Journal of Preventive Medicine and Holistic Health 2023;9(2):106–112

Content available at: https://www.ipinnovative.com/open-access-journals

Journal of Preventive Medicine and Holistic Health

Journal homepage: https://www.jpmhh.org/

Original Research Article

Study of *Escherichia coli* and *Klebsiella oxytoca* isolated from edible leafy greens

Ankush Kabiraj[1], Sayan Bhattacharyya[1]*, Shibani Lahiri[1]

[1]*Dept. of Microbiology, All India Institute of Hygiene and Public Health, Kolkata, West Bengal, India*

ARTICLE INFO

Article history:
Received 21-09-2023
Accepted 18-10-2023
Available online 14-11-2023

Keywords:
Enteropathogens
Tulsi
Mint
Spinach

ABSTRACT

Introduction: Tulsi, Mint and Spinach all are leafy greens. They have tremendous medicinal properties thus they are used as culinary herbs or sometimes consumed as raw. Although sometimes they caused health issues due to presence of some enteropathogenic bacteria.
Aims: In this study we are tried to identify the enteropathogens *Escherichia coli* and *Klebsiella oxytoca* through different types of laboratory examinations.
Data collection: We looked for the enteropathogens *Escherichia coli* and *Klebsiella oxytoca* in Tulsi, mint and spinach leaves via different types of laboratory techniques. Many of these leaves contained th3ese pathogens which can be harmful if ingested by the oral route.
Conclusion: Enteropathogenic bacteria can often be found in edible leafy greens and this should be of public health concern.

1. Introduction

Tulsi, mint and spinach all are herbs and very important as well as very beneficial for our health. However sometimes they contain some harmful bacteria and parasites that may cause foodborne infections. Tulsi is sometimes used as divine offering or Prasad. Not many studies have tried to find out the load of pathogenic bacteria, parasitic cysts and ova causing foodborne infections, in these leafy vegetables. In this study we have been tried to find the pathogenic microorganisms present on these herbs and their detrimental effects on the health of consumers.

1.1. Tulsi

Holy basil (*Ocimum sanctum*) is commonly known as *Tulasi* in Sanskrit or *Tulsi* in Hindi.[1] Holy basil is a perennial flowering plant from the family called *Lamiaceae.* While this plant is native to the Indian subcontinent, it may also grow throughout Southeast Asia.[2] Tulsi has a special place in Ayurveda as well as the homes of Hindus in India. It is considered sacred by Hindus and worshipped by them and is also used medicinally to relieve symptoms like cough and cold. Three main types of Tulsi are seen growing in India:

1. Bright green leaves called Ram Tulsi
2. Purplish green leaves called Krishna Tulsi
3. Common wild Vana Tulsi

Tulsi has used as prasad or offering to God and also proved to be highly effective in protecting our body from various infections and diseases of the liver, skin, kidney, respiratory tract and other sites.

Tulsi is known to have many medicinal properties like:

It might be an antipyretic (relieves fever) and anti-inflammatory activity, an antiemetic (prevents vomiting), might help lower the blood sugar (antidiabetic), may act as a hypotensive (lowers blood pressure). It might also have hypolipidemic (lowers cholesterol) activity, might act as an analgesic (relieves pain), may have anti-asthmatic activity.

* *Corresponding author.*
E-mail address: sayantheboss@yahoo.co.in (S. Bhattacharyya).

https://doi.org/10.18231/j.jpmhh.2023.019

It might be an hepatoprotective (liver-protective) agent, may help reduce stress (anti-stress), might be a potent expectorant (expels mucous), It might have anticancer potential and might be a diaphoretic (induces sweating).

Owing to its numerous health benefits, Tulsi is rightly called the 'Queen of Herbs'.[3]

In spite of having several beneficial roles, Tulsi may contain several harmful bacteria like- Enterobacterales (a previous large family of Gram-negative bacteria that includes a number of pathogens such as *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Serratia* spp. and other species), *Pseudomona* s spp[4] and parasites like- *Cyclospora* spp.[5]

1.2. Pudina

Mint, scientifically known as *Mentha spicata*, is an aromatic herb belonging to the family Lamiaceae. It is called Spearmint in English and Pudina in Hindi. It is widely used in Indian kitchen for flavour. The herb originated in Europe.[6] Fresh and dried plant and essential oils derived from Pudina are widely used in the food, cosmetic, confectionery, chewing gum, toothpaste, and pharmaceutical industries.[7] It is grown worldwide for commercial uses as a flavouring agent for food and to be used in medicines.[8]

The properties of the Pudina are given below-

It may act as carminative (for removal of gas from the stomach), may show the antispasmodic property (used to relieve muscle spasms), may show diuretic activity and may show antibacterial property also. It may additionally show antifungal property, act as antioxidant agent (minimize the damage caused by oxidants).[7]

It may show anti-diarrhoeal and anti-asthmatic activity.

It may also act as an antidote (medication given to counteract a particular poison).[8]

There is still no information about the presence of any bacteria and parasites in pudina leaves, so we will search for it.

1.3. Spinach

Spinach (*Spinacia oleracea*) is a green leafy flowering plant native to central and western Asia. It belongs to the family Amaranthaceae. It is consumed either fresh, or after storage using preservation techniques by canning, freezing or dehydration. It may be eaten cooked or raw, and the taste differs considerably; the high oxalate content may be reduced by steaming.[9]

The beneficial properties of spinach are as follows:

1. Promotes weight management (reduce weight gain risk by up to 82%)[10]
2. Reduces cancer risk[11]
3. Protects eye health (rich in Vit A & C which helps to prevent age related degeneration)[12,13]
4. Prevents hair loss (rich in iron)[14]
5. Prevention of anaemia (rick in Vit K & iron)[15–17]
6. High in antioxidants (scavenging free radicals and prevent oxidative stress)[18]

However, spinach is very much beneficial for our health, but a variety of bacteria like- *Salmonella*, *Shigella* etc and parasites like- *Ascaris lumbricoides* eggs, *Entamoeba histolytica* cysts, *Giardia lamblia* cysts may be present in it.[19–25]

Tulsi, pudina and spinach both are used as food as well as medicinal purpose. These bacteria present in these edible leaves may contain many harmful pathogens and they may be multi-drug resistant also. Leafy vegetables like spinach, lettuce and pudina are good sources of dietary fibre and vitamins like Vitamin K. Tulsi (*Ocimum sanctum*) is also used as food rarely and also commonly as offering to God in temples for prasad. Leafy vegetables have many antimicrobial properties and are also good source Vitamin K, Dietary fibers, mineral and Vitamin K. However, they can also contain many pathogenic microorganisms which can infects us by feco-oral route. Many researchers have found parasitic eggs and cysts along with pathogenic bacteria in leafy vegetables. High concentrations of thermotolerant coliforms (ThC), intestinal helminth eggs, and protozoa have been found in a wastewater-fed lake where water spinach was grown. Water spinach samples can contain *Cyclospora* (8%), *Giardia* cysts (56%), *Cryptosporidium* (17%).[26] Many workers have detected Cyclospora in vegetables in the local agricultural markets in the Central Valley of Costa Rica. The highest proportion of faecal coliforms was identified during the rainy season. *Cyclospora* spp. have been identified only in lettuce during the dry season.[27]

In other studies, protozoa *Giardia duodenalis* cysts have been found on lettuce.[27] In fact, fresh produce, in particular, as it is eaten with minimal preparation, is a very strong vehicle for transmission of bacterial and parasitic infections, and *G. duodenalis* cysts have been detected in such produce in many countries.[27]

VTEC (Verotoxigenic *Escherichia coli*) strain, *E. coli* O157:H7 'Sakai,' has also been found to colonize the roots and leaves of four leafy vegetables: spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), vining green pea (*Pisum sativum*), and prickly lettuce (*Lactuca serriola*).[28] This contamination is related to irrigation pattern, use of portable toilets and the field worker personal hygiene. various factors like contaminated soil, fertilizer (manure/compost), wildlife, and irrigation water also contribute to presence of *Escherichia coli* in produce.[29] This can be a dangerous pathogen as it may also cause Haemolytic uremic syndrome (HUS). High burden of *Salmonella* contamination has been seen in leafy vegetables, including mint leaves, parsley and

lettuce.[30] Moreover, these leaves are consumed raw or by making sauce (e.g., Mint leaves).

Spinach leaf has also been found to contain harmful oocysts of coccidia like *Cryptosporidium parvum*.[31] Many foodborne illnesses in the United States are caused by germs on vegetables and fruits that people eat raw. Harmful germs sometimes found on leafy greens include E. coli**,** norovirus, Salmonella, Listeria, and Cyclospora.[32] The enteropathogenic bacteria present in these edible leafy greens may be multi-drug resistant also, which should also be studied because in case of gut infection due to these pathogens, empirical antibiotics are needed and one needs to know about the extant susceptibility pattern.

So keeping these things in mind, there are lots of pathogenic bacteria and parasites found in these leaves which can infect us and we tried to assess the burden of *Escherichia coli* and *Klebsiella oxytoca* in these edible leaves.

2. Materials and Methods

2.1. Time of study

Beginning of May 2023 to end of September 2023 (5 months)

2.2. Place of study

Department of Microbiology, BN campus, All India Institute of Hygiene and Public Health, Kolkata.

2.3. Type of study

Laboratory based observational study.

2.4. Sample size

One hundred and twenty one leaf samples (121) were collected from the open and online markets of different zones in Kolkata (east, west, north & south) and if possible, from other places of West Bengal. Sample size was 120, calculated by method of convenience.

2.5. Methodology proper

Samples was collected in sterile universal container or if packed, then in packet. Then samples were transported to the laboratory for testing. Samples was weighed and specific weight or volume was cultured on the following media.

1. MacConkey agar with neutral red as pH indicator (constituents:- Peptone, Neutral red, Agar agar, Lactose, Sodium taurocholate, deionized water) for culturing aerobic LF or NLF bacteria.
2. Xylose Lysine Deoxycholate (XLD) media {Agar, Sucrose, Lactose, Sodium thiosulphate, L-Lysine, NaCl, xylose, Yeast extract, Sodium deoxycholate, Ferric ammonium citrate} for culturing aerobic bacteria like *Shigella* and *Salmonella* spp.
3. Robertson's cooked meat medium (RCM) {Cooked meat medium, Peptic digest of animal tissue, Dextrose, Sodium chloride, yeast extract, Iron filings, Hemin, Vitamin K} for culturing anaerobic bacteria. RCM has been incubated for 2 days and then Gram stain has been done to find anaerobic bacteria.
4. Selenite F broth has been used for enrichment and later after 1 day of incubation at 37 degree C, 1 has been subcultured from it on to XLD agar and MacConkey agar again. This has been done to find *Shigella* spp. and *Salmonella* spp.
5. Normal saline and Lugol's Iodine has been used to detect the trophozoites and cysts of protozoa, and egg & larvae of helminths by making wet mount.

One gram of dry sample was mixed in 1 ml normal (0.9%) saline in a sterile universal container, vortexed and transferred to 10 ml test tube. Then it has been centrifuged at 5000 rpm for 2 minutes, and with deposit, saline mount, Lugol's Iodine mount and Gram stain also done. Modified ZN (Ziehl Neelsen) stain has been carried out with 4% Sulphuric acid (H_2SO_4) as decolorizer Also culture on the aforesaid media has been performed from the deposit. The colonies of the bacteria that has been appeared on the culture media after overnight incubation, has been identified by Gram stain, Catalase, Oxidase and other standard biochemical tests.

Also, antibiotic susceptibility was checked for some of these bacterial isolates of different species, by Kirby-Bauer's disk diffusion method, by using the following antibiotic disks: -

1. Cotrimoxazole: - 25 μg.
2. Tetracycline: - 30 μg
3. Ciprofloxacin: - 5 μg.
4. Ampicillin: - 10 μg

3. Results

The following tables show the presence of Escherichia coli and Klebsiella oxytoca in Tulsi, spinach and mint.

Table 1: Tulsi leaves

Escherichia coli	**Klebsiella oxytoca**
Sample no. AK 69	Sample no. AK43
Sample no. AK 72(Indole negative)	-
Sample no. AK 81	-

So, we carried out antibiotic susceptibility of 3 isolates by Kirby-Bauer's disk diffusion method. One *Klebsiella oxytoca* isolate was MDR or Multidrug resistant (resistant to 3 or more different classes of antibiotics). Among these *E.coli* isolates all were indole positive except one.

Figure 1: Image of ordinary Tulsi leaf. Source: author

Figure 2: Image of krishna/black Tulsi leaf. Source: internet

Figure 3: Spinach leaf.
Source: https://www.amazon.in/Fresh-Produce-Palak-Leaf-Bunch/dp/B01G6OOMQG0pt)

Figure 4: Mint/pudina leaf.
Source: **https://www.goodhousekeeping.com/health/diet-nutrition/a47308/health-benefits-mint/**

Table 2: Mint leaves

Escherichia coli	**Klebsiella oxytoca**
AK 111(EHEC:0157:H7)	AK 3
AK 112	AK 30
-	AK 110

Table 3: Spinach leaves

Escherichia coli	Klebsiella oxytoca
AK 34(non EHEC O157:H7)	AK 27
-	AK 85
-	AK 116
-	AK121

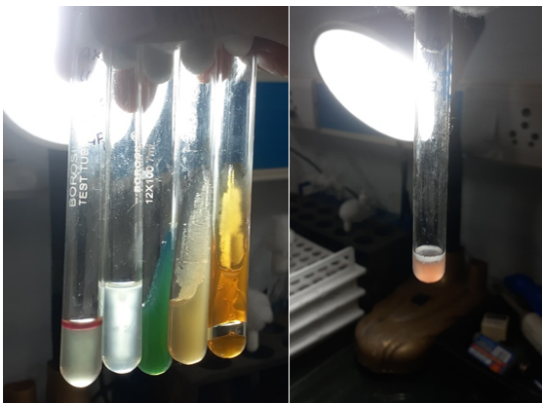

Figure 5: Biochemical reactions of *Escherichia coli* (EHEC O157:H7) from Mint

Figure 6: Biochemical reactions of *Escherichia coli* (Indole Negative) from Tulsi

Figure 7: Biochemical reactions of *Klebsiella oxytoca* from Spinach

Table 4: Data about antibiotic resistance of *E. coli* and *K. oxytoca* **:-**

Sample	Name of the Bacteria	Sensitive to Antibiotics	Resistant to Antibiotics
AK110 (Mint)	*Klebsiella oxytoca*	-	Cotrimoxazole, Ciprofloxacin, Ampicillin, Tetracycline
AK111(Mint)	*Escherichia coli* (EHEC: O157:H7)	Cotrimoxazole, Ciprofloxacin, Tetracycline	Ampicillin
AK116(Spinach)	*Klebsiella oxytoca*	Cotrimoxazole, Ciprofloxacin, Tetracycline	Ampicillin

4. Discussion

Green leafy vegetables are an excellent source of nutrients like Vitamins A, C, E, D, B Complex and iron. They are also a very good source of folate which is necessary food good heart health. These nutrients present in vegetables also have antioxidant effects which help to reduce the risk of cancer.[33] However, they also may contain many pathogenic microorganisms like bacteria, oocysts of protozoa and ova of helminths. Sufficient fresh vegetable consumption is an essential dietary need for individuals worldwide. Due to the changes in dietary habits, the propensity to consume raw vegetables is on the rise. In particular, spinach is noted to be an excellent source of many different

nutrients, like phenolic compounds, beta carotene, vitamin C, and Potassium. However, fresh greens also allow the growth of various pathogenic bacteria, namely *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella spp*., and *Shigella spp*., because of their wide surface area and potential for pathogen internalization in structures like the stomata. Additionally, in 2008, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) ranked leafy green vegetables as the highest anxiety product group from microbial safety perspective.[34] Furthermore, many outbreaks related to foodborne pathogens like ***E. coli*** O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in fresh spinach have been reported recently (34). In 2006, multiple outbreaks of *E. coli* O157:H7 infection associated with contaminated baby spinach resulted in 205 confirmed cases of serious illnesses, 31 cases of haemolytic uremic syndrome (HUS), and three deaths Hence if we consume these leafy vegetables raw or unprepared, it can spread many gut infections caused by bacteria, protozoa as well as helminths. These are of great public health importance. In a study from the USA, common bacteria retrieved from fresh cut spinach were Pseudomonas fluorescens, Aeromonas caviae and *Staphylococcus xylosus*.[35] However, no pathogens such as Listeria monocytogenes and *Salmonella* could be detected. In this study, the microorganisms were not present on the surface of healthy unbroken leaves. Antibiotic resistance also has been studied for the bacterial isolates, which is important from clinical and one health viewpoint. This is a neglected area of public health research and hence the findings of this study should be very importance. Pseudomonas aeruginosa isolates were found in all three types of samples. These leaves or samples can be consumed raw, and hence there is a risk of gastrointestinal discomfort arising due to *Pseudomonas aeruginosa* in the consumers. *Pseudomonas aeruginosa* has been found to be an important cause of gastritis. Baby spinach is rich in Calcium, Folate, Iron, Magnesium, Phosphorus, Vit A, Vit $B_{6,}$ Vit C, Vit K, Vit E and Zinc. This is usually consumed as salad with apples and others fruits hence there is a risk of acquiring infections due to enteropathogens.[36] Green Leafy vegetables (Tulsi, Mint and Spinach) grown in organic and conventional systems were collected from various areas in Korea. These leafy vegetables were analysed for levels of indicator bacteria (aerobic bacteria, coliforms, and Escherichia coli) and the prevalence of the pathogens Staphylococcus aureus, E. coli O157:H7, Listeria monocytogenes, Bacillus cereus, and Salmonella. Aerobic bacteria and coliforms were detected in all vegetable types, but non-pathogenic E. coli was below the limit of detection in all samples. B. cereus was the most prevalent pathogen, found in spinach samples.[37] We have isolated *Pseudomonas aeruginosa* from many leaf samples. These isolates were mostly susceptible to the antibiotics tested. These implies that in cases of possible gastroenteritis due to *Pseudomonas aeruginosa* occurring after ingesting such leaves, the antibiotic that we tested can be used for empirical therapy.

We found that bacteria like *Proteus vulgaris, Proteus mirabilis, Stenotrophomonas maltophilia, Shewanella putrefaciens, Edwardsiella tarda, Plesiomonas shigelloides, Delftia tsuruhatensis, Staphylococcus epidermidis, Edwardsiella tarda and Oligella urethralis* were not found in Tulsi leaves. This means that Tulsi can have antibacterial effect against these bacteria. We found that bacteria like *Lactococcus* spp., *Bacillus cereus, Clostridium* non-*perfringens, Leclercia* spp., *S. epidermidis* and *Oligella* spp. were not found in Mint leaves. This means that Mint can have antibacterial effect against these bacteria.

As far as we know, this type of study has not been carried out this part of India but this study should be of tremendous public health importance. *E. coli* and *Klebsiella oxytoca* are hence commonly found in the edible leafy greens, and can rarely be multi drug resistant also. More such studies are needed.

5. Conclusion

Hence at the end of the study we have been delineate the pathogenic microorganisms like *E. coli* (even EHEC O157:H7)and *Klebsiella oxytoca* which can be present in Tulsi, Spinach and Pudina, which will usher in a new vista of public health research in food microbiology.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Jamshidi N, Cohen MM. The clinical efficacy and safety of tulsi in humans: a systematic review of the literature. *Evid Based Complement Alternat Med*. 2017;p. 9217567. doi:10.1155/2017/9217567.
2. Petruzello M. holy basil. Available from: https://www.britannica.com/plant/holy-basil.
3. Garg P. Tulsi (Holy Basil): Health Benefits, Uses and Nutritional Value. Available from: https://pharmeasy.in/blog/health-benefits-of-tulsi/.
4. Wick AR. Basil - Pseudomonas. Available from: https://ag.umass.edu/greenhouse-floriculture/photos/basil-pseudomonas#:~:text=Pseudomonas%20cichorii%20causes%20a%20bacterial.
5. Friedman LF. Basil From Mexico Is Likely Cause of Cyclospora Food Poisoning Outbreak. Available from: https://www.consumerreports.org/food-safety/basil-from-mexico-is-likely-cause-of-cyclospora-food-poisoning-outbreak/.
6. Mentha spicata (Mint, Spearmint) | North Carolina Extension Gardener Plant Toolbox. Available from: https://plants.ces.ncsu.edu/plants/mentha-spicata/.
7. Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, De Feo V. Mentha spicata Essential Oil: Chemical Composition, Antioxidant and Antibacterial Activities against Planktonic and Biofilm Cultures of Vibrio spp. Strains. *Molecules*. 2015;20(8):14402–24. Available from: https://pubmed.ncbi.nlm.nih.gov/26262604/.

8. Mahendran G, Verma SK, Rahman LU. The traditional uses, phytochemistry and pharmacology of spearmint (Mentha spicata L.): A review. *J Ethnopharmacol*. 2021;278:114266. doi:10.1016/j.jep.2021.114266.
9. Available from: https://en.m.wikipedia.org/wiki/Spinach.
10. Nour M, Lutze SA, Grech A, Farinelli MA. The relationship between vegetable intake and weight outcomes: A systematic review of cohort studies. *Nutrients*. 2018;10(11):1626. doi:10.3390/nu10111626.
11. Vaňková K, Marková I, Jašprová J. Chlorophyll-mediated changes in the redox status of pancreatic cancer cells are associated with its anticancer effects. *Oxid Med Cell Longev*. 2018;p. 4069167. doi:10.1155/2018/4069167.
12. National Institutes of Health Office of Dietary Supplements. Vitamin A: Fact sheet for health professionals. Available from: https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/.
13. Age-related macular degeneration. Available from: https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/age-related-macular-degeneration.
14. Park SY, Na SY, Kim JH, Cho S, Lee JH. Iron plays a certain role in patterned hair loss. *J Korean Med Sci*. 2013;28(6):934–42.
15. Bai X, Long J, He X, Yan J, Chen X, Tan Y, et al. Overexpression of spinach non-symbiotic hemoglobin in Arabidopsis resulted in decreased NO content and lowered nitrate and other abiotic stresses tolerance. *Sci Rep*. 2016;6:26400. doi:10.1038/srep26400.
16. National Institutes of Health Office of Dietary Supplements. Iron: Fact sheet for health professionals. Available from: https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/.
17. National Institutes of Health Office of Dietary Supplements. Vitamin K: Fact sheet for health professionals . Available from: https://ods.od.nih.gov/factsheets/VitaminK-HealthProfessional/.
18. Pizzino G, Irrera N, Cucinotta M. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev*. 2017;p. 8416763. doi:10.1155/2017/8416763.
19. Abadias M, Usall J, Anguera M, Solsona C, Vinas I. Microbiological quality of fresh minimally processed fruit and vegetables, and sprouts from retail establishments. *Int J Food Microbiol*. 2008;123:121–9.
20. Oliveira MA, Oliveira VM, Souza AMM, Bergamini ECP. Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control*. 2011;22:1400–3.
21. Tambekar DH, Mundhada RH. Bacteriological quality of salad vegetable sold in Amravati city (India). *J Biol Sci*. 2006;6(1):28–30.
22. Bello ABA, El-Shewy CS, Abdulla K. The prevalence of parasites in commonly used leafy vegetables in South Western Saudi Arabia. *Saudi Med J*. 2006;27:613–6.
23. Daryani A, Ettehad GH, Sharif M, Ghorbani L, Ziaei H. Prevalence of intestinal parasites in vegetables consumed in Ardabil. *Iran Food Control*. 2008;19:790–4.
24. Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control*. 2010;21(5):760–2.
25. Fallah AA, Pirali-Kheirabadi K, Shirvani F, Saei-Dehkordi SS. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Food Control*. 2012;25:617–20.
26. Ortega YR, Sanchez R. Update on Cyclospora cayetanensis, a food-borne and waterborne parasite. *Clin Microbiol Rev*. 2010;23(1):218–52.
27. Cook N, Nichols RA, Wilkinson N, Paton CA, Barker K, Smith HV. Development of a method for detection of Giardia duodenalis cysts on lettuce and for simultaneous analysis of salad products for the presence of Giardia cysts and Cryptosporidium oocysts. *Appl Environ Microbiol*. 2007;73(22):7388–91.
28. Crozier L, Hedley PE, Morris J, Wagstaff C, Andrews SC, Toth I, et al. Whole-Transcriptome Analysis of Verocytotoxigenic Escherichia coli O157:H7 (Sakai) Suggests Plant-Species-Specific Metabolic Responses on Exposure to Spinach and Lettuce Extracts. *Front Microbiol*. 2016;12(7):1088. doi:10.3389/fmicb.2016.01088.
29. Park S, Navratil S, Gregory A. Generic Escherichia coli contamination of spinach at the preharvest stage: effects of farm management and environmental factors. *Appl Environ Microbiol*. 2013;79(14):4347–58.
30. Sarré FB, Dièye Y, Seck AM, Fall C, Dieng M. High Level of Salmonella Contamination of Leafy Vegetables Sold around the Niayes Zone of Senegal. *Horticulturae*. 2023;9(1):97. doi:10.3390/horticulturae9010097.
31. Macarisin D, Bauchan G, Fayer R. Leaf Stomata Harboring Cryptosporidium parvum Oocysts: a Potential Threat to Food Safety. *Appl Environ Microb*. 2010;76(2):555–9.
32. Available from: https://www.cdc.gov/foodsafety/foods-linked-illness.html#:~:text=Leafy%20greens%20are%20sometimes%20contaminated.
33. Yan L. Available from: https://www.ars.usda.gov/plains-area/gfnd/gfhnrc/docs/news-2013/dark-green-leafy-vegetables#:~:text=Dark%20green%20leafy%20vegetables%20are.
34. Jae JY, Won J. Combined treatment of UV-A radiation and acetic acid to control foodborne pathogens on spinach and characterization of their synergistic bactericidal mechanisms. *Food Control*. 2019;106:106698. doi:10.1016/j.foodcont.2019.06.024.
35. Babic I, Roy S, Watada AE, Wergin WP. Changes in microbial populations on fresh cut spinach. *Int J Food Microbiol*. 1996;31(1-3):107–19.
36. Kachuei V, Abadi TB, Rahimi A, Forootan F. Colonization by Pseudomonas aeruginosa and Staphylococcus aureus of Antral Biopsy Specimens from Gastritis Patients Uninfected with Helicobacter Pylori. *Infect Drug Resist*. 2020;13:1411–7.
37. Zelman KM. Health Benefits of Spinach; 2023. Available from: https://www.webmd.com/diet/health-benefits-baby-spinach#:~:text=You%20can%20enjoy%20baby%20spinach.

Author biography

Ankush Kabiraj, Student of MSc Applied Nutrition

Sayan Bhattacharyya, Associate Professor https://orcid.org/0000-0001-7741-0866

Shibani Lahiri, Director Professor

Cite this article: Kabiraj A, Bhattacharyya S, Lahiri S. Study of *Escherichia coli* and *Klebsiella oxytoca* isolated from edible leafy greens. *J Prev Med Holistic Health* 2023;9(2):106-112.