10.5005/jp-journals-10068-0016

ORIGINAL ARTICLE

Transmission of Enteropathogens through Fresh Herbs and Method of using them as Safe Condiments

[1]Rumpa Saha, [2]Himanshi Grover, [3]Malvika Singh, [4]Sungtila T Jamir, [5]Bhoomika Saxena, [6]Amir M Khan, [7]Narendra P Singh

ABSTRACT

Background and objectives: As an indispensable dietary component, fresh fruits and vegetables, which are often consumed raw, have been reported to harbor large microbial populations often culminating in enteric disease outbreaks. The present study aimed to assess the microbial quality of coriander and mint and find the efficacy of potassium permanganate ($KMnO_{4)}$ in effectively removing such microbes.

Materials and methods: Ten grams each of fresh, unstored green coriander (n = 50) and mint (n = 50) samples were examined for their parasitic and bacterial content before and after washing with tap water and with an aqueous solution of 0.001% $KMnO_{4.}$ The different species of bacteria, as well as their main burden in these herbs, were also estimated along with the parasites they harbored.

Results: Only 22% unwashed herbs harbored parasites which were reduced to a significant number on washing with tap water. One hundred percent of the herb samples before washing, and 98% of tap water washed herb samples were contaminated with bacteria. A total of 33 different species of bacteria were isolated, from members of the family *Enterobacteriaceae* (56.3%) *Pseudomonadaceae* (15.6%), *Vibrionaceae* (9.4%,), and *Acinetobacteriaceae* (6.2%). These bacteria were significantly removed after treatment with $KMnO_4$.

Conclusion: The study reveals the presence of harmful enteric bacteria and parasites in fresh green coriander and mint which are liable to cause enteric diseases. We recommend that all the fresh produce which is consumed raw should be treated with 0.001% $KMnO_4$ after tap water washing to render them safe for consumption.

Keywords: Coriander, Enteropathogens, Fresh produce, Mint.

How to cite this article: Saha R, Grover H, Singh M, Jamir ST, Saxena B, Khan AM, Singh NP. Transmission of Enteropathogens through Fresh Herbs and Method of using them as Safe Condiments. J Gastrointest Infect 2018;8(1):22-27.

[1]Professor, [2]Student, [3]Senior Demonstrator, [4,5]Postgraduate Student, [6]Associate Professor, [7]Director Professor

[1-5,7]Department of Microbiology, University College of Medical Science and Guru Teg Bahadur Hospital, Delhi, India

[6]Department of Community Medicine, University College of Medical Science and Guru Teg Bahadur Hospital, Delhi, India

Corresponding Author: Rumpa Saha, Professor, Department of Microbiology, University College of Medical Science and Guru Teg Bahadur Hospital, Delhi, India, Phone: 9212116906, e-mail: rumpachatterjee@yahoo.co.in

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Attention to fresh fruits and vegetables as vital dietary components is noteworthy and has been linked to maintenance of well-being of individuals and have protective properties.[1] Fresh green coriander and mint are such leafy fresh produce known by their scientific name *Coriandrum sativum L* and *Mentha arvensis* respectively. Indians are familiar with these herbs as *dhania* and *pudina*. These are usually consumed raw as garnishing agent on culinary dishes, like salads and dips (*chutney*), etc. They not only enhance the aroma of food but also supplement its nutritional and medicinal values.[2-8] Therefore it is essential to include these herbs in our daily meals. Besides their benefits, they have been shown to anchor important human pathogens which can be transmitted during the consumption of such raw vegetables.[9,10] The larger and uneven surface of leafy fresh produce enable microbes to attach easily to their surfaces. Unfortunately, the increase in consumption of such raw fresh produce has been correlated with an increased frequency of outbreaks of enteric illness. According to a report in 2013 by Jonathan et al.,[11] pathogens like *Escherichia coli, Salmonella, Vibrio cholerae*, etc., have been found in fresh produce like lettuce, peppers, and others, increasing the probability of outbreaks through their raw consumption.[12,13]

Although production practices, growth conditions and location of the edible part during growth can affect their microbial status, nevertheless these diverse microbiomes can pass through stomach to the gut where they establish specific associations with the host resulting in various effects on human health of the consumers.[14] Recently, interesting relationships have been found between gut microbiota and obesity, malnutrition, cancer, etc., in which microbial balance is critical for maintaining the healthy state.[14,15]

Studies have reported the use of potassium permanganate (KMnO) to disinfect water for drinking purpose.[16] Due to its oxidizing properties, it can kill pathogenic organisms. Owing to its antiseptic properties, it has been incorporated in mouthwash for treating gingivitis and is also being used for vaginal and urethral irrigation.[17] Maximum permissible limit of $KMnO_4$ to be used is 0–4 mg/kg aqueous solution. Ingestion of small amounts up to 200 mg/L (approximately 4 mg/kg aqueous solution of $KMnO_4$) has been associated with gastrointestinal distress.[16]

To the best of our knowledge, there are no reports in the literature regarding the microbial population harbored by these fresh herbs. Whether routine washing of these herbs with tap water, (which is usually done in every household) is sufficient to free them from microbes has also not been documented. This limitation in knowledge prompted the present study with the aims (i) to evaluate the microbial quality of fresh herbs and (ii) to assess the efficacy of $KMnO_4$ treatment for removal of the microbes from the herbs.

MATERIALS AND METHODS

This four-month (April–July 2017) cross-sectional study was done in the Department of Microbiology of our tertiary care hospital after clearance from Institutional Ethics Committee. The samples of fresh coriander and mint (10 g each) were obtained from the same grocer at a thrice-weekly interval, placed in sterile plastic bags and taken immediately to the laboratory. Only aerial parts of the herbs were used for the analysis. The microbiological status of 50 samples each of fresh unstored coriander and mint were examined and the effective removal of their microbial content was assessed by treating with 0.001% $KMnO_4$.

Assessment of the Presence of Microorganisms

(a) *In samples of unwashed fresh herbs:* Ten gram each of the herbs were washed directly in 50 mL of sterile distilled water in a sterile beaker for one minute to find out the microorganisms (parasite/bacteria) present. Water left after washing the herb was centrifuged at 756 g for 15 minutes. The deposit thus formed was used for wet mount microscopy to look for the presence of parasites and culture was done for isolation of bacteria as follows:

Saline and iodine wet mount were prepared from all the centrifuged deposits and looked for the presence of parasites. Giemsa stain was done for confirmation wherever necessary.

Deposits were directly plated onto blood agar, MacConkey agar, xylose lysine deoxycholate (XLD) agar and Bile salt agar (BSA) and incubated at 37°C in the ambient aerobic atmosphere.

Deposits were also inoculated in enrichment media (Selenite F broth and alkaline peptone water) and incubated at 37ºC for 4 hours before subculturing onto XLD and BSA respectively.

The growth was assessed after 24 hours of incubation and isolated bacterial colonies were processed by using Microscan Walk Away system (Beckman Coulter, USA) for identification of bacterial species.

(b) *In fresh herb samples after washing them with tap water:* For assessment after washing, the same sample of herb was washed under running tap water for 15 seconds and then washed in a sterile beaker containing 100 mL of sterile distilled water for one minute to assess the effectiveness of household washing process for removal of these microorganisms. For this, 50 mL of above water was again centrifuged at 756 g for 15 minutes and the deposit similarly used for evaluation of parasites and bacterial culture as described above.

(c) *In fresh herb samples after washing them with $KMnO_4$:* In the remaining 50 mL of the above water, 0.0005 g of $KMnO_4$ (0.001% KMnO4) was added and kept for one minute. Next, this water was centrifuged at 756 g for 15 minutes, and the deposit was used to assess the efficacy of $KMnO_4$ in removing the microorganisms as described already.

After completion of the work all the strains were discarded as per BMW guidelines 2016.[18]

Statistical Tools

The results from the study were loaded onto a excel sheet and data analysis was performed using R software version 3.4.2 (R Core Team 2017).[19] McNemar's Chi-squared test with continuity correction was used to evaluate the correlation between the results obtained after analysis of bacterial and parasitic content in unwashed, tap-water washed and $KMnO_4$ treated samples. A *p*-value of <0.05 was regarded as significant.

RESULTS

A total of 100 samples of herbs (coriander N = 50; mint N = 50) were assessed for their parasitic and bacterial status. Overall five different parasites and 33 different bacterial species were found in the herbs.

Microorganisms Isolated

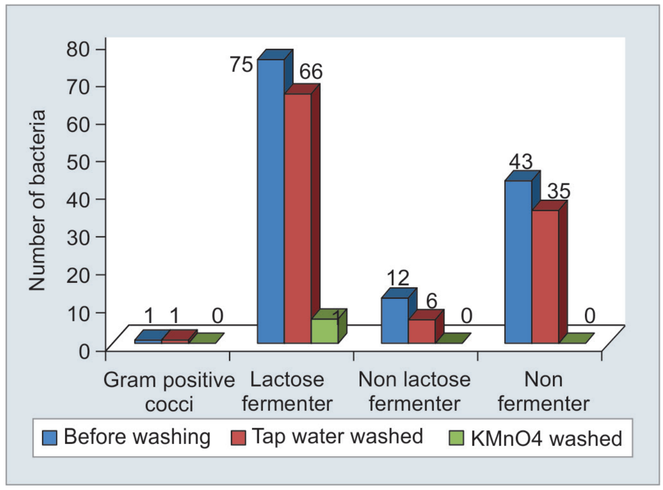
(a) *From unwashed fresh herb samples:* Twenty-two of the 100 unwashed herb samples showed the presence of 11 parasites of 5 different types and 33 different bacterial species.

(b) *From herbs washed with tap water:* Only one sample of tap water washed herb yielded a single type of parasite. There was a significant reduction ($p = 0.0044$, McNemar's test) in the parasitic count in the tap water washed samples compared to unwashed samples. Ninety-eight of the 100 tap water washed herb samples yielded presence of 23 different bacterial species. Tap water washing did not reduce the bacterial load significantly.

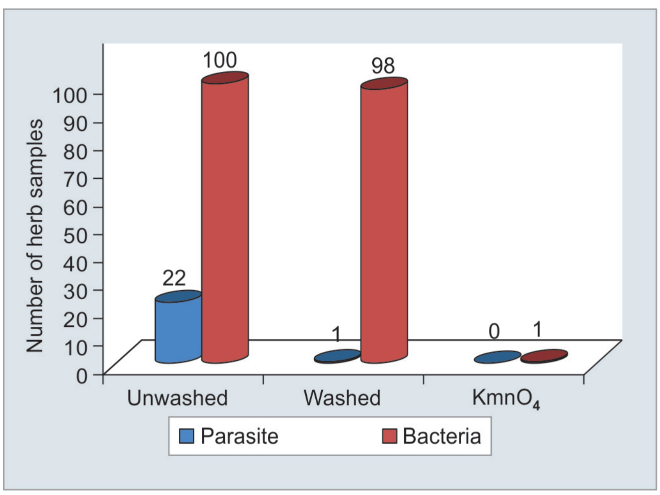
(c) *From fresh herbs samples washed with $KMnO_4$:* Only one of the 100 $KMnO_4$ treated herb samples showed the presence of a single gram-negative bacterial species. None of the $KMnO_4$ treated samples yielded the presence of any parasite. $KMnO_4$ treatment was able to significantly reduce the bacterial contents of herbs from 98% to 1% (p <0.001, McNemar's test).

One hundred percent of unwashed and 98% of tap water washed samples contained bacteria whereas 22% of the unwashed and 1% of the tap water washed samples were contaminated with parasites (Graph 1). The parasites stained with iodine and/or with Giemsa are depicted in Figure 1. Tap water washing did not reduce the bacterial load of the herbs to a great extent, whereas treatment with $KMnO_4$ reduced the bacterial load significantly (Graph 2). Amongst all the 33 different species of bacteria isolated from the herbs samples, 57.57% were found to be enteric pathogens of which 25% were coliforms. Predominantly enteric bacteria were found in all the samples before washing and after washing with tap water (Graph 3).

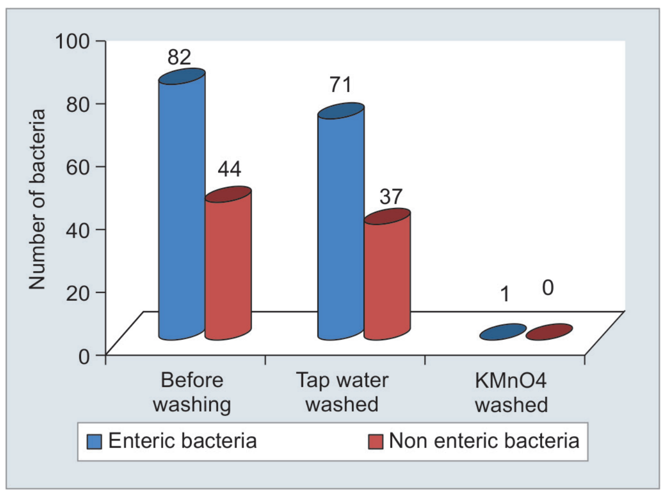
The microbes before and after washing the herbs were compared. The absolute number of different parasitic ova/cyst/larva that was found in the herb samples before and after washing is shown in Table 1. When bacterial contamination in the herb samples was checked more than 50% of the bacteria were retained even after washing with tap water. Gram-negative bacilli (GNB) were found in the majority (96.9%) of the herbs samples of which *E. coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* were found in very high



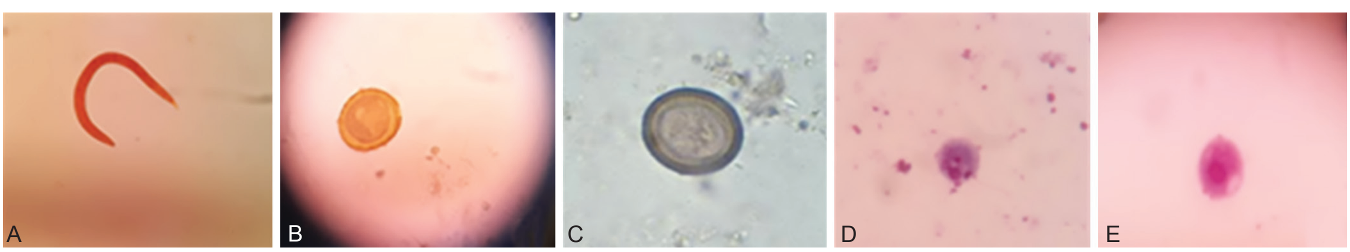
Graph 2: Number of different types of bacteria found in herbs before and after washing



Graph 1: Distribution of organisms found in the herb samples



Graph 3: Enteric bacteria found in herbs before and after washing



Figs 1A to E: Parasites found on fresh unwashed herbs. (A) Strongyloides larva in iodine wet mount (40X); (B) Ascaris egg in iodine wet mount (40X); (C) Taenia egg saline wet mount (40X); (D) Chilomastix trophozoite on Giemsa stain (100X); (E) Blastocystis hominis on giemsa stain (100X)

Table 1: Parasites found from herb samples before and after washing

Herb sample	*Parasite* *Strongyloids*	*Ascaris*	*Taenia*	*Chilomastix*	*Blastocystis hominis*	*Total*	*McNemar's test*
Unwashed	2	4	2	1	2	11	
Washed	0	0	0	0	1	1	p = 0.0044
KMnO	0	0	0	0	0	0	–

numbers. From the total different species of bacteria isolated, members of the family *Enterobacteriaceae*, *Pseudomonadaceae*, *Vibrionaceae*, and *Acinetobacteriaceae* were found in 56.3%, 15.6%, 9.4%, and 6.2% respectively. The main enteric pathogens isolated from the present study were *E. coli, Plesiomonas shigelloides, V. cholera, Aeromonas hydrophila, Yersinia enterocolitica, Shigella dysenteriae* in that order (Table 2).

The stool sample of the particular vendor from which the herb samples were procured did not reveal any enteric pathogens during the study period.

DISCUSSION

The present study aimed to evaluate the microbial status of the coriander and mint which are commonly consumed raw as condiments. The current study revealed the presence of parasites in 22% of unwashed herb samples. Studies from Sudan report lower (13.5%)[20] rate of protozoa and helminthic parasites in their fresh produce whereas those from Nigeria (36%)[21] and Ethiopia (54.4%) [22] document higher contamination rate. The present study further reports the most common parasite contamination in fresh unwashed herbs by eggs of *Ascaris*

Table 2: Bacterial species isolated from herb samples before and after washing

	Organisms	*Unwashed*	*Washed*	*$KMnO_4$ treated*
GPC	MRSA	0	1	0
	MSSA	1	0	0
	Total	1	1	0
GNB LF	Citrobacter freundi	12	10	0
	Klebsiella pneumoniae	32	26	0
	Escherichia coli	14	12	0
	Citrobacter koserii	1	1	0
	Enterobacter aerogenes	5	7	0
	Pantoea agglomerans	4	4	0
	Kluyvera cryocrescens	1	1	0
	Enterobacter cloaca	3	1	1
	Enterobacter intermedius	1	0	0
	Escherichia hermannii	0	1	0
	Enterobacter amnigenes	1	0	0
	Klebsiella oxytoca	0	1	0
	Citrobacter diversus	1	2	0
	Total	75	66	1
GNB NLF	Vibrio cholera O1 Ogawa	2	1	0
	Aeromonas hydrophila	2	1	0
	Proteus rettgeri	1	1	0
	Yersinia enterocolitica	1	0	0
	Shigella dysenteriae	1	0	0
	Plesiomonas shigelloids	3	3	0
	Burkholderia cepacia	1	0	0
	Cedeceae lapagei	1	0	0
	Total	12	6	0
GNB NF	*Pseudomonas aeruginosa*	24	22	0
	Alcaligenes faecalis	6	3	0
	Acinetobacter lwofii	1	1	0
	Bordetella bronchiseptica	1	0	0
	Ralstonia paucula	1	0	0
	Pseudomonas putida	1	0	0
	Pseudomonas stuarti	0	1	0
	Pseudomonas oryzihabitans	1	1	0
	Achromobacter xylosoxidans	0	1	0
	Acinetobacter baumannii	8	6	0
	Total	43	35	0

lumbricoides which is comparable to studies from Ethiopia[22] and Philippines.[23] The ubiquitous distribution and tough character of eggs of this parasite that enables them to survive unfavorable conditions (absence of oxygen, desiccation for 2 to 3 weeks, 5 to 10°C for 2 years) might account for its dominance over other parasites.[24] The absence of hookworm ova in our study is analogous to accounts from Ethiopia[22] and Egypt[25] probably because of the very short lifespan of this ova in the soil. However, its presence in fresh produce in Nigeria[21] may be attributed to differences in geographical location, climatic conditions, and soil types.

The present study also found contamination of bacteria in 100% of unwashed and 98% of tap water washed herb samples. From the total 33 different bacterial species isolated, this study has identified 57.57% of them to be enteric bacterial pathogens in unwashed herb samples of which 25% of them were coliforms. This is comparable to a recent study from Lome done on lettuce samples reporting similar findings (20%) of coliforms.[26] The present study detected bacteria in all the 100 unwashed samples and 98 washed samples of herbs. Bacteria detected belonged to family *Enterobacteriaceae* (56.3%), *Pseudomonadaceae* (15.6%), *Vibrionaceae* (9.4%), *Acinetobacteriaceae* (6.3%) in the herb samples. A study from Colorado, USA, shows comparable reports of microbes belonging to *Enterobacteriaceae* family from strawberries whereas higher percentages (87.6%) of bacteria from the same family were detected in sprouts.[11] The same study documents low bacterial content from lettuce belonging to *Pseudomonadaceae* (4.6%) and *Vibrionaceae* family (0.7%). Although the above study did not report any bacteria from *Acinetobacteriaceae* family from lettuce, 9% bacteria from the same family were found in grapes in their study.[11] A 2017 study from Sweden reports Pseudomonadales as the dominant order of bacteria found in their samples of baby spinach and mixed ingredient salad regardless of storage temperature.[27]

From our herb samples, no *Salmonella* species were detected, although it has been reported from a Florida study on fresh produce. Twelve fruits and vegetables from various countries were analyzed in a multi-centric study from Oman which documented organisms from *Enterobacteriaceae* in 60% fruits and 91% vegetables.[28] The same study also reported *Enterococci* in 20% fruit samples and *S.aureus* in 7% vegetables.[28] In the present study *Staphylococcus aureus* was found in 3.1% only and no other Gram-positive cocci were perceived in the herb samples.

Many of the bacteria from different fresh produce reported from various studies are enteropathogenic and can have ill effects on the consumer's health. Although irradiation seems to be a more efficient method for making these herbs pathogen free, chemical disinfection has also shown to partially reduce the initial bacterial contamination.[29] Satpathy et al.[30] in a study in 2012 reported use of various disinfectants like 0.9% NaCl, 0.1% NaHCO3, 0.1% acetic acid, 0.001% KMnO4, 0.1% ascorbic acid 0.1% malic acid for disinfecting raw vegetables. Since KMnO4 has antibacterial properties in water purification, we decided to use 0.001% KMnO4 in our study. The maximum permissible limit of $KMnO_4$ to be used in drinking water is 0–4 mg/kg aqueous solution.[16] Accordingly 0.001% (10 mg/L) $KMnO_4$ was used in the study. The results of the present study demonstrated that washing of herbs with an aqueous solution of 0.001% $KMnO_4$, for one minute removed bacteria significantly, whereas, washing with tap water removed only a small proportion (2%) of these bacteria. Hence 0.001% $KMnO_4$ can be termed as an effective disinfectant for fresh produce.

Apart from direct disease causation, these microbes found in the herbs may have other swerving impacts on human health. Exposure to non-pathogenic microbes associated with plants may influence the development of allergies.[31] Moreover, the consumption of raw fresh produce may represent an important means by which new lineages of commensal bacteria are introduced into the human gastrointestinal system.[31] Further, produce-associated microbes may also affect the rates of food spoilage, and many of the microbes found on kitchen surfaces appear to come from produce sources.[32,33]

This is a pilot study with fresh herbs and further studies should be carried out with other fresh produce consumed raw with a larger sample size. Even a lower percentage of KMnO4 can be tried as a disinfectant for fresh produce.

CONCLUSION

The present study found 22% parasites in fresh green coriander and mint which were removed to a significant extent by washing them with tap water. However, 100% of these herb samples showed bacteria mainly belonging to family *Enterobacteriaceae, Pseudomonadaceae* and *Vibrionaceae,* both in the unwashed herb samples as well as after washing with tap water (98%). The gut microbial balance is critical not only for maintaining a healthy state but can also lead to outbreaks of enteric illness after consumption of such fresh produce. This study further demonstrated that one-minute treatment of these herbs with an aqueous solution of 0.001% $KMnO_4$ removed these bacterial pathogens significantly. Hence we recommend that all the fresh produce especially those consumed raw should be treated for one minute with 0.001% $KMnO_4$ after tap water washing to make them safe for consumption in the raw state.

REFERENCES

1. Sharma V, Hussainm S, Gupta M, Saxena AK. In vitro anticancer activity of extracts of Mentha spp. against human cancer cells. Indian J Biochem Biophy 2014;51:35-38.
2. Bhat S, Kaushal P, Kaur M, Sharma HK. Coriander (*Coriandrum sativum* L.): Processing, nutritional and functional aspects. African J Plant Sci 2014;8(1):25-33.
3. Darughe F, Barzegar M, Sahari MA. Antioxidant and antifungal activity of Coriander (*Coriandrum sativum L.*) essential oil in cake. Int Food Res J 2012;19(3):1253-1260.
4. Dhiman R, Aggarwal N, Aneja KR, Kaur M. In vitro antimicrobial activity of species and medicinal herbs against selected microbes associated with juices. Int J Microbiol 2016;1:4-6.
5. Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and internodal callus extracts of Mentha arvensis. Asian Pac J Trop Med 2011;1:197-200.
6. Kumar P, Mishra S, Sharma AK. Antifungal efficacy of plant essential oils against stored grain fungi of Fusarium species. J Food Sci Technol 2016;53:17-20.
7. Lin KH, Yang Y, Yang CM, Huang MY, Lo F, Liu KC, *et al*. Antioxidant activity of herbaceous plant extracts protect against peroxide induced DNA damage in human lymphocytes. BMC Res notes 2013;6:2-4.
8. Londonkar RL, Poddar PV. Studies on activity of various extracts of Mentha arvensis Linn against drug induced gastric ulcer in mammals. World J Gastrointest Oncol 2009;1(1):82-88.
9. Oliveira M, Usall J, Vinas I, Anguera M, Gatius F. Microbiological quality of fresh lettuce from organic and conventional production. Food Microbiol 2010;27:679–684.
10. Rastogi G, Sbodio A, Tech JJ, Suslow TV, Coaker GL. Leaf microbiota in an agro ecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. ISME Journal. 2012;6(10):1812.
11. Jonathan WL, Noah F. Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables. PLoS ONE 2013;8(3):e59310.
12. Fatica MK, Schneider KR. Salmonella and produce: Survival in the plant environment and implications in food safety. Virulence 2011;2:573-579.
13. Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr Rev* Food *Sci* Food Saf 2003;2:78-141.
14. Berg G, Erlacher A, Smalla K, Krause R. Vegetable microbiomes: is there a connection among opportunistic infections, human health and our 'gut feeling'? Microbiol Biotech 2014; 7(6): 487-495.
15. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489(7415):220-230.
16. Willhite C, Bhat V, Ball G, McLellan C. Emergency: Do Not Consume/Do Not Use concentrated form of potassium permanganate in drinking water. Hum Exp Toxicol 2013;32(3):257-298.
17. Stalder JF, Fleurym M, Sourissem M, Allavoinem T, Chalametm C, Brosset P, *et al*. Comparative effects of two topical antiseptics (chlorhexidine vs KMnO4) on bacterial skin flora in atopic dermatitis. Acta Derm-Venereol Suppl (Stockh) 1992;176: 132-134.
18. mpcb.gov.in – Biomedical waste management guidelines 2016.
19. R Core Team . R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2017.
20. Mohamed MA, Siddig EE, Elaagip AH, Edris AMM, Nasr AA. Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. Ann Clin Microbiol Antimicrob 2016;15:17.
21. Damen JG, Banwat EB, Egah DZ, Allanana JA. Parasitic Contamination of Vegetables in Jos, Nigeria. Ann African Med 2007;6:115-118.
22. Bekele F, Tefera T, Biresaw G, Yohannes T. Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. Infect Dis Poverty 2017;6:19.
23. Sia SuGL, Mariano CMR, Matti NSA, Ramos GB. Assessing parasitic infestation of vegetables in selected markets in Metro Manila, Philippines. Asian Pac J Trop Dis 2012;2(1):51-54.
24. Haburchak DR. Medical Parasitology. Austin (Texas): Landes Bioscience;2009.p38.
25. Eraky MA, Rashed SM, Nasr ME, El-Hamshary AM, Salah El-Ghannam A. Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. Journal of parasitology research. 2014;2014.
26. Adjrah Y, Karou DS, Djeri B, Anani DB, Soncy K, Ameyapoh K, *et al*. Hygienic quality of commonly consumed vegetables and perception about disinfecting agents in Lomé. Int Food Res J 2011;18(4):1499-1503.
27. Söderqvist K, Osman OA, Wolff C, Bertilsson S, Vågsholm I, Boqvist S. Emerging microbiota during cold storage and temperature abuse of ready to-eat salad. Infect Ecol Epidemiol 2017;7:1-9.
28. Al-Kharousi ZS, Guizani N, Al-Sadi AM, Al-Bulushi IM, Shaharoona B. Hiding in fresh fruits and vegetables: opportunistic pathogens may cross geographical barriers. International journal of microbiology. 2016;2016.
29. Nguyen C, Carlin F. The microbiology of minimally processed fresh fruits and vegetables. Crit Rev Food Sci Nutr 1994; 34: 371-401.
30. Satpathy G, Tyagi YK, Gupta RK. Removal of Organophosphorous (OP) Pesticide Residues from Vegetables Using Washing Solutions and Boiling. J Agric Sci 2012;4(2):69-78.
31. Hanski I, Von Hertzen L, Fyhrquist N, Koskinen K, Torppa K, Laatikainene T, *et al.* Environmental biodiversity, human microbiota, and allergy are interrelated. Proc Natl Acad Sci U.S.A. 2012;109:8334–8339.
32. Gram L, Ravn L, Rasch M, Bruhn J B, Christensen A B, Givskov M. Food spoilage–interactions between food spoilage bacteria. Int J Food Microbiol 2002; 78: 79-97.
33. Flores GE, Bates ST, Caporaso JG, Lauber CL, Leff JW, Knight R, *et al.* Diversity, distribution and sources of bacteria in residential kitchens. Environ Microbiol 2012;15(2):588-596.