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Microbiological quality of ready-to-eat fresh vegetables and their link to food safety environment and handling practices in restaurants

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ABSTRACT

The increased consumption of ready-to-eat salads outside homes as a result of a fast paced lifestyle, awareness on their nutritional attributes and enhanced processing technology is well documented. This study aimed to determine the microbiological quality of fresh-cut salads vegetables in small and medium sized foodservice establishments (SMEs) and to identify risk factors and handling practices through observational assessment in order to investigate if an association between microbiological quality and visual assessment (inspection) scores can be established.

A total of 118 samples fresh-cut vegetable salads were collected from 50 inspected locations and analysed microbiologically, in addition to 49 swabs of knives and cutting boards. There was no statistically significant correlation between visual assessment scores and bacteriological counts on vegetables or cutting boards. Nonetheless, the consistent relationship between inspection ratings on cross-contamination and cleaning components and *Listeria* spp. levels was statistically significant. This study demonstrated that overall visual assessment scores would not directly reflect the safety of salad vegetables and that the significance of microbiological assessment should be considered in relation to individual inspection components. It is necessary to place

effective control measures on cleaning standards and risk of cross-contamination to improve the microbiological safety of fresh salad vegetables in SMEs.

1. INTRODUCTION

Fresh vegetables are rich sources of water-soluble vitamins and other nutrients essentials to improve the nutritional status and decrease the risk of cardiovascular disease (Su & Arab, 2006). However, when they are not carefully prepared, they can be subjected to pathogenic contamination and become hazardous to health particularly when eaten raw (WHO, 2008).

Outbreak investigations often indicate that food service establishments (FSE) greatly contribute to foodborne illnesses involving fresh produce (Jones & Angulo, 2006; Sodha et al., 2011). Multiple studies revealed that food workers were frequently engaged in unsafe food handling (Clayton & Griffith, 2004; Manning, 1994; Rajagopal & Strohbehn, 2013; Sneed, Strohbehn, & Gilmore, 2004) and that microbial contamination of ready-to-eat (RTE) foods typically occurred in FSEs with food handlers as asymptomatic carriers of pathogenic microorganisms or with poor personal hygiene being involved (McEvoy et al., 2004; Todd et al., 2008). Equipment or surfaces that have not been effectively cleaned or remained wet between cleaning and use also serve as direct routes for contamination of ready to eat foods (Evans et al., 2004; Gill et al., 2001), besides inappropriate storage temperatures, and insufficient cooking (Jones et al., 2008; WHO, 2007).

Less information is available on the relative health risks attributed to handling practices and preparation procedures of raw salad vegetables in SMEs, while other RTE foods and meats have attracted more attention.

Inspection tools are essential for capturing information on the general hygiene standards and food handlers' practices Although private or local authorities 'inspections are an

effective mechanism to assure compliance to food safety standards, there is no a clear indication of a correlation between risk of foodborne illnesses and inspection scores. There have been many cases when restaurants scored high on inspections and were still having critical violation in food safety(Jones et al., 2004). The significance of association of microbiological quality of RTE vegetables to hygiene inspection scores has not been fully investigated and not sufficiently addressed by researchers. Earlier attempts to establish direct relationship between the results on microbiological analysis of food and visual inspections have not been successful and were mostly based on foods of animal origins(Powell & Attwell, 1995; Tebbutt & Southwell, 1989; Wyatt & Guy, 1980).

This study aimed at conducting observational assessment of the fresh produce handling processes from the receiving stage until display and service to identify risk factors that may be associated with the microbial safety of fresh produce in SMEs which will provide further insights to devise effective preventive measures.

2. MATERIAL AND METHODS

2.1 Observational survey

A convenience sample of fifty SMEs located in Beirut were observationally assessed for hygiene standards and handling practices of food handlers during the salad vegetable preparation. The survey checklist comprised 6 constructs of 2-7 components for analysis in which the good hygienic practices (GHP) and other prerequisites proposed by the Codex Alimentarius (CAC/RCP 1, 1969) were considered for the visual assessment (Table 1). Additional components in relation to salad preparation practices were also included. The criteria for each component were defined to specify limits for classification. (Supplementary materials).

A reliability analysis test was performed to measure the internal consistency in the survey questionnaire. Cronbach's Alpha was 0.928 which indicates a high level of internal consistency for our scale.

2.2 Additional information

Additional 8 questions on handling practices of fresh vegetables during receiving, washing and storage were posed to food handlers (n=80) via face-to-face interviews that were conducted in our earlier study on food safety knowledge, attitudes and practices (Faour-Klingbeil et al., 2015). The questions were ranked on a five points rating scale (never = 1, rarely = 2, sometime = 3, often = 4 and always =5).

To ensure consistency and unbiased data records, the data collection and visual assessment were carried out by one of the authors (Dima Faour-Klingbeil).

2.3 Collection of RTE fresh-cut salads vegetables samples

2.3.1 Management of samples

A total of 118 samples of various fresh cut RTE salad vegetables (lettuce, parsley, arugula, coriander, cucumber, tomato and radish) prepared in 50 restaurants were collected after washing and cutting/chopping. On average, 3 types of vegetables were sampled from each restaurant, being subjected to availability and preparation plans at times of visits. They were placed in a sterile bag by food handlers at the end of the preparation process by means of utensils or tools typically used when bringing them into display or storage containers, taking care that they would not touch the inside of the bags.

2.3.2 Swabs of cutting boards and knoves

Before cutting/chopping vegetables, surfaces of cleaned cutting boards and knives (normally cleaned by assigned cleaners in well-established restaurants, or food workers

in less developed restaurants) were swabbed by moistened cotton-tip in buffered peptone water (BPW) (Bio-rad laboratories Ltd, Hemel Hempstead, UK) in three different directions: left to right, top to bottom, and diagonal over a 50 cm^2 area for cutting boards and a length of ca. 10cm on knives. The swabs were placed in tubes of 5 ml buffered peptone water for subsequent analysis.

2.3.3 Microbiological analysis of samples

Samples of salad vegetables were analysed for the presence of pathogens and hygiene indicators organisms commonly isolated from RTE fresh vegetables, i.e., *S. aureus*, *Salmonella* spp., *Listeria* spp., *L. monocytogenes*, in addition to total viable counts (APC), *E. coli* and TCs(Nguz et al., 2005; Sagoo et al., 2001). 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. Aliquots of 0.1 ml of appropriate dilutions were spread in duplicates on suitable media. APC were enumerated on plate-count agar, as for *E. coli* and TC, 1 ml 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. *Coagulase-positive Staphylococci* were enumerated on RAPID'*Staph* Agar supplemented with egg yolk. 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). For the isolation 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 37ºC for

24h (± 2h). While for *L. monocytogenes*, 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. *Listeria* spp. were enumerated and typical *L.* monocytogenes colonies were afterwards selectively identified and by *Listeria* strips (bioMérieux, Marcy l'Etoile, France). *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.

The counts were reported as means of colony-forming units (CFU) per g and were converted into Log CFU/g.

Additionally, for statistical purposes, *Listeria* spp were ranked into 3 levels (Above 100 CFU/g, Below 100 CFU/g, and Not detected).

2.3.4 Swab tests

The swabs in 5 ml tube of BPW were vortexed vigorously for 1 min. Tenfold serial dilutions were spread-plated onto duplicate plates of PCA, RAPID'*Staph* agar supplemented with egg yolk and RAPID'*E. coli* 2 agar.(Sneed, Strohbehn, Gilmore, et al., 2004). Counts were expressed as log CFU/swabbed area.

3. DATA HANDLING AND STATISTICAL ANALYSIS

All data were analysed using the IBM SPSS Statistics (SPSS) version 22.

Observational assessment of each of the 26 components was rated on three units scale (adequate=3, incomplete=2, inadequate=1). The sum of the total awarded units on adequacy level (visual assessment scores) was converted to 100 points.

Frequency of levels in compliance (adequacy level) for each visually inspected component was obtained. Bacterial levels differences among different compliance levels were compared using One-way ANOVA, and independent t-test was performed to compare results between two groups.

The association between bacterial counts and overall visual assessment scores was assessed by Pearson correlation and multiple linear regression analysis; binomial regression was performed for *S. aureus*.

The percentage variances in bacterial counts (Log CFU/g) explained by individual inspection components were determined by correlation ratio ETA^2 (η^2 ratio). In the case *Listeria* and *S. aureus*, Spearman's rho and cross-tabulations Somer'd tests were also applied.

4. RESULTS

4.1 Overall results on food handlers 'practices and hygiene conditions on premises

Results of the visual inspections of FSEs and food handlers' practices during the preparation of fresh salads vegetables indicated structural inadequacies and insufficient fulfilment of hygiene prerequisites with a mean score on overall adequacy level of 55.5 ± 19.0 over 100 possible points (Figure 1), with the majority of locations being below scores of 50-70. Over half (54%) of the food premises failed to fulfil the basic hygienic requirements for clean floors, equipment and food contact surfaces, while a third had limitations in the structural conditions (Figure 2). Recorded incompliances included open drains, gaps and holes on windows and walls and evidence of pests (cockroaches) at the time of the survey. Furthermore, 22% had not a completely well maintained premise. More than a half (52%) of the FSEs had space limitations compromising the preparation of food safely, whereas only 22% of premises had taken measures to

167 separate areas for the preparation of raw meats and RTE foods. It was notable that the
168 inappropriate sanitation measures were not applied in 60% of the premises (Figure 2).
169 Only 8% of FSEs had cleaning schedules, and showed evidence of temperature
170 monitoring records of salads display and cold storage.
171 In addition, a large percentage of food businesses (64%) lacked hand washing sinks; or
172 designated sinks for washing fresh fruits and vegetables were either absent (32%) or if
173 fitted, it was not clean and used for others purposes such as washing hands or
174 implements used with raw meat and cooked foods (40%). More concerning, gloves were
175 used correctly and appropriately during the salad preparation in just a fifth (20%) of the
176 premises.

177 Risks of cross-contamination were detected in 48% of the premises, for example by the
178 presence of heavily chipped or unclean cutting boards, unfamiliarity of food handlers
179 with the concept of color-coding or separate use of utensils and cutting boards for raw
180 meat and fresh vegetables. There was misuse of colour-coded cutting boards in 18% of
181 FSE's where colour-coded cutting boards were used for several types of food. The
182 component "frozen foods are thawed properly" was not observed in 74% of the
183 premises visited, yet it was inadequately performed in 14% of the locations where
184 frozen fish or chicken soaked in water were noted at the time of the visit.

185 **4.2 Handling practices and the process of salads vegetables preparation**

186 Fresh vegetables were received during the mornings (7-9 a.m.) in plastic crates transported
187 on open trucks or in vans. The great majority (95%) reported that they received fresh
188 produce in uncooled vehicles (Table 2). In some cases, the person in charge or business
189 owner purchased the daily needs from the central market or nearby groceries. More than
190 two thirds of the respondents reported sourcing the fresh produce from the same

supplier (68.4%), and washing the vegetables before cutting (77%). In general, preparation started early, particularly with bundles of parsley which were finely chopped for serving later in the day in traditional salads and appetizers. Parsley leaves were chopped before washing in 34% of FSEs, which is consistent with the typical preparation sequence at homes (Figure 3), aiming to keep the texture of the leaves longer, as they would becoming soggy if they are washed ahead of time. About a third of the food businesses did not sanitize fresh vegetables, and used only water to wash them. However, a large proportion (84%) reported that the wash water was neither treated nor filtered. With long-standing shortages of potable water in Lebanon, restaurants, and homes, purchase water, often of uncertain quality and source, which is then stored in tanks. Out of the 56% using sanitizers, 21% used sodium dichloroisocyanurate (NaDCC) and more than a third (45%) applied a post-sanitization water rinse to remove the remaining taste or odour, respectively. It was noted during inspection discussions and observations that automated systems regulating the concentrations of chemical sanitizers in addition to water filters were in place, in some corporate-managed restaurants. On other places (24 %), incorrect dilutions of sanitiser was observed, typically as haphazard mixing of vinegar or NaDCC tablets in water. The majority reported that fresh produce was kept in cold storage, whereas this was actually only observed in 38% of the premises, with inadequate alternatives including stairways, kitchen floors of spaces in crowded production areas.

4.3 The microbiological quality of fresh salads vegetables

Results on microbiological analysis of fresh-cut salad vegetables are presented in (Table 3 and 4).

The mean APC levels ranged from 2.90 to 7.38 Log CFU/g, with counts above 10^7CFU/g recorded for 17% of the samples. `The prevalence rate was substantially high

in TCs (79.6%, 94/118). TCs were found between 1.72 - 6.40 Log CFU/g, of which 38% were >4 Log CFU/g. Whereas, *E.coli* was isolated from 31.3% (37/118), with bacterial loads ranging from less than 1.00 to 7.15 Log CFU/g, and the incidence rate was 64.8% of the positive samples (24/37) for counts higher than 100 CFU/g.

More than two thirds (41.5%) of the samples were found to contain *S. aureus*. In addition, *Listeria* spp. were isolated from 70.6% of the samples. The overall incidence level was 53% for counts above 100 CFU/g, with an average of 3.24 Log CFU/g. *L. monocytogenes* had a prevalence rate of 3.7 % mainly in arugula, parsley and lettuce, whereas *Salmonella* was detected in 0.9%, (lettuce).

Results on recovered microorganisms from contact surfaces (cutting boards and knives) are presented in Table 5. The microbial levels varied from below detection limits (10 CFU/swabbed area) to generally high levels. *E.coli* was isolated from 30.6% (15/49) of contact surfaces (knives and cutting boards); of those, the mean values were found between 2.70 - 7.02 Log CFU/swabbed area, whereas the incidence rate in TCs was higher (53.0%, 26/49) with levels between 4.88 - 8.40 Log CFU/swabbed area. There was no statistically significant correlation between the microbial counts recovered from contact surfaces and the ratings on the adequacy level of sanitation of work surfaces ($p>0.05$).

Overall, the analysis of data shows no statistical significant differences and inconsistent trends in bacterial counts of different visual assessment rankings for each individual inspection component ($p>0.05$). For instance, higher counts of TCs were observed on lettuce and parsley obtained from premises with inadequate sanitary conditions and unsafe handling practices, however this was not the case with cucumbers (Table 6). Also, the frequency in the distribution of bacterial levels on lettuce and parsley in relation to hygiene scores shows that high concentration levels were grouped at lower

scores (Figure 4). Likewise, the mean levels of coagulase-positive *Staphylococcus* spp. were higher on all vegetables prepared on premises lacking handwashing sinks (Figure 5).

There was no correlation between total visual assessment scores and bacterial levels ($p>0.05$). However, independent t-test still reveals a significant difference ($t=-2.198$, 81, $p=0.03$), between inspection scores for premises with *Listeria* counts above 100 CFU/g (53.44± 18.39) and those where the organism was not detected (64.48 ±26.12). When Eta correlation and non-parametric tests were further performed for this organism, no significant correlations of microbial results with all individual inspection component ($p>0.05$) were shown, while correlation tests and cross tabulations somer'd test revealed a significantly low and moderate association of *Listeria* levels with the inspection components related to cross contamination, handling practices, zoning and availability of handwashing sinks ($p<0.05$) (Figure 6). This association level was consistent with linear regression establishing that *Listeria* spp levels may be predicted by the visual assessment scores ($F1,103)=11,614$, $p=0.001$, but the score accounted for only 10.5% (R^2) of the explained variability in *Listeria* levels in vegetables. Given the small value of R^2, the prediction model using the visual assessment scores is not accurate. However and more interestingly, as we considered each inspected component individually, Eta^2 coefficients showed higher percentage in variations in *Listeria* spp. counts (30-34%) which were explained and attributed to cross contamination and cleaning operations components ($p<0.05$).

5. DISCUSSION

5.1 Food safety practices and microbial quality of fresh salads vegetables

A number of food safety practices concerns were identified in this study. The general lack of cleaning and sanitization procedures combined with a clear evidence of cross-contamination opportunities were generally reflected in the overall unsatisfactory quality of RTE vegetables. The majority of SMEs seemed to be unaware of the significance of applying control measures when handling vegetables and of the fundamental requirements for separate handwashing and vegetables washing sinks. APC were above the specified limits for RTEs, 7 Log CFU/g, in 17% of the analysed samples. when APC count is $>10^6$ CFU/g, it may not necessarily relate to food safety hazards; in many of these cases, there is a predominant microorganism from an environmental source (PHLS, 2000) such as the processing stages involving handling, cutting, slicing and improper storage as well as display conditions (Abadias et al., 2012); Nguz et al. (2005) showed that chlorine treated fresh-cut organic mixed vegetables were still found to harbour high levels of TCs (5.9 Log CFU/g) and it was proposed that high loads of coliforms in RTE vegetables at retails levels is directly influenced by intense use of untreated manure during pre-harvest, and extensive handling during postharvest (Aycicek et al., 2006). In our earlier study, TCs≥5 Log CFU/g were isolated from more than two third of the fresh vegetables (69%) coming from locations with alarming deficits at harvest and post-harvest washing, storage and distribution stages (Faour-Klingbeil et al., 2016).

According to the EC legal food safety criteria and the UK Public Health Laboratory Service (PHLS) microbiological guidelines for RTE foods sampled at the point of sale, for category 5 fresh vegetables (HPA, 2009; PHLS, 2000), our study results on microbial contamination levels of more than half of the RTE salad vegetables were unsatisfactory due to *E. coli* and *Listeria* spp. counts that exceeded the criteria limits

288 >10^2 CFU/g indicating poor hygienic practices and sanitary conditions (Gilbert et al.,
289 2000).
290 *Listeria* spp. are rarely implicated in illnesses involving produce, however, they may
291 indicate a significant failure of hygiene standards in the preparation and /or storage of
292 fresh vegetables(Gilbert et al., 2000) which in turn are considered hazardous for
293 *L.monocytogenes* contamination (Ponniah et al., 2010). Presence of *L.monocytogenes*
294 and *Salmonella* spp. were traced back to samples obtained from restaurant that had no
295 handwashing sinks, fresh vegetable washing sinks, or adequate preparation and storage
296 areas or surfaces and the corresponding visual assessment score recorded 32 over 100
297 possible points.
298 The lacking of handwashing sinks explained the fact that proper handwashing before
299 and after use of gloves were not commonly observed, although many other factors could
300 interfere as well. High frequency of *S. aureus* indicates poor hygiene practices of food
301 handlers, the latter being known to be carriers of this pathogen (Todd et al., 2008) and
302 may contribute in direct contamination of RTE fresh vegetables and contact surfaces via
303 the hands (Todd et al., 2008).
304 **5.2 Food contact surfaces**
305 The PHLS recommended guidelines for cleaned contact surfaces specified levels of
306 total viable microorganisms less than 80 CFU/cm^2 as satisfactory, 80-10^3CFU/cm^2 is
307 borderline, and over 10^3CFU/cm^2 is unsatisfactory been associated with poor hygiene
308 practices (Herbert et al., 1990). PCA counts ≥10^3CFU/cm^2 was recorded for 33/49
309 swabbed surface. The overall incidence rate of *E.coli* was 15/49 with counts ≥ 1
310 CFU/cm^2, whereas *E. coli* counts ≥10^3CFU/cm^2 were recorded for 10/49 of swabs. TCs
311 and *Staphylococcus* spp. were found in 26/49 and 39/49 of swabs with counts
312 ≥10^3CFU/cm^2. In this regard, the high microbial population size on contact surfaces

offered an additional assumption for the actual contamination observed on the washed salad items, particularly that sanitization and cleaning operations were lacking in a great majority of locations. Sneed, Strohbehn, Gilmore, et al. (2004) indicated that inadequate sanitation and recontamination problems were actually related to high aerobic plate counts recovered from cutting boards. Non-sanitized and scratched cutting surfaces, combined in some cases with misuse of sanitizers dilution, are an appropriate environment for harbouring pathogens that have the propensity to form biofilm on surfaces (Pui et al., 2011) and resist washing processes (Ravishankar et al., 2010).

As RTE fresh vegetables were obtained after washing, the existing microbiological characteristics do raise further doubts as to the implication of water quality. It is well recognized that natural resources and water supply in Lebanon endures a high risk of chemical and microbial pollution (Houri & El Jeblawi, 2007; Jurdi, 1992), at the same time, it is substantiated that washing with water of unsatisfactory microbial quality can serve as a vehicle for dispersion of microorganisms (Holvoet et al., 2013) and was the primary cause for the homogenous spread of *Salmonella* Enteritidis to fresh-cut vegetables during processing (Perez-Rodriguez et al., 2014). The quality of water used for washing or in post-sanitization rinsing process in SMEs should be addressed in future studies as a critical element to maintain fresh vegetables safety specially when more restaurants nowadays rely on purchasing water of unknown sources, usually coming in tankers collected from spring water but may or may not be chlorinated, to compensate for the shortage in water supply..

5.3 Association of microbial counts to visual assessment scores and inspection components

Our data revealed an inconsistent association between the bacterial counts and visual assessment scores of handling practices and hygiene conditions. As we also studied the

possibility of association to each single inspection component, the microbiological quality of salad vegetables did not show any direct correlation with each individual inspected component. It was found that the cell counts were either corresponding or conflicting in trend across ranking on adequacy level and types of produce. The complexity of the interfering factors during sampling of RTE fresh vegetables from different operational conditions (e.g., environment and storage temperature, receiving and pre-receiving conditions of fresh vegetables, preparation stages of fresh cut vegetables, sampling methods) challenges the possibility to detect a clear cut trend and association. Add to this, large number of samples might be needed to investigate such a trend. Our findings are in accordance with a study by Powell and Attwell (1995) where a link between the total viable counts and *S.aureus* on turkey and ham and the compliance rate to different inspection components was not established. Findings of earlier studies did not as well confirm such an association with the microbiological quality of foods of meat origin (Tebbutt & Southwell, 1989; Wyatt & Guy, 1980). Kuri et al. (1996) found that microbial indicators in meats, including pathogen prevalence, were not correlated to total hygiene scores of meat retailers, nor to temperature of samples, but they were related to type of retailer or origin of product.

We actually noted higher population size of hygiene indicators on some samples prepared under inadequate hygiene conditions, although a statistically significant correlation with the inspection scores failed. According to our results, it may be reasonable to consider that low visual assessment scores on the hygiene standards and handling practices probably indicate unsatisfactory microbial quality and likelihood for risks of salad vegetables contamination with *L.monocytogenes*, however, this association was only significant in relation to individual components related to cross-contamination and effective cleaning. The total visual assessment score can be affected

363 by a number of possible combinations of ranking levels of the 26 variables; a low
364 inspection score might not necessarily indicate low ratings of all the critical components
365 that have direct impact on the microbiological quality of vegetables. Hence, inspections
366 should focus upon factors most likely to be responsible for foodborne infection or high
367 microbial levels associated with RTE vegetables.
368

369 **6. CONCLUSION**

370 Links between the visual assessment scores on the overall food safety performance and
371 the microbiological quality of RTE fresh vegetables are not simple to establish and were
372 not clearly correlated. The total visual assessment scores per se would not directly
373 indicate the microbiological safety of RTE vegetables in restaurants. However,
374 variations in microbial counts and a significant correlation of high *Listeria* levels with
375 the inadequate cleaning performances and cross-contamination preventive measures
376 were recorded, which imply that shortfalls in those particular practices may possibly
377 indicate pathogenic contamination of fresh vegetables.
378 Also, this study found high microbial loads in RTE vegetables that could serve as an
379 indicator for the need to promote awareness on the critical areas commonly identified in
380 SMEs and as guidance for local authorities to target those that may mostly affect the
381 safety of fresh vegetables. It underscored the considerable requisite for improvement in
382 sanitary and good hygienic practices and for vigilant cleaning and sanitation procedures
383 to reduce or eliminate contamination and cross-contamination risks that may occur at
384 pre-farm gate and throughout the supply chain stages. Therefore, applications of critical
385 control points for the preparation of fresh salad vegetables and personnel training on the
386 hazards associated with their preparation are fundamentals to improve the food safety of
387 fresh produce particularly when prepared in small working facilities in SMEs.

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Table 1. The six different constructs comprised in the visual assessment survey in SMEs

Inspection constructs	Individual Inspection Components
Construct 1: Structural compliance	• General maintenance conditions and evidence of pest in the production environment • Zoning (separation of fresh produce from raw meat and poultry) • All major pieces of equipment such fridges, freezers ovens, hot holding equipment, cold holding equipment are fitted with working temperature monitoring gauges • Availability of proper handwashing sink
Construct 2: Personal Hygiene	• Wearing hair cap • Appropriately clean personnel protective clothing
Construct 3: Sanitation	• Clean floors, walls, overall facilities and implements • Waste containers are covered, kept clean • Sanitisers for work surfaces readily available for use during food preparation • Containers used to drain vegetables are kept clean
Construct 4: Evidence of procedures and management system control	• Records keeping for verification of temperature monitoring and system audits (during cooking, cooling, storing) • Cleaning system and schedule • Where a chemical sanitiser is used , there are records to show levels are maintained
Construct 5: Contamination and Cross contamination control measures	• Staff cleaning tools are stored in appropriate manner and not at risk of contaminating food or equipment during preparation • Staff personal belongings are stored in appropriate manner and not at risk of contaminating food or equipment during preparation? • Received fresh vegetable are stored in protected areas • Washing sink designated for fresh produce only • Unprocessed raw vegetables are prepared so that contamination and cross- contamination does not occur (separate cutting boards and utensils) • Visitors or unauthorized staff are granted protective clothing upon entry • Entry for authorized personnel only
Construct 6: Safe and hygienic handling practices	• Appropriate use of gloves and handwashing • Frozen food is properly thawed • Vegetable sanitizers are made up correctly • Food on hold is covered

Table 2 Frequency of self-reported handling practices of fresh vegetables in foodservice establishments

Process	Frequency of handling practices N (%)				
	Always	Often	Sometimes	Rarely	Never
Are fresh vegetables delivered from one supplier/source?	52(68).	17 (22)	5 (7)	1 (1)	1 (1)
Are fresh leafy vegetables or/and pre-cut vegetables delivered cooled?	2 (3)	0 (0)	2 (3)	0 (0)	72 (94)
Is the washing water used for fresh vegetables and fruits chlorinated?	13(17)	0 (0)	0 (0)	0 (0)	64(83)
Do you wash the vegetables before cutting?	51 (77)	1 (1)	1 (1)	0 (0)	13 (20)
If applicable: how often you record the temperature of the display salad bar?	12 (35)	0 (0)	0 (0)	0 (0)	22 (65)
The received fresh vegetables are kept in the cold storage room/fridge	67 (93)	0 (0)	1 (1)	0 (0)	4 (6)
The washed and cut vegetables for salads and garnishes are held at room temperature before preparation/service	17 (26)	0 (0)	2 (3)	0 (0)	47 (71)

Table 3. Microbial loads of different fresh salads vegetables

Produce	**N**	**PCA**[†]	**Coliforms**[†]
Lettuce	30	5.50 ± 1.55	3.89 ±2.19
Parsley	34	5.42 ± 1.32	4.48 ± 2.16
Cucumber	18	4.60 ± 2.01	3.52 ± 2.10
Radish	9	5.09 ± 2.20	1.72 ± 2.68
Mint	11	3.92 ± 2.74	3.93 ± 2.75
Coriander	1	7.38 ± 0.00	6.40 ± 0.00
Aragula	5	3.99 ± 2.44	3.30 ± 3.06
Tomato	3	2.90 ± 2.57	2.13 ± 2.20
Lettuce	4	5.35 ± 1.59	3.20 ±1.49
Iceberg	3	4.54 ± 0.77	1.46 ± 2.53

†Values are mean Log CFU/g ± standard deviation.
The minimum detection limit was 10 CFU/g.
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Table 4.Mean levels of *E.coli* and coagulase–positive *Staphylococcus* spp. on salads vegetables

Produce	**N**	***E. Coli*** Log CFU/g ±SD (min-max)	***Staphylococcus* spp.** Log CFU/g ±SD (min-max)
Lettuce	30	0.92± 1.80 (<1.00 -7.15)	2.89 ± 2.28 (<1.00 – 7.76)
Parsley	34	0.70 ± 1.50 (<1.00 - 5.40)	2.93 ± 187 (<1.00 – 6.16)
Cucumber	18	1.30 ± 1.43 (<1.00 - 3.40)	2.01 ± 1.99 (<1.00 – 5.45)
Radish	9	0.35 ± 0.88 (<1.00 -2.65)	2.84 ± 2.37 (<1.00 – 6.48)
Mint	11	1.36 ± 1.78 (<1.00 - 4.91)	2.69 ± 2.08 (<1.00 – 5.62)
Coriander	1	1.30 ± 0.91 (<1.00 - 1.30)	4.04
Aragula	5	0.92 ± 1.45 (<1.00 - 3.30)	2.76 ± 1.67 (<1.00 – 4.15)
Tomato	3	<1.00	2.00 ± 2.00 (<1.00 – 4.00)
lettuce	4	<1.00	4.47 ± 1.73 (2.30 – 6.00)
Iceberg	3	0.33± 0.58 (<1.00 – 1.00)	1.83 ± 1.58 (<1.00 – 2.78)

The minimum detection limit was 10 CFU/g.

Table 5. Bacterial counts recovered from two contact surfaces

Contact surface	N	Mean log CFU/swabbed area (min-max)			
		PCA	*Staphylococcus* spp	*E.coli*	Total coliforms
Chopping board†	29	4.99 (<1.00-8.40)	4.42 (<1.00-8.40)	1.19 (<1.00-6.02)	2.62 (<1.00-8.40)
Knife*	20	5.62 (<1.00-8.40)	4.62 (<1.00-7.98)	1.13 (<1.00-5.95)	4.31 (<1.00-8.40)

†Cuttinng board swabbed area of 50 cm^2

*Knife (no defined area – ca.10-20 cm^2)

Table 6. Distribution of the mean Log CFU/g of bacterial loads on fresh produce according to adequacy level of control measures

			Prevention of cross-contamination	Sanitation	Protected, clean storage of fresh produce	
Microorganism	**Rating†**	**N**	**Mean ± SD**	**Mean ± SD**	**N**	**Mean ± SD**
Coliforms						
Lettuce	Adequate	9	3.84 ± 3.09	3.67 ± 2.93	11	3.81 ± 2.59
	Inadequate	17	3.86 ± 1.68	4.20 ± 1.98	13	4.42 ± 1.68
Parsley	Adequate	10	3.80 ± 2.20	3.97 ± 2.23	14	3.95 ± 1.94
	Inadequate	20	4.68 ± 2.19	5.35 ± 2.39	13	4.46 ± 2.69
Cucumber	Adequate	6	4.15 ± 2.42	3.92 ± 2.48	7	3.84 ± 2.35
	Inadequate	9	3.79 ± 1.82	3.47 ± 1.99	7	3.61 ± 2.06
E.Coli						
Lettuce	Adequate	9	1.46 ± 2.50	1.18 ± 2.17	11	1.19 ± 2.31
	Inadequate	17	085 ± 1.54	1.23 ± 1.77	13	0.85 ± 1.56
Parsley	Adequate	10	0.54 ± 0.97	0.79 ± 1.55	14	1.15 ± 2.05
	Inadequate	20	0.65 ± 1.48	0.81 ± 1.83	13	0.63 ± 1.15
Cucumber	Adequate	6	1.96 ± 1.47	1.79 ± 1.47	7	1.68 ± 1.53
	Inadequate	9	1.29 ± 1.43	0.91 ± 1.47	7	1.36 ± 1.53
PCA						
Lettuce	Adequate	9	6.14 ± 1.71	6.10 ± 1.54	11	5.41 ± 1.63
	Inadequate	17	5.21 ± 1.40	5.07 ± 1.32	13	5.41 ± 1.63
Parsley	Adequate	10	5.51 ± 1.51	5.48 ± 1.29	14	5.31 ± 1.28
	Inadequate	20	5.49 ± 1.21	5.30 ± 1.29	13	5.42 ± 1.55
Cucumber	Adequate	6	5.87 ± 1.22	4.36 ± 2.72	7	5.84 ± 1.11
	Inadequate	9	4.09 ± 1.82	4.84 ± 1.11	7	3.87 ± 1.96
Staphylococcus						
Lettuce	Adequate	9	2.83 ± 1.73	3.36 ± 2.13	11	3.20 ± 1.91
	Inadequate	17	2.67 ± 2.43	2.53 ± 2.55	13	2.84 ± 2.90
Parsley	Adequate	10	2.85 ± 2.17	3.16 ± 1.87	14	3.18 ± 1.89
	Inadequate	20	2.95 ± 1.78	2.26 ± 1.97	13	2.13 ± 2.08
Cucumber	Adequate	6	1.80 ± 2.02	1.56 ± 1.82	7	1.91 ± 1.87
	Inadequate	9	2.53 ± 2.12	3.24 ±.1.97	7	2.86 ± 2.12

†"Incomplete" ranking was omitted for easier presentation of data

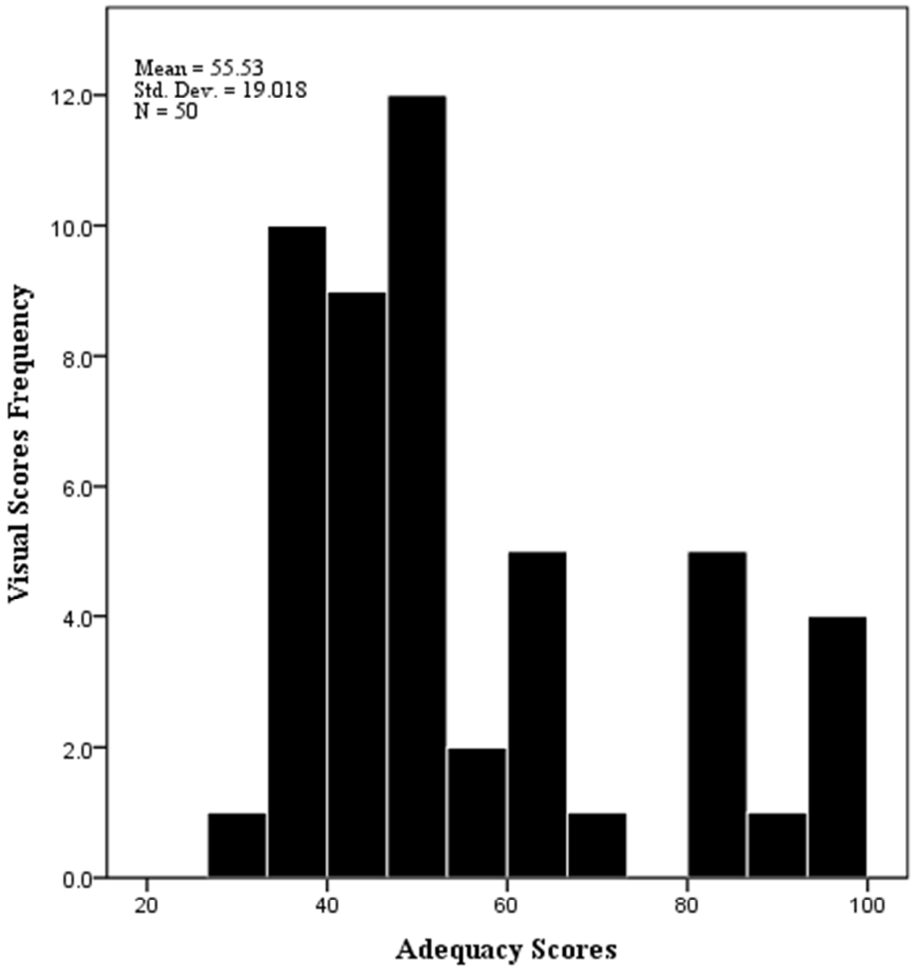

Figure 1. The distribution of total score obtained from the overall visual assessment of hygiene conditions and handling practices.

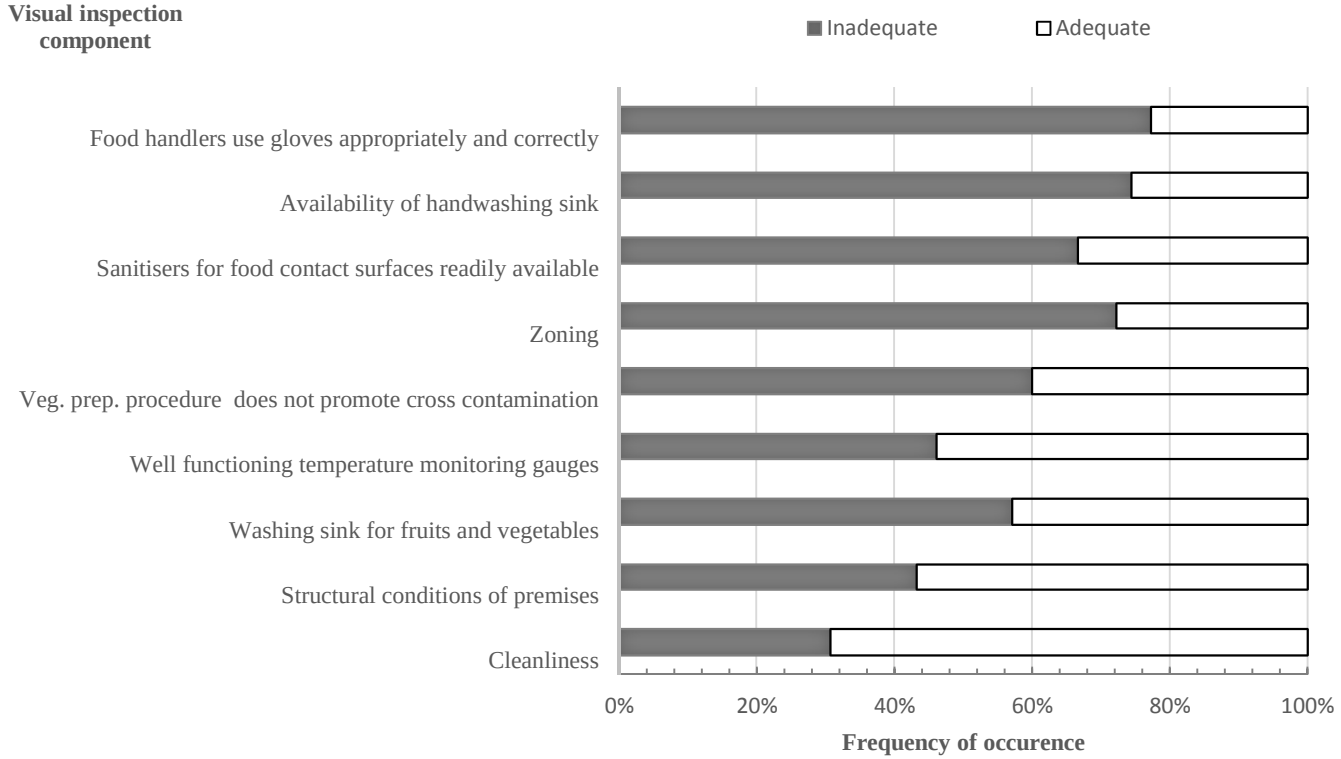

Figure 2. Distribution of food businesses' compliance with basic hygiene requirements and control measures

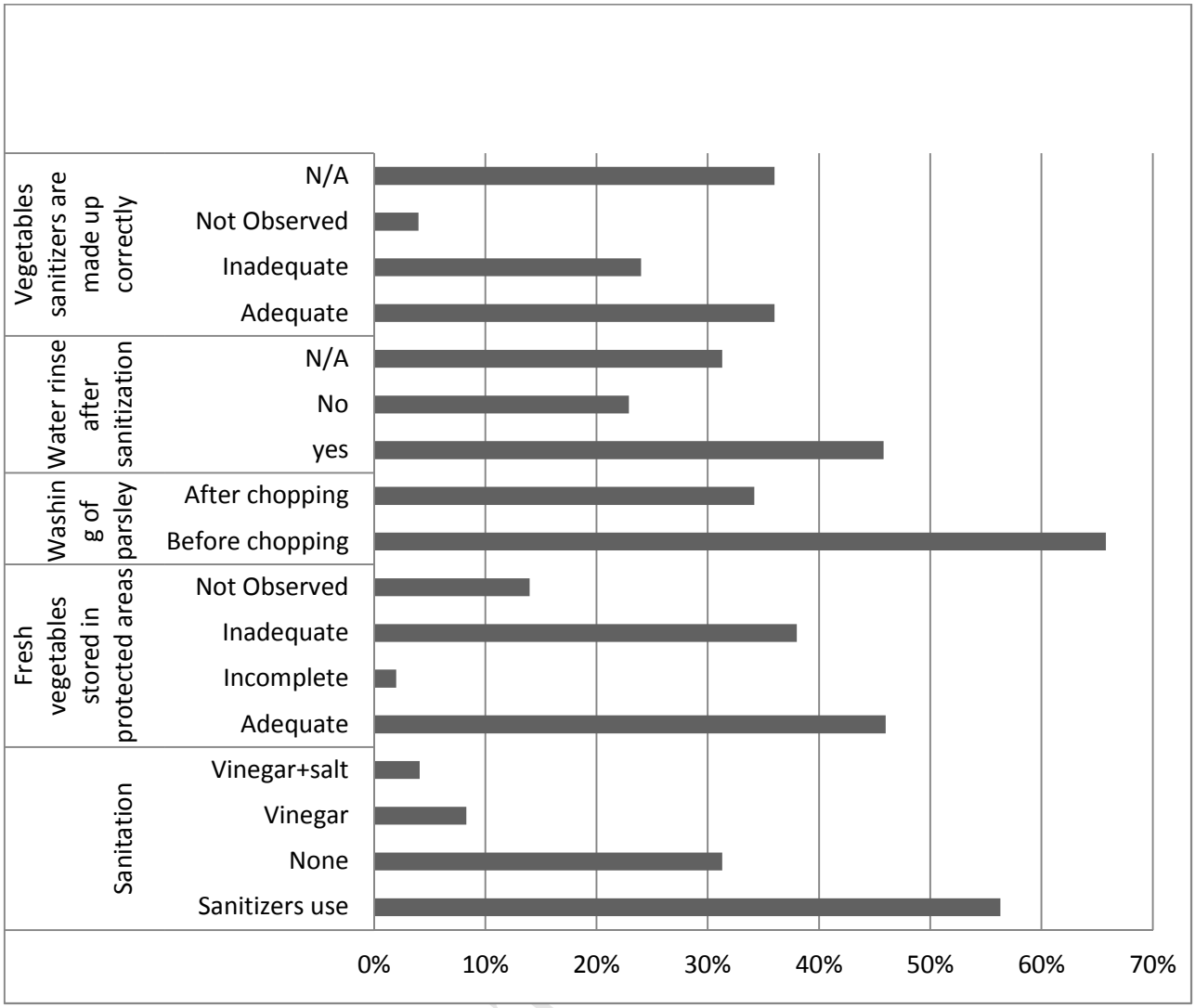

Figure 3. Distribution of food businesses' adequacy level in relation to washing and storing practices of fresh salads vegetables

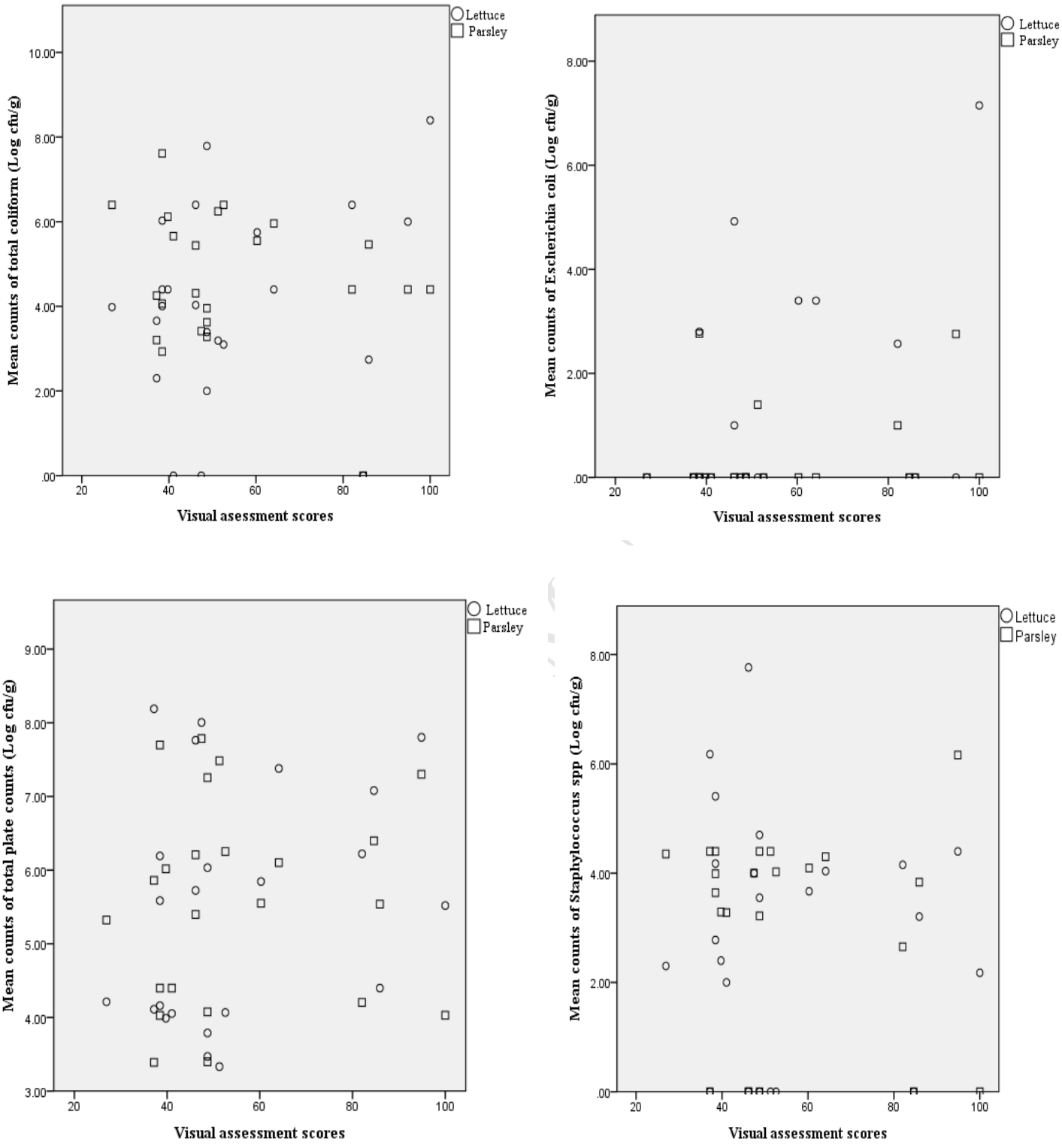

Figure 4. The distribution of microorganism levels on fresh vegetables in relation to the different values of visual assessment scores obtained on all inspected components

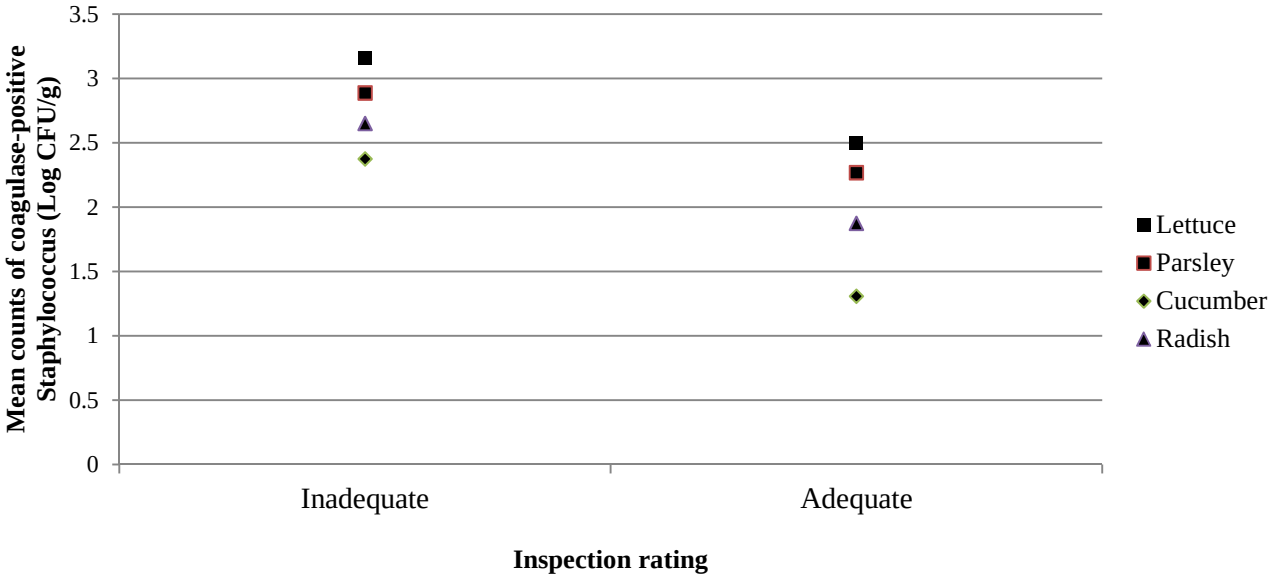

Figure 5 Distribution of mean levels of *Staphylococcus* spp. in relation to component "Availability of handwashing facilities"

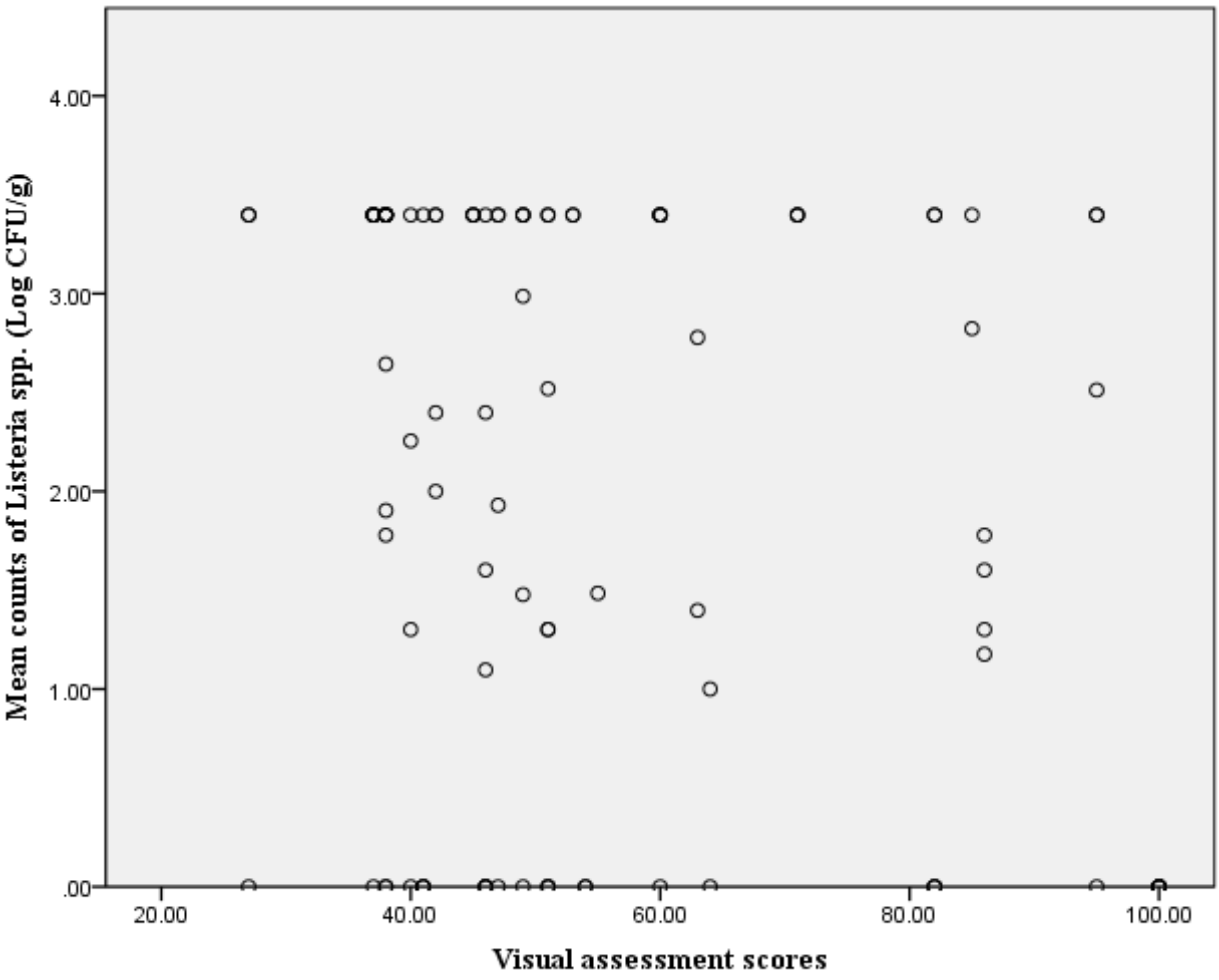

Figure 6. Distribution of Listeria spp. in relation to the visual assessment scores on all inspected components during salad vegetables preparation.

The association of microbiological quality and handling practices of ready-to-eat fresh salad vegetables with food safety environment in restaurants: Case study in Lebanon

Highlights

1. Microbial loads on salads vegetables, hygienic conditions/practices were assessed.
2. Association of microbiological quality with visual assessment scores was tested.
3. *Listeria monocytogenes* and *Salmonella* spp. were detected.
4. There was no significant relationship with the total visual assessment scores
5. Correlation of cross-contamination components to *Listeria* levels was significant
6. Poor cleaning can possibly be linked to *Listeria* levels in salads vegetables.