Al-Musawi et al | Article 8544
J Pure Appl Microbiol. 2023;17(2):1214-1220. doi: 10.22207/JPAM.17.2.51
Received: 03 March 2023 | Accepted: 12 May 2023
Published Online: 31 May 2023

JPAM JOURNAL OF PURE AND APPLIED MICROBIOLOGY
P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Fecal Coliform Bacteria in Vegetable Salads Prepared in Baghdad Restaurants

Adil Turki Al-Musawi[1]*, Raafat Ahmed Abu-Almaaly[1] and Haider Shannon Kareem[2]

[1]Department of Commodity Evaluation and Service Performance, Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq.
[2]Central Public Health Laboratory, Ministry of Heath, Iraq.

Abstract

This study aimed to evaluate the presence of coliforms in 50 samples (25 ready-to-eat vegetable salads and 25 handlers' hands) collected randomly from restaurants in Baghdad. The total coliform count in the samples of vegetable salads from Al-Sadria and Hay al-Amel reached 4.78 and 4.32 log cfu/g, respectively, whereas those in the swab samples of handlers' hands from the same areas reached 3.70 and 3.90 log cfu/g, respectively. The percentages of fecal coliform bacteria in the salad samples from Al-Sadria and Hay al-Amel were 35% and 32%, respectively, whereas those in the hand swabs from the same areas were 41% and 36%, respectively. Two isolates of the serotype *Escherichia coli* O157:H7 were detected in the study samples from the same areas, where the rates of *E. coli* and fecal coliform bacteria increased. Considering the virulence of this bacterial serotype and its direct impact on consumer safety, we highly recommend implementing quality programs in ready-to-eat vegetable salad production chains, raising the cultural level and health awareness of restaurant owners and workers preparing these salads, and raising public awareness of the potential health risks of consuming contaminated food products.

Keywords: Vegetable Salads, Workers' Hands, Fecal Coliform Bacteria, Baghdad Restaurants

***Correspondence:** adilalmusawi@mracpc.uobaghdad.edu.iq

Citation: Al-Musawi AT, Abu-Almaaly RA, Kareem HS. Fecal Coliform Bacteria in Vegetable Salads Prepared in Baghdad Restaurants. *J Pure Appl Microbiol.* 2023;17(2):1214-1220. doi: 10.22207/JPAM.17.2.51

© The Author(s) 2023. **Open Access**. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License which permits unrestricted use, sharing, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

The World Health Organization (WHO), through its specialists in food, nutrition, and public health, has given special attention to the basic nutritional elements and components related to human health and safety, emphasizing the need for nutrients that are eaten for growth, bodybuilding, and health maintenance.[1] Vegetables are an important part of a healthy diet. Leafy vegetables, such as lettuce, spinach, cabbage, kale, and bok choy, and diet rich in fresh produce prevent certain chronic diseases, such as diabetes, cancer, cardiovascular disease, hypertension, and obesity.[2,3]

Vegetables contain a high percentage of vitamins and minerals, such as iron and calcium; it is also rich in fiber, which aids in the digestion and metabolism of the body. If we take into account the simple and quick preparation steps and this is characterized by its availability in the market for the cheap price of its primary food requirements.[4]

However, vegetables have become a major concern worldwide for facilitating the growth of microorganisms and parasites that cause food poisoning and diseases and the transmission of infectious diseases that threaten public health.[5]

Fresh vegetables can be contaminated with microorganisms through several mechanisms, such as using soil contaminated with animal waste and irrigation water contaminated with wastewater; other sources of contamination are related to agricultural practices, such as the fertilizers and pesticides used and the harvesting, storage, and transportation techniques employed.[6] Eighty-five multistate outbreaks linked to fresh produce occurred from 2010 to 2017. These events have been largely attributed to cross-contamination within the distribution chain and poor agricultural practices, along with the production of sprouts and importation of fresh produce.[7]

The method of display and sale in markets and use of poor-quality vegetables and spices for preparing restaurant salads are other reasons for food contamination.[8]

The outbreak of many diseases caused by bacteria, viruses, and parasites is linked to the consumption of various types of vegetables and even fruits. Fresh vegetables that are not heat treated may be contaminated with pathogenic bacteria, such as *Salmonella* spp., *Shigella* spp., *Shiga* toxigenic *Escherichia coli, Listeria monocytogenes,* and *Campylobacter* spp.[7] A previous study found that fresh spinach, lettuce, and tomatoes used in some fast food restaurants in California are contaminated with *Salmonella* and *E. coli*, particularly the virulent O157:H7 serotype.[9] These studies suggest that the contamination of some frozen foodstuffs with pathogenic bacteria is linked to poor food handling and preparation by workers. The U.S. Food and Drug Administration (FDA) Food Safety Modernization Act, signed into law in 2011, allows the FDA to not only respond to food contamination events and foodborne outbreaks but also to focus on prevention-based strategies.[10]

This study aimed to investigate the presence of fecal coliform (FC) bacteria on food handlers' hands and its association with the microbiological contamination of ready-to-eat vegetable salads in Baghdad restaurants.

MATERIALS AND METHODS

Chemicals

The following chemicals were used: phosphate buffered saline (PBS; BDH Company, UK), peptone water (PW), MacConkey agar medium, tryptic soy broth (TSB), and eosin methylene blue (EMB) agar, cefixime tellurite sorbitol MacConkey (CT-SMAC) agar, and tryptic soy agar (TSA). Except for PBS, all other reagents were supplied by Hi-media (India). Gram staining was performed using the set supplied by SYR Bio Company (Switzerland).

Sample Modeling

Fifty samples, including 25 samples of ready-to-eat vegetable salads and 25 swab samples of handlers' hands, were collected randomly from restaurants in various areas (Mansour, Karrada, Kadhimiya, Palestine Street, Al-Sadriya, and Hay Al-Amel) in Baghdad from September to October 2021.

After collection, the 25 samples of salads were placed in sterile plastic bags in a cool box and then transported to the laboratory. The 25 swab samples were placed inside sterile tubes containing 3 ml of PBS and then transported to the laboratory in a refrigerated box. The modeling process was carried out with three replications, with a period not exceeding 2–4 h from the time of sample collection.

Microbial Examinations

Sample Activation

The microbial examinations of the vegetable salad were carried out inside a sterile laminar air flow cabin. The culture media used in each examination were sterilized and prepared following manufacturer's instructions. Tests were conducted as previously described.[11] Samples (10 g) were transferred to stomacher bags containing 90 ml of PW.

The samples were mixed at a speed of 2000 rpm for 2 min to reach a dilution of 10^{-1} and then incubated for 24 h.

Total Coliform Count

Following a method previously described,[1] a series of dilutions up to 10^{-8} was conducted by transferring 0.1 ml of the initial 10^{-1} dilution into pre-sterilized tubes containing 9.9 ml of physiological saline, transferring 0.1 ml of the 10^{-6} dilution to the prepared MacConkey agar medium, and spreading it evenly with a sterile L-shape vector. The dishes were incubated at a temperature of 37°C for 24 h. Coliform bacteria were identified by their pink colonies fermenting lactose and counted as colony-forming units per ml (CFU/ml). Colorless colonies, which indicate that the bacteria did not ferment lactose, were neglected.

Isolation of FC

A 1 ml aliquot of the initial dilution was transferred to pre-sterilized glass tubes containing 9 ml of TSB and then incubated at 37°C for 24 h. With the use of a sterile vector, it was spread on EMB agar and then incubated at 37°C for 24 h.[3] A differential medium was used because *E. coli* appear in small colonies with a metallic blue–green color.

Isolation of *E. coli* O157:H7

E. coli O157:H7 was isolated following a previously described method.[12] Colonies were selected and subjected to diagnostic tests for serotyping. They were distinguished from other strains of non-sorbitol-fermenting *E. coli* by growing them on CT-SMAC agar. Hand swab samples were cultured directly on fortifying culture media, including blood base agar supplemented with 7% sheep blood and TSA. Mixed colonies and those positive for Gram-negative bacteria were independently collected and spread through the stripping method on blood and MacConkey agar media and then incubated at 37°C for 24 h. The process was repeated several times to further purify the colonies, which were then transferred to differential culture media for later use in biochemical tests and serotyping.

Statistical Analysis

The Statistical Analysis System[13] program was used to determine the effect of different factors on the vegetable salad samples. Least significant difference and analysis of variation were used to compare the significance between means.

RESULTS AND DISCUSSION

As shown in Table 1, the salad samples from the different areas significantly differed ($P \leq 0.05$) in the logarithm of the total count of coliform bacteria (log cfu/g). Specifically, the total coliform counts in the salad samples from Al-Sadria and Al-Amel neighborhood reached 4.78 and 4.32 log cfu/g, respectively, whereas those in the hand swab samples in the same areas were 3.70 and 3.90 log cfu/g, respectively. In Mansour and Palestine Street, the total coliform counts in the salad samples were 1.35 and 1.37 log cfu/g, respectively, whereas those in the hand swab samples were 1.10 and 1.15 log cfu/g, respectively. The hygiene, health control, preparation method, presentation, and compliance to health measures in restaurants all influence the presence of pollutants and pathogens in food, including salads.[14] A previous study[6] indicated that the methods of dealing with vegetables by sellers and restaurant workers clearly affect the percentage of fresh lettuce contamination with microorganisms. In fact, the coliform counts are lower (2.04–3.00 log cfu/g) in lettuce washed twice with sterile running water than in unwashed lettuce (2.84–3.60 log cfu/g). Other studies in Cameroon[4] and India[1] indicated that fresh, unwashed vegetables contain large numbers of coliform bacteria. The first study recorded 2.99 log cfu/g in green turnips, 2.78 log cfu/g in red turnips, 3.06 log cfu/g in lettuce, and 2.85 log cfu/g in carrots, whereas the second study recorded 3.17 log cfu/g in tomatoes, 3.21 log cfu/g in carrots, 3.32 log cfu/g in turnip greens, and 3.24 log cfu/g in cucumbers. Moreover, another study[15] found that coliform counts are greater when displaying unwashed fresh vegetables in open retail stores (4.11 log cfu/g) than when displaying them after washing and drying in supermarkets (1.26 log cfu/g).

Table 1. Total count of coliform bacteria (log cfu/g) in ready-to-eat salads and hand swabs of workers in sampling areas

Sample collection areas	Coliform total count (log cfu/g)	
	Ready-to-eat salads	Workers hand swabs
Mansour	1.35 ±0.07 c	1.10 ±0.05 c
Karrada	2.20 ±0.11 c	1.90 ±0.12 bc
Kadhimiya	3.45 ±0.19 b	2.35 ±0.17 b
Palestine street	1.37 ±0.04 c	1.15 ±0.08 c
Al-Sadria	4.78 ±0.27 a	3.70 ±0.19 a
Hay Al-Amel	4.32 ±0.20 ab	3.90 ±0.23 a
LSD value	1.073 *	0.996 *

Means having with the different letters in same columns differed significantly. * (P≤0.05)

As shown in Table 2, the contamination rates of fecal coliform bacteria in the vegetable salad and hand swab samples significantly differed (P ≤ 0.05) depending on the area where the samples were collected. Specifically, the contamination rates in the salad samples from Al-Sadriyah and Al-Amel districts were 35.93% and 32.81%, respectively, whereas those in the hand swab samples from the same areas were 41.46% and 36.58%, respectively. These rates were higher than those in the other study areas. In addition, the total number of fecal coliform bacteria in Al-Sadriyah and Al-Amel exceeded the permissible limits of 1×10^2 cfu/g in fresh vegetables for human consumption as recommended by the Iraqi specification for microbial limits.[16]

This result can be ascribed to the fact that the water used during cultivation or harvesting can be highly contaminated with fecal matter from sewage or to poor hygiene.[17] A previous study[18] attributed the increased contamination rate to the diversity of pollution methods for vegetables and fruits, which include the use of organic waste in agricultural lands as fertilizers; contamination of irrigation water with fecal matter; direct contamination by livestock, wild animals, and birds; and post-harvest issues, such as worker hygiene, transport containers, and harvesting equipment.

E. coli bacteria are among the most isolated bacteria from food, and their presence indicates product contamination with feces.[10]

Previous studies[15,19] found that the method of displaying and handling vegetables

Table 2. Percentage of fecal coliform bacteria in ready-to-eat salad samples and workers' hand swabs in sample collection areas

Sample collection areas	Ready-to-eat salads		Workers hand swabs	
	No. of isolates	Percentage (%)	No. of isolates	Percentage (%)
Mansour	2	3.12 d	0	0 d
Karrada	5	7.81 d	3	7.31 cd
Kadhimiya	11	17.18 c	6	14.63 c
Palestine street	2	3.12 d	0	0 d
Al-Sadria	23	35.93 b	17	41.46 b
Hay Al-Amel	21	32.81 b	15	36.58 b
Total	64	99.97 a	41	99.98 a
LSD value	---	11.378 *	---	10.861 *

Means having with the different letters in same columns differed significantly. * (P≤0.05).

clearly influences the rate of contamination with fecal coliform bacteria. Specifically, the rates are higher in open shops (4.11 and 4.63 log cfu/g) than in closed shops (1.26 and 3.35 log cfu/g).

As shown in Table 3, significant differences (P ≤ 0.05) in the percentage of *E. coli* O157:H7 were found depending on the area where the samples were collected. Five isolates of *E. coli* O157:H7 were obtained in the salad samples collected from Kadhimiya, Al-Sadriya, and Hay Al-Amel, with percentages of 20%, 40%, and 40%, respectively. Meanwhile, four isolates were obtained from the hand swab samples from Al-Sadriya and Hay Al-Amel, each with a percentage of 50%. All isolates were confirmed to be *E. coli* O157:H7 following previously described diagnostic methods.[12] All the isolates identified to be *E. coli* O157:H7 were distinguished by their inability to ferment sorbitol, colorless to pale circular shape in CT-SMAC medium, small size and smoothness, and pink appearance on MaconConkey agar medium due to their ability to ferment lactose.

A previous study[20] reported that CT-SMAC medium is commonly used to isolate *E. coli* O157:H7 because of its biochemical characteristics, including its inability to ferment sorbitol, compared to more than 90% of *E. coli* bacteria that can ferment this sugar. Moreover, this medium is fast, cheap, and simple to use. Adding rhamnose sugar to this medium has contributed to increasing the transparency of pale colonies due to the inability of serotype O157:H7 to ferment this sugar. Moreover, O157:H7 is resistant to tellurite, which can affect other types of colored bacteria when added in the form of salts to the medium.

Table 3. Percentage of *E. coli* O157:H7 bacteria in ready-to-eat salad samples and workers' hand swabs in sampling areas

Sample collection areas	Ready-to-eat salads		Workers hand swabs	
	No. of isolates	Percentage (%)	No. of isolates	Percentage %
Mansour	0	0 d	0	0 c
Karrada	0	0 d	0	0 c
Kadhimiya	1	20 c	0	0 c
Palestine street	0	0 d	0	0 c
Al-Sadria	2	40 b	2	50 b
Hay Al-Amel	2	40 b	2	50 b
Total	5	100 a	4	100 a
LSD value	--	14.54 *	---	16.79 *

Means having with the different letters in same columns differed significantly. * (P≤0.05).

Consistent with the findings of the present study, the results of a previous study[21] showed that the isolation percentages of *E. coli* O157:H7 in ready-to-eat salad samples from shops of Gaza schools in Palestine are 1.2%–2.%. Meanwhile, the isolation percentage of this serotype in fresh cabbage in India is 3.3%.[1]

In a previous study,[15] the isolation rate of this bacteria is 2.59% in vegetables sold in open markets while less than 1.4% in washed and packaged vegetables displayed in closed vegetable stores. Another study[22] found that washing the vegetables used in salads with a saline solution can significantly reduce the proportion of *E. coli* O157:H7; specifically, the contamination rate is 0.12% in vegetables washed with water only, whereas none of these bacteria are isolated in vegetables washed with saline solutions.

Several studies[9,14,23] indicated that the salads are exposed to several sources of contamination, such as the vegetable shops, in which the products are covered with worn-out cloths and sprayed constantly to maintain freshness, the hands of workers who do not follow health conditions and personal hygiene requirements, and cutting and preparation utensils and equipment that were previously used in meat preparation. Flavors and spices with poor quality, damage, or high microbial contamination also play a role in pollution. Moreover, unhygienic display methods, flies and other insects, dust, and other inappropriate conditions contribute to the contamination of food, especially those that are offered fresh to the consumer without being cooked. A previous study[24] reported that high temperatures promote the growth and reproduction of microorganisms in ready-to-eat vegetables, whereas preserving vegetables at 4 and 10°C can minimize the total number of bacteria to 2.212–3.154 log cfu/g. Another study[25] in Nigeria confirmed that the percentage of coliform bacteria isolation increased significantly on the outer surface of vegetables when the summer temperature rose from 20°C to 30°C. Specifically, it increased from 8.3% to 35% in tomatoes, 1.7% to 46.2% in cabbage, and 1.5% to 20% in carrots. Previous research[26] indicated a positive correlation between the total number of fecal coliform bacteria present in vegetables and their number in irrigation water contaminated with wastewater. This study also reported that some vegetables, such as carrots, are less polluted than others, whereas cabbage recorded the highest levels of contamination. Previous studies[4,17] revealed that the presence of pathogens, such as fecal coliform bacteria, especially *E. coli* O157:H7, in vegetables cannot be underestimated because it represents a serious threat to health, especially when these vegetables are consumed raw. Common reasons for the contamination of vegetables with pathogens are either growing them in soil contaminated with animal waste, irrigating them with water contaminated with sewage, or washing them with contaminated water.

CONCLUSION

The study showed the direct potential risks to consumer health of the increased total coliform counts in ready-to-eat salads and hand swabs of food handlers. Coliforms can contaminate, grow, and multiply in vegetables used to prepare the salads offered by restaurants in the study areas. The serotype *E. coli* O157:H7 was observed in some study samples and in the same areas where the rates of *E. coli* and fecal coliform bacteria increased. Considering the virulence of *E. coli* O157:H7 and its direct impact on consumer safety, we highly recommend implementing quality programs in ready-to-eat vegetable salad production chains, raising the cultural level and health awareness of restaurant owners and workers preparing these salads, and raising public awareness of the potential health risks of consuming contaminated food products.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Mritunjay SA, Kumar V. A study on prevalence of microbial contamination on the surface of raw salad vegetables. 3 *Biotech.* 2017;7(1):13. doi: 10.1007/s13205-016-0585-5
2. DenisN, Zhang H Leroux A, Trudel R, Bietlot H. Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. *Food Control.* 2016;67:225-234. doi: 10.1016/j.foodcont.2016.02.047
3. WHO. Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint FAO/WHO Expert Consultation. WHO Technical Report Series. 2003;916. Available from: https://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf;sequence=1 (accessed Februrary 20, 2019).
4. Akoachere J-FTK, Tatsinkou BF, Nkengfack JM. Bacterial

and parasitic contaminants of salad vegetables sold in markets in Fako Division, Cameroon and evaluation of hygiene and handling practices of vendors. *BMC Res Notes.* 2018;11(1):100. doi: 10.1186/s13104-018-3175-2

5. Ava AI, Noor R. Microbiological analysis of commonly consumed vegetable: A review on the ongoing studies. *Qeios.* 2022;17:1-12. doi: 10.32388/2NEQK1
6. Arienzo A, Murgia L, Fraudentali I, Gallo V, Angelini R, Antonini G. Microbiological Quality of Readyto- Eat Leafy Green Salads during Shelf-Life and Home-Refrigeration. *Foods.* 2020;9(10):1421. doi: 10.3390/foods9101421
7. Carstens C K , Salazar J K, Darkoh C. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Front Microbiol*. 2019;10:2667. doi: 10.3389/fmicb.2019.02667
8. Maiwore J, Baane MP, Leopold TN, et al. Microbiological Quality of Lettuce (*Lactuca Sativa*) Consumed on the Streets Maroua (Cameroon): Effect of Disinfecting Agents Used by Some Vendors. *Int J Microbiol Res.* 2017;9(8):913- 918.
9. Mohammed J, Al - Mousawi A T, Abu-Almaaly RA. Investigation of Bacterial Contaminants in Freezers Keeping Frozen Food in Local Markets. *Journal of Market Research and Consumer Protection.* 2017;9(1): 85-91.
10. FDA, Food Safety and Modernization Act (FSMA). Available from: https://www.fda.gov/food/guidance-regulation-food-and-dietary-supplements/food-safety-modernization-act-fsma (accessed May 13, 2019).
11. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis. Microbiological Food Testing. Chapter (17).USA. 2005.
12. Abu-Almaaly R A, Al-Musawi AT. Detection of *Escherichia coli* O157:H7 in Meat and its Products Available in Local Markets. Conference: The 1st International Conference of Biological Science (22-24/09/2015) At: College of Science for Women in the University of Baghdad. *Iraqi Journal of Biotechnology.* 2015;2(14):2015.
13. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.16h ed. SAS. Inst. Inc. Cary. N.C. USA. 2018.
14. Al-Allaf M A, Al-Rawi AM, Shehab AS. Bacteriological Study of some Locally Prepared Salads in some Restaurants in Mosul City. *Rafidain Journal of Science.* 2014;25(4):70-80. doi: 10.33899/rjs.2014.88661
15. Srisamran J, Atwill E R, Chuanchuen R, Jeamsripong A. Detection and analysis of indicator and pathogenic bacteria in conventional and organic fruits and vegetables sold in retail markets. *Food Quality and Safety.* 2022;6:1-10. doi: 10.1093/fqsafe/fyac013
16. IQS. 2270. Microbial limits in foods, Part 7, Microbial limits of vegetables and their products. Central Organization for Standardization and Quality Control (COSQC), Ministry of Planning, Republic of Iraq. 2006. https://old.mop.gov.iq/news/view/details?id=278.
17. Weldezgina D, Muleta D. Bacteriological Contaminants of Some Fresh Vegetables Irrigated with Awetu River in Jimma Town, Southwestern Ethiopia. *Advances in Biology.* 2016;1526764. doi: 10.1155/2016/1526764
18. Maikai BV, Akubo DO. Coliform count and isolation of *Escherichia coli* in fresh fruits and vegetables sold at retail outlets in Samaru, Kaduna State, Nigeria. *Niger Vet J.* 2018;39(4):327- 331. doi: 10.4314/nvj.v39i4.5
19. Abakari G, Cobbina SJ, Yeleliere E. Microbial quality of ready-to-eat vegetable salads vended in the central business district of Tamale, Ghana. *Int J Food Contam.* 2018;5:3. doi: 10.1186/s40550-018-0065-2
20. Seran T, Aysegul E, Sahsene A. Prevalence of *Escherichia coli* O157 in red meat and meat products determined by VIDAS ECPT and Light Cycler PCR. *J. Vet. Anim. Sci.* 2012;36(3):305-310. doi. 10.3906/vet-1107-38
21. Elmanama AA, ArafaM HZ, Abu Owda SM. Bacteriological Quality of Fresh Vegetables Salad Sold in Schools Canteens and Restaurants in Gaza Strip-Palestine. *IUG Journal of Natural Studies.* 2017;25(1):1-12.
22. Coniglio MA , Faro G, Marranzano M. The Importance of the Microbiological Quality of Ready-to-Eat Salads from a Public Health Perspective. *Journal of Food Processing & Technology.* 2016;7(4):1000577. doi: 10.4172/2157-7110.1000577
23. Khalil R, Gomaa M. Evaluation of the microbiological qual¬ity of conventional and organic leafy greens at the time of purchase from retail markets in Alexandria, Egypt. *Pol J Microbiol*. 2014;63(2):237-243. doi: 10.33073/pjm-2014-031
24. Caldera L, Franzetti L. Effect of storage temperature on the microbial composition of ready-to-use vegetables. *Curr Microbiol.* 2014;68:133-139. doi: 10.1007/s00284-013-0430-6
25. Chinakwe EC, Nwogwugwu NU, Ajugwo GC, et al. Coliform bacteria profile of the surface of raw salad vegetables sold in open markets in Owerri metropolis, south eastern Nigeria. *South Asian J Res Microbiol.* 2022;13(2):19-25.doi: 10.9734/sajrm/2022/v13i230293
26. Berhanu L, Abebe M, Gizeyatu A, Berihun G, Teshome D, Walle Z. Evaluation of the Effect of Wastewater Irrigation on the Microbiological Quality of Vegetables in Northeast Ethiopia: Implication for Food-Borne Infection and Intoxications. *Environ Health Insights.*2022;16:1-7. doi: 10.1177/11786302221127856