market-place, and agricultural fields in Beheira Governorate (Damanhour, El-Mahmodya, Abo Homous and Kafr El Dawar) (during April 2016 through March 2017). All samples were randomly collected in sterile plastic bags, labeled and transported for analysis Figure 1.

During collection, samples were picked randomly from different locations and immediately put into sterile zip-lock bags without washing and were transported to the microbiology laboratory using ice box [8]. The sample size was from 300 to 500 g for small vegetables while samples of head lettuce consisted of the entire head; all samples were kept at 4°C for microbial analyses within 24 hrs of samples collection<sup>9</sup>.

### Microbiological analyses

#### Total coliform count using colony count technique

Ten gram of fresh produce sample was weighed and transferred into sterile stomacher bag containing 90 ml of sterile 1% buffered peptone water. The sample was homogenized in

a stomacher followed by 1 ml was transferred to make two-fold dilutions. Then 1 ml was inoculated and evenly distributed over a dry surface of sterile violet red bile (VRB) agar by a bented glass rod. After sufficient spreading a cover layer (tempered promptly to about 45°C) of (VRB) agar was poured over all plates and incubated at 37°C for 24 hrs. Clearly visible purple colonies surrounded by a purple halo were counted. The results were calculated and recorded as total coliform count per gram<sup>10</sup>.

#### Confirmatory test for coliforms

Inoculate five colonies of each doubtful type into tubes of lactose bile brilliant green broth. Incubate the tubes in the incubator set at 30°C for (24 ± 2) hrs. Consider colonies which show gas formation in the Durham tube, as coliforms<sup>11</sup>.

#### Detection of *Salmonella* spp.

Twenty-five gram of fresh produce sample was weighed, washed with tap water and homogenized in sterile stomacher bag containing 225 ml lactose broth, followed by incubation at 37° C for 24 hrs. One milliliter of lactose pre-enrichment broth was then transferred to 50 ml tetrathionate broth and incubated at 37°C. After 18 to 24 hrs bacterial cultures were streaked over xylose lysine deoxycholate agar and incubated for 24 hrs at 37°C. The red colonies with black center were then transferred into triple sugar iron agar slants and incubated for 24 hrs. at 37°C for further identification using biochemical analyses<sup>9</sup>.

#### Detection of *L. monocytogenes*

Twenty-five gram of fresh produce sample was weighed, washed with tap water and homogenized in sterile stomacher bag containing 225 ml *Listeria* enrichment broth. Followed by incubation from 24 to 48 hrs at 30° C. *Listeria* was isolated using *Listeria* selective agar (Oxford Formulation) and incubated for 24 hrs at 37°C. Typical colonies were further identified by using biochemical analyses<sup>9</sup>.

#### Microbiological Detection of *E. coli* O157:H7 [12]

Twenty-five gram of fresh produce sample was weighed, washed and homogenized in sterile stomacher bag containing 225 ml of Tryptic Soy broth (TSB) supplemented with novobiocin, followed by incubation at 38°C for 12–16 hrs. After incubation, 0.1 ml was plated on Sorbitol MacConkey agar supplemented with cefixime

and potassium tellurite (CT-SMAC). Plates were incubated at 38 °C for 18 hrs typical colonies are transparent and almost colorless with a pale yellowish-brown appearance and a diameter of approximately 1 mm.

#### Microbiological Detection of fecal *E. coli*

Twenty-five gram of fresh produce sample was weighed, washed and homogenized in sterile stomacher bag containing 225 ml brain heart infusion (BHI) broth, followed by incubation from 3 hrs at 35°C. One milliliter of the pre-enrichment is then transferred to 225 ml of tryptone phosphate (TP) broth and incubated at 44 °C for 20 hrs. After incubation, 0.1 ml was streaked over eosin-methylene blue agar and incubated for 24 hrs at 37°C. Colonies that showed green metallic sheen on EMB media were considered as *E. coli*<sup>13</sup> and was further identified by using biochemical analyses.

#### Proteomic identification of the isolated bacteria

All the isolated bacterial strains were further identified by Proteome-based bacterial identification using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)<sup>14</sup>.

#### Detection of multi-drug resistant food-borne pathogens

##### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing and results interpretation were performed according to

the recommendation of the Clinical Laboratory and Standards Institute (CLSI)<sup>15</sup>. The antibiotic (µg/disc) used in the present study were: Enrofloxacin (5 µg), Pefloxacin (5 µg), Amoxycillin (10 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Amoxycillin (30 µg), Oxytetracycline (30 µg), Cephadrine (30 µg). The disc was firmly placed on the agar plates previously streaked with the test organisms with the aid of a sterile forceps and incubated at 37 °C for 18-24 hours. Susceptibility or resistance of the isolates to different antibiotics was indicated by the appearance or nonappearance of clear zones of inhibition, these were measured to the nearest millimeter using a calibrated ruler.

##### Double-disk synergy test (DDST)

Disks containing cephalosporins (cefotaxime or ceftriaxone, ceftazidime, cefepime) are applied next to a disk with clavulanic acid (amoxicillin-clavulanic acid or ticarcillin-clavulanic acid). The positive result is indicated when the inhibition zones around any of the cephalosporin disks are augmented in the direction of the disk containing clavulanic acid. The distance between the disks is critical and 20 mm center-to-center has been found to be optimal for cephalosporin 30¼g disks<sup>16</sup>.

##### Statistical Analysis

Data analysis was done using the SPSS computer software version 19.0. Two-way and one

**Table 1.** Levels of coliform contamination in the collected fresh produce

Produce Varieties	Total Coliform Count (Mean log CFU/g ± SD)			Total
	Agricultural Field	Market	Grocery	
Parsley	4.85±0.27	5.06±0.25	5.28±0.02	5.04±0.27
Radish	5.01±0.04	5.11±0.11	5.36±0.04	5.15±0.15
Arugula	5.15*	5.13±0.15	5.35±0.08	5.19±0.15
Tomato	-	4.64±0.19	4.89±0.01	4.76±0.18
Green pepper	-	-	-	-
Cucumber	-	4.63±0.44	4.55±0.44	4.59±0.40
Lettuce	4.73±0.17	5.11±0.13	5.35±0.06	5.07±0.26
Spinach	-	5.11±0.09	5.19±0.06	5.15±0.08
Total	4.90±0.21x	4.99±0.27x	5.14±0.31x	

p-value > (0.05)

X indicates statistically highly significant differences between total counts of fresh produce from an agricultural area, marketplace grocery, and restaurant.

-: This level combination of factors is not observed; thus, the corresponding population marginal mean is not estimable.

\*: No standard deviation because only one sample of arugula from agricultural field that has coliform count

way ANOVA was used to compare mean values among fresh produce samples. The criteria for statistical significance was based on a ( $p < 0.05$ ).

**RESULTS AND DISCUSSION**

In a trial to test the microbiological quality of collected produce; coliform bacteria were detected 62 out of 200 fresh produce samples that

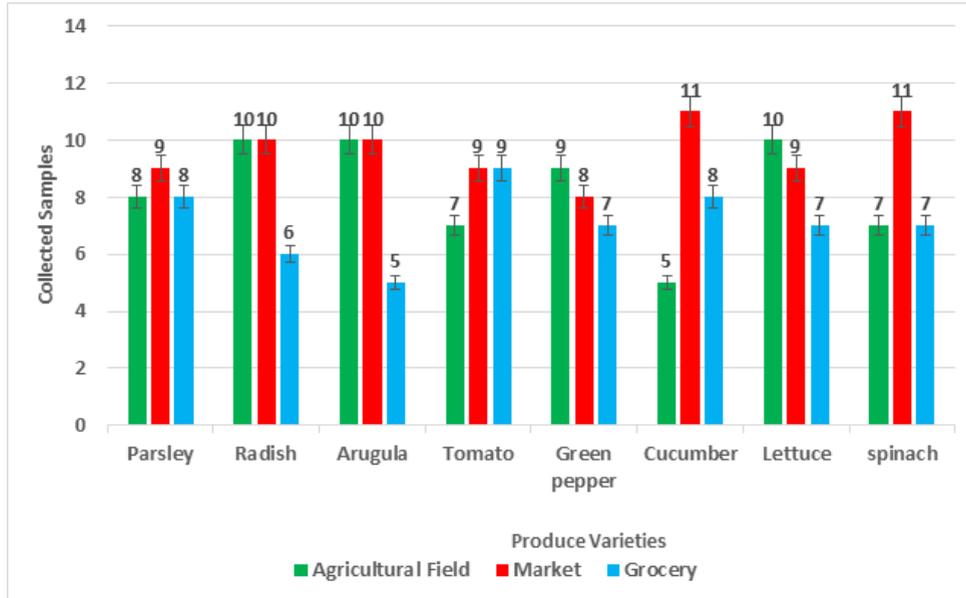


Fig. 1. Distribution of the collected samples according to produce varieties and origin

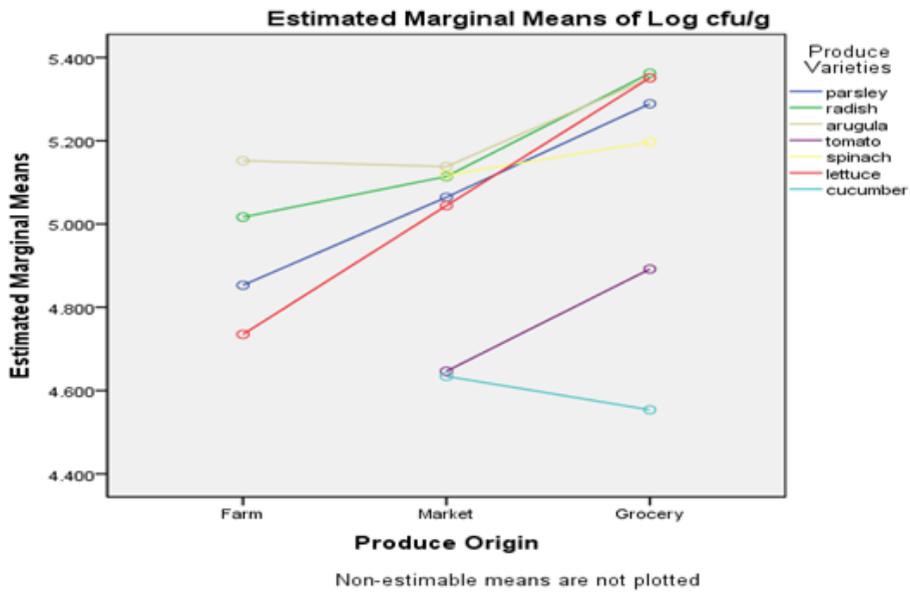


Fig. 2. Profile Plots resulted from two-way ANOVA showing the effect of fresh produce origin and produce varieties on mean log CFU/g, where  $p$ -value  $> (0.05)$

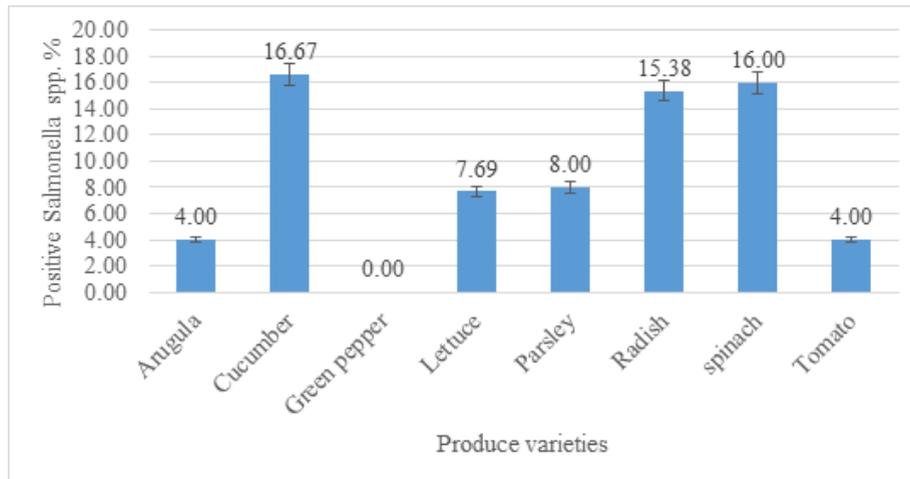
represent 31% of all the samples. The total mean log CFU/g ± SD of fresh produce collected from the grocery are the highest coliform contaminated sources (5.14±0.30) followed by marketplace (4.99±0.27) then by agricultural field (4.90±0.21)

**Table 2.** Incidence of *Salmonella* isolates in fresh produce under test according to produce origin

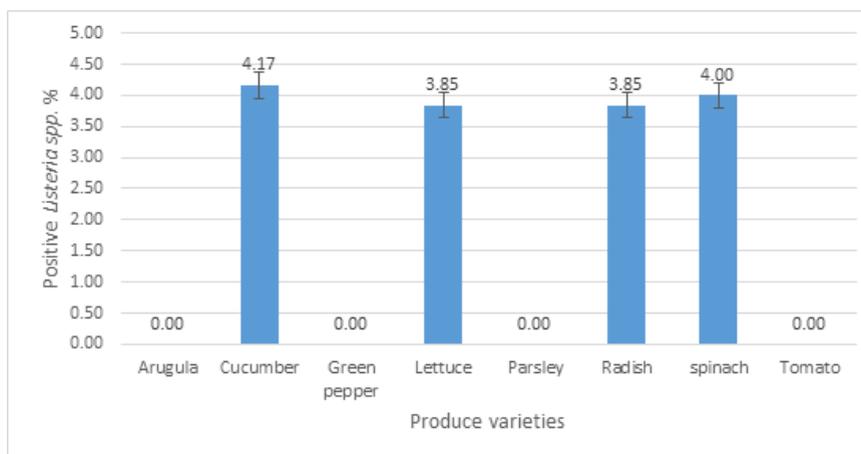
Produce origin (No.)	Positive <i>Salmonella</i> isolates samples %, (No.)
Agricultural Field (66)	3.03 (2)
Market (77)	10.39 (8)
Grocery (57)	14.04 (8)
Total (200)	9.00 (18)
p-value < (0.05)	

Table 1. The total mean log CFU/g ± SD of arugula, spinach and radish were the highest coliforms contaminated fresh produce among all the tested fresh produce samples (5.19±0.15, 5.15±0.08 and 5.15±0.15), respectively. While cucumber was the lowest coliform contaminated fresh produce among all the tested fresh produce samples with total mean log CFU/g ± SD (4.59±0.40) Figure 2.

On the other hand, the mean log CFU/g ± SD of cucumber coliform count collected from marketplaces and grocery stores were (4.63±44) and (4.55±0.44), respectively. The mean log CFU/g ± SD of coliform count detected in lettuce collected from agricultural fields, marketplaces and grocery stores were (4.73±0.17), (5.05±0.13) and (5.35±0.06), respectively. On the other hand, the



**Fig. 3.** Prevalence of *Salmonella* isolates in fresh produce under test according to produce varieties



**Fig. 4.** Prevalence of *Listeria* spp. in fresh produce under test according to produce varieties

mean log CFU/g  $\pm$  SD of coliform count detected in parsley collected from agricultural fields, marketplaces and grocery stores were (4.85 $\pm$ 0.27), (5.06 $\pm$ 0.25) and (5.28 $\pm$ 0.02), respectively. Among all collected fresh produce samples from agricultural fields, arugula showed the highest level of coliform count log CFU/g (5.15) followed by mean log CFU/g  $\pm$  SD of radish (5.01 $\pm$ 0.04) followed by mean log CFU/g  $\pm$  SD of parsley (4.85 $\pm$ 0.27) followed by mean log CFU/g  $\pm$  SD of lettuce (4.73 $\pm$ 0.17). Arugula, spinach and radish reported the highest level of coliform contamination among all the samples collected from marketplaces with mean log CFU/g  $\pm$  SD of (5.13 $\pm$ 0.15), (5.11 $\pm$ 0.09) and (5.11 $\pm$ 0.11), respectively, followed by lettuce (5.04 $\pm$ 0.13), parsley (5.06 $\pm$ 0.25), cucumber (4.63 $\pm$ 0.44) and tomato was (4.64 $\pm$ 0.19). Radish, lettuce and arugula reported the highest level of coliform contamination among all the samples collected from grocery places with mean log CFU/g  $\pm$  SD of (5.36 $\pm$ 0.04), (5.35 $\pm$ 0.06) and (5.35 $\pm$ 0.08),

respectively followed by parsley (5.28 $\pm$ 0.02), spinach (5.19 $\pm$ 0.06), tomato (4.89 $\pm$ 0.01) and cucumber that showed (4.55  $\pm$ 0.44). Among all the level of coliform contamination, cucumber collected from grocery stores represented the lowest means log CFU/g  $\pm$  SD of (4.55 $\pm$ 0.44 SD). The variations in coliform counts within the different types of fresh produce and different sample origin were obvious and statistically significant ( $p < 0.05$ ). The coliform count on most of the fresh produce under test ranged from 4 to 6 log CFU/g Figure 2.

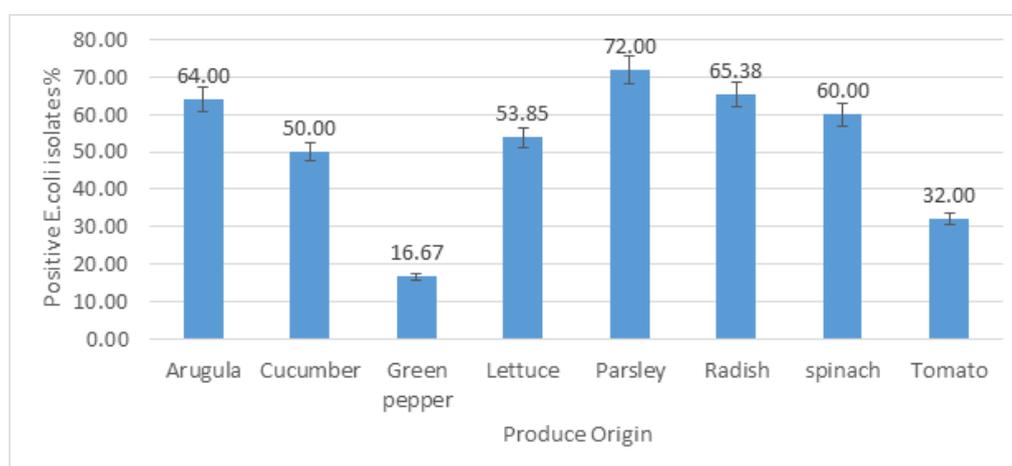
Results of the present study were consistent with that reported by Ruiz-Cruz *et al*<sup>17</sup> whereas microbial levels in carrot samples, collected from local wholesale market in México, ranged from 10<sup>4</sup> to 10<sup>6</sup> log CFU/g, but exceeded the recommended coliform levels by WHO and International Commission on Microbiological Specifications for Food standards, ranged from 10 to 10<sup>2</sup> log CFU/g<sup>18</sup>. Also, results of the present

**Table 3.** Incidence of *L.monocytogenes* in fresh produce under test according to produce origin

Produce origin (No.)	Positive <i>L.monocytogenes</i> samples %, (No.)
Agricultural Field (66)	1.52 (1)
Market (77)	2.60 (2)
Grocery (57)	1.75 (1)
Total (200)	2.0 (4)
p-value < (0.05)	

**Table 4.** Incidence of *E.coli* in fresh produce under test according to produce origin

Produce origin (No.)	Positive <i>E.coli</i> samples %, (No.)
Agricultural Field (66)	34.85 (23)
Market (77)	75.00 (44)
Grocery (57)	57.14 (37)
Total (200)	52.00(104)
p-value < (0.05)	



**Fig. 5.** Prevalence of *E.coli* in fresh produce under test according to produce varieties

study were different than other studies in the southern United States that examined microbial load ranging from 2 to 3 log CFU/g on some tested items of fresh produce namely: arugula, parsley and spinach<sup>9</sup>. The level of total coliforms in the present study was relatively high this might be due to variation in sample type, methods of analysis, type of irrigation water used, habits of farmers and sellers in handling with fresh produce and sampling location. Conditions and measures were taken during pre-harvest, harvest and post-harvest stages might affect the microbial contamination of fruits and vegetables. During post-harvest phase, treatment of fresh produce such as handling, storage, transportation and cleaning, these practices factors might lead to cross-contamination of the product from other agricultural materials or from the workers. In the present study, the total fresh produce originating from agricultural lands were the lowest coliform contaminated with mean log CFU/g $\pm$  SD (4.90 $\pm$ 0.21). However, the level of contamination increase during the post-harvest phase such as handling, transportation, and distribution resulting in an increase in the level of contamination with mean log CFU/g $\pm$  SD (4.99 $\pm$ 0.27) in the marketplaces. Lynette *et al.* reported a significant increase in the fecal coliform load for both root crops and leafy vegetables from farm to market<sup>9</sup>. This level was the highest in grocery places due to long storage period with mean log CFU/g $\pm$  SD (5.14 $\pm$ 0.30). There is a possible explanation for the relatively high total coliform counts in arugula, radish, spinach, lettuces, and parsley. These vegetables are leafy greens with large surface areas and folds. This makes them more susceptible to bacterial contamination and adhesion; and when untreated manure is utilized as soil fertilizers in the farm, it is also easy for their open leaves to contact with irrigation water and soil, thus result in microorganisms transfer on to their leaves<sup>19</sup>.

In the present study, isolated bacteria that were identified by proteomics using MALDI-TOF MS were *S.typhimurium*, *S.enterica Dublin*, *S.enterica Anatum*, *S.enterica Hadar*, *S.enterica Enterica*, *S.enterica Diarizonae*, *S.enterica Gallinarum* and *Listeria monocytogenes*.

In the present study, *Salmonella* isolates were 18 out of 200 that represent 9% of the fresh produce samples collected from the agricultural

field, marketplaces and grocery places. The variations in *Salmonella* isolates contamination within the different fresh produce types and with a different origin, were obvious and not statistically significant ( $p>0.05$ ). The cucumber, spinach, and radish were the highest load of *Salmonella*-contaminated fresh produce 4 out of 24 samples (16.67%), 4 out of 25 samples (16.0%) and 4 out of 26 samples (15.38%), respectively, followed by parsley, lettuce, arugula and tomato that showed slight *Salmonella* contamination 2 out of 25 samples (8%), 2 out of 26 samples (7.69%), 1 out of 25 samples (4.0%) and 1 out of 25 samples (4.0%), respectively. However, green pepper was completely free of *Salmonella* contamination (0%) Figure 3.

The fresh produce collected from grocery stores showed high level of *Salmonella* contamination 8 out of 57 samples (14.04%), followed by fresh produce collected from marketplace 8 out of 77 samples (10.39%) and fresh produce collected from agricultural fields that showed a lower *Salmonella* contamination 2 out of 66 samples (3.03%) Table 2.

In the present study, *Listeria monocytogenes* isolates were 4 out of 200 that represents as 4% of the fresh produce samples collected from the agricultural field, marketplaces and grocery stores. The variations in *Listeria monocytogenes* isolates contamination within the different fresh produce types and with a different origin, were obvious and not statistically significant ( $p>0.05$ ). The cucumber and spinach were the highest load *L.monocytogenes* contaminated fresh produce 1 out of 24 samples (4.17%) and 1 out of 25 samples (4.0%) respectively. Lettuce and radish showed slight *L.monocytogenes* contamination 1 out of 26 samples (3.85%) and 1 out of 26 samples (3.85%), respectively. However, arugula, green pepper, and tomato were completely free from *L.monocytogenes* contamination (0%) Figure 4.

The fresh produce collected from marketplace showed high load of *L.monocytogenes* contamination 2 out of 77 samples (2.6%), followed by fresh produce collected from grocery stores 1 out of 57 samples (1.75%) and fresh produce collected from agricultural fields showed a lower *L.monocytogenes* a contamination 1 out of 66 samples (1.52%) Table 3.

In the present study, *E.coli O157:H7* was

not detected in all collected fresh produce samples and this in agreement with a study that tested 106 imported fresh produce samples across Canada<sup>1</sup>, another study surveying 1,183 samples of Ontario grown organic and conventional produce<sup>20</sup> and agree with a separate larger-scale studies surveying 1003 imported and 1028 domestic produce samples in the United States<sup>21</sup>. Within all the studies that surveyed microbial loads in produce, *E. coli* O157:H7 was not detected in any the samples despite the incidence of other foodborne pathogens such as *Salmonella*<sup>20</sup>.

In the present study, the variations in *E. coli* contamination within the different fresh produce types and with a different origin were obvious and not statistically significant ( $p > 0.05$ ). Parsley, radish, arugula, and spinach showed high load of *E. coli* fresh produce contamination 18 out of 25 samples (72%), 17 out of 26 samples (65.38%), 16 out of 25 samples (64%) and 15 out of 25 samples (60%), respectively, followed by lettuce and cucumber 14 out of 26 sample (53.85%) and 12 out of 24 sample (50%), respectively. Tomato and green pepper showed slight *E. coli* contamination 8 out of 25 sample (32%) and 4 out of 24 sample (16.67%), respectively, Figure 5.

The fresh produce collected from marketplaces showed high level of *E. coli* contamination 44 out of 77 samples (75%), followed by fresh produce collected from grocery stores 37 out of 57 samples (57.14%) and fresh produce collected from agricultural fields showed lower *E. coli* contamination 23 out of 66 samples (34.85%) Table 4.

On the contrary, Cardenas *et al*<sup>22</sup> reported that a survey in Mexico found that 1.25% of RTE tomato and peppers were positive for *Salmonella*. Santos *et al*<sup>23</sup> reported that the positive rate for *Salmonella* and *L. monocytogenes* in Portugal was 1.99% and 0.66%, respectively that was lower than the present study. Badosa *et al*<sup>24</sup> reported that 0.74% and 1.48% for *Salmonella* and *L. monocytogenes* contamination, respectively in the tested raw vegetables in Spain. While Froder *et al*<sup>25</sup> reported that the leafy salad samples in Brazil showed 3% and 0.6% of *Salmonella* and *L. monocytogenes* contamination respectively. Sait Aykut *et al*<sup>26</sup> showed that the prevalence of contamination by *Salmonella* and *L. monocytogenes* were 14 and 8.5% , respectively on leafy green

vegetables grown around Ankara which higher than the present study. Direct or indirect contact with feces, leakage of sewage, surface water, and untreated irrigation affect greatly the quality of fresh produce contamination by *S. enterica*<sup>27</sup>. The absence of *L. monocytogenes* in some vegetables can also be explained by an antibacterial effect of some components of vegetables<sup>28</sup>. Furthermore, the background flora present on the produce might have an inhibitory effect on *L. monocytogenes*. Soriano *et al*<sup>29</sup>, reported that the prevalence of *E. coli* of ready-to-use lettuce served at university restaurants in Brazil was 25%. The percentage of *E. coli*-positive samples found in a survey of conventionally grown fresh vegetables in Japan including cabbage, lettuce, onions, spinach, and celery was 2%<sup>30</sup>. However, in the present study, *E. coli* was found in 52% fresh produce. For decades, *E. coli* has been used as the reference indicator for fecal contamination, and a number of surveys have reported its isolation from fresh fruits and vegetables<sup>31</sup>. The quality of water used for irrigation can be a significant source of pathogenic contamination and plays an instrumental role in all stages from pre-harvest, to harvest, to post-harvest, to being sold in retail markets or served in food service establishments<sup>13</sup>. In Egypt, some farmers after harvest and sellers during the selling process were used to wash fresh produce with contaminated water to remove soil debris and this could infect vegetables with pathogenic bacteria such as *Salmonella* and this might occur in Beheira Governorate due to unhygienic practices of water and sewage water leakage. Cross contamination could be a good contaminated source for fresh produce with fecal or human pathogens and this contamination could result through handling by farmers and sellers that may introduce contamination to post-harvest produce through the distribution stages. There are many poultry farms in Beheira Governorate that could spread *Salmonella* through the agricultural field by manure resulted from these farms. *Salmonella sp.* is widely dispersed in nature. Poultry and other meat products, eggs and dairy products, are the most commonly implicated sources of outbreaks involving *Salmonella*<sup>32</sup>, however, fresh produce has also been implicated as the source of major outbreaks, particularly in recent times<sup>33</sup>.

In the recent years, the occurrence of multi-

drug resistance in pathogenic and opportunistic bacteria has been increasingly recognized<sup>34</sup>. However,  $\beta$ -lactamases continues to be the leading cause of resistance to  $\beta$ -lactam antibiotics in Gram-negative bacteria<sup>35</sup>. In the present study, *Salmonella* isolates showed resistance to cephadrine (CE-30 $\mu$ g), oxytetracycline (OT-30 $\mu$ g) and amoxicillin (AMC-10 $\mu$ g). As long as, 12 out of 13 (92.3%) isolates were considered as resistant to chloramphenicol (C-30 $\mu$ g) and 1 out of 13 (7.3%) isolates were considered as resistant to pefloxacin (PEF-5 $\mu$ g). On the other hand, all of them showed sensitivity to amoxicillin / clavulanic acid (AMC, 20/10), streptomycin (S-10 $\mu$ g) and enrofloxacin (ENR-5 $\mu$ g). It was noticed that all the isolated *Salmonellae* were ESBL positive. In the present study, the isolates were resistant to cephadrine, oxytetracycline, and amoxicillin. As long as, 12 out of 13 (92.3%) isolates were considered as resistant to chloramphenicol and 1 out of 13 (7.3%) isolates were considered as resistant to pefloxacin. This might be due to indiscriminate use of these antibiotics which are always resorted to for medication in the treatment of *Salmonella* infections especially in developing countries like Egypt. Pui *et al.*,<sup>36</sup> reported that *Salmonella* strains resistant to one or more antibiotics have increased in worldwide. This scenario has always been linked to the increased and uncontrolled use as well as easy accessibility to antibiotics in many countries of the world<sup>37</sup>. In Enterobacteriaceae, resistance to cephalosporins is generally attributed to the production of large spectrum beta-lactamases such as ESBL and AmpC beta-lactamase<sup>38</sup>. In the present study, ESBL production was detected in 100% (13/13) of the *Salmonella* isolates. The antimicrobial resistance patterns of *L.monocytogenes* isolates under test were investigated. It was revealed that, 4 (100%) of *L.monocytogenes* isolates, showed sensitivity to cephadrine (CE-30  $\mu$ g), oxytetracycline (OT-30  $\mu$ g), chloramphenicol (C-30  $\mu$ g), amoxicillin / clavulanic acid (AMC,20/10), streptomycin (S-10  $\mu$ g), amoxicillin (AMC-10  $\mu$ g), pefloxacin (PEF-5  $\mu$ g) and enrofloxacin (ENR-5  $\mu$ g). These results provide useful information on antibiotic resistance of *L.monocytogenes* strains isolated from foods, and can potentially be used to develop antibiotic treatments to guard against the hazards associated

with *L.monocytogenes* in ready-to-eat vegetables and other food products.

## CONCLUSION

The present study showed that the prevalence of *Salmonella* was high in fresh produces especially those from grocery stores origin. In addition, acidified products may limit microbial growth or survival, and the extent of this survival depends on the types of microorganisms harbored in the food, the amount of acid and the storage temperature. MALDI TOF spectrometry is high significant protein-based bacterial identification.

## REFERENCES

1. Allen, K.J., *et al.*, Microbiological survey of imported produce available at retail across Canada. *Int J Food Microbiol*, 2013. **162**(2): p. 135-42.
2. Barth, M., *et al.*, Microbiological spoilage of fruits and vegetables, in Compendium of the microbiological spoilage of foods and beverages. 2009, Springer: p. 135-183.
3. Olaimat, A.N. and R.A. Holley, Factors influencing the microbial safety of fresh produce: a review. *Food microbiology*, 2012. **32**(1): p. 1-19.
4. James, J.B., T. Ngarmasak, and R. Rolle, Processing of fresh-cut tropical fruits and vegetables: A technical guide. RAP Publication (FAO) eng no. 2010/16, 2010.
5. Amoah, P., *et al.*, Irrigated urban vegetable production in Ghana: microbiological contamination in farms and markets and associated consumer risk groups. *Journal of water and health*, 2007. **5**(3): p. 455-466.
6. Lynch, M.F., R.V. Tauxe, and C.W. Hedberg, The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology & Infection*, 2009. **137**(3): p. 307-315.
7. Calvin, L., Outbreak linked to spinach forces reassessment of food safety practices. *Amber Waves*, 2007. **5**(3): p. 24.
8. Khatib, A. and G. Khawaja, Shiga toxin-producing *E. coli* (STEC) associated with lebanese fresh produce. *International Journal of Current Microbiology and Applied Sciences*, 2015. **4**(2): p. 481-496.
9. Johnston, L.M., *et al.*, A field study of the

- microbiological quality of fresh produce. *Journal of food protection*, 2005. **68**(9): p. 1840-1847.
10. No, I.S., 4832 (2006) Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of coliforms—colony-count technique. International Organization for Standardization, Geneva, 2006.
  11. Puniya, A.K. and S. Vij, Practical Manual on Food and Industrial Microbiology. 2010, NDRI, KARNAL.
  12. ISO, E., 16654: 2001-Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli* O157. International Organization for Standardization, 2001.
  13. Hanjra, M.A., *et al.*, Wastewater irrigation and environmental health: implications for water governance and public policy. *International journal of hygiene and environmental health*, 2012. **215**(3): p. 255-269.
  14. Carbonnelle, E., *et al.*, MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clinical biochemistry*, 2011. **44**(1): p. 104-109.
  15. Jorgensen, J.H., *et al.*, New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria. *Clinical infectious diseases*, 2007. **44**(2): p. 280-286.
  16. Drieux, L., *et al.*, Phenotypic detection of extended spectrum  $\beta$  lactamase production in Enterobacteriaceae: review and bench guide. *Clinical Microbiology and Infection*, 2008. **14**(s1): p. 90-103.
  17. Ruiz-Cruz, S., *et al.*, Efficacy of sanitizers in reducing *Escherichia coli* O157: H7, *Salmonella* spp. and *Listeria monocytogenes* populations on fresh-cut carrots. *Food Control*, 2007. **18**(11): p. 1383-1390.
  18. Foods, I.C.o.M.S.f., Microbial Ecology of Food Commodities. Vol. 6. 1998: Blackie Academic & Professional.
  19. Aycicek, H., U. Oguz, and K. Karci, Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health*, 2006. **209**(2): p. 197-201.
  20. Arthur, L., *et al.*, Microbial survey of selected Ontario-grown fresh fruits and vegetables. *Journal of food protection*, 2007. **70**(12): p. 2864-2867.
  21. León, J.S., L.A. Jaykus, and C.L. Moe, Food safety issues and the microbiology of fruits and vegetables. *Microbiologically safe foods*, 2009: p. 255-281.
  22. Control, C.f.D. and Prevention, Update on multi-state outbreak of *E. coli* O157: H7 infections from fresh spinach, October 6, 2006. Retrieved November, 2006. **20**: p. 2006.
  23. Santos, M., *et al.*, Evaluation of minimally processed salads commercialized in Portugal. *Food Control*, 2012. **23**(1): p. 275-281.
  24. Badosa, E., *et al.*, Microbiological quality of fresh fruit and vegetable products in Catalonia (Spain) using normalised plate counting methods and real time polymerase chain reaction (QPCR). *Journal of the Science of Food and Agriculture*, 2008. **88**(4): p. 605-611.
  25. FROeDER, H., *et al.*, Minimally processed vegetable salads: microbial quality evaluation. *Journal of food protection*, 2007. **70**(5): p. 1277-1280.
  26. Aytac, S.A., *et al.*, Evaluation of *Salmonella* and *Listeria monocytogenes* contamination on leafy green vegetables. *J Food Agric Environ*, 2010. **8**: p. 275-279.
  27. Takeuchi, K., *et al.*, Comparison of the attachment of *Escherichia coli* O157: H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Pseudomonas fluorescens* to lettuce leaves. *Journal of food protection*, 2000. **63**(10): p. 1433-1437.
  28. Nguyen the, C. and B.M. Lund, An investigation of the antibacterial effect of carrot on *Listeria monocytogenes*. *Journal of Applied Microbiology*, 1992. **73**(1): p. 23-30.
  29. Soriano, J., *et al.*, Assessment of the microbiological quality and wash treatments of lettuce served in University restaurants. *International Journal of Food Microbiology*, 2000. **58**(1): p. 123-128.
  30. Kaneko, K.-I., *et al.*, Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *Journal of food protection*, 1999. **62**(6): p. 644-649.
  31. Jay, J., Modern food microbiology. Maryland. 2000, Aspen Publishers, Inc.
  32. Lund, B., T.C. Baird-Parker, and G.W. Gould, Microbiological safety and quality of food. Vol. 1. 2000: Springer Science & Business Media.
  33. Fung, J., *et al.*, Microbiological quality of urban-vended salad and its association with gastrointestinal diseases in Kumasi, Ghana. *International Journal of Food Safety, Nutrition and Public Health*, 2011. **4**(2-4): p. 152-166.
  34. Jones, M.E., *et al.*, Emerging resistance among bacterial pathogens in the intensive care unit—a European and North American Surveillance study (2000–2002). *Annals of Clinical Microbiology and Antimicrobials*, 2004. **3**(1): p. 14.
  35. Bradford, P.A., Extended-spectrum  $\beta$ -lactamases in the 21st century: characterization,

- epidemiology, and detection of this important resistance threat. *Clinical microbiology reviews*, 2001. **14**(4): p. 933-951.
36. Pui, C., *et al.*, Salmonella: A foodborne pathogen. *International Food Research Journal*, 2011. **18**(2).
37. Yoke Kqueen, C., *et al.*, Characterization of multiple antimicrobial resistant Salmonella enterica subsp. enterica isolated from indigenous vegetables and poultry in Malaysia. *Letters in applied microbiology*, 2008. **46**(3): p. 318-324.
38. de Jong, A., *et al.*, Antimicrobial susceptibility of Salmonella isolates from healthy pigs and chickens (2008–2011). *Veterinary microbiology*, 2014. **171**(3): p. 298-306.