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Understanding the routes of contamination of ready-to-eat vegetables in the Middle East

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Highlights:

- A high prevalence of *E.coli* and total coliforms was found from farms to market
- *E.coli* and total coliforms counts significantly increased in post-harvest washing
- Pathogenic organisms were detected on fresh produce along the supply chain
- Post-harvest wash process is identified as a pathway of pathogens on fresh produce
- Untreated chicken manure are likely to be a source of the high occurrence of S.a*ureus*

Abstract

In the developing countries, inaccessibility to safe water, lack of agricultural infrastructures and limitations to implementing good agricultural practices (GAP) are persistent challenges. To understand the spread of hazards and identify critical areas of transmission in the food chain, a total of 90 samples of raw salad vegetables (parsley, lettuce, radish) were collected from farms and post-harvest washing facilities (n=12) in an extensively cultivated area in Lebanon, the Bekaa Valley and from wholesale market stalls traced back to surveyed fields. Our results showed high geometric mean indicator levels ranging from <0.7 to 7 log CFU/g (*Escherichia coli*), 1.69-8.16 log CFU/g (total coliforms), <0.7 - 8.39 log CFU/g (*Staphylococcus aureus*). The mean counts of total coliforms and *Escherichia coli* on fresh produce followed an increasing trend from fields to the markets indicating potential sources of faecal contamination throughout the food chain. Of more concern was the presence of pathogens *Listeria monocytogenes* (14%) and *Staphylococcus aureus* (45.5%) in fresh produce from harvest to retail, and *Salmonella* spp. was detected in 6.7% of the raw vegetables from the post-harvest washing areas. These results along with our observations highlight shortfalls in hygienic farming and postharvest practices, including the use of inappropriately treated manure and chicken litter to fertilize the crops on the fields which contributed to the high levels of *S. aureus* in the product at retail. Unregulated use of wash water, inadequate transportation and storage conditions with risks of cross

contamination was also identified. Suggested control measures should mitigate the risks at the source and put emphasis on developing strict policies on monitoring the safety of water sources and on the application of the good agricultural and hygienic practices (GAP, GHP) on primary production stages, washing, transportation and storage at retail.

Keywords: Leafy greens; fresh produce supply chain; irrigation water**;** post-harvest wash water; *Salmonella*; *Staphylococcus aureus*

1. Introduction

In the Middle East, many types of vegetables are eaten raw in salads or used as garnishes in appetizers and traditional dishes, and also increasingly because of their perceived healthy attributes. Yet, they have been in recent years a major contributor to foodborne illnesses in other parts of the world (Callejón et al., 2015; Lynch et al., 2009; Painter et al., 2013). In the United States (U.S.), leafy greens were identified at the top of the 10 riskiest foods regulated by the Food and Drug Administration (FDA) accounting for almost 40% of foodborne outbreaks based on data derived from the Centers for Disease Control and Prevention (CDC)(CSPI, 2009). Pathogens identified as hazards on fresh vegetables include S*higella* spp., *Listeria monocytogenes*, *Staphylococcus aureus, Aeromonas hydrophila* and the spore-formers *Bacillus cereus*, *Clostridium botulinum* and *C. perfringens*. However, the ones implicated in most outbreaks involving fresh fruits and vegetables are *Salmonella*, *E. coli* O157:H7 (Buck et al., 2003; European Commission, 2002) with reported doses as low as 10 cells and 2–2,000 cells, respectively (Harris et al., 2003; Kisluk et al., 2012). Norovirus is also among the pathogens of greatest concern that are associated with fresh produce outbreaks (Todd & Greig, 2015) and the

high likelihood of inflicting illnesses is attributed to its low infectious doses10-100 viral particles as reported by D'Souza and Su (2010) and Barrabeig et al. (2010). The reportedly held rationale that increased consumption of fresh vegetables is actually the reason for the increased foodborne illnesses has been challenged in a American Society for Microbiology (2008) report stating that the proportion of outbreaks due to leafy greens has increased beyond what can be explained by increased consumption. This leads us to focus on the primary production stages on farms and subsequent processing as the main contamination sources, although no doubt coupled with enhanced epidemiological and surveillance programs (CSPI, 2009) and the expanded interaction of the local and international markets of fresh produce.

Perishable fruits and vegetables are now transported long distances from growing to retail markets with a wide product distribution range to meet consumer demand. Thus, any associated illnesses could be widely dispersed within or beyond national borders, requiring sophisticated surveillance tools like PulseNet to identify these, while traceability to origin remains a challenge in such an extended supply chain (Sivapalasingam et al., 2004). This may be beyond the resources of many developing countries including those in MENA (Middle East North Africa), where illnesses related to leafy greens may be underestimated or rarely reported. In this Region prompt concerted research efforts to understand, prevent and control risks of illnesses arising from consumption of contaminated salad vegetables and fruits are lagging behind those in other regions. Throughout the farm to fork continuum, fresh produce is subjected to numerous opportunities for microbial contamination due to a range of handling, processing, storage and transportation activities which in the event of unfavorable conditions may lead to the presence of microbial hazards (Gil et al., 2015).

Water is recognized as one of the most important vectors of enteric human pathogens on vegetable crops (Park et al., 2012), This is exacerbated by the fact that water scarcity impacts the quality of the water used for irrigation coming from uncertain sources which may harbor pathogens (Leifert et al., 2008). Facing multiple challenges, i.e., political, economic, climate change, unfortunately many developing countries are increasingly reverting to the use of untreated wastewater for irrigation and processing of vegetables (Aiat Melloul & Hassani, 1999; AL-Jaboobi et al., 2013; Castro-Rosas et al., 2012; De Bon et al., 2010; Ensink et al., 2007; Thurston-Enriquez et al., 2002). One example for this is the produce industry in Lebanon, where agricultural production is concentrated in the Bekaa Valley, both the most cultivated area and the most affected by water pollution (Halablab et al., 2011; Jurdi, 1992). Almost 146 farms use the surface water of the Litani River to irrigate various vegetables as reported in 2011 in local news (retrieved from http://english.al-akhbar.com/node/2617) .This river is frequently polluted by untreated sewage, domestic solid waste, and industrial effluents (Houri & El Jeblawi, 2007) and as result, leafy greens in that area have been found to pose health risks to consumers (Halablab et al., 2011). In addition, export potential for produce may be increasingly at risk because importing countries are demanding higher standards. Despite the fact that risks of foodborne illness are likely to be higher in the developing countries of the MENA regions where the waste water treatment is still underdeveloped and use of untreated water for irrigation is illegal, most research on the microbiological safety of fresh vegetables and fruits has been carried out in developed nations (Allen et al., 2013; Johnston et al., 2005; Lehto et al., 2011; Seow et al., 2012; Wood et al., 2015). Certainly, very little has been done in Lebanon (Halablab et al., 2011; Khatib et al., 2015), maybe because the surveillance data for foodborne illness is lacking, and partly because of lack priority for research funding. There can be no doubt that foodborne infections originating

from contaminated fruits and leafy green vegetables do occur in the MENA region including Lebanon, based on surveillance data from other regions since they are frequently eaten at most meals (EFSA, 2014; European Commission, 2002; Painter et al., 2013).

To address this lack of understanding of what and how microorganisms of concern are transmitted across the food chain, we conducted a study of risk factors contributing to microbial contamination of vegetables eaten raw, represented by flat leaf parsley (*Petroselinum crispum*. var. *neapolitanum*), romaine lettuce (*Lactuca sativa* L. var. *longifolia*), and small red radish (*Raphanus sativus*) from farms in the Bekaa Valley, Lebanon, to the central market of fresh vegetables in Beirut, and recommended mitigation strategies.

2. Materials and methods

2.1 Study design and sample collection

Sampling sites comprised 10 major farms in the Bekaa Valley, 2 crop washing facilities and the wholesale market in Beirut which receives most of farmers' crops and a major supply point of fresh raw vegetables for supermarkets, distributors, groceries and restaurants in Beirut. Target commodities included leafy greens and radish.

The study was planned to obtain samples from different points of the chain to reflect the farm-to-retail contamination and microbial growth potential.

Table 1 shows samples distribution across different sampling locations.

Samples of lettuce, parsley and radish (n=90) were collected in July-August 2013 and July 2014, a relatively hot and dry season in the Bekaa. A whole head of lettuce, and a bundle of parsley or radishes was considered as one sample; sampling of each type was done from different points of the same field. Water samples (n= 5 of 1 litre-samples each collected in 250 ml portions from different points of the crop washing ponds or in 1 litre bulk from the wells and n=6 of 100 ml

samples from water streams) were collected in polystyrene sterile bottles/cup. We noted in our on-farm assessment survey that non-potable river water was used for irrigation and post-harvest washing. However, when water sources declined in the summer, farmers were forced to use private wells for irrigation and filling the washing ponds. In two of the farms, sewage water was used both as irrigation and nutrient fertilizer for economic reasons.

Samples were placed in insulated coolers with ice-packs and transported 135 km to the laboratory the same day. Logistically it was not feasible to process all the food samples on the same day, and these were stored in freezers at -18ºC to be analysed on subsequent days, whereas the water samples were analysed that day.

Table 1. Summary of fresh produce and water samples collected from different points of the agro-food environment

Sample sources	Type of samples	Label*	N (%)
Farms fields	Fresh produce†	F-FP	35 (38.9)
Post-harvest washing ponds	Fresh produce	PHW-FP	15 (16.7)
Wholesale market	Fresh produce	WSM-FP	40 (44.4)
Total			**90 (100)**
Wells	Irrigation water	W-WI[1]	30 (53.6)
Post-harvest washing ponds	Crops washing water	PHW-W[1]	20 (35.7)
Water streams	Irrigation water	Water streams	6 (10.7)
Total			**56 (100)**

[1]Water samples analysed in 100 ml volumes

*****The abbreviations listed under "Label" are used in subsequent tables and texts

†Type of fresh produce samples included lettuce, parsley and radish

2.2 Bacteriological analysis

For irrigation and wash water microbiological assessment, *Escherichia coli* designated as Hygiene Criterion indicating faecal contamination (EFSA, 2014) and total coliforms (TC) were tested. The group TC comprises the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*, indicator organisms that indicate the general sanitary level of water and possible contamination by different pathogens (Pachepsky et al., 2011; WHO, 2006). The enumeration of bacteria was performed according to the filtration method following EN ISO 9308-1:2000 using selective enrichment and RAPID'*E. coli* chromogenic media (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK).

Fresh produce samples were analyzed for the presence of pathogens and hygienic indicator organisms, i.e., *Staphylococcus aureus* , *Salmonella* spp., *Listeria monocytogenes*, and for total plate counts (APC) and. *E. coli* and TC (WHO, 1989, 2006). APC were included as an indicator of any microbiological pollution and of existing favourable conditions for the multiplication of microorganisms. This parameter is useful to indicate efficient applications of good hygienic practices (GHP) and temperature control during processing, transportation, and storage (Aycicek et al., 2006). Given a reported high counts of *S. aureus* on vegetables cultivated near the Litani River (Halablab et al., 2011), its frequent recovery from waste water and abundance in the animal production environment particularly in chicken litter in other countries (Hashem et al., 2013; Schilling et al., 2012), *S. aureus* was also considered in this study.

For microbiological analysis, all the media used were obtained from Bio-Rad Laboratories Ltd., Hemel Hempstead, UK unless otherwise mentioned and samples were analysed according to ISO 16140. Briefly, 10 g of the samples was weighed into sterile stomacher bags and homogenized with 90 ml sterile peptone buffered water (BPW) for 2 min at

medium speed. Samples of 0.1 ml of each of the 10^{-1}, 10^{-3} and 10^{-5} dilutions were spread on in duplicates on appropriate media. APC were enumerated on plate-count agar at 37ºC for 48 hours. As for *E. coli* and TC, 1 ml from each decimal dilution was dispensed into petri dishes for enumeration by pouring technique using RAPID'*E. coli* 2 agar. The plates were incubated at 37ºC for 48 h. For the detection of *S. aureus*, typical presumptive colonies with clear halo resulting from proteolysis of egg yolk were further tested using a latex agglutination test (Pastorex Staph Plus). *Staphylococcus aureus* was enumerated on RAPID'*Staph* Agar supplemented with egg yolk. Typical colonies on the plates were enumerated and colony counts in 1 g sample were determined. The counts were reported as means of colony-forming units (CFU) per g and were converted into log CFU/g. *Salmonella* spp. and *L. monocytogenes* was reported as present or absent.

2.3 Detection of pathogens

For the isolation of *Salmonella* spp. and *L. monocytogenes*, the pre-enrichment/enrichment selective plating method was used according to ISO 16140. In the case of *Salmonella* spp., selective enrichment was performed in Rappaport-Vassiliadis-soya broth to be incubated at 41.5ºC. After 24 h of incubation, a 0.1 ml sample was plated on RAPID *Salmonella* agar and plates were incubated at 37 C for 24h (± 2h). While for *L. mono*cytogenes, Fraser ½ broth was used in the selective enrichment and after incubation for 1 h at 20ºC, 0.1 ml of the homogenate was transferred onto RAPID'*L. monocytogenes* agar plates to be incubated at 37ºC for 24–48h. Typical *L. monocytogenes* colonies were afterwards selectively identified. *Salmonella* spp. colonies were identified biochemically by the lysine iron agar and tryptic sugar iron agar slants biotyping technique. Additional confirmation for positive *Salmonella* spp. colonies and for *E.*

coli was done by the API 20E bacterial identification test strip (bioMérieux, Marcy l'Etoile, France).

3. Statistical analysis

Descriptive and frequency tests were performed using version 21.0 of the SPSS software package. Bacterial counts across different points of the supply chain and in different types of produce were analysed. Kurtosis Levene's test for homogeneity variance showed normality within the distribution of the CFU counts, except for *E. coli* that showed non normality which violates one of the assumptions underlying analysis of variance (ANOVA). In this case, Kruskal–Wallis tests was used for groups comparison, while when it is tenable, the mean values were compared by one-way analysis of variance (ANOVA) and subject to Tukey test to determine any statistically significant difference ($P < 0.05$) among the means(Granato et al., 2014). Chi-square Fisher exact test and non-parametric correlation (Spearman's rho test) were applied to test associations and correlations among bacterial counts and categorical variables. Linear regression analysis was performed to test the predicting power of agricultural water of the hygiene criteria in fresh produce.

E. coli prevalence was calculated by using the number of samples tested positive for *E. coli,* and then dividing that number by the total number of samples.

4. Results

4.1 The Microbiological quality of fresh produce

Overall, the APC ranged from a geometric mean of 3.50 to 8.39 log CFU/g (Table 2), with parsley and radishes having the highest levels (Table 3). Two-thirds of the raw vegetables (62%) had APCs above 6 log CFU/g. TC was observed in all vegetable samples, with counts ranging

183 from 1.69 to 8.16 log CFU/g (with 69% having counts ≥5 log CFU/g). *E. coli* was present in
184 almost half (45.5%) of the raw vegetables, with levels ≥2 log CFU/g in more than a third (37%);
185 counts on parsley were significantly higher compared to lettuce and radish. *Staphylococcus* spp.
186 and *S. aureus* were isolated from 91% and 45.5% of all produce types, respectively. In general,
187 the geometric mean *S. aureus* counts was relatively high 4.80 log CFU/g (Table 2) and highest
188 for parsley and radishes.

Table 2. Mean (log CFU/g) [a] of selected parameters of contamination across the different sampling sources, from fields to wholesale market

Microorganism	Source	N	Mean (Range)
S. aureus	F-FP	18	5.50 (3.32-8.39)
	PHW-FP	5	4.51 (3.64-6.38)
	WSM-FP	18	4.18 (< 0.7-6.23)
	Total	**41**	**4.80** (< 0.7-**8.39)**
E. coli	F-FP	35	1.28[a] (< 0.7-7.00)
	PHW-FP	15	2.24[a] (< 0.7-6.78)
	WSM-FP	40	2.10 (< 0.7-5.32)
	Total	**90**	**1.80 (< 0.7-7.00)**
TC	F-FP	35	5.13[b] (1.69-7.60)
	PHW-FP	15	6.04[b] (5.30-7.60)
	WSM-FP	40	5.92 (3.55-8.16)
	Total	**90**	**5.63 (1.69-8.16)**
APC	F-FP	35	6.52 (3.96-8.39)
	PHW-FP	15	6.23 (5.50-8.27)
	WSM-FP	40	6.39 (3.50-7.88)
	Total	**90**	**6.43 (3.50-8.39)**

F-FP=.Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce, W-WI=Well, water for irrigation, PHW-W= Post-harvest washing water, TC = total coliforms, APC = total plate counts.

[a] minimum detection limit of 0.7 log CFU/g was included in statistical analysis in the event of no visual growth.

Similar superscript letters above the means in the same column indicate significant difference at $p<0.05$.

Table 3. Mean (log CFU/g) of selected parameters of contamination in different types of fresh produce

		E. coli	*S. aureus*	TC	APC
	Count(N)	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Lettuce	45	1.71^{a}±1.58	3.85±1.55	5.25^{a}±0.97	6.00^{ab}±0.87
Parsley	35	2.17^{b}±1.69	4.69±1.63	6.38^{ab}±1.05	6.87^{a}±1.01
Radish	10	0.96^{ab}±0.56	4.94±2.10	4.74^{b}±1.59	6.87^{a}±1.01

Similar superscript letters above the means in same column indicate significant difference at $p<0.05$ by ANOVA and Tukey test . For *E. coli*, significance was determined by Games-Howell test assuming non-variance and Kruskal-Wallis test.

4.2 Comparative analysis of sanitation and hygienic handling indicators on raw vegetables from the fields to the whole sale market

To identify the critical risk factors along the fresh produce supply chain, a comparative analysis of the bacterial loads on raw vegetables across the interrelated sampling locations was performed. The flow diagram of leafy greens and radish supply chain and identified risk factors is presented in Figure 1 (A),(B),(C).

The results of the hygienic parameters analysis demonstrated that the population size of APC and *S. aureus* was the highest on produce in the fields, 6.52 log CFU/g and 5.50 log CFU/g, respectively, and that APC almost remained constant throughout the market. The slight decreasing trend of APC levels was apparent from samples taken from farms and at the post-harvest washing stage, while a slight increase was observed thereafter, in the wholesale market. However, *S. aureus* concentration levels on raw vegetables from farms and washing ponds were always higher than on vegetables on display (Table 2)

On the contrary, Kruskal-Wallis test showed that *E. coli* mean levels were significantly different across categories of sample sources. Furthermore Spearman's rho demonstrated a significant correlation between TC and *E. coli* and the sampling sources at $p<0.05$ and $p<0.01$, respectively (Figure 2). We noted that the TC and *E. coli* levels on raw vegetables increased

213 significantly ($p<0.05$) from the farms (means, 5.13 and 1.28 log CFU/g, respectively) to post-
214 harvest washing and packing for subsequent distribution (means, 6.04 and 2.24 log CFU/g,
215 respectively).. Although there was a slight decrease of TC and *E. coli* levels from market
216 samples (means, 5.92 and 2.10 log CFU/g, respectively), these were still higher counts than at
217 harvest.

218 ***4.3 Pathogen detection***

219 The prevalence of *L. monocytogenes* was 20% in vegetables in the fields and after washing in the
220 post-harvest areas, but decreased to 8% by the time they reached the retail markets, but in each
221 case at low levels. The overall prevalence of *L. monocytogenes* was 14%. Its prevalence was
222 detected in each sampling location, with equal levels of 20% on vegetables from each, the fields
223 and the post-harvest areas and only about 8% at WSM (Figure 3). About half of the ready-to-eat
224 vegetables in the fields (51%) contained *S. aureus.* Although the prevalence decreased slightly
225 (33%) in the PHW stage, it rose again as vegetables reached the WSM (45%). In contrast, the
226 study found only one sample (parsley) out of 15 obtained from the washing pond contaminated
227 with *Salmonella* spp. resulting in an overall prevalence rate of 6.7% for vegetables sampled from
228 the washing area.

229 ***4.4 Microbiological quality of irrigation and wash water***

230 The mean count of *E. coli* in wells water and wash water samples ranged from <0.7 -135
231 CFU/100 ml and 15-140 CFU/100 ml, respectively (Table 4) and the TC was too numerous to
232 count per100 ml analysed samples. Furthermore, water from wells and from river streams used
233 for post-harvest washing and crop irrigation in 5 farms contained unacceptable levels of TC and
234 *E. coli* > 100 cells/ 100 ml, of each. In our analysis of the impact of water quality used in

235 irrigation on vegetables traced back to its sources, Chi square analysis showed significant
236 association between *E. coli* counts on raw vegetables and the microbial quality of agricultural
237 and wash water. By simple linear regression, a significant regression equation was found (F (1,
238 44) = 77,174, p<.000), with an R^2 of 0.637. *E. coli* counts on fresh produce increased 0.799 for
239 each CFU/100ml of water used. The regression analysis showed that the microbiological quality
240 of agricultural and wash water obtained from same sampling locations of fresh produce is a
241 useful predictor explaining 64% of the *E. coli* variations on raw vegetables that were traced to
242 their sources (Table 4).

Table 4. The *E. coli* counts on fresh produce in the market traced back to farmers' fields, agricultural water quality and post-harvest washing areas

Farmers	Samples location-type	N	Mean (range)†	Median
Farm IB	F- FP	3	2.80 (<0.70-7.00)	<0.70
	PHW-FP[1,2]	6	1.49 (<0.70-2.88)	0.95
	WSM- FP	11	1.86 (<0.70-5.20)	1.00
	W-WI	10	36.20 (13.00-80.00)	25.50
Farm S				
	F- FP	10	1.01 (<0.7-3.84)	0.99
	WSM-FP[2]	13	2.09 (<0.70-4.45)	2.20
	PHW-W	10	83.00 (50.00-140.00)	80.00
Farm B	F- FP	3	3.54 (<0.70-6.00)	4.60
	PHW- FP[2]	3	2.73 (<0.70-6.78)	0.70
	W-WI	6	50.00 (20.00-135.00)	30.00
Farm J	PHW-FP	6	2.76 (<0.70-4.40)	2.77
	WSM,FP[2]	5	1.32 (<0.70-2.22)	1.30
	PHW-W	10	25.80 (15.00-50.00)	25.00
	W-WI	10	0.70 (<0.70-6.00)	<0.70

*F-FP=.Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce, W-WI=Well, water for irrigation, PHW-W= Post-harvest washing water

† CFU/g for fresh produce samples and *E. coli* cells/100 ml for irrigation and wash water samples

[1,2] *Salmonella* and *Listeria monocytogenes* detected on produce from this farm

[2] *Listeria monocytogenes* detected in this farm

5. Discussion

In general, the bacterial loads of the raw salad vegetables exceeded the ICMFS (ICMSF, 1998) acceptable limits of 10^3 to 10^5 coliforms (TC) in 100 g of vegetables usually eaten raw. Moreover, the European Union (2007) established for pre-cut fruit and vegetables (ready-to-eat), a limit value m of 100 *E. coli*/g and a limit value M of 1000 *E. coli* /g. In this context, many of the samples (37%) did not meet acceptable limits for *E. coli* in our study. The overall prevalence level of *E. coli* (45.5%) showed a comparable result to a previous study (42.30%) of vegetables grown in the Bekaa (Halablab et al., 2011). These results are also consistent with a study in Yemen by AL-Jaboobi et al. (2013) which demonstrated that 35% of raw vegetables irrigated with waste water contained *E. coli*. Similar results have been reported in developing countries beyond the MENA Region. Maffei et al. (2013) reported *E. coli* in 40.0% of leafy vegetables harvested in Brazil, and Castro-Rosas et al. (2012) reported faecal coliforms in 99% and *E. coli* in 85% of RTE 130 salad samples originating from vegetables in Mexico irrigated with untreated sewage water. The occurrence level of TC (>5 log CFU/g) in our study (69%) was slightly higher than the prevalence rate reported in Singapore (50%, n=125) (Seowa et al., 2012), and it was isolated from all the samples (100%) . In contrast, data from western countries reported substantially lower levels of enteric pathogens contamination, such as 8.2% of *E. coli* was recovered from fresh produce in Canada (Bohaychuk et al., 2009), and from only five samples (n=890) in Norway (Johannessen et al., 2002). In the U.S., the range of TC and *E. coli* in leafy greens and herbs, respectively, was <1 - 4.4 log CFU/g and <1 - 1.5 CFU/g, in a study by (Johnston et al., 2005). In our samples, parsley accounted for the highest overall geometric mean for TC and *E. coli* compared to lettuce and radishes (Table 3). The common use of sprinkle irrigation observed in our study (unpublished data), a mode of irrigation frequently linked to

increased risk for crop contamination and to higher faecal counts (FDA/CFSAN, 2001; Jung et al., 2014), together with the parsley leaf surface form which could enhance contamination and survival by favouring bacterial attachment and its persistence in curly leaves and crevices (Harapas et al., 2010).

We were surprised by the high levels of *S. aureus* in all the produce items (up to 5 log CFU/g). The contamination level of fresh produce on fields with *S. aureus* did not exhibit a notable change in the post-harvest washing stage. Overall, the high levels showed consistency with some local and international studies (Halablab et al., 2011; Viswanathan & Kaur, 2001), being due to improper handling at harvest (Beuchat, 1995; Sabbithi et al., 2014; Viswanathan & Kaur, 2001). Local environmental conditions could also have contributed to the contamination of the surface vegetables with the survival of *S. aureus* for several weeks (Erkan et al., 2008). Such sources could be from wild or domestic animal faeces, such as sheep pasturing the fields after harvest and before the next seeding, or sewage- polluted irrigation water. However, one major source is inadequately-treated chicken litter which is used as fertilizer by some farms. In this regard, our data concurs with Halablab et al. (2011) who demonstrated that this pathogen was predominant in raw vegetables obtained from areas irrigated with Litani River (51.5%) compared to those in other areas downstream (26.6%). Nevertheless, *S.aureus* might represent public health hazard when growth exceeds 10^5-10^6 CFU/g given optimum conditions or as a result of cross-contamination during handling processes. Similarly, AL-Jaboobi et al. (2013) recorded high counts of *S. aureus* ≥5 log CFU/g on vegetables irrigated with untreated waste water and polluted river water. Interestingly, a recent study in Ghana further highlight the predominance of this bacterial species (50%) on vegetables from cultivated gardens irrigated with waste water and from the market, with mean CFU of around 10^6 CFU/g from each sampling location (Pesewu et

al., 2014). More evidently, high level of methicillin-resistant *S aureus* was isolated from the raw sewage of examined treatment plants (Pattillo, 2013) and in the wash water of crops (Ofor et al., 2009). Thus, unlike in studies of vegetables in western countries, *S. aureus* may represent a pathogen of concern that can reach consumers phase in some developing countries.

The variations of microbial population throughout the supply chain were in parallel with previous studies that reported identical levels of APC in the production and retail levels (Chau et al., 2014; Johnston et al., 2005; Ruiz et al., 1987) and the distribution stage (Johnston et al., 2005). There was also a large increase in APC and *Staphylococcus* on carrots as they travelled further through the distribution chain (Ghosh et al., 2004). Although a reduction in bacterial counts could be expected following the washing process, we noted an increase in TC and *E. coli* counts from farms to post-harvest washing, likely originating from the contaminated wash water, based on our observations and consistent with the results of Gagliardi et al. (2003) and Johnston et al. (2005). The high range of *E. coli* levels on washed vegetables (Figure 1) is probably because of different water quality experienced during sampling days resulting from inconsistent and unregulated frequencies of wash water replenishments; together with the variable microbial loads of mixed types of produce dipped into the ponds. Therefore, cross-contamination can be explained by transfer from contaminated to clean batches during washing operations in the ponds with no disinfection or sanitization steps (wash-dip for parsley and radishes, or the spray-wash applied on lettuce whilst stacked in open crates on trucks prior to distribution to the wholesale market). Thus, we were not surprised to find *Salmonella* on vegetables packed in crop washing areas. This would explain the higher levels of TC and *E. coli* on produce at wholesale markets (WSM) than at farms, but compounded by lack of cold chain during transportation and retailing, use of non-sanitized equipment for packing, storage and transportation, and inadequate hygienic

conditions at the markets, consistent with Uyttendaele et al. (2014), who found that improper hygiene of sellers at open market stalls in Egypt resulted in higher levels of faecal coliforms in produce.

On the other hand, the detection of *L. monocytogenes* on produce from field to the market, also reported by Johnston et al. *(*2006) and Prazak et al. *(*2002). This pathogen has been implicated in listeriosis outbreaks worldwide but not yet in the MENA Region (Todd & Notermans, 2011), and more recently linked to consumption of salad vegetables (Cordano & Jacquet, 2009; Ponniah et al., 2010). The 2011 outbreak of *L. monocytogenes* in cantaloupes with 147 illnesses and 33 deaths in 28 U.S. states, where unhygienic conditions and improper cooling played a role, highlights this risk (McCollum et al., 2013). As it can be found in the agro-environment through shedding by domestic animals, (Ivanek et al., 2006; Weiss & Seeliger, 1975), it is not surprising it can also be recovered from river water and ponds used for irrigation, as can *Salmonella* (Combarro et al. (1997); Johnson et al. (1997) Greene et al. (2008)). However, we observed conditions that would exacerbate contamination. Crop washing operations took place in unprotected open areas, a risky practice as stated by (WHO/FAO, 2008), and fresh produce was kept in open areas in unwashed plastic baskets until used for the next consignment. We also observed wash water turbid from overuse of washing successive batches of produce (replenishment with fresh water supply was based on a subjective visual degree of turbidity). High turbidity levels are often associated with higher levels of pathogenic organisms (U.S.EPA., 2000). Since irrigation and washing of fresh produce can be vectors of pathogenic microorganisms (Ibenyassine et al., 2006; Solomon et al., 2002), water used for post-harvest operations should ideally be potable (Hernandez-Brenes, 2002) and not to exceed 10^3 CFU/ml F.C. /100 ml for the irrigation of raw eaten crops (unrestricted irrigation) (Blumenthal et al.,

2001; Probst et al., 2012; WHO, 1996). However, other national and federal guidelines, such as DIN 19650 (German standards), enforce stricter limits considering the water quality is the same as drinking water quality with no *E. coli* or faecal streptococci should be present (Pfleger, 2010) and according to U.S. Environmental Protection Agency and British Columbia, a limit of *E. coli* less than or equal to 77 CFU/100 ml is defined (British Columbia MoE, 2001; U.S.EPA, 2001). It was noted that on one farm wash water ponds derived from well water with no detectable TC and *E. coli* became contaminated to levels similar to that of nearby river water, indicating that inadequate control allows unacceptable environmental contamination on these farms.

6. Conclusion and recommendations

To our knowledge, this is the first attempt in Lebanon and the Middle East region to provide baseline information on critical risk factors associated with the microbial quality and on the prevalence of pathogens on fresh produce from the farm to the market. It is apparent that shortfalls in the good agricultural practices (GAP), the lack of clear hygienic guidelines for processing and retailing most likely contributed to the contamination of raw vegetables with *S. aureus* (from chicken litter), TC and *E. coli* and *L. monocytogenes* (from environmental sources). Although *Salmonella* spp. was only found in one sample, an overall prevalence of 1.1% is unacceptable considering the high volume of raw vegetables eaten locally. The crop washing stage showed to be an evident risk area for pathogens transmission to fresh produce and one possible source of crop contamination. The fact that organisms indicative of faecal contamination were frequently found in levels with the potential for pathogens to be present and surviving on vegetables right up to the consumption stage as raw, should raise concerns (Srikanth & Naik, 2004). Though the knowledge of the precise sources of contamination were not the objective of this study, they are likely the same as have been identified in other regions,

e.g., faecal contamination from farms including untreated manure, wild animal reservoirs, human sewage, and infected food workers (European Commission, 2002), especially as it is well-known that the river water used for irrigation and washing is well documented as containing faecal contaminants (Houri & El Jeblawi, 2007) and that cold chain and proper storage and sanitation conditions were largely lacking from farm to the market. Although the current study is not based on representative samples of water and all fruits and vegetables throughout the country or region, the use of contaminated water for irrigation and washing for produce is widespread, and our results are likely valid for many growing areas in the Middle East. The poor handling practices as well as conditions of transportation and storage facilities of fresh produce in the MENA region is documented, although countries may vary in their standards and enforcement (Kader et al., 2011) . There, results on the assessment of crops losses in the region indicated existing lack or poor status of the cold chain infrastructure and basic hygiene along the chain. Consequently, as the developing countries are confronted with stringent requirements of the international market, governments have a pivotal role to set national GAP standards that comply with the recommended requirements of Codex Alimentarius (CAC, 2003) and to create enabling environment to ensure compliance of stakeholders.

This study underscores the importance of informing stakeholders and consumers on the associated risks with current practices and of applying vigilant sanitation measures, GHP and risk-based preventive measures from farm to fork to mitigate the risk of cross-contamination. Relevant government authorities should give a high priority to improve and maintain storage and transportation conditions essential for the fresh produce safety and to ensure the implementation of adequate sanitation during the post-harvest washing processes. Equally important, they should enforce an overall water policy in Lebanon (and in other MENA countries) to provide potable

water for both urban and agricultural use (The Lebanese Center for Policies Studies http://www.lcps-lebanon.org/featuredArticle.php?id=27). In this context, it is advisable that government initiatives and the technical and financial support of international organizations consider the provisions of incentives schemes for farmers who may prefer using nutrient-rich polluted waters to fertilize as well as irrigate crops and are conducive to incorporate strategic solutions for using treated grey water and on-farm wastewater treatment in order to address the economic and water scarcity challenges that jeopardize the safety of the fresh produce

7. Limitations of the study

Our study faced one main limitation that challenged our effort to continue this work in the Bekaa Valley owing to security risks that prevented us from collecting a sufficiently representative sample of vegetables and untreated waste water used for irrigation throughout the Valley. As it was not the aim of this study, we did not consider the assessment of the seasonal effect. This study was limited to demonstrate conditions in selected areas of the Bekaa Valley and may not be generalized to other parts of Lebanon and MENA countries. However, it does provide good baseline data on common gaps in hygiene practices along the fresh produce chain and for building on risk factors where poultry litter and polluted waters are used for crops. Due to such logistical limitations, analysing the food samples within 24 hrs of collection was not possible, and these were frozen and thawed before analysis. From this we understand that the freezing and thawing likely led to some decline in the reported bacterial counts, which could have been higher than we actually documented. In addition, we did not look for norovirus which undoubtedly was present from any human sewage sources, and would present a further health risk to consumers (Todd & Greig, 2015).

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Farming-Harvesting

- Untrained labourers and farmers on GAP and GHP
- Unsafe water sources for irrigation
- lack of sanitary conditions and labourers lodgings on fields
- Unawareness on workers' health and personal hygiene requirements
- Inappropriate handling of manure
- Access of domestic and grazing animals to fields crops and streams
- Proximity to landfills or animal production farms

Some farms rely on sewage directed into irrigation pools or streams

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Transportation

↓

Crop washing processes

Figure 1(A). Flow diagram of leafy greens and radish supply chain and identified risk factors from farms to crop washing areas

Crop washing processes

- Lack in monitoring measures and policies to ensure the use of safe water sources
- Shortfalls in washing practices and in maintaining clean water supply
- Basic washing method for a large volume of mixed batches of fresh produce
- Inadequate structural facilities
- Non-sanitized storage implements

Stored in open areas of a washing facility for next consignments

Storage and Packing

Figure 1(B). Flow diagram of leafy greens and radish supply chain and identified risk factors based on an on-farm assessment survey – From Crop washing areas to storage

- Exposure to external environment and improper temperature conditions
- Inadequate structural facilities
- Non-sanitized storage implements

Storage areas for domestic market

Wholesale market

Transportation

- General lack of cold chain along the supply continuum
- Plastic crates are overused, non-sanitized and kept piled on the market entrance after distribution

Eateries, groceries

Retails

Distributors

Figure 1(C). Flow diagram of leafy greens and radish supply chain and identified risk factors based on an on-farm assessment survey- From storage to retails

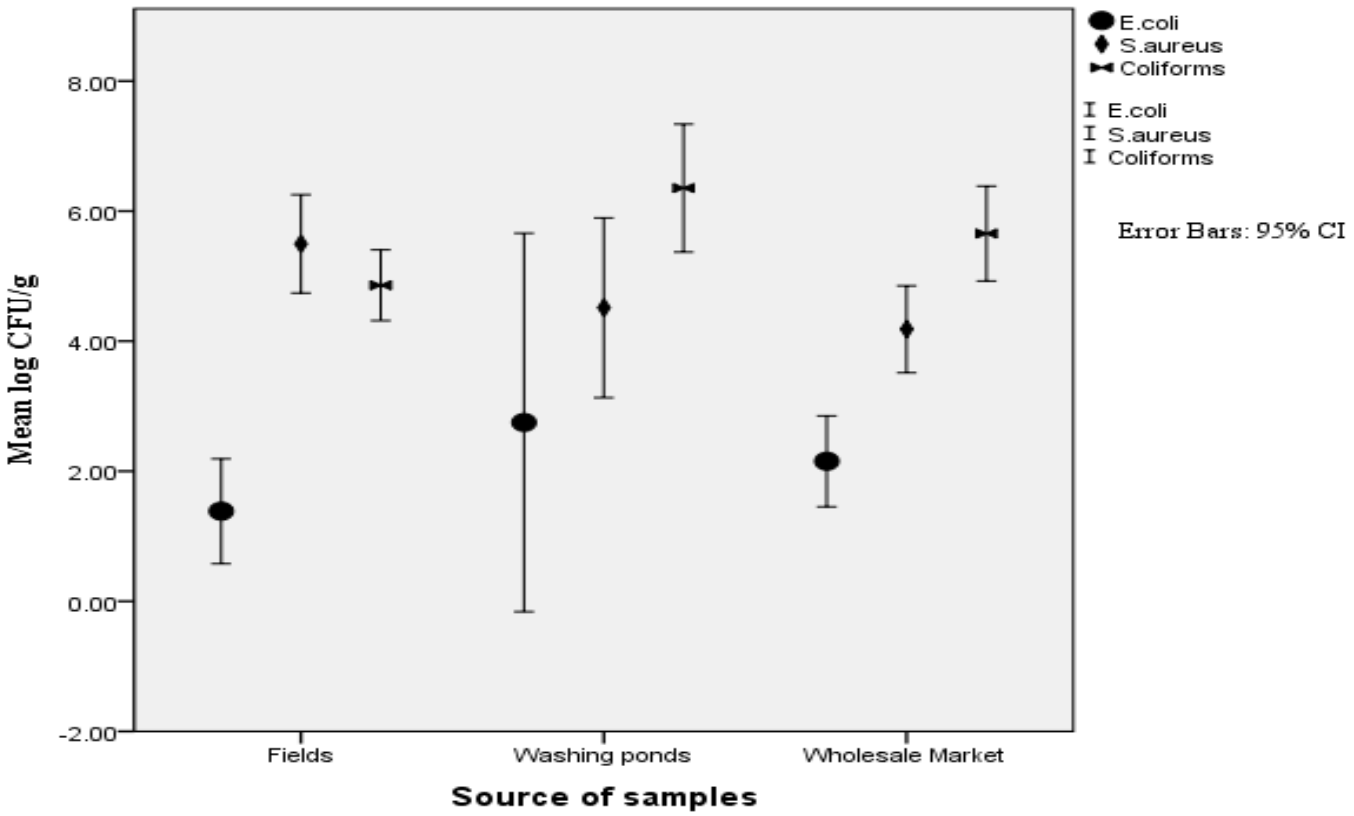

Figure 2. Distribution of the mean log CFU/g of *S. aureus, E. coli* and TC on raw vegetables according to sampling sources along the fresh produce supply chain. Higher values of the mean log CFU/g ±SD in hygiene indicators are demonstrated on fresh produce obtained from the post-harvest washing ponds.

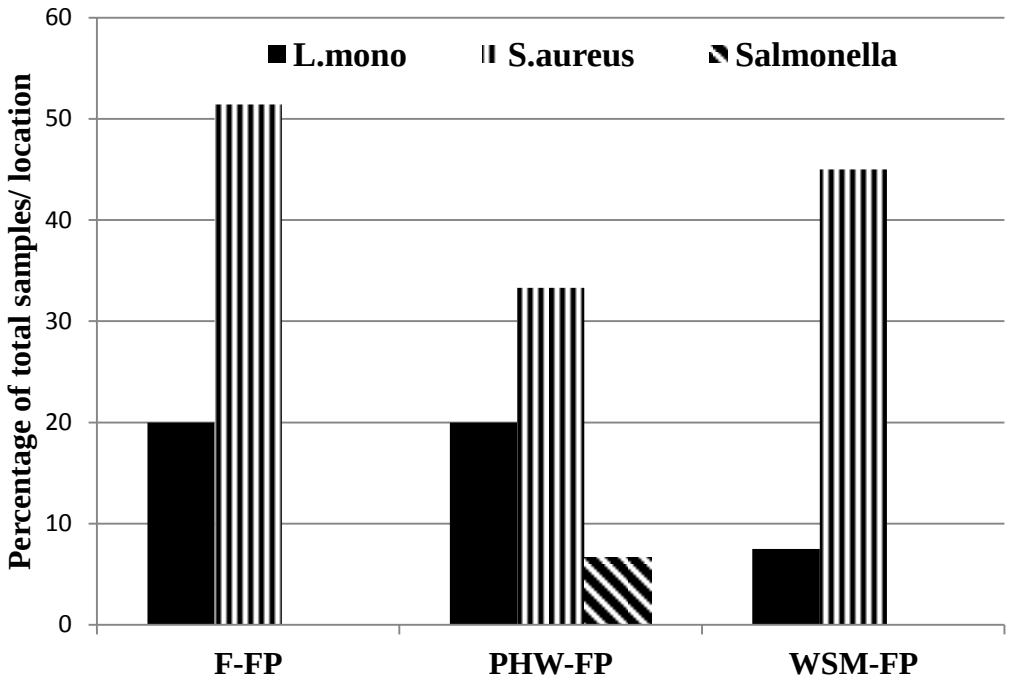

Figure 3. The prevalence of pathogens on fresh produce, calculated as the percentage of total samples in each sampling location. F-FP=.Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce.