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Evaluation of *Salmonella* and *Listeria monocytogenes* contamination on leafy green vegetables

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Abstract

The objective of this study was to monitor *Salmonella* and *Listeria monocytogenes* contamination of leafy green vegetables produced in urban and periurban agricultural fields with natural water irrigation. A total of 164 samples comprising of different leafy green vegetables (8 basils, 15 dills, 20 garden cresses, 16 kales, 12 lettuces, 19 mints, 19 parsleys, 18 purslanes, 1 radish, 20 rockets, 14 scallions and 2 spinaches) were randomly collected from nearby agricultural fields in the close vicinity of Ankara Stream over an 8-month period between April 2007 and November 2007. Samples were examined for *Salmonella* and *L. monocytogenes* using ISO methods with immunomagnetic separation (IMS) and then polymerase chain reaction (PCR). No *Salmonella* and *L. monocytogenes* was detected from the 25-g radish, spinach and scallion samples tested. However, 23 samples (1 basil, 2 dills, 1 garden cress, 1 kale, 2 lettuces, 4 mints, 3 parsleys, 5 purslanes and 4 rockets) were positive for *Salmonella* and 14 samples (3 basils, 1 dill, 1 garden cress, 2 kales, 1 lettuce, 1 mint, 2 parsleys, 1 purslane and 2 rockets) were positive for *L. monocytogenes*. Overall, important contamination of leafy green vegetables by *Salmonella* and *L. monocytogenes* was observed. Therefore, this study will draw attention to the production of freshly consumed leafy green vegetables and so will aid in the development of control measures for these pathogens before harvesting and will highlight the importance of their intake which may cause a communicable disease and so pose a serious threat to both environment and human health.

Key words: Contamination, detection, immunomagnetic separation (IMS), leafy green vegetable, *Listeria monocytogenes*, polymerase chain reaction (PCR), *Salmonella.*

Introduction

Although there has been an increase in the consumption of leafy green vegetables within the past decade because of public awareness of the benefits of healthy nutrition, recent outbreaks of salmonellosis and listeriosis which have been traced to the consumption of contaminated leafy green vegetables, raised a growing public concern toward the safety of freshly consumed leafy green vegetables and gave consumers a cause of apprehension of being infected by *Salmonella* and *Listeria* [1, 5, 17].

Salmonellae are generally associated with food products of animal origin like eggs, poultry and beef but leafy green vegetables have been catching up with them as a major source of *Salmonella* due to the recent outbreaks [9]. Likewise the most recent outbreaks of listeriosis have been traced back to contaminated poultry and meat products as well as contaminated fresh vegetables since *Listeria monocytogenes* is widely distributed in nature [16]. A recent outbreak of *Salmonella enterica* subsp. *enterica* serovar Senftenberg (*S.* Senftenberg) in the United Kingdom has been linked to the consumption of contaminated basil [4, 24]. The outbreaks of *S.* Aberdeen, *S.* Saint Paul and *S.* Stanley in 2005 and *S.* Hvittingfoss in 2007 in Finland were all related to the consumption of contaminated spinach imported from Thailand [9]. The outbreak involving 57 *S.* Typhimurium DT104 infections occurred in the United Kingdom and Finland in 2005, was associated with iceberg lettuce imported from Spain, after wastewater was used to irrigate the crop [30]. The outbreak of *S.* Thompson infections in Norway in 2004 reflected a large international (Sweden-Denmark-Slovenia) outbreak caused by rockets imported from Italy [22]. The outbreak of *S.* Newport in England and Northern Ireland in 2004 was related to the consumption of contaminated lettuce [13]. In 2000, there was an outbreak involving 361 *S.* Typhimurium infections that was linked to contaminated lettuce [19]. The consumption of biologically grown vegetables was found as the source of a small *L. monocytogenes* outbreak, which infected 9 newborns with their mothers and 2 other adults in Austria in 1986 [2].

Besides, these leafy green vegetable-associated *Salmonella* and *Listeria* outbreaks, other vegetables have also been found contaminated with *Salmonella* and *L. monocytogenes*. Contaminated Roma tomatoes were found as the source of the multi-state outbreak of *S.* Braenderup diarrhea infections, occurred in 18 states of USA and in Canada during the summer 2004 [15]. In 2002 and 2005, tomatoes grown on the eastern shore of Virginia (USA) which were contaminated with S. Newport, caused illnesses in 510 patients in 26 states and in at least 72 patients in 16 states, respectively [14]. *L. monocytogenes* was isolated from 2 samples among a total of 402 vegetable samples in South Korea [7].

Although there are many potential sources of *Salmonella* and *L. monocytogenes* contamination of leafy green vegetables in the field such as manure usage as fertilizer, contaminated irrigation water is considered as the major source of contamination [9]. The risk of contamination by irrigation water is increased in recent

years as untreated natural water sources like streams and rivers, which are nearby the agricultural fields, are commonly used for the irrigation of leafy green vegetables, like in Ankara, Turkey. Ankara Stream has also been continuously contaminated with faecal material and other inputs such as sewage in each passing day since it runs by big residential locations and by areas where industrial establishments are also present.

Salmonella infections can lead to septicaemia and sometimes death [12]. Nevertheless, *L. monocytogenes* infections range from a mild flu-like illness to a severe septicaemia and meningitis with a high fatality rate, particularly in pregnant women, newborns, the elderly and the immunocompromised people [8]. People in recent years have become infected by freshly consuming contaminated leafy green vegetables. Therefore, the objective of this study was to evaluate the prevalence of *Salmonella* and *L. monocytogenes* on leafy green vegetables grown around Ankara, Turkey, and to emphasize the importance of maintaining the safety of freshly consumed leafy green vegetables.

Materials and Methods

Collection of leafy green vegetable samples: All leafy green vegetable samples were collected from various nearby agricultural fields in the close vicinity of Ankara Stream, over an 8-month period between April 2007 and November 2007 (Table 1). All samples, which were directly collected from the soil, were brought on ice to our food microbiology laboratory and analyses were initiated after the soil was removed from the samples by shaking, within 3 h of sample collection.

Isolation of Salmonella and L. monocytogenes: Unless otherwise stated, all media were obtained from Merck KGaA (Darmstadt, Germany). Isolation of *Salmonella* and *L. monocytogenes* was individually carried out by ISO 6579:2002 and ISO 11290-1:1998 methods, respectively, with immunomagnetic separation (IMS). Twenty-five g subsamples of each leafy green vegetable sample were weighed and transferred into two separate sterile Stomacher bags, one containing 225 ml of buffered peptone water (BPW) for *Salmonella* analysis and the other containing 225 ml of Half Fraser broth for *L. monocytogenes* analysis and were homogenized for 1 min at maximum speed in a Stomacher® 400 (Seeward, Norfolk, UK), followed by incubation at 37°C for 24 h for *Salmonella* analysis and at 30°C for 24 h. *L. monocytogenes* analysis. One millilitre portions of each aliquot of pre-enriched sample were then subjected to IMS, where 20 µl Dynabeads® anti-Salmonella and 20 µl Dynabeads® anti-Listeria (Dynal, Oslo, Norway) were separately incubated with 1 ml of these aliquots at room temperature for 10 min with continuous mixing (Dynal MX3; Dynal, Oslo, Norway) so that the specific antibodies coated onto the beads would bind the target bacteria. The bead-bacteria complexes were subsequently separated using a magnetic particle concentrator (Dynal MPC-M®; Dynal, Oslo, Norway). Afterwards, 1 ml of washing buffer (0.15 M NaCl, 0.01 M sodium phosphate buffer [pH 7.4], 0.05% Tween 20) was added to wash the complexes and the steps of separation were repeated three times for removing food debris and other microorganisms. Then, 100 µl of washing buffer was added to resuspend the beads again. Next, resuspended anti-Salmonella bead-bacteria complexes were transferred into 10 ml of Rappaport-Vassiliadis (RVS) broths and incubated at 42°C for 24 h. Finally, the 24 h RVS cultures (100 µl) were streaked onto Xylose Lysine Deoxycholate (XLD) agar and Rambach® agar and the plates were cultivated at 37°C for 18-24 h for *Salmonella* analysis and resuspended anti-Listeria bead-bacteria complexes were streaked onto Oxford agar, Palcam agar, and chromogenic *Listeria* agar (OCLA) (Oxoid, Hampshire, UK) and the plates were cultivated at 30°C for 48 h for *L. monocytogenes* analysis. When present, at least two or more typical colonies were transferred to Tryptone Soy (TS) agar slants, followed by DNA isolation for the generic identification of *Salmonella* and *L. monocytogenes*.

Confirmation of Salmonella and L. monocytogenes: Isolates giving typical results by ISO methods were further confirmed by PCR. In PCR unless otherwise stated, all molecular reagents were obtained from Roche Diagnostics, Mannheim, Germany. For PCR, DNA of all presumptive isolates was extracted using the high pure PCR template preparation (HPPTP) kit in accordance with manufacturer recommendations. Five µl of lysozyme solution (10 mg/ml lysozyme in 10 mM Tris-HCl [pH 8.0]) was added to each 200-µl resuspended bead-bacteria suspension and this suspension was then incubated at 37°C for 15 min. Next, 200 µl of binding buffer (6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% [v/v] Triton X-100, pH 4.4) and 40 µl of proteinase K were added and this suspension was incubated at 70°C for 10 min. After mixing with 100 µl of isopropanol, each suspension was applied to the combined filter-collection tube. Following centrifugation at 8000 x g for 1 min (Mikro 200 Hettich, Tuttlingen, Germany), the filter was washed once with 500-µl inhibitor removal buffer (5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6) and twice with 500-µl wash buffer (20 mM NaCl, 2 mM Tris-HCl, pH 7.5). Final DNA extract was eluted in 200-µl pre-warmed (70°C) elution buffer (10 mM Tris, pH 8.5) and was centrifuged at 8000 x g for 1 min. The resulting solution was the template DNA and was subjected to PCR.

Salmonella-specific primers [139: (5'-GTG AAA TTA TCG CCA CGT TCG GGC AA-3') and 141: (5'-TCA TCG CAC CGT CAA AGG AAC C-3')] [26] were directed to *invA* gene and *L.* monocytogenes-specific primers [*inlA*-1: (5'-ACT ATC TAG TAA CAC GAT TAG TGA-3') and *inlA*-2: (5'-CAA ATT TGT TAA AAT CCC AAG TGG-3')] [6, 11] were directed to *inlA* gene. A total reaction volume of 50 µl for each PCR reaction contained, 1.5 µl aliquot of DNA, 1XPCR buffer, 0.5 µM of each primer (MWG Biotech, Ebersberg, Germany), 200 µM of each dNTP, 2 mM of $MgCl_2$ and 0.025 U/µl FastStart Taq DNA polymerase. Amplifications were carried out using a Primus 96 thermocycler (MWG Biotech, Ebersberg, Germany). Initial denaturation was at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 52°C for 45 s and extension at 72°C for 60 s, with a final extension at 72°C for 7 min. Electrophoresis of amplification products was on 1.5% agarose gel containing 1 mg/ml ethidium bromide with a 100-base pair GeneRuler™ DNA ladder plus (ready-to-use, Fermentas, Vilnius, Lithuania) as molecular weight marker, in TBE buffer (10.8 g Tris-base, 5.5 g boric acid, 4 ml 0.5 M EDTA [pH 8.0] in 1 litre of distilled water) at 165 V for 60 min and visualised at 302 nm under UV illumination at InGenius gel visualisation and analysis system (Syngene, Cambridge, UK). The purified DNA from *S.* Enteritidis (ATCC 13076) and *L. monocytogenes* (1462) (provided from University of Veterinary Medicine-Institute for Milk Hygiene and Milk Technology, Austria) were used as positive controls, while ultra pure water and a *Salmonella* and *L. monocytogenes*-

free spinach sample were used as negative controls. Positive and negative controls were included in each run. The *Salmonella*-specific band, which is a positive result, was indicated by a fluorescent band at the 284 base-pair level. The *L. monocytogenes*-specific band, which is a positive result, was indicated by a fluorescent band at the 250 base-pair level.

Results and Discussion

A total of 164 leafy green vegetable samples were collected from various nearby agricultural fields in the close vicinity of Ankara Stream from April 2007 to November 2007. Twelve types of leafy green vegetable samples were collected, including basils, dills, garden cresses, kales, lettuces, mints, parsleys, purslanes, radish, rockets, scallions and spinachs. Table 1 specifies the number of samples of each type and indicates the collection locations of the agricultural fields. Some types of leafy green vegetables could not be collected from every agricultural field since they had not been found in those fields, such as basil in the fields of Yenikent (Local 2) and Çubuk (Local 5), radish in the fields of Yenikent, İvedik (Local 3), Sincan (Local 4) and Çubuk, and spinach in the fields of Eryaman (Local 1), İvedik and Çubuk.

The presence of *Salmonella* and *L. monocytogenes* in the tested samples were presented in Figs 1 and 2, respectively. In Fig. 1, the agarose gel analysis of amplified DNAs obtained from *Salmonella*-contaminated 25-g leafy green vegetable samples and in Fig. 2, the agarose gel analysis of amplified DNAs obtained from *L. monocytogenes*-contaminated 25-g leafy green vegetable samples, were shown. As seen in both figures, the obtained molecular weights for the PCR products amplified from *Salmonella* Enteritidis (ATCC 13076) and *Listeria monocytogenes* (1462) pure cultures were 284 bp and 250 bp, respectively (lanes 2 and 18 in both figures), and suspected isolates were confirmed either *Salmonella* positive or *L. monocytogenes* positive. In the same figures, as might have been expected, no amplifications were obtained in the presence of ultra pure water in lanes 3 and 19. Likewise in lane 30 in Fig. 1 and in lane 21 in Fig. 2, *Salmonella* and *L. monocytogenes*-free spinach sample was observed as negative regarding when these *Salmonella*-specific and *L. monocytogenes*-specific primer pairs were used. Mercanoglu and Griffiths [21] checked the specificity of the 139 and 141 primers to several *Salmonella* strains and strains belonging to other common foodborne bacterial genera and reported no cross-reactivity with the strains other than *Salmonella*. The specificity of the *inlA*-1 and *inlA*-2 primers used for *L. monocytogenes* had also previously been demonstrated by Gaillard *et al.* [11].

In other words, ISO methods with IMS and PCR for the isolation and confirmation of *Salmonella* and *L. monocytogenes* were performed on 164 leafy green vegetable samples, of which 23 (1 of the 8 basils, 2 of the 15 dills, 1 of the 20 garden cresses, 1 of the 16 kales, 2 of the 12 lettuces, 4 of the 19 mints, 3 of the 19 parsleys, 5 of the 18 purslanes and 4 of the 20 rockets) were *Salmonella* positive and of which 14 (3 of the 8 basils, 1 of the 15 dills, 1 of the 20 garden cresses, 2 of the 16 kales, 1 of the 12 lettuces, 1 of the 19 mints, 2 of the 19 parsleys, 1 of the 18 purslanes and 2 of the 20 rockets) were *L. monocytogenes* positive while all of the 1 radish, 14 scallion and 2 spinach samples were found as both *Salmonella*

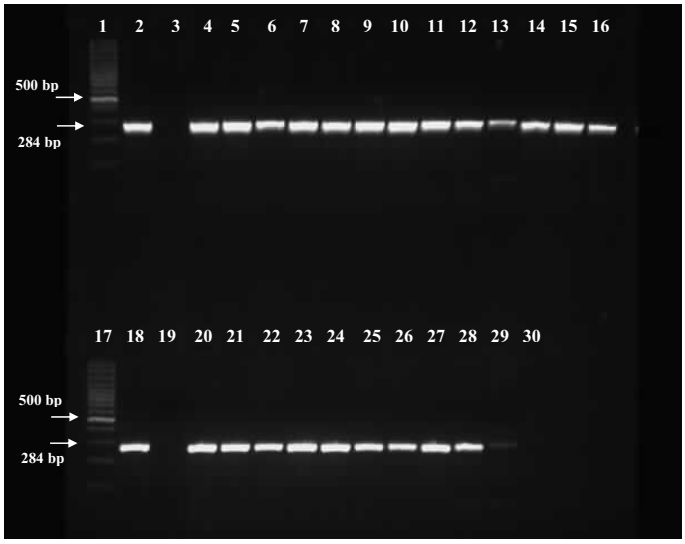

Figure 1. Electrophoresis of PCR products on 1.5% agarose gel stained with ethidium bromide: 100-bp molecular weight marker (lanes 1 and 17); amplification product from DNA of *Salmonella* Enteritidis (ATCC 13076) as positive control (lanes 2 and 18); sterile ultra pure water as negative control (lanes 3 and 19); amplication product from basil samples (lane 24); amplication product from dill samples (lanes 14 and 23); amplication product from garden cress samples (lane 13); amplication product from kale samples (lane 16); amplication product from lettuce samples (lanes 10 and 29); amplication product from mint samples (lanes 7, 9, 15 and 21); amplication product from parsley samples (lanes 4, 6 and 26); amplication product from purslane samples (lanes 5, 8, 11, 25 and 27); amplication product from rocket samples (lanes 12, 20, 22 and 28); *Salmonella*-free spinach sample as internal amplification control (IAC) (lane 30).

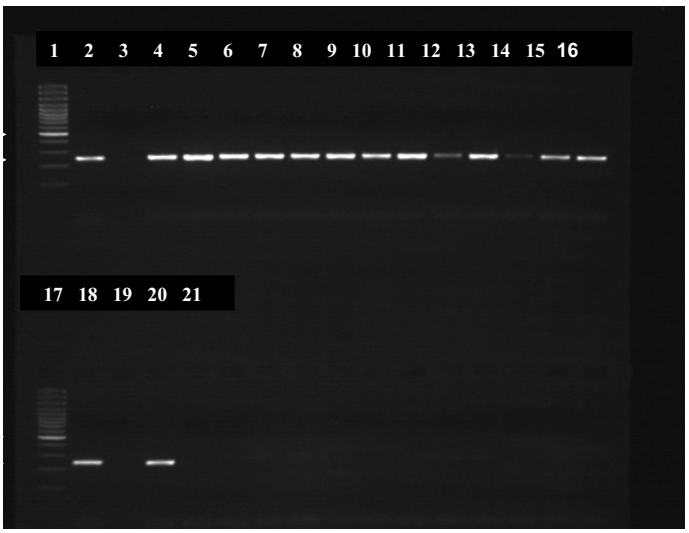

Figure 2. Electrophoresis of PCR products on 1.5% agarose gel stained with ethidium bromide: 100-bp molecular weight marker (lanes 1 and 17); amplification product from DNA of *Listeria monocytogenes* (1462) as positive control (lanes 2 and 18); sterile ultra pure water as negative control (lanes 3 and 19); amplication product from basil samples (lane 7, 12 and 15); amplication product from dill sample (lanes 20); amplication product from garden cress sample (lane 9); amplication product from kale samples (lanes 11 and 13); amplication product from lettuce sample (lane 14); amplication product from mint sample (lane 4); amplication product from parsley samples (lanes 8 and 10); amplication product from purslane sample (lane 16); amplication product from rocket samples (lanes 5 and 6); *Listeria monocytogenes*-free spinach sample as internal amplification control (IAC) (lane 21).

Table 1. Number and collection location of leafy green vegetable samples.

Sample Type	Sample number by collection location					Number (n)
	Local 1	Local 2	Local 3	Local 4	Local 5	
Basil	4	-	2	2	-	8
Dill	6	3	4	1	1	15
Garden cress	10	3	5	1	1	20
Kale	6	3	3	3	1	16
Lettuce	3	3	1	4	1	12
Mint	7	6	3	2	1	19
Parsley	8	3	4	3	1	19
Purslane	3	5	5	4	1	18
Radish	1	-	-	-	-	1
Rocket	9	4	3	3	1	20
Scallion	4	1	5	3	1	14
Spinach	-	1	-	1	-	2
Total number (N)						164

Table 2. Number of leafy green vegetable samples positive and negative for *Salmonella* and *Listeria monocytogenes*, with sample no(s) of contaminated leafy green vegetables.

Sample type	Sample No(s)	*Salmonella* spp. (a/N)*	Sample No(s)	*Listeria monocytogenes* (b/N)*
Basil	76	1/8	96, 107, 116	3/8
Dill	53, 75	2/15	127	1/15
Garden cress	51	1/20	51	1/20
Kale	67	1/16	67, 132	2/16
Lettuce	49, 149	2/12	49	1/12
Mint	46, 48, 60, 72	4/19	48	1/19
Parsley	43, 45, 110	3/19	45, 55	2/19
Purslane	44, 47, 50, 99, 141	5/18	50	1/18
Radish		0/1		0/1
Rocket	52, 70, 74, 144	4/20	61, 128	2/20
Scallion		0/14		0/14
Spinach		0/2		0/2
Total		23/164		14/164

***a/N:** number of *Salmonella* contaminated-leafy green vegetable sample(s)/total number of leafy green vegetable sample(s).
***b/N:** number of *Listeria monocytogenes* contaminated-leafy green vegetable sample(s)/total number of leafy green vegetable sample(s).

and *L. monocytogenes* negative (Table 2).

In this study, information on the prevalence of contamination of *Salmonella* (14%) and *L. monocytogenes* (8.5%) on leafy green vegetables grown around Ankara, Turkey, was provided. These results showed that leafy green vegetables grown around Ankara have more contamination percentage of *Salmonella* and *L. monocytogenes* than the tested raw vegetables in Catalonia, Spain (0.74% *Salmonella*, 1.48% *L. monocytogenes*) [3], and the tested leafy salad samples in Sao Paulo, Brazil (3% *Salmonella*, 0.6% *L. monocytogenes*) [10]. Moreover, Lin *et al.* [20] did not find any *Salmonella* spp. in vegetable samples examined and Abadias *et al.* [1] did not find any *L. monocytogenes* in whole and fresh-cut vegetable samples, except lettuce and mixed salads. However, the prevalence of these pathogens reported here were lower than those reported for various fresh vegetables in different countries. Thunberg *et al.* [31] brought out that *Salmonella* spp. are often isolated from fresh vegetables, including most commonly lettuce, sprouts, spinach and tomatoes. Levels of *Salmonella* between 35 and 20% have been reported in Zambia and Malaysia [23, 27]. *L. monocytogenes* has been found at levels above 10% [18, 28].

The prevalence of *L. monocytogenes* in this study were found as high in lettuce samples (8.3%) in accordance with the results of published data, which reported prevalences of *L. monocytogenes* in lettuce of 2.5% [29] and of 3.4% [1]. Also, Abadias *et al.* [1] showed the prevalence of *Salmonella* as 3.4% in lettuce samples examined. On the other hand, the prevalence of *L. monocytogenes* was not found as high as in garden cress samples (5.0%) in accordance with the prevalence of 18% [31]. Moreover, in this study, neither *Salmonella* nor *L. monocytogenes* were found in both spinach and in radish samples whereas Pingulkar *et al.* [25] exhibited the presence of *L. monocytogenes* in 2 out of 4 spinach samples and Heisick *et al.* [18] isolated *L. monocytogenes* from radishes which showed significant amount (30.3%) of contamination.

In spite of this variable prevalence of these pathogens on leafy green vegetables, these products become a matter of concern as a potential source of *Salmonella* and *L. monocytogenes* and a potential risk to public health from a food safety perspective. Ankara Stream has been continuously contaminated in each passing day, by receiving the water discharge of various industrial plants in which advanced treatment of wastewater is completely lacking and therefore the wastewater after treatment is being used in nearby agricultural areas by local farmers. The pathogens identified in this study can cause mild to severe communicable diseases and probably the local farmers and the local people may not aware of these risks associated with eating contaminated leafy green vegetables.

Conclusions

The results show that leafy green vegetables grown around Ankara are vehicles for pathogens such as *Salmonella* and *L. monocytogenes* and constitute potential health risks. The findings from this study indicate that there is a need for advanced treatment of surface water before its usage in agricultural areas around Ankara or impermissible of agricultural use of this contaminated surface water, like in several countries, to minimize or counter the risk. The most of all, it is important to educate the local farmers on the risk involved in the usage of this contaminated surface water for irrigation of agricultural areas around Ankara and the local people on the risk involved eating these contaminated vegetables.

This study also highlighted likely problem of agricultural areas in other countries.

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References

[1]Abadias, M., Usall, J., Anguera, M., Solsona, C. and Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables and sprouts from retail establishments. Int. J. Food Microbiol. **123**:121-129.

[2]Allerberger, F. and Guggenbichler, J. P. 1989. Listeriosis in Austria: A report of an outbreak in 1986. Acta Microbiol. Hung. **36**:149-152.

[3]Badosa, E., Trias, R., Parés, D., Pla, M. and Montesinos, E. 2008. Microbiological quality of fresh fruit and vegetable products in Catalonia (Spain) using normalised plate-counting methods and real time polymerase chain reaction (QPCR). J. Sci.Food Agr. **88**:605-611.

[4]Berger, C. N., Shaw, R. K., Brown, D. J., Mather, H., Clare, S., Dougan, G., Pallen, M. J. and Frankell, G. 2009. Interaction of *Salmonella enterica* with basil and other salad leaves. The ISME J. **3**:261-265.

[5]Beuchat, L. R. 1996. *Listeria monocytogenes*: Incidence on vegetables. Food Control **7**:223-228.

[6]Chatterjee, S. S., Otten, S., Hain, T., Lingnau, A., Carl, U. D., Wehland, J., Domann, E. and Chakraborty, T. 2006. Invasiveness is a variable and heterogeneous phenotype in *Listeria monocytogenes* serotype strains. Int. J. Med. Microbiol. **296**:277-286.

[7]Cho, S. Y., Park, B. K., Moon, K. D. and Oh, D. H. 2004. Prevalence of *Listeria monocytogenes* and related species in minimally processed vegetables. J. Microbiol. Biotechn. **3**:515-519.

[8]Farber, J. M. and Peterkin, P. I. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55**:476-511.

[9]Franz, E. and van Bruggen, A. H. C. 2008. Ecology of *E. coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. Crit. Rev. Microbiol. **34**:143-161.

[10]Froder, H., Martins, C. G., de Souza, K. L. O., Landgraf, M., Franco, B. D. G. M. and Destro, M. T. 2007. Minimally processed vegetable salads: Microbial quality evaluation. J. Food Protect. **70**:1277-1280.

[11]Gaillard, J. L., Berche, P., Frehel, C., Gouin, E. and Cossart, P. 1991. Entry of *L. monocytogenes* into cells is mediated by internalin a repeat protein reminiscent of surface antigens from Gram-positive cocci. Cell **65**:1127-1141.

[12]Garcia-del Portillo, F. 2000. Molecular and cellular biology of *Salmonella* pathogenesis. In Cary, J. W., Linz, J. E. and Bhatnagar, D. (ed.). Microbial Foodborne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis. Technomic Publishing Company, USA, pp. 1-51.

[13]Gillespie, I. A. 2004. Outbreak of *Salmonella* Newport infection associated with lettuce in the UK. Eurosurveillance Weekly 8. Available at: http://www.eurosurveillance.org/ew/2004/041007.asp.

[14]Greene, S. K., Daly, E. R., Talbot, E. A., Demma, L. J., Holzbauer, S., Patel, N. J., Hill, T. A., Walderhaug, M. O., Hoekstra, R. M., Lynch, M. F. and Painter, J. A. 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiol. Infect. **136**:157-165.

[15]Gupta, S. K., Nalluswami, K., Snider, C., Perch, M., Balasegaram, M., Burmeister, D., Lockett, J., Sandt, C., Hoekstra, R. M. and Montgomery, S. 2007. Outbreak of *Salmonella* Braenderup infections associated with Roma tomatoes, northeastern United States, 2004: A useful method for subtyping exposures in field investigations. Epidemiol. Infect. **135**:1165-1173.

[16]Hanning, I. B., Johnson, M. G. and Ricke, S. C. 2008. Precut prepackaged lettuce: A risk for listeriosis? Foodborne Pathog. Dis. **5**:731-746.

[17]Heaton, J. C. and Jones, K. 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. J. Appl. Microbiol. **104**:613-626.

[18]Heisick, J. E., Wagner, D. E, Nierman, M. L. and Peeler, J. T. 1989. *Listeria* spp. found on fresh market produce. Appl. Environ. Microb. **55**:1925-1927.

[19]Horby, P. W., O'Brien, S. J., Adak, G. K., Graham, C., Hawker, J. I., Hunter, P., Lane, C., Lawson, A. J., Mitchell, R. T., Reacher, M. H., Threlfall, E. J. and Ward, L. R. 2003. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. Epidemiol. Infect. **130**:169-178.

[20]Lin, C., Fernando, S. Y. and Wei, C. 1996. Occurrence of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* and *E. coli* O157:H7 in vegetable salads. Food Control **7**:135-140.

[21]Mercanoglu, B. and Griffiths, M. W. 2005. Combination of immunomagnetic separation with real-time PCR for rapid detection of *Salmonella* in milk, ground beef and alfalfa sprouts. J. Food Prot. **68**:557-561.

[22]Nygård, K., Lassen, J., Vold, L., Andersson, Y., Fisher, I., Löfdahl, S., Threlfall, J., Luzzi, I., Peters, T., Hampton, M., Torpdahl, M., Kapperud, G. and Aavitsland, P. 2008. Outbreak of *Salmonella* Thompson infections linked to imported rucola lettuce. Foodborne Pathog. Dis. **5**:165-173.

[23]Nguz, K., Shindano, J., Samapundo, S. and Huyghebaert, A. 2005. Microbiological evaluation of fresh-cut organic vegetables produced in Zambia. Food Control **16**:623-628.

[24]Pezzoli, L., Elson, R., Little, C., Fisher, I., Yip, H., Peters, T., Hampton, M., de Pinna, E., Coia, J. E., Mather, H. A., Brown, D. J., Nielsen, E. M., Ethelberg, S., Heck, M., de Jager, C. and Threlfall, J. 2007. International outbreak of *Salmonella* Senftenberg in 2007. Eurosurveillance **12**:E070614.3.

[25]Pingulkar, K., Kamat, A. and Bongirwar, D. 2001. Microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads: An evidence of inhibition of *Listeria monocytogenes* in tomatoes. Int. J. Food Sci. Nutr. **52**:15-23.

[26]Rahn, K., de Grandis, S. A., Clarke, R. C., McEwen, S. A., Galan, J. E., Ginocchio, C., Curtis III, R. and Gyles, C. L. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method for detection of *Salmonella*. Mol. Cell Probe. **6**:271-279.

[27]Salleh, N. A., Rusul, G., Hassan, Z., Reezal, A., Isa, S. H., Nishibuchi, M. and Radu, S. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. Food Control **14**:475-479.

[28]Simon, M., Tarregó, C. and Ferrer, M. D. 1992. Incidence of *Listeria monocytogenes* in fresh foods in Barcelona (Spain). Int. J. Food Microbiol. **16**:153-156.

[29]Szabo, E. A., Scurrah, K. J. and Burrows, J. M. 2000. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. Lett. Appl. Microbiol. **30**:456-460.

[30]Takkinen, J., Nakari, U. M., Johansson, T., Niskanen, T., Siitonen, A. and Kuusi, M. A. 2005. Nationwide outbreak of multiresistant *Salmonella* Typhimurium in Finland due to contaminated lettuce from Spain, May 2005. Eurosurveillance **10**:E050630.1.

[31]Thunberg, R. L., Tran, T. T., Bennett, R. W. and Matthews, R. N. 2002. Research Note: Microbial evaluation of selected fresh produce obtained at retail markets. J. Food Prot. **65**:677-682.