Journal of Food
Processing & Technology
ISSN: 2157-7110

Uyttendaele et al., J Food Process Technol 2014, 5:3
DOI: 10.4172/2157-7110.1000308

Research Article Open Access

Microbiological Safety of Strawberries and Lettuce for Domestic Consumption in Egypt

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Abstract

Food borne outbreaks with fresh produce are increasingly being reported in developed countries. Food borne illness is an important threat for human health also in developing countries, but data on food safety, in particular related to microbial food safety in fruits and vegetables, the developing world are scarce. In the present study, the sanitary quality and safety of Egyptian lettuce and strawberries, obtained from either primary production or domestic retail market, was assessed by enumeration of faecal indicators organism *Escherichia coli* and coliforms and the detection of *Salmonella* spp. Twelve farms in three different regions of Egypt were visited and apart from strawberries (18) and lettuce (18) also samples of soil (12) and irrigation water (12) were obtained. Furthermore, three different types of domestic retail outlets, i.e. open markets, shops and hypermarkets were sampled for strawberries (30) and lettuce (30). *Salmonella* prevalence in Egyptian domestic fresh produce was very high, namely 42% (20/48) in lettuce and 29% (14/48) in strawberries. The presence of *Salmonella* was associated with elevated levels of *E. coli* and coliforms. Observations from this restricted data set suggest that the microbiological quality and safety of lettuce and strawberries in the primary production of Egypt is subjected to considerable regional differences, presumably related to differences in irrigation water quality. Moreover, the microbiological quality and safety of retail lettuce and strawberries increased in accordance with the scale and organization level of the retail outlet.

Keywords: Egypt; *Salmonella*; Lettuce; Strawberry

Introduction

Food borne outbreaks with fresh produce are increasingly important due to the increased consumption, larger scale production and distribution, and a growing awareness of the problem on the part of public health officials [1,2]. Produce associated outbreaks have increased the past decades in the United States [3,4], in Europe [5-8], and in Australia [9-11]. The most important bacterial pathogens associated with fresh produce were *Salmonella* and human pathogenic verotoxin producing *Escherichia coli* (including *E. coli* O157) and the most frequently implicated produce items included leafy greens (such as lettuce), tomatoes, melons, crucifers, carrots, berries and sprouts [12,13,3,4]. Most of the reported food borne outbreaks originates from Europe, North America, Australia and New Zealand, as these locations have well developed epidemiological surveillance systems. Such systems are often only to a limited extent or not available in much of the developing world. Different institutions are often dealing with food safety issues in developing countries, leading to overlapping responsibilities and coordination problems [14]. Food safety inspection activities typically involve only end product testing, while the resources and the number of qualified inspectors are limited. Moreover, regulatory decisions and standards are usually not made based on risk assessment. As a result, the level of food safety monitoring in developed countries is usually poor.

Data on food safety in most developing countries is scarce [15]. From the few reports available on food safety of fresh produce in these countries, prevalence of pathogens seems to be high [16]. No information on non-human isolates of *Salmonella* in Egypt is currently available [17], except for a recent study on Egyptian meat products, in which *Salmonella* was found in 6 of 40 (15%) samples of chicken, 2 of 40 (5%) beef, 1 of 40 (2.5%) milk, 1 of 40 (2.5%) Kushary sausages, and 4 of 40 (10%) Sogok sausages [18]. *Salmonella* in fresh produce is also expected to be an important pathogen-commodity combination in Egypt, since *Salmonella* was the predominant food borne pathogen in Saudi Arabia between 1995 and 2002 [19]. Despite the lack of data, food safety is also very important for the developing world, since food borne illness is an important threat for human health and economic growth and development [20]. As in developed countries, fresh fruits and vegetables are of (growing) importance in developing countries [21-24]. As the standard of living rises, people tend to show more interest in fresh produce, e.g. increased fruit consumption was observed in South-Africans, among other less healthy preferences [25]. Contamination of fresh produce can occur at multiple stages in the farm-to-fork pathway. Especially the sanitary quality of the manure and the irrigation and washing water influences the microbiological quality and safety of the fresh produce [26-29]. Also in Egypt, farmers are reported to re-use waste and agricultural water from drainage canals, despite the official prohibition of such practices, due to the limited availability water for irrigation [30].

This study was performed to assess the current microbiological quality and safety status of Egyptian domestic fresh produce, namely strawberries (*Fragaria x ananassa* Duch.) and lettuce (*Lactuca sativa* L.), by enumeration of the faecal indicator *Escherichia coli* and detection of the enteric pathogen *Salmonella* on the primary production level in different regions and on the domestic market, as available from different types of retail outlets.

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Received February 18, 2014; **Accepted** March 24, 2014; **Published** April 03, 2014

Citation: Uyttendaele M, Moneim AA, Ceuppens S, Tahan FE (2014) Microbiological Safety of Strawberries and Lettuce for Domestic Consumption in Egypt. J Food Process Technol 5: 308. doi:10.4172/2157-7110.1000308

Materials and Methods

Sampling

Two lettuce farms and two strawberry farms were sampled in 3 different regions (governorates) of Egypt (Suez, Ismailia and Behira) in spring (March, April and May) and autumn (end of September, October, November and first two weeks of December) of 2011 (Figure 1A-1C). At each farm, samples of soil (1), water (1) and the fresh produce (3) was taken, resulting in 12 samples of soil, 12 of water, 18 of lettuce and 18 of strawberries at primary production. Soil samples of approx. 500 g were taken from five different places on the field and pooled in large stomacher bags of 1 L, transferred to lab and mixed well before taking a representative sample for further microbiological analyses (10 g for indicator *E. coli* and coliforms enumerations and

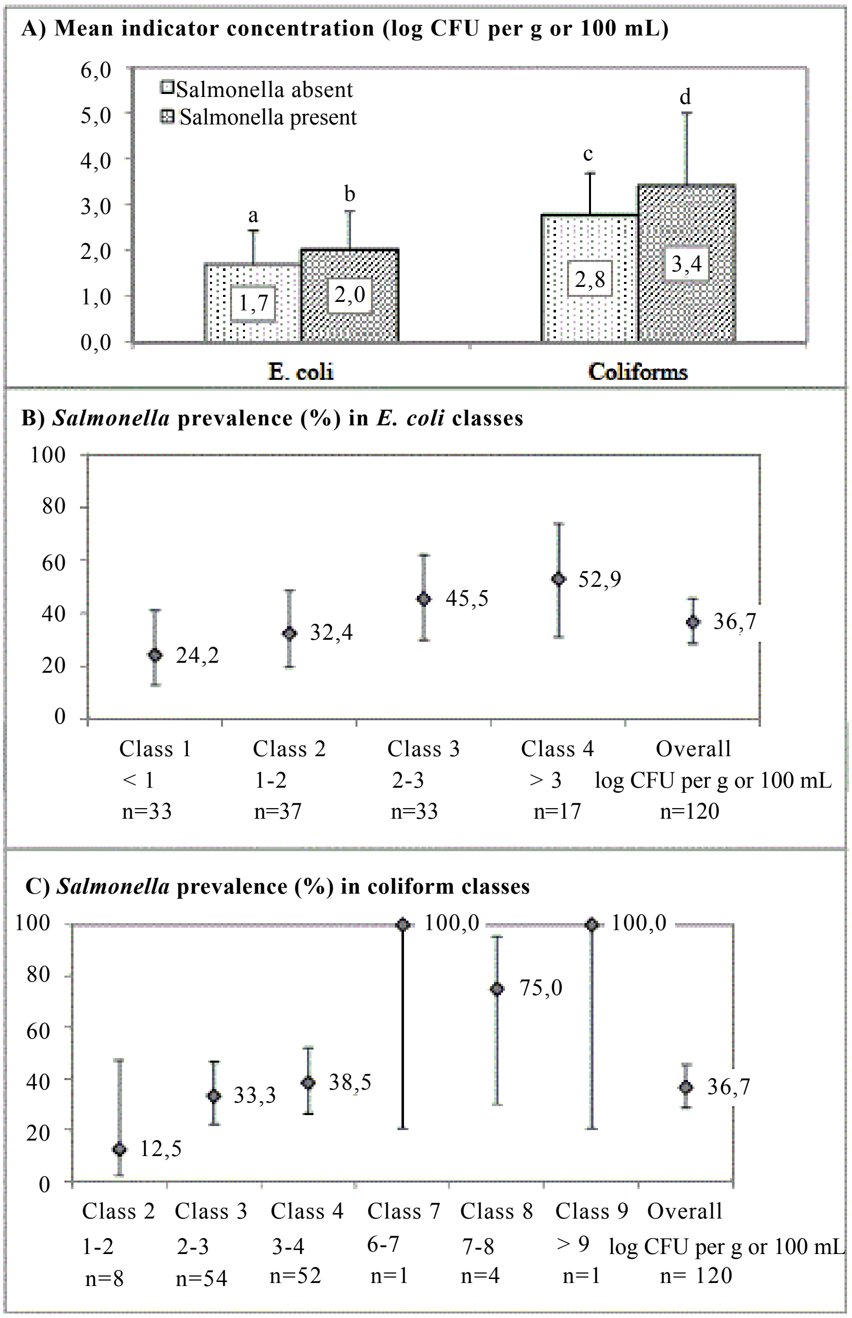

Figure 1: (A) Mean bacterial concentrations (in log CFU/g or 100 mL) of samples without (dotted bars) and with *Salmonella* (hatched bars), error bars indicate the standard deviations and different letters indicate statistically significant differences within one microbiological parameter.
(B) *Salmonella* prevalence in samples belonging to different *E. coli* concentrations, grouped into classes of tenfold differences, error bars indicate the 95 % confidence intervals.
(C) *Salmonella* prevalence in samples belonging to different coliforms concentrations, grouped into classes of tenfold differences, error bars indicate the 95 % confidence intervals.

25 g for *Salmonella* detection). Two water samples of 1 L were taken from the irrigation water source of the farm in a sterilized bottle by submersion and inversion of the bottle in the water in case of an open water reservoir and by collection from the tap in case of a closed reservoir. Fresh produce samples consisted either of 5 crops of lettuce or 1 kg of strawberries, which were cut and mixed before microbiological analysis of subsamples of 10 g for *E. coli* and coliforms and 25 g for *Salmonella*. Retail lettuce (5 crops) and strawberries (1 kg) were also sampled in Great Cairo (Cairo, Giza and Qaulobia) from 10 shops each belonging to 3 different types of markets, namely open markets (street vendors), small indoor shops for fruits and vegetables and large modern hypermarkets, resulting in a total of 30 lettuce and 30 strawberry samples analyzed at the retail level [31-33].

Microbial analyses

All microbial analyses were performed in the Royal International Inspection Laboratories (RIIL). RILL is recognized as an affiliated Lab for Food safety authority in Egypt and accredited from DAP (Deutsches Akkreditierungs system Prüfwesen) Germany in the field of food microbiology analysis, aflatoxin analysis and heavy metal analysis. Soil, lettuce and strawberry samples of 10 g were tenfold diluted maximum recovery diluent (MRD, Oxoid) and enumeration of *E. coli* was done on violet red bile agar with methylum belliferyl-b-D-glucuronide (MUG) (VRBA, Oxoid) according to the modified NMKL 125 2005 method and coliforms on VRBA (Oxoid) according to ISO 4832/2005. Water samples of 100 mL were filtered (cellulose membrane filters of 47 mm diameter and 0.22 µm pore size, Millipore) and subsequently analyzed for the number of *E. coli* on m FC (Difco) according to Standard Method 9222 B and coliforms on m Endo (Difco) according to Standard Method 9222 D. The presence of *Salmonella* spp. was determined per 25 g of soil and fresh produce according to ISO 6579/2002 and per 1 L of water according to ISO 6340-1995 by non-selective pre-enrichment in buffered peptone water (BPW, Oxoid) at 37°C for 16 to 20 h, followed by selective enrichment in Rappaport-Vassiliadis broth (RSV, Oxoid) broth at 42°C for 24 h and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth, Oxoid) at 37°C for 24 h. The enrichment is plated on xylose lysine desoxycholate agar (XLD, Oxoid) and Hektoen enteric agar (HEK, Oxoid) and incubated at 37°C for 24 h. Typical and/or suspect colonies are streaked on nutrient agar (NA, Oxoid) and confirmed by following biochemical tests: triple sugar/iron test, urea test, L-lysine decarboxylation test, β-galactosidase test, Voges-Proskauer test and indole test.

Statistical analyses

Despite the small size of the survey, many positive samples for *Salmonella*, *E. coli* and coliforms were obtained, enabling statistical analysis of the data. All analyses were performed with SPSS Statistics version 21 at a significance level of 5% ($p = 0.050$). The 95% confidence intervals for *Salmonella* prevalence were calculated according to the Wilson score method without continuity correction [34]. Comparison of the *Salmonella* prevalence per governorate, per sample type and per market types was done using the likelihood ratio calculated in the chi-squared test of independence with the Monte Carlo option (10,000 repetitions, 99% confidence level). Correlations between the indicators and the presence of *Salmonella* were calculated using the Mann-Whitney U test. The performance of enumeration of indicator microorganisms and subsequent classification as a screening tool to identify samples with pathogens was evaluated by Receiver Operating Characteristic (ROC) curve analysis in SPSS. Comparison of the other microbiological parameters between the different governorates, market types and sample types was determined by the Kruskal-Wallis 1-way ANOVA test. If significant differences were found, Mann-Whitney U tests were performed on pair wise selections of the data to identify the significant differences between individual categories. In case of n pair wise comparisons, Dunn-Sidak correction was applied, resulting in adjusted individual p' values: $p' = 1-(1-p)^{1/n}$, in which $p = 0.050$ to obtain a family-wise error rate of 5%.

Results and Discussion

The presence of *Salmonella* in Egyptian fresh produce was very high: the overall prevalence was 37% (44/120). The overall prevalence of *Escherichia coli* and coliforms was also very high: 73% (87/120) and 100% (120/120), respectively. In the primary production, *Salmonella* was found in 39% of the lettuce, 28% of the strawberries, 42% of soil and 42% of water samples (Table 1). In retail, *Salmonella* was present in 43% of lettuce and 30% of strawberries sampled. Despite the limited sample size of this survey (n = 120), a relatively large amount of positive samples were obtained for *Salmonella*, indicating a food safety issue for on the domestic Egyptian market for strawberries and lettuce, both examples of ready-to-eat fresh produce. Furthermore, the high prevalence allowed statistical analysis of the observed trends and to draw conclusions from this small dataset.

Specific detection of pathogens in food is expensive, time consuming, complex, and impractical because food products may contain several pathogens. Therefore, indicator organisms such as *Escherichia coli,* coliforms and enterococci are routinely used instead to verify effective implementation of good agricultural practices and good manufacturing practices and the risk of the presence of pathogens [35,36]. As expected, the presence of *Salmonella* was associated with an increased average *E. coli* and coliform concentrations (Mann-Whitney U test, $p = 0.016$ and $p = 0.037$, respectively) (Figure 2). The performance of the indicators *E. coli* and coliforms to predict *Salmonella* presence was evaluated by Receiver Operating Characteristic (ROC) curve analysis. *E. coli* and coliforms counts had more significant predictive power ($p = 0.029$ and $p = 0.028$, respectively) than the random 50/50 chance of predicting pathogen presence/absence over all sample types. *E. coli* and coliform counts had an Area Under the Curve (AUC) of respectively 0.627 and 0.619 with standard errors of 0.054 and 0.057, respectively. These AUC are very low, suggesting that none of these indicators have any substantial predictive power. *E. coli* numbers were the most useful classifiers of *Salmonella* presence in Canadian surface waters [37]. When *Salmonella* was detected (per liter) in the water sample the *E. coli* median value was 365 cfu/100 ml, whereas in case of samples with no *Salmonella* detection, the *E. coli* median value was 54 cfu/100 ml. In contrast, no relation between the presence of *Salmonella* and the numbers of *E. coli* was observed in leafy greens at retail in the United Kingdom [38]. It is to be noted that in the present study even if the threshold value for acceptable sanitary quality in lettuce and

Level	Sample	*E. coli* presence	*Salmonella* presence
Primary production	Lettuce	10/18	7/18
	Strawberry	13/18	5/18
	Soil	11/12	5/12
	Water	12/12	5/12
	Total	**46/60**	**22/60**
Domestic retail	Lettuce	21/30	13/30
	Strawberry	20/30	9/30
	Total	**41/60**	**22/60**

Table 1: Overview of the number of samples from which generic *E. coli* and *Salmonella* were isolated in relation to the total number of samples analyzed per sample type in the primary production of lettuce and strawberries and in these fresh produce items sold in domestic retail establishments.

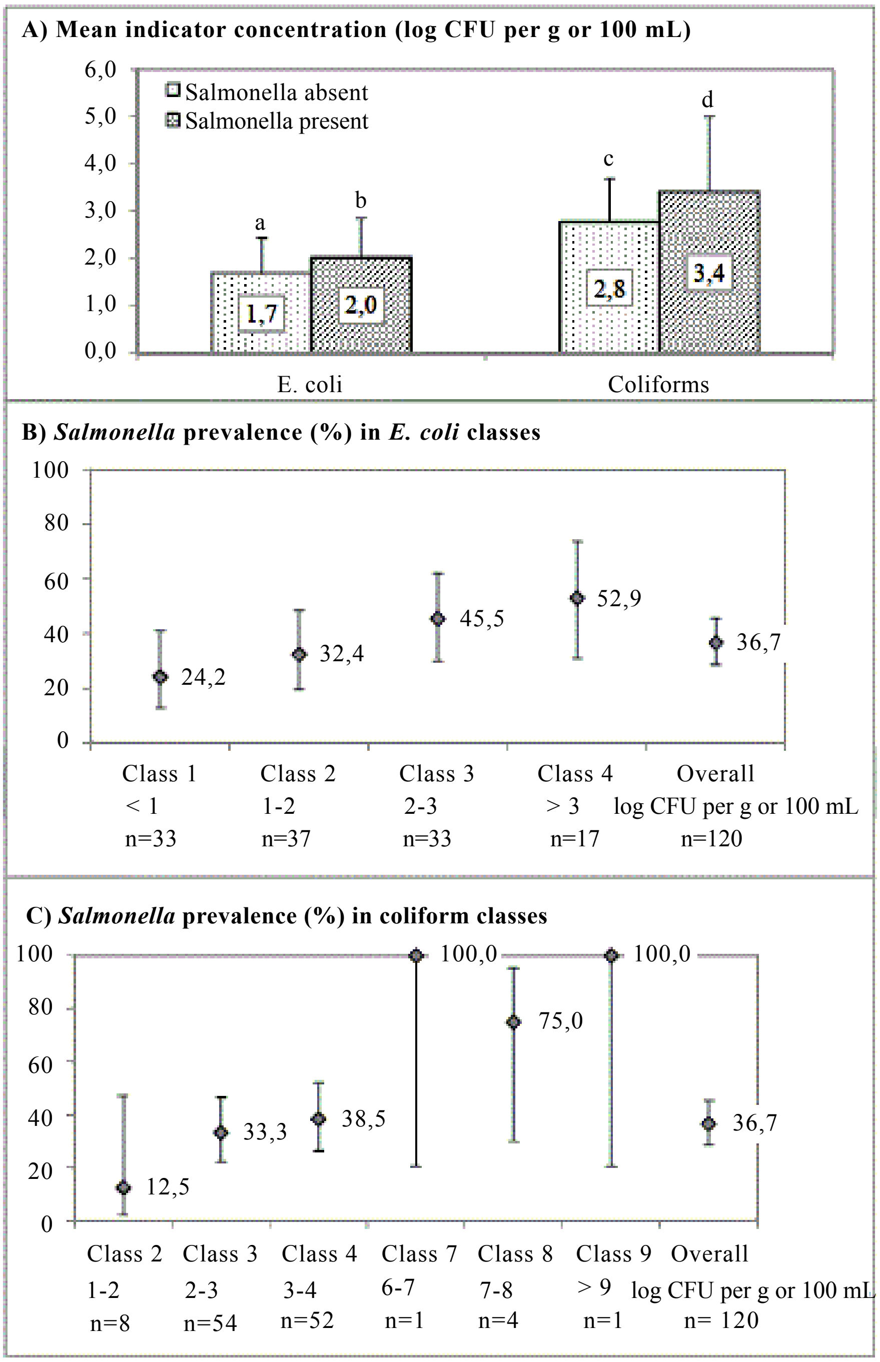

Figure 2: Mean concentrations of the bacterial indicators in log CFU/g or 100 mL with error bars indicating the standard deviations (A, C, E) and *Salmonella* prevalence in % with error bars indicating the 95 % confidence interval (B, D, F) are presented for (A, B) different sample types: soil (white bars), water (horizontal striped bars), lettuce (vertical striped bars) and strawberries (black bars), (C, D) different governorates in Egypt: Behira (white bars), Ismailia (horizontal striped bars) and Suez (black bars) and (E, F) different retail types in Egypt: open markets (white bars), shops (horizontal striped bars) and hypermarkets (black bars). Different letters indicate statistically significant differences between factors within one microbiological parameter.

strawberries would be set at < 10 CFU/g (i.e. the limit of detection of the conventional *E. coli* enumeration method) such an indicator threshold value would still be associated with a low sensitivity for guaranteeing food safety. The associated false negative rate would be undesirably high, i.e. *Salmonella* was present in the absence of *E. coli* (< 10 cfu/g) in 14% of the sampled lettuce and 30% of the sampled strawberries. In addition, the majority of the tested samples would be rejected due to non-compliance with the threshold value of < 10 CFU/g *E. coli*, i.e. 69% of the lettuce and 65% of the strawberries. Although elevated *E. coli* and coliform levels were found to be meaningful risk indicators of *Salmonella* in Egyptian lettuce and strawberries, no practically applicable screening test could be devised due to unacceptably low sensitivity and/or specificity. As a consequence of the high pathogen prevalence, direct detection of *Salmonella* is more cost-effective and useful than an indicator screening test in this particular situation. Therefore, in this case priority should be given to target in food safety monitoring *Salmonella* (rather than *E. coli*) in order to protect human health.

Despite the small size of this survey, high *Salmonella* prevalence was observed in all sample types, indicating a widespread environmental contamination of the agricultural environment and products with this pathogen (Figure 2). Moreover, regional differences were observed, with Ismailia showing better microbiological quality and safety results, i.e. the lowest *Salmonella* prevalence and lowest *E. coli* concentrations than the other two regions (Figure 2a and 2b). Contamination of surface water with faecal indicators and *Salmonella* is correlated with human population density and urbanisation [39]. Population density is highest in the Behira governorate (559 inhabitants/km²), followed by Ismailia (220 inhabitants/km²) and finally the lowest in Suez (66 inhabitants/km²). The population densities were calculated by dividing the total population estimate for 1/1/2013 of the governorate by its surface area (data obtained from GeoHive, available at http://www.geohive.com/cntry/egypt.aspx). Furthermore, flood irrigation with River Nile water through sewage ducts was practiced on farms in the Behira region. In contrast, drip irrigation was used in Ismailia and Suez with resp. ground water and River Nile water. Direct contact with irrigation water facilitates the transfer of pathogens to the fresh produce, so the flood irrigation techniques pose the highest risk. This general finding was confirmed in an Egyptian study which assessed the risk of re-using waste water for produce irrigation in Egypt, indicating that drip irrigation was the safest and thus recommends way of applying irrigation water [40]. In addition, natural disinfection of the surface water occurred due to passage of the Nile water through a large desert canal (the Ismailia canal) between Cairo and Ismailia. As a consequence of the lower population density, the natural disinfection of the irrigation water and the drip irrigation with groundwater practiced in the Ismailia region probably contributed simultaneously to the observed decreased *Salmonella* prevalence and lower *E. coli* concentrations in samples of the fresh produce and agricultural environment in comparison with the other governorates.

To the best of our knowledge, this is the first report on *Salmonella* occurrence on fresh produce in Egypt. Until now, no data on *Salmonella* prevalence in fruits and vegetables in Egypt was publicly available. Data on *Salmonella* prevalence in developing countries and in fresh produce world-wide from the scientific literature is presented in Table 2. The level of contamination of food products with *Salmonella* in Egypt appears to be higher than reported otherwise worldwide, but as for many countries in the Middle East region, other data on *Salmonella* prevalence in fresh produce are simply lacking. More analyses of Egyptian fresh produce are thus warranted to confirm the high contamination levels of lettuce and strawberries with *Salmonella* in small farms producing for domestic sale and to elucidate its sources. Based on the preliminary data generated in this study, application of (faecally) contaminated surface water for irrigation and untreated manure as natural fertilizers appear as potential sources of *Salmonella* in the primary production of fresh produce, which are primary risk factors for introducing pathogens to fresh produce [41]. Surface water and manure may contain various human pathogens including *Salmonella* spp. and pathogenic *Escherichia coli* strains, so its application on agricultural field without prior treatment, such as proper composting, poses health risks due to the potential contamination of the primary production environment (soil, irrigation water and cultivated vegetables) with these pathogens. According to the World Health Organization (WHO), the five keys practices to grow safe fruits and vegetables are: (1) Practice good personal hygiene; (2) Protect fields from animal faecal contamination; (3) Use treated faecal waste; (4) Evaluate and manage risks from irrigation water; (5) Keep harvest and storage equipment clean and dry [42]. Food safety has to

Country	*Salmonella* Prevalence (%)	Sample	Level	Reference
Iran	9.4	Mixed ready-to-eat fresh herbs	Retail	25
Iran	5.6	Mixed fresh-cut vegetable salads	Retail	25
Morocco	0.9	Meat and meat products	Processing and retail	10
Lebanon	47.5	Shawarma sandwiches	Catering	28
Senegal	6	Lettuce	Primary production	34
Zambia	23.1	Fresh-cut organic vegetables	Processing	36
Mexico	5.8	Vegetables	Retail	42
Mexico	2.8	Tomatoes	Primary production	38
Mexico	5	Mung bean sprouts	Retail	13
Mexico	7.5	Ready-to-eat raw vegetable salads	Catering	24
Mexico	4	Ready-to-eat cooked vegetable salads	Catering	7
India	38	Fruit	Street vendors	53
India	33	Vegetables	Street vendors	53
India	4	Sprouts	Street vendors	53
Singapore	1.5	Minimally processed vegetables	Retail	49
Singapore	< 3.0	Fresh vegetables	Retail	49
Korea	< 3.9	Sprouts and seeds	Retail	30
Japan	0.1 – 0.2	Vegetables and fruits		27
Japan	< 0.9	Iceberg lettuce	Retail	31
EU	0.6	Vegetables, fruits and herbs	Retail	16
Ireland	3.6	Herbs and spices	Retail	16
the Netherlands	1.6	Herbs and spices	Retail	16
Spain	1.3	Fresh and minimally processed fruit and vegetables	Retail	1
Portugal	< 2.5	Minimally processed salads	Retail	47
Switzerland	< 1.6	Ready-to-eat lettuce, fresh-cut fruit, and sprouts	Retail	4

Table 2: *Salmonella* prevalence in various food samples, focussing on neighbouring countries and fresh produce.

be integrated along the entire food chain from farm to table, with the government (preferably one single food safety agency), industry and consumers sharing the responsibility [43].

The microbial quality of retail lettuce and strawberries differed significantly among the investigated retail types (Figure 2c and 2a). The lowest mean concentration of faecal indicators was found in lettuce and strawberries in hypermarkets, followed by intermediate counts in retail lettuce and strawberries in shops and finally the highest concentrations were observed in products sold in open markets (2 (Mann Whitney, all $p \leq 0.012$; Figure 2c). A similar but statistically non-significant trend was observed for fresh produce safety, i.e. the lowest *Salmonella* prevalence in hypermarkets (25%, 5/20), intermediate in shops (35%, 7/20) and the highest in open markets (50%, 10/20) (Chi-Square test, Likelihood ratio, $p = 0.300$;). Open markets constitute street vended foods of the lowest microbial quality, which can be explained by the fact that these street vendors have no cooling or other facilities to ensure product quality. This is in accordance with previous studies, which showed that street foods are often of poor microbial quality in developing countries and thus constitute an important cause of food borne illnesses [44,45]. Street vendors are often poorly educated, unlicensed, untrained in food hygiene and working under unsanitary conditions [46]. In addition, the lack of access to clean water to wash hands and utensils, properly functioning sanitary facilities and waste disposal will contribute to spread of pathogens on produce by insufficient hygienic practices [47,48]. In particularly in water stressed regions, insufficiently treated wastewater or sewage contaminated surface waters are being used as a source for crop washing. Consequently, enteric pathogens such as *Salmonella* can also be present in such waters used for fresh produce washing and hence enter the food chain [49]. Moreover, small scale street vendors also lack the awareness and the opportunity to make selections with regard to the sanitary quality in the fresh produce they buy from the farmers. In contrast, lettuce and strawberries of the best sanitary quality were found in large modern hypermarkets. These big retailers usually have their own standards for product selection and for hygiene during transport and handling of the products. For example, hypermarkets have their products delivered by trucks and use cooling during transport, storage and display of their fresh produce. Products of intermediate quality were present in small indoor shops for fruits and vegetables, which are also intermediate in terms of scale, organization and resources in comparison to open markets and hypermarkets. During a small survey (n =18) of imported Egyptian strawberries in Belgium, no *Salmonella* and no *E. coli* (< 10 CFU/g) were detected, indicating a quality gap between domestic and foreign markets. Since the late 1990, supermarkets have been installed in the developing world with increasing success due to consumer demand for foods with higher convenience and higher quality as a consequence of increased income, urbanization, infrastructure and favourable domestic policies [50,51]. For example, supermarkets grew from a niche retail market in Latin America, comprising 10 to 20% of the national food retail sales in the 1980s, to the dominant form constituting 50 to 60% of the national sales in 2000 [52]. Supermarkets impose private stringent grades and standards of quality and safety in their procurement of fresh fruits and vegetables from local farmers in developing countries [53,54]. These quality requirements of supermarkets are beneficial for the local consumers, since overall GAP and GHP in local agriculture and food traders is stimulated [55]. For example, Global GAP certification of green bean and mango exporting firms in Senegal increased the investment in training of workers required for Global GAP certification, as well as an increase in daily wages and employment periods for workers [56].

Conclusion

The very high prevalence of *Salmonella* in Egyptian fresh produce observed in our study shows that the current food safety level in small scale farmers and the domestic market in developing countries is not yet at an acceptable level. In the developed world, Good Agricultural Practices (GAP) are well understood and generally applied, but in developing countries there still exists a strong need for more information and training in (Global) GAP for small farmers. The quality and safety of the fresh produces increases along with the level of modernization at the retail level, so investment in infrastructure and training for the local shop keepers may also be appropriate to increase the quality and safety of the produce for the local consumers to a similar level as that for the foreign consumers which receive high quality export products.

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