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*Research Article*

# Microbiological survey of vegetables from farm to export and their contamination during washing process

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## Abstract

For Thailand, fresh produce is a major export agricultural commodity to Europe, however over the last five years, fresh produce companies have encountered the problem of *Salmonella* spp. and *Escherichia coli* contamination on exported produce which has in turn had a negative impact on the Thai business chain. The sources of contamination on vegetables were identified as farm environments, equipment and poor practices in packing house, including washing process without any application of sanitizers. Since there was no sanitizer used and the use of repeat water during washing step observed, the microbiological survey showed *Salmonella* spp. and *E. coli* counts increased on produce after washing ranged from 0.96 to 2.94 log CFU/g. Thus further experiments were conducted to determine the possibility of cross contamination when using repeat water compared to water with and without 50 ppm sodium hypochlorite. *Enterobacter aerogenes* was used as the surrogate of *Salmonella* spp, while basil and coriander were used as the fresh produce model. After washing, the inoculated basil and coriander in tap water (without shaking for 5 min) resulted in 0.60 and 0.51 log unit reduction (from the initial load 4.17 and 4.21 log CFU/g), respectively. *E. aerogenes* cells transferred to wash water by 2.97 to 3.05 log CFU/ml. The second, third and fourth washing were done with un-inoculated vegetables and *E. aerogenes* counts in repeat water (without chlorine) were 3.01, 3.01 and 3.02 log CFU/ml, respectively. *E. aerogenes* was transferred by repeat water to un-inoculated basil by 2.23 to 2.58 log CFU/g. In the case of coriander, the *E. aerogenes* counts in repeat water were 3.05, 3.03 and 3.05 log CFU/ml, respectively, while *E. aerogenes* was transferred to produce by 2.31 to 2.60 log CFU/g. The

addition of 50 ppm sodium hypochlorite in wash water, noticeably resulted in reduction of *E. aerogenes* in inoculated basil and coriander by 1.25 and 1.03 log CFU/g, respectively. No detection of *E. aerogenes* in un-inoculated basil, un-inoculated coriander and repeat chlorinated water (remained free chlorine 32.6 ppm) was observed. This experiment showed the advantage of adding chlorine as the sanitizer in washing process which significantly kill pathogenic bacteria and prevent the cross contamination between contaminated vegetables through the washwater to clean vegetables.

**Keywords:** *Enterobacter aerogenes*, *Salmonella*, sodium hypochlorite, pathogenic bacteria, sanitizers, Thailand.

## Introduction

Fresh produce is one of the most exported agricultural products from Thailand and there is a great potential market for health food. The range of microorganisms associated with contamination outbreaks linked to fresh vegetables encompasses bacteria, viruses and parasites. Mostly, it has been reported as bacteria particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* spp. and *Escherichia coli* O157:H7 in sprouted seeds and fruit juice are of particular concern. Fruit and vegetables normally carry a non-pathogenic epiphytic microflora. However, many factors contribute to the microbiological contamination of these products with pathogens. Contamination can arise as a consequence of cultivation (soil, organic fertilizers and irrigation water), post-harvest (trimming, cutting, washing) and transporting [1, 2, 3, 4]. Another aspect contributing to the microbial risk for the consumer is consuming raw vegetables [5].

During 2005-2006, fresh produce exported from Thailand, including coriander, curry leaves and four varieties of basil were detected as having *Salmonella* spp. contamination by the Health Protection Agency (HPA) at the point of entry (Border Inspection Post) in London and a pan-London retail markets [6, 7]. In addition, in 2007, ready-to-eat fresh herbs (3,760 samples) were collected from retail premises in the UK for determination of the presence or absence of *Salmonella* spp. and level of *E. coli*. Fifteen samples of Thai exported fresh herbs were collected and the results showed that 4 samples were contaminated with *E. coli* over the limitation (2 log CFU/g) but *Salmonella* spp. was absent [8]. That particular incident of pathogen contamination in exported fresh produce had a detrimental effect on the export economy of Thailand.

Microorganism decontamination by using antimicrobial agents in fruit and vegetables has been widely reported [9, 10, 11, 12]. Various antimicrobial agents can be used to reduce the microbial load in fruit and vegetables. The most common antimicrobial agents used are chlorine-based compounds with free chlorine concentrations of 50–100 ppm [13]. In much of the research work, surrogate microorganisms were used due to pathogen manipulation. For instance, *Enterobacter aerogenes* was used since it is a non pathogenic surrogate with attachment characteristics similar to *Salmonella* spp. [14, 15]. Thus this work was conducted to study 1) the microbiological quality of Thai exported fresh produce from farm to export and 2) the contamination between washing process by using *Enterobacter aerogenes* as a model during washing process with and without sodium hypochlorite.

## Materials and Methods

### *Collection of samples*

The samples (n=330) were collected from farm environment (seeds, soil, irrigation water, manure), packing house environment (gloves/hands, scissors, washwater, tables), transporting process (basket, cover material) and vegetables (sweet basil and coriander) at various steps (after trimming,

after washing, after transporting to factory and after transporting to airport). The farms and factories were located in Nakhon Pathom Province, Thailand.

***Microbial analysis***

The environment (25 ml of water, 10 $cm^2$ of surface tested) and vegetable samples (25 g) were analyzed for the amount of *Salmonella* spp. by using the PCR technique in combination with the MPN method. To count *E. coli*, the *E. coli*/Coliform Count Plates Petrifilm (3M Petrifilm) was applied.

***Strain isolation***

To identify *Salmonella* spp., the samples were mixed with buffered peptone water (BPW, Difco, Detroit, Mich) and incubated at 37$^o$C for 18 to 24 h. One ml. of enriched broth was transferred to Rappaport-Vassiliadis broth (RV broth, Difco, Detroit, Mich) then incubated at 42$^o$C for 18 to 24 h. After that, the selective culture was streaked onto Xylose Lysine Desoxycholate Agar and Hektoen Enteric Agar (XLD and HE, Difco, Detroit, Mich) and incubated at 37$^o$C for 18 h. The suspect colonies were tested with biochemical test. Serological tests were then performed when the results showed positive for *Salmonella* spp. at SAP laboratory (Thailand).

***Bacteria strains and inoculum preparation***

The nonpathogenic food-grade strain of *Enterobacter aerogenes* that is resistant to nalidixic acid, which allows it to be quantified in the presence of other microorganisms in food and of resident bacteria on the vegetables, was used in this work. *E. aerogenes* cells were grown overnight (18 to 24 h) at 37$^o$C in tryptic soy broth (Difco,Detroit, Mich.) containing 50 μg/ml. nalidixic acid (Sigma Chemical Co., St. Louis. Mo.). Cells were harvested by centrifugation (Micro 7: Fisher Scientific, Pittsburgh, Pa.) at 5,000Xg for 3.5 min and washed three times in phosphate buffered saline (0.1 M, pH 7.2) (Fisher Scientific). Cell pellets were re-suspended in phosphate buffered saline. The initial concentration of solution was 8 log CFU/ml. Finally, the bacteria suspension was adjusted to 4-5 log CFU/ml.

***Preparation of antimicrobial solution***

Solution with 50 mg/l active chlorine was prepared by adding the appropriate volume of a concentrated solution of sodium hypochlorite (NaClO) to water. pH of the sodium hypochlorite solution was adjusted to 6.8 using hydrochloric acid (HCl). Washing solutions were prepared one day before application. The initial free chlorine concentration was measured by using an iodine-sodium thiosulfate redox titration (Oxidizer Kit 322, Ecolab).

***Background checking***

For detection of *Enterobacter aerogenes*, fresh basil and coriander were obtained from a supermarket. Damaged leaves and stems were removed. Representative 10 gram samples of each item were tested to confirm the absence of the test strain by adding 90 ml of peptone water and homogenizing for 1 min in a masticator. 10-flod serially diluted technique was applied and spread plated onto MacConkey agar (Difco) containing 50 μg/ml nalidixic acid. Agar plates were incubated at 37°C for 24 h.

***Contamination on fresh vegetables***

Fresh basil (*Ocimum basilicum)* or coriander *(Coriandrum sativum*) was completely submerged into the bacteria suspension (~$10^4$ for basil and ~$10^5$ for coriander CFU/ml) and manually shaken for 5 min at room temperature. After inoculation, the samples were dried in a biosafety cabinet for 30 min. Inoculated air-dried samples were stored in a sterilized bag at 4°C for 24 h before use. The appropriate level of *E. aerogenes* on basil and coriander was $10^4$ CFU/g**.**

To study the possibility of cross contamination between washing process during washing, an experiment was conducted with and without sodium hypochlorite, while basil and coriander were used as the fresh produce model**.**

First washing process: Ten grams of inoculated basil and coriander were washed by submerging into tap water or chlorinated water at room temperature for 5 min. After 5 min, the wash water and the inoculated basil or coriander were taken and immediately neutralized by adding 0.05% sterile sodium thiosulphate ($Na_2S_2O_3$) by weight of sample/volume of neutralized solution ratio 1:5 for stopping any residual bacteriostatic or bactericidal activity for 1 min. After that 40 ml of peptone water was added and mixed for 1 min. The serially diluted technique was then applied and plated onto MacConkey agar containing 50 µg/ml nalidixic acid and incubated at 37°C for 24 h.

Second, third and fourth washing process: Ten gram samples of un-inoculated basil or coriander were washed by submerging into the reused water for 5 min. The wash water and the un-inoculated basil or coriander were taken and immediately neutralized by adding 0.05% $Na_2S_2O_3$ for 1 min. After that 40 ml of peptone water was added and mixed for 1 min. The serially diluted technique was then applied and plated onto MacConkey agar containing 50 µg/ml nalidixic acid. Agar plates were incubated at 37°C for 24 h.

***Statistical analysis***

All data were analyzed at $p<0.05$ for significant differences by ANOVA and Duncan's multiple range tests (Statistical Analysis System). The experiment included 3 replications.

**Results and Discussion**

From sweet basil farm environments, *Salmonella* spp. and *E. coli* were detected in each source (Table 1). For *Salmonella* spp., the prevalence of *Salmonella* spp. was high in the irrigation water and seed, whereas the prevalence of *E. coli* was high in soil and seed. The number of *Salmonella* spp. was also high in soil and seed. For *E. coli*, the high number was found from seed and soil. However, the prevalence of *E. coli* was also high in soil and irrigation water. Thus soil and irrigation water might be considered as the sources of contamination at farm environment.

**Table 1. Incidence of *Salmonella* spp. and *E. coli* in sweet basil farm environments.**

| Samples | *Salmonella* spp. | | *E. coli* | | | |
|---|---|---|---|---|---|---|
| | Positive | Number (MPN/g,ml) | Positive | Number (log CFU/g,ml) | | |
| Soil (n=15) | (5/15) | <30 - 30 | (11/15) | 0 | - | 3.31 |
| Manure (n=15) | (4/15) | <3 - 15 | (1/15) | 0 | - | 0.7 |
| Irrigation water (n=15) | (8/15) | <3 - 15 | (9/15) | 0 | - | 2 |
| Seed (n=3) | (2/3) | <30 - 30 | (3/3) | 4.81 | - | 4.86 |

For coriander farm environments (Table 2), the amounts of *Salmonella* spp. and *E. coli* contamination in irrigation water, fertilizer and seed were detected in low quantities because of their source. Underground water was used as irrigation water and composted manure was used as fertilizer. For coriander, when commercial seed was used for cultivation the amounts of *Salmonella* spp. and *E. coli* contamination were low. Thus soil might be one of the sources of *E. coli* contamination. The positive number of *Salmonella* spp. in manure and irrigation water was higher than positive number of *E. coli* because of the sensitivity of detection method, as mentioned above.

**Table 2. Incidence of *Salmonella* spp. and *E. coli* in coriander farm environments.**

| Samples | *Salmonella* spp. | | *E. coli* | | | |
|---|---|---|---|---|---|---|
| | Positive | Number (MPN/g,ml) | Positive | Number (log CFU/g,ml) | | |
| Soil (n=15) | (7/15) | <3 – 15 | (8/15) | 0 | - | 3.00 |
| Manure (n=15) | (14/15) | <3 – 15 | (0/15) | 0 | - | 0 |
| Irrigation water (n=15) | (7/15) | <3 – 20 | (0/15) | 0 | - | 0 |
| Seed (n=3) | (0/3) | <30 | (0/3) | 0 | - | 0 |

For sweet basil and coriander packing house and field environments, washing water showed high amounts of *Salmonella* spp. and *E. coli* (Tables 3 and 4). It might be the potential source of contamination because the wash water was not changed during processing and the disinfectant was not applied for reducing the microbial load in vegetables or contamination between wash water and vegetables. Moreover, baskets for containing basil and coriander after washing were contaminated with high levels of *Salmonella* spp. and *E. coli*. Additionally, *Salmonella* spp. and *E. coli* were found from table and gloves with high levels.

**Table 3. Incidence of *Salmonella* spp. and *E. coli* in sweet basil packing house and field environments**

| Samples | *Salmonella* spp. | | *E. coli* | |
|---|---|---|---|---|
| | Positive | Number (MPN/g, ml) | Positive | Number (log CFU/cm$^2$,ml) |
| Tables (n=6) | (6/6) | 0.06 – 2.40 | (4/6) | 0 - 2.98 |
| gloves (n=8) | (7/8) | <0.30 – 1.50 | (6/8) | 0 - 3.04 |
| Scissors (n=10) | (3/10) | <0.30 – 1.60 | (6/10) | 0 - 1.48 |
| Wash water (n=3) | (3/3) | <0.30 – 2.80 | (3/3) | 1.18 - 3.08 |
| Baskets (n=5) | (3/5) | <0.30 – 2.80 | (5/5) | 1.70 - 2.56 |
| Cover material (n=5) | (5/5) | 0.06 – 4.20 | (1/5) | 0 - 1.70 |

**Table 4. Incidence of *Salmonella* spp. and *E. coli* in coriander packing house and field environments.**

| Samples | *Salmonella* spp. | | *E. coli* | |
|---|---|---|---|---|
| | Positive | Number (MPN/g, ml) | Positive | Number (log CFU/cm$^2$,ml) |
| Table (n=10) | (9/10) | <0.06 – 5.80 | (5/10) | 0 - 3.76 |
| Hand (n=10) | (7/10) | <0.06– 0.56 | (7/10) | 0 - 3.76 |
| Wash water (n=10) | (9/10) | <3.00 – 35 | (9/10) | 0 - 3.22 |
| Baskets (n=5) | (5/5) | <0.06- 0.20 | (5/5) | 2.00 - 3.78 |
| Cover material (n=6) | (4/6) | <0.30 – 4.30 | (6/6) | 2.28 - 3.81 |

The prevalence and number of *Salmonella* spp. and *E. coli* contamination in sweet basil and coriander were as shown in Figures 1 and 2, respectively. The amounts of *Salmonella* spp. contamination in vegetables before washing were lower than after washing. The cross contamination in washing process occurred due to reusing the washing water. From this result, it showed that the wash water not only could not reduce the microbial level but also be the source of contamination.

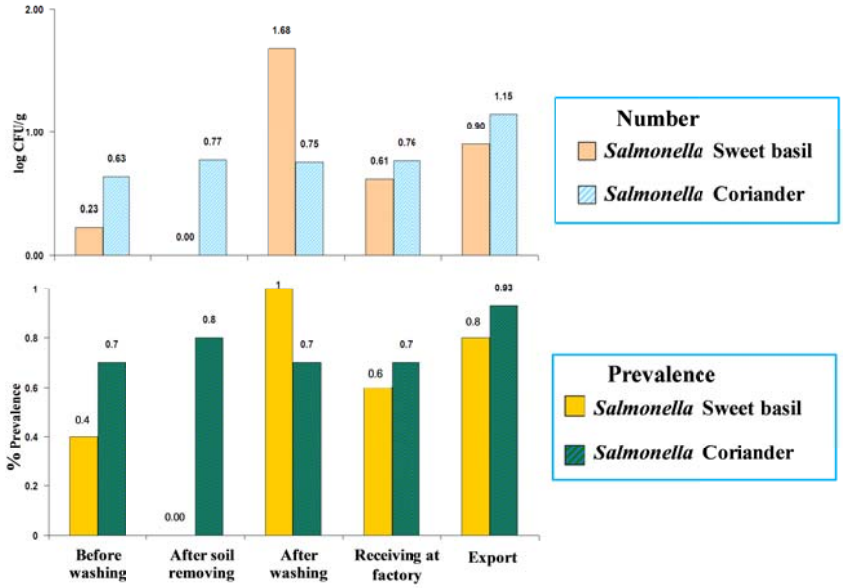


**Figure 1. Number and prevalence of *Salmonella* spp. in vegetables.**

Kim and Harrison [16], also showed that ice can be carrier by ice contaminated with the pathogen or by transferred from lettuce surfaces via melting ice.

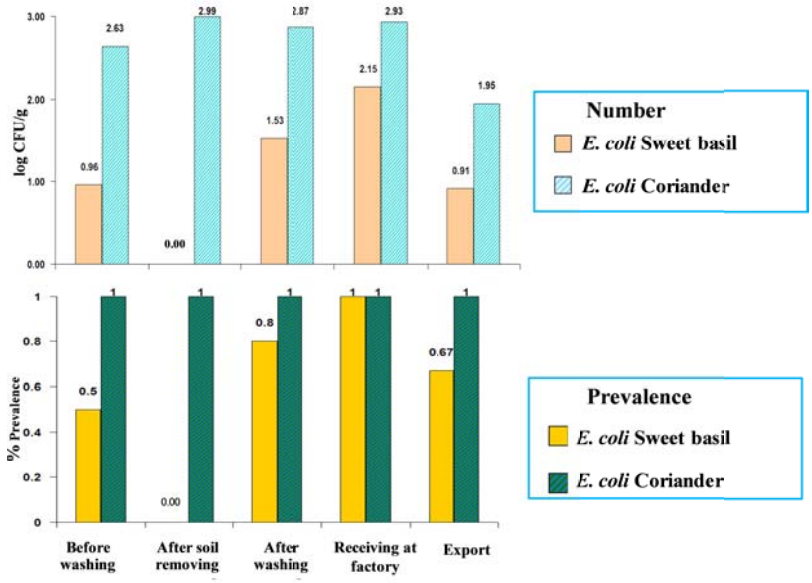


**Figure 2. Number and prevalence of *E. coli* in vegetables.**

To identify the serotype of *Salmonella* contamination in sweet basil and coriander, the results are shown in Table 5. For sweet basil, *Salmonella* Hvittingfoss (group I) was found in samples before

washing. Singapore (group C) and also Weltevreden (group E) serotype were found in export sweet basil, as well as Aberdeen (group F) and Bovismorbificans (group C) in gloves and tables. Also, group J II 17: g,t: - (O: 17) was found from the baskets. For coriander, Augustenborg (group C) were found in samples before washing, after soil removal, after transport to factory and in washing water. In addition, Singapore (group C) was isolated from export coriander. Serotype Newport (group C), Albany (group C) and IIIb 48: 1, V: 1, 5, 7 (O: 48) were found in coriander samples after washing, in irrigation water and tables, respectively.

**Table 5. Isolation of *Salmonella* spp. from environments, sweet basil and coriander.**

| Type of vegetables | Sources | Serotype |
|---|---|---|
| **Sweet basil** | Hands | *Salmonella* Aberdeen (group F) |
| | | *Salmonella* Bovismorbificans (group C) |
| | Tables | *Salmonella* Aberdeen (group F) |
| | | *Salmonella* Bovismorbificans (group C) |
| | Basket | *Salmonella* II 17 :g,t:- (O:17)(group J) |
| | Sweet basil before washing | *Salmonella* Hvittingfoss (group I) |
| | Exported sweet basil | *Salmonella* Singapore (group C) |
| | | *Salmonella* Weltevreden (group E) |
| **Coriander** | Irrigation water | *Salmonella* Albany (Group C) |
| | Wash water | *Salmonella* Augustenborg (Group C) |
| | Table | *Salmonella* IIIb 48 : 1, V : 1, 5, 7 (O:48) |
| | Coriander before washing | *Salmonella* Augustenborg (Group C) |
| | | *Salmonella* IIIb 48 : 1, V : 1, 5, 7 (O:48) |
| | Coriander after soil removing | *Salmonella* Augustenborg (Group C) |
| | Coriander after washing | *Salmonella* Newport (Group C) |
| | Coriander at factory | *Salmonella* Augustenborg (Group C) |
| | Exported coriander | *Salmonella* Singapore (Group C) |

The cross contamination in vegetables and reused water during washing process when using reused water with and without sodium hypochlorite is shown in Table 6. The initial *E. aerogenes* levels of inoculated basil and coriander before washing were 4.17 ± 0.14 and 4.21 ± 0.22 log CFU/g, respectively. After washing in tap water without shaking for 5 min, *E. aerogenes* counts in inoculated basil and coriander were reduced by 0.63 and 0.51 log CFU/g, respectively. *E. aerogenes* cells transferred to wash water by 2.97 (basil) and 3.05 (coriander) log CFU/ml. For investigating the cross-contamination between wash water and herbs, 3 branches of un-inoculated basil and coriander, each branch was washed in the reused water for 5 min (previously washed water). The levels of *E. aerogenes* on un-inoculated basil and un-inoculated coriander after washing in reused water for 5 min reached 2.23 and 2.31 log CFU/g, respectively. After washing each for 5 min, the second, third and fourth washing process were done with un-inoculated vegetables, *E. aerogenes* counts in repeat water (without chlorine) were 3.01, 3.01 and 3.02 log CFU/ml, respectively with no significant difference ($\alpha$=0.05). *E. aerogenes* was transferred by repeat water to un-inoculated basil by 2.23 (second) to 2.58 (fourth) log CFU/g. In case of coriander, the *E. aerogenes* counts in the second, third and fourth repeat water were 3.05, 3.03 and 3.05 log CFU/g, respectively with no significant difference ($\alpha$=0.05). When adding 50 ppm sodium hypochlorite in wash water, there was a noticeable reduction of *E. aerogenes* in inoculated basil and coriander by 1.25 and 1.03 log CFU/g, respectively. No detectable pathogens in un-inoculated basil, un-inoculated coriander and repeat chlorinated water were observed, due to the available chlorine still remaining in wash water (remaining available chlorine 32.6 ppm).

**Table 6. Cross contamination in vegetables and washwater during washing process when using recycled water with and without sodium hypochlorite**.

| Sequence of washing | Samples | | Mean ± SD *Enterobacter aerogenes* population (log CFU/g,ml) | | | |
|---|---|---|---|---|---|---|
| | | | Sweet basil | | Coriander | |
| | | | 0 ppm NaOCl | 50 ppm NaOCl | 0 ppm NaOCl | 50 ppm NaOCl |
| I | Vegetables | Washing inoc. veg | 3.54 ± 0.28 | 2.92 ± 0.12 | 3.70 ± 0.52 | 3.18 ± 0.07 |
| II | | 1st washing un-inoc. veg | 2.23 ± 0.32 | ND | 2.31 ± 0.28 | ND |
| III | | 2nd washing un-inoc. veg | 2.27 ± 0.35 | ND | 2.25 ± 0.13 | ND |
| IV | | 3rd washing un-inoc. veg | 2.58 ± 0.25 | ND | 2.60 ± 0.18 | ND |
| I | Wash water | After washing inoc. veg | 2.97 ± 0.10 | ND | 3.05 ± 0.05 | ND |
| II | | After 1st washing un-inoc. veg | 3.01 ± 0.16 | ND | 3.05 ± 0.01 | ND |
| III | | After 2nd washing un-inoc. veg | 3.01 ± 0.13 | ND | 3.03 ± 0.04 | ND |
| IV | | After 3rd washing un-inoc. veg | 3.02 ± 0.22 | ND | 3.05 ± 0.14 | ND |

ND: not detected (Detection limit: wash water = 1 CFU/ml, vegetables = 0.1 CFU/g).

## Conclusion

The results indicated that washing vegetables with 50 ppm sodium hypochlorite could prevent the cross contamination between wash water and un-inoculated vegetables. Chlorine washing is enough for preventing *E. aerogenes* transference from inoculated vegetables to wash water and from inoculated vegetables to un-inoculated vegetables via wash water. In addition, chlorinated water is not enough to eliminate *E. aerogenes* attachment on vegetables but it showed to be more efficient for pathogen removal on the contaminated vegetables. Adding chlorine as the sanitizer was needed in washing process for killing pathogenic bacteria and preventing the cross contamination between contaminated vegetables through the wash water to clean vegetables.

## References

1. Beuchat, L.R. (1996). Pathogenic microorganisms associated with fresh produce. ***Journal of Food Protection,*** 59(13):204-16.

2. Doyle, M.P. and Erickson, M.C. (2008). Summer meeting 2007 – the problems with fresh produce: an overview. ***Journal of Applied Microbiology***, 105(2):317-30.

3. Keller, S.E. (2009). Microbial contamination of fresh produce. In: Taher FA, Jackson L, DeVries JW, editors. Intentional and unintentional contaminants in food and feed. American Chemical Society. p 25-45.

4. Taormina, P.J., Beuchat, L.R., Erickson, M.C., Ma, L., Zhang, G. and Doyle, M.P. (2009). Transfer of *Escherichia coli* O157:H7 to iceberg lettuce via simulated field coring. ***Journal of Food Protection,*** 72(3):465-72.

5. [EC] European Commission. (2002). Report of the Scientific Committee on Food, risk profile on the microbiological contamination of fruits and vegetables eaten raw. Brussels, Belgium. Available from: www.europa.eu.int/comm/food/fs/sc/scf/out125_en.pdf. Accessed Feb 5.

6. Surman-Lee, S., Murphy, N., Pathak, K., Clements, J. and Pinna, E.D. (2008). A pan London study of the microbiological quality of fresh herbs. Abstract 262 at Health Protection 2008. Available from: www.hpa-events.org.uk/hp2008. Accessed Aug 28.

7. Johannessen, G.S. and Cudjoe, K.S. (2009). Regulatory issues in Europe regarding fresh fruit and vegetable safety. In: Gerald MS, Ethan BS, Karl RM, editors. The produce contamination problem: Causes and solutions. California: Elsevier Science Publishing. p 331-52.

8. Elviss, N.C., Little, C.L., Hucklesby, L., Sagoo, S., Surman-Lee, S., Pinna, E.D. and Threlfall, E.J. (2009). Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. ***International Journal of Food Microbiology,*** 134(1-2):83-8.

9. Han, Y., Sherman, D.M., Linton, R.H., Nielsen, S.S. and Nelson, P.E. (2000). The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157: H7 to green pepper surfaces. ***Food Microbiology,*** 17(5):521-33.

10. Keskinen, L.A., Burke, A. and Annous, B.A. (2009). Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves. ***International Journal of Food Microbiology,*** 132(2-3):134-40.

11. Zhang, G., Ma, L., Phelan, V.H. and Doyle, M.P. (2009). Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. ***Journal of Food Protection,*** 174(72):1392-7.

12. López-Gálvez, F., Gil, M.I., Truchado, P., Selma, M.V. and Allende, A. (2010). Cross-contamination of fresh-cut lettuce after a short-term exposure during pre-washing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite. ***Food Microbiology,*** 27(2):199-204.

13. [WHO/FAO] World Health Organization and Food and Agriculture Organization of the United Nations. (2008). Microbiological hazards in fresh leafy vegetables and herbs : Meeting report. Microbiological risk assessment series 14. 151p. Available from: World Health Organization.

14. Zhao, P., Zhao, T., Doyle, M.P., Rubino, J.R. and Meng, J. (1998). Development of a model for evaluation of microbial cross-contamination in the kitchen. ***Journal of Food Protection,*** 61:960-3.

15. Chen, Y., Jackson, K.M., Chea, F.P. and Schaffner, D.W. (2001). Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. ***Journal of Food Protection,*** 64(1):72-80.

16. Kim, J.K. and Harrison, M.A. (2008). Transfer of *Escherichia coli* O157:H7 to romaine lettuce due to contact water from melting ice. ***Journal of Food Protection,*** 71(2):252-256.