GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 06 | Issue: 03

On 30th September 2020

http://www.research.lk

Author: Piyumi Buddhika Dissanayake, Supeshala Kotalawala

(Business Management School, Sri Lanka)

GARI Publisher | Microbiology | Volume: 06 | Issue: 03

Article ID: IN/GARI/ICID/2020/101 | Pages: 19 - 30 (11)

ISSN 2659-2193 | ISBN 978-955-7153-02-5

Edit: GARI Editorial Team | Received: 20.08.2020 | Publish: 30.09.2020

DETERMINATION OF ESCHERICHIA COLI IN SPINACIA OLERACEA (SPINACH) BY CULTURE, BIOCHEMICAL TESTS AND CHROMOGENIC MEDIA

[1]Piyumi Buddhika Dissanayake, [2]Supeshala Kotalawala

Department of Biomedical Science, BMS, Sri Lanka

[1]piyumi30.1996@gmail.com, [2]supeshala@bms.edu.lk

ABSTRACT

The consumption of leafy vegetables such as Spinacia oleracea (Spinach) has been expanded in recent years due to the healthy life style recommendations. However, spinach is highly susceptible to cause foodborne diseases as it is usually consumed raw or minimally cooked. To prevent foodborne diseases, it is required to limit bacterial contamination during the pre-harvesting and post-harvesting time periods. This study is mainly focused on detecting the presence of Escherichia coli in spinach samples because E. coli is the most common bacteria involved for causing wide range of infections. For this study, twenty-one fresh spinach samples were collected from local markets and supermarkets located at Maharagama, Boralesgamuwa, Dehiwala and Nugegoda areas of Sri Lanka. The samples were analysed by culturing on selective McConkey agar and biochemical tests, then cultured on chromogenic media for further confirmation of E. coli. As found from this study, 15.91% of E. coli were present in the purchased samples of all four regions. The highest E. coli percentage was identified from Dehiwala (31%) and E. coli were not present in the samples purchased from Nugegoda. Moreover, Citrobacter spp. (22.73%), Klebsiella spp. (22.73%), Erwinia spp. (20.45%), Enterobacter spp. (15.91%) and Serratia spp. (2.27%) were detected from the samples of all four regions. Although, samples were purchased from local markets and supermarkets under different storage conditions, significant difference was not observed in bacterial distribution. The hygiene and environmental conditions and handling practices had a direct impact for contaminating spinach with bacteria.

Key words: Spinach, Escherichia coli, Culture

INTRODUCTION

The foodborne diseases manifest infectious or toxic nature in the body due to the entering of harmful agents through the ingestion of food or water (Gunasekara, Baumann and Muralitha, 2017). Food borne diseases caused due to the consumption of contaminated vegetables are frequently occur in developing nations like Sri Lanka. However, outbreaks are usually get undetected due to the absence of food borne disease examinations and standard diagnostic measures in Sri Lanka (De Silva, Abayasekara and Dissanayake, 2013). The amount of food borne outbreaks related to vegetables have been expanded in recent years due to healthy life style recommendations. It also influenced by changes in vegetables cultivation and processing practices. Considerable numbers of foodborne disease outbreaks have been aroused due to the consumption of minimally processed fresh leafy vegetables such as

spinach (Smith, Fratamiko and Gunther, 2014).

Spinacia oleracea (spinach) is generally considered as a useful leafy green vegetable due to its different nutritional components, which comprises vitamins, minerals, phytochemicals and bio-actives that provide health benefits beyond the basic nourishment (Roberts and Moreau, 2016). However, spinach is vulnerable to get contaminated with foodborne pathogens due to animal faeces contaminated fertilizer and water suppliers utilized to irrigate crops (Yanamala et al., 2011). Usually spinach is consumed raw or minimally cooked and contain less hindrance against microbial growth as not much amount of salt or food preservatives are added while preparing, thus contaminated spinach is highly susceptible to cause foodborne illnesses (Mritunjay and Kumar, 2015). Considering these reasons, this study will be performed by using Spinacia oleracea as the sample to detect certain food borne pathogens including Escherichia coli, which is a type of bacteria provide signs of faecal contamination in water and food samples (Hoang, Quy and Chi, 2018).

The Escherichia coli was first isolated by the German scientist Theodor Escherich in 1885. It is gram-negative, rod-shaped and lactose fermenting bacterium (Mondal, 2013). E. coli is a facultative anaerobic bacterium, but it unable to survive at extreme temperatures and pH levels. These microorganisms benefit its mammalian host by producing vitamin K and vitamin B12 (Blount, 2015). E. coli is a major inhabitant of the gastrointestinal tract of humans and animals. Most E. coli strains are harmless but few strains are pathogenic and they are causing bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection, severe diarrhoea, hemorrhagic colitis and hemolytic-uremic syndrome in human beings. Furthermore, E. coli is associated with enterobacteriaceae family and has a close correlation with pathogenic bacteria such as Klebsiella, Enterobacter, Serratia and Citrobacter which also referred as coliform bacteria due to shared properties (Guentzel, 1996).

According to the reports, developed countries reflect less mortality rates compared to developing countries (Croxen et al., 2013). The main reason for this could be better food processing practices carried out in developed countries, for example; in most of the developed countries classify spinach cultivars in to three groups as savoy, semi-savoy and flat or smooth leaved based on the varieties in the leaf blade. After that, savoy and semi savoy cultivars are subjected for processing while smooth leaved cultivars are directed to the market due to the simplicity of cleaning, but such categorization or processing is not properly carried out in developing countries such as Sri Lanka (Carder et al., 2010). Despite of maintaining well systematized processing methods, in 2006 and 2010 two major foodborne disease outbreaks were occurred in US due to the consumption of pathogenic E. coli contaminated spinach, causing number of hospitalizations and deaths. Hence, there is a higher risk for arising food borne diseases due to the consumption of leafy vegetables in developing countries including Sri Lanka as the processing practices are not efficient to prevent foodborne outbreaks (Grant et al., 2008; Leonard et al., 2015).

This study determines lactose fermenting bacteria by culturing on MacConkey selective media and then suspicious colonies are subjected to series of biochemical tests according to the Bergey's manual to identify E. coli and other lactose fermenting bacteria. Finally, the suspected E. coli colonies will be cultured on chromogenic media to confirm the presence of Escherichia coli.

METHODOLOGY

Sample Collection

Twenty-one Spinacia oleracea samples were collected to sterile zip lock bags from different local markets and supermarkets around Colombo (Maharagama, Boralesgamuwa, Nugegoda, Dehiwala), Sri Lanka. The properly sealed Spinacia oleracea samples were transported to the laboratory within 24 hours and did the sample preparation in the same day.

All steps were carried out under sterile environment using sterile equipment.

Sample Preparation

A 11.2g of spinach was measured and finely chopped aseptically. Then extract was filtered through muslin cloth and added 100ml of peptone broth, thereby 50ml was added to a labelled falcon tube and incubated at 37°C for 16-18 hours. The same procedure was followed to extract all the samples.

MacConkey Agar Plates Preparation and Streaking

A 500ml of MacConkey agar medium was prepared and autoclaved. The medium was poured in to labelled petri dishes while gently stirring and allowed to solidify. Prior to the streaking, inoculation loop was kept in the flame until red hot. After cooling down, a loop full of extracted sample was taken and quadrant streaking was done on the agar plate. Agar plates were streaked with different extracted spinach samples and incubated at 37°C for 18 hours. After observing bacterial growth, the plates were stored at 4°C.

Subculture in Nutrient Broth

A 5ml of autoclaved nutrient broth was added to twenty-six labelled falcon tubes (15ml). Then one or two dark pink colonies with different morphologies were selected from each petri dish and sub cultured in nutrient broth under sterile conditions and incubated at 37°C for 24 hours.

Biochemical Tests for Lactose Fermenting Bacteria

According to the Bergey's manual isolated pure, lactose fermenting bacterial samples were subjected for following biochemical tests to identify E. coli and other bacteria types.

Kovac's Indole Test

A 5ml of autoclaved peptone broth was added to each of twenty-six test tubes. The grown colonies in the nutrient broth were obtained aseptically and respectively introduced into the each labelled test tubes. The samples were incubated at 37°C for 24 hours. As the final step, 1-2ml of Kovac's indole reagent was added to each sample and the results were noted.

Simmon's Citrate Test

A 5ml of autoclaved citrate agar was added to each of twenty-one test tubes and slanted on a support media until get solidified. The inoculation loop was kept in the flame until red hot and allowed to cool. Afterwards, the bottom of the slants was streaked with bacterial samples and incubated at 37°C for 24 hours. The results were observed after the incubation.

Methyl Red and Voges Proskauer (MR-VP) Test

A 5ml of autoclaved MRVP broth was added to each of five test tubes. After that, grown colonies in the nutrient broth were aseptically obtained and respectively introduced into the each labelled test tubes. The samples were incubated at 37°C for 24 hours. Afterwards, 1ml of the broth was removed to separate test tubes for VP testing. Remaining 4ml of the broth were re incubated for an extra two days for the methyl red test. Then, nine drops of 5% α-neophthol reagent was added to 1ml of broth and gently mixed. Afterwards, three drops of 40% potassium hydroxide was added to 1ml of broth and shaken the tube gently for 30 seconds and results were observed.

After 48 hours of incubation, two drops of methyl red were added to 4ml of broth

and shaken gently. The results were interpreted immediately.

Lysine Decarboxylase Test

A 5ml of autoclaved lysine decarboxylase broth was added to a labelled test tube. The bacteria were aseptically obtained from the nutrient broth and introduced to the test tube. Then a thin layer of mineral oil was poured on to the lysine decarboxylase broth and incubated at 37°C for 48 hours. The colour change was observed every 24 hours.

Chromogenic Media Preparation and Streaking

Autoclaved highrome medium was poured aseptically to sterilized petri dishes and left to solidify. The plates were divided to two regions and labelled the sample code and the date. Afterwards, aseptically bacteria were obtained from two citrate negative bacteria samples and streaked on each region according to zigzag pattern. Then, all the plates were incubated at 37°C for 24hours and results were interpreted after the incubation.

RESULTS

Colony Morphology Exhibited on MacConkey Agar

Figure 1. Colonies formed on MacConkey agar.

Lactose fermenting bacterial colonies appear pink in colour. Different shades of pink colonies with different textures were observed on all the petri plates. When selecting colonies for biochemical tests, priority was given for dark pink coloured, dome shaped colonies as E. coli strains grown on the MacConkey agar usually show bright pink colonies with dome shaped appearance (Barcella, Barbaro and Rogolino, 2016).

Biochemical Tests

Kovac's Indole Test

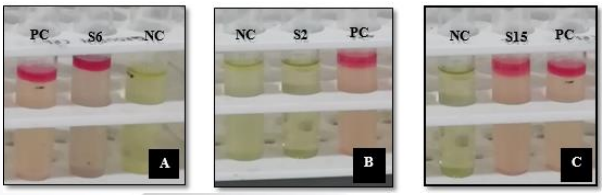

Figure 2. Positive control (PC), negative control (NC) and three samples (A2-S6, B2-S2, C2-15) results of indole test.

The positive control formed a pink colour layer on top of the peptone broth and negative control formed a yellow colour layer on top of the peptone broth upon the addition of Kovac's indole reagent. Therefore, S6 and S15 were positive, S2 was negative.

Simmon's Citrate Test

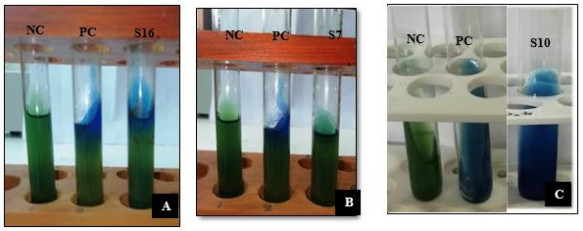

Figure 3. Positive control (PC), negative control (NC) and three samples (A3-S16, B3-S7, C3-S10) results of citrate test.

The slant of the positive control turned to blue colour and the slant remained green colour in the negative control. Therefore, S10 and S16 were positive, S7 was negative.

Voges Proskauer Test

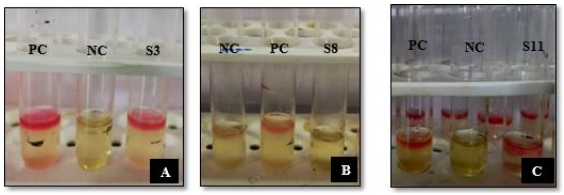

Figure 4. Positive control (PC), negative control (NC) and three samples (A3-S3, B3-S8, C3-S11) results of voges proskauer test.

The positive control formed a pinkish red colour band at the interface and the negative control formed a yellow colour band at the interface. Therefore, S3 and S11 were positive, S8 was negative.

Methyl Red

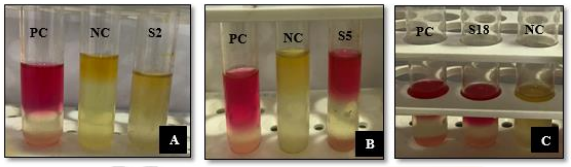

Figure 5. Positive control (PC), negative control (NC) and four samples (A2-S2, B2-S5, C2-18) results of methyl red test .

The positive control formed a bright red colour band at the interface and the negative control formed a yellow colour band at the interface. Therefore, S5 and S18 were positive and S2 was negative.

Lysine Decarboxylase Test

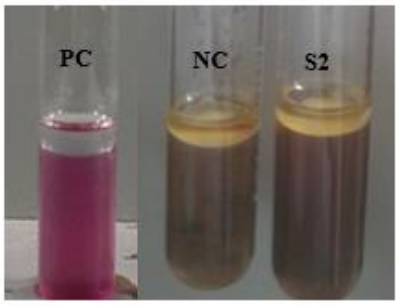

Figure 6. Positive control (PC), negative control (NC) and S2 sample results of lysine decarboxylase test.

The positive control turned to purple color and the negative control turned to brown color upon the incubation. Therefore, S2 was negative.

Chromogenic Media

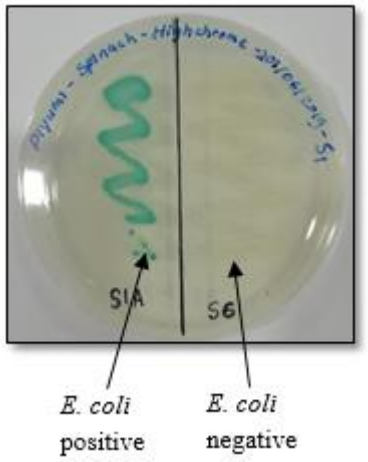

Figure 7. 'A' shows an E. coli positive result on chromogenic media eliciting the presence of green colonies. 'B' shows an E. coli negative result on chromogenic media eliciting the absence of green colonies

Figure 8. The results of few samples which yielded positive results on the chromogenic agar confirming the presence of E. coli in samples S13, S15, S19 and S21.

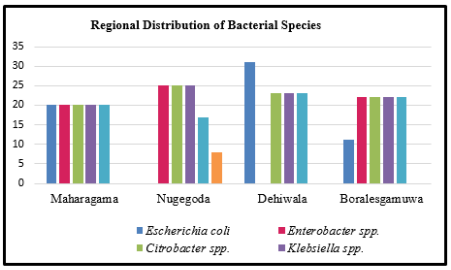

Figure 9. Regional distribution of bacterial contamination in *Spinacia oleracea.*

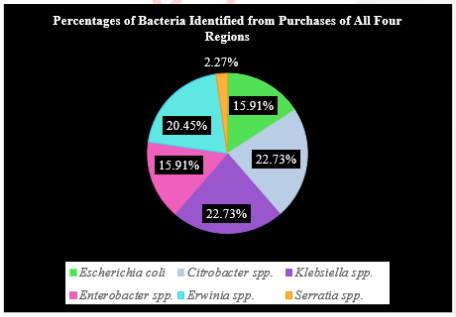

Figure 10. Percentages of bacteria identified from purchases of all four regions.

DISCUSSION

All the spinach samples were collected from the southern part of Colombo metropolitan which is an urbanized environment, but samples were at different storage conditions when purchasing from local markets and supermarkets. Supermarkets are indoors, normally air conditioned to maintain a particular temperature and vegetables are arranged properly in the shelves. Local markets are typical open air markets where food is sold at ambient temperature without covering. As suggested by Sudershan et al., (2012), highly crowded and urbanized areas in developing countries are more prone to bacterial contaminations due to the polluted water and poor hygienic conditions, this was further proven from this study as the bacteria distributions were considerably higher in all four study regions. Moreover, spinach leaves were used for this study because literature suggest that spinach phylloplane (leaf surface) is more prone to retain microorganisms (Mitra, 2009).

According to this study, different bacteria types were detected from all 21 samples, hence this study emphasises that although the consumption of green leafy vegetables such as spinach is important for healthy life, it could contaminate with different varieties of food-borne pathogens including E. coli. As revealed from the study, highest Escherichia coli distribution (31%) was identified from the samples purchased from Dehiwala and Escherichia coli were not detected from the samples purchased from Nugegoda. Moreover, Escherichia coli (15.91%), Citrobacter spp. (22.73%), Klebsiella spp. (22.73%), Erwinia spp. (20.45%), Enterobacter spp. (15.91%) and Serratia spp. (2.27%) were detected from the purchases of all four regions.

The green leafy vegetables can get contaminated with microorganisms either in pre-harvesting period or post-harvesting period. Pathogenic microorganisms establish on growing crops in the pre-harvesting period, then hazard can be enhanced during the post-harvesting period due to further direct contamination. In the pre-harvesting stage, leafy vegetables can be contaminated due to irrigation water, human handling, contaminated containers and animal waste fertilizers. In the post-harvesting stage, leafy vegetables can be contaminated due to improper storage, post-harvest washing, improper packaging and contaminations from other foods in food preparation area (Mritunjay and Kumar, 2015). Outbreaks were reported in 2002 and 2006 from Salinas and Salleys San Juan valleys of California, United States due to the consumption of E. coli 0157 contaminated spinach, through the investigations it was recognised that outbreaks were mainly implicated due to sewage released from the farms in those areas (Cooley et al., 2017).

In Sri Lanka, usually untreated irrigation water and sewage fertilizer are used for cultivations and it could be a main reason for contaminating leafy vegetables with bacteria at the pre-harvesting stage (De Silva, Abayasekara and Dissanayake, 2013). Some studies have discovered that pathogenic E. coli contaminated water dropped on the spinach leaves directly contribute to uptake and internalization of pathogen and it also reveals that pathogen is unable to internalize to the spinach plant through contaminated soil (Mitra et al., 2009). It means, post harvesting washing also plays a major role in both contamination and pathogenesis of food-borne bacteria. Rathnasiri and Manage, (2015), demonstrated a study to evaluate water quality in South Colombo region and revealed that almost all the wells in this particular area are contaminated with faecal coliform bacteria. As the vendors usually use well water for cleaning and sprinkling vegetables, there is a high possibility to contaminate vegetables with coliforms.

When purchasing spinach especially from local markets, they were under wet condition and the water sprinkled on to the spinach could be contaminated. Also, spinach leaves were bundled and kept along with other vegetables which enhances susceptibility to contaminate from other vegetables. Although, samples were purchased from local and supermarkets under different storage conditions, significant difference was not observed in bacterial distribution through this study. However, Ananchiapattana et al., (2012), has carried out a Thailand based study and recovered 44% and 15% of E. coli respectively from the leafy vegetable samples collected from local markets and supermarkets representing considerable difference in percentages. Therefore, it can be suggested that bacteria presentation would be drastically changed by place to place with their hygienic and environmental conditions. Normally, majority of the leafy vegetables supplied to the local and supermarkets in South Colombo area through Dambulla and Pettah whole sale market at where have poor sanitary conditions. Therefore, there is a high chance to contaminate spinach even at the post-harvesting stage. (Perera, Kodithuwakku and Weerahewa, 2004).

Escherichia coli, Enterobacter spp, Citrobacter spp, Klebsiella spp and Erwinia spp. were detected from the samples purchased from Maharagama, Boralesgamuwa, Nugegoda and Dehiwala. The Serratia spp. was found only from the samples purchased from Nugegoda. According to studies, Serratia spp. are rarely isolated from the environmental sources such as vegetables. The Serratia spp. are sporadically perceived as a reason for medical clinics acquired infections (Khanna, Khanna and Aggarwal, 2013). Bacterial soft rot of spinach could observe from certain leaves of the samples. Erwinia spp. is the one of the main bacteria type involved for causing soft rot of vegetables during harvesting or storing at ambient temperature (Rawat, 2015).

From the samples collected from Dehiwala, 31% of Escherichia coli, 23% of Citrobacter spp., 23% of Klebsiella spp. and 23% of Erwinia spp. were identified (Figure 10). E. coli distribution was higher in Dehiwala compared to other regions. The Dehiwala markets were close to drainage lines with polluted water and there were lot of flies around the vegetable cages. Insects are also a possible cause of bacterial contamination. Some studies have shown that contaminated flies are able to directly transfer bacteria to vegetables or green leaves (Fels-Klerx, 2018). Escherichia coli is a type of bacteria which provide signs of faecal contamination in water and leafy vegetables, it means spinach samples were directly or indirectly exposed to faeces. Poor hygiene of vendors also could

contribute for the contamination of spinach (Hoang, Quy and Chi, 2018).

This study suggests that spinach should be properly cleaned with clean water and disinfectants and appropriately cooked before consumption to avoid food-borne diseases. Usually, people use salt solutions, vinegar, and turmeric water as disinfectants to clean green leafy vegetables while cooking, but according to previous studies, these common disinfectants have low efficacy to eliminate faecal coliforms (Subramanya, 2018). However, Amoah et al., (2007) has found that 0.001% Potassium permanganate solution is an effective disinfectant to reduce bacterial load in ready to eat green leafy vegetables.

CONCLUSION

According to the results obtained from biochemical tests and chromogenic media, fresh ready-to-eat spinach were highly contaminated with Escherichia coli (15.91%), Klebsiella spp (22.73%), Citrobacter spp (22.73%), Erwinia spp (20.45%) and Enterobacteria spp (15.91%). Therefore, it can be concluded that spinach should be properly cleaned before consumption with strong disinfectants such as very diluted 0.001% Potassium permanganate to reduce the risk of bacterial infections. The main objectives of this study were accomplished successfully, but further tests can be carried out to identify the presence of other bacterial species in spinach including non-lactose fermenting bacteria such as Salmonella spp.

REFERENCES

Amoah, P., Drechsel, P., Abaidoo, R. and Klutse, A. (2007) 'Effectiveness of common and improved sanitary washing methods in selected cities of West Africa for the reduction of coliform bacteria and helminth eggs on vegetables', Tropical Medicine & International Health, 12, pp.40-50, [Online]. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3156.2007.01940.x (Accessed: 22 August 2019).

Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongsawat, S., Latiful, B.M., Isobe, S. and Inatsu, Y. (2012) 'Prevalence of foodborne pathogens in retailed foods in Thailand', Foodborne Pathogens and Disease, 9(9), pp.835-840, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/22953752 (Accessed: 21 August 2019).

Becerra, S.C., Roy, D.C., Sanchez, C.J., Christy, R.J. and Burmeister, D.M. (2016) 'An optimized staining technique for the detection of gram positive and gram negative bacteria within tissue', BMC Research Notes, 216, [Online]. Available at: https://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-016-1902-0 (Accessed: 22 August 2019).

Blount, Z.D. (2015) 'The unexhausted potential of E. coli', eLife, 4, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4373459/ (Accessed: 21 August 2019).

Carder, P.A., Ponder, M.A., Welbaum, G.E., Williams, R.C. and Freeman, J.H. (2010) 'Microbial communities of spinach at various stages of plant growth from seed to maturity', [Online]. Available at: https://pdfs.semanticscholar.org/9ba7/1adbe04befae640f26ec986ced6488f46d35.pdf (Accessed: 22 August 2019).

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J. and Mandrell, R.E. (2007) 'Incidence and tracking of Escherichia coli O157:H7 in a major produce production region in California', PLoS One, 2(11), pp.1159, National Center for

Biotechnology Information, [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2174234/ (Accessed: 24 August 2019).

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B. (2013) 'Recent advances in understanding enteric pathogenic Escherichia coli', Clinical Microbiology Reviews, 26(4), pp.822-880, [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3811233/ (Accessed: 21 August 2019).

De Silva, A., Abayasekara, C. and Dissanayake, D. (2019) 'Freshly Eaten Leafy Vegetables: A Source of Food Borne Pathogens?', Ceylon Journal of Science, 42(2), pp.95-99, [Online]. Available at: https://pdfs.semanticscholar.org/365c/121f9c31731e785c207587b598ff97449b26.pdf (Accessed: 23 August 2019).

Fels-Klerx, H., Camenzuli, L., Belluco, S., Meijer, N. and Ricci, A. (2018) 'Food Safety Issues Related to Uses of Insects for Feeds and Foods', Comprehensive Reviews in Food Science and Food Safety, 17(5), pp.1172-1183, [Online]. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/1541-4337.12385 (Accessed: 23 August 2019).

Grant, J., Wendelboe, A.M., Wendel, A., Jepson, B., Torres, P., Smelser, C. and Rolfs, R.T. (2008) 'Spinach-associated Escherichia coli O157:H7 outbreak, Utah and New Mexico, 2006', Emerging Infectious Diseases, 14(10), pp.1633-1636, National Center for Biotechnology [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2609868/ (Accessed: 21 August 2019).

Guentzel, M.N. (1996) Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus, Medical Microbiology, [Online]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK8035/ (Accessed: 23 August 2019).

Gunasekera, U., Muralithas, M. and Baumann, M. (2017) 'Food safety monitoring and surveillance system for Sri Lanka', Sri Lanka Veterinary Journal, 63(2), pp.31, [Online]. Available at: https://www.researchgate.net/publication/312548036_Food_safety_monitoring_and_surveillance_system_for_Sri_Lanka (Accessed: 21 August 2019).

Hoang, H. A., Quy, N.T.C. and Chi, N.V.T. (2018) 'Detection of Escherichia coli in ready-to-eat fresh vegetables using broad-host-range recombinant phages', Journal of Applied Microbiology, 124(6), pp.1610-1616, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/29341389 (Accessed: 23 August 2019).

Jeddi, M.Z., Yunesian, M., Gorji, M.E., Noori, N., Pourmand, M.R. and Khaniki, G.R.J. (2014) 'Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets', Journal of Health, Population and Nutrition, 32(3), pp.391-399, National Center for Biotechnology Information, [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4221445/ (Accessed: 21 August 2019).

Khanna, A., Khanna, M. and Aggarwal, A. (2013) 'Serratia Marcescens- A rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients', Journal of Clinical and Diagnostic Research, 7(2), pp.243-246, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3592283/ (Accessed: 24 August 2019).

Leonard, S., Mammel, M., Lacher, D. and Elkins, C. (2015) 'Application of Metagenomic Sequencing to Food Safety: Detection of Shiga Toxin-

Producing Escherichia coli on Fresh Bagged Spinach', Applied and Environmental Microbiology, 81(23), pp.8183-8191, [Online]. Available at: https://aem.asm.org/content/81/23/8183 (Accessed: 25 August 2019).

Mitra, R., Cuesta-Alonso, E., Wayadande, A., Talley, J., Gilliland, S. and Fletcher, J. (2009) 'Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen Escherichia coli O157:H7 in spinach', Journal of Food Protection, 72(7), pp.1521-1530, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19681281 (Accessed: 21 August 2019).

Mondal, A. (2013) 'Isolation and characterization of Escherichia coli from raw vegetables', pp.9-82, [Online]. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.916.504&rep=rep1&type=pdf (Accessed: 21 August 2019).

Mritunjay, S.K. and Kumar, V. (2015) 'Potential hazards of microbial contamination associated with raw eaten salad vegetables and fresh produces', Middle-East Journal of Scientific Research, 23(4), pp.741-749, [Online]. Available at: https://pdfs.semanticscholar.org/bf48/bb9d729e1a8b3f31ff006dc8545e09eacd97.pdf?_ga=2.32185721.613169018.1564640536-1971077110.1564513998 (Accessed: 23 August 2019).

Perera, M., Kodithuwakku, S. and Weerahewa, J. (2011) 'Analysis of Vegetable Supply Chains of Supermarkets in Sri Lanka', Sri Lankan Journal of Agricultural Economics, 6(1), pp.67, [Online]. Available at: https://www.researchgate.net/publication/237447199_Analysis_of_Vegetable_Supply_Chains_of_Supermarkets_in_Sri_Lanka (Accessed: 21 August 2019).

Rathnasiri, P.A.S.A. and Manage, P.M. (2015) 'Evaluation of groundwater quality in five grama divisions of Maharagama urban area using groundwater quality index (WQI)', International Journal of Multidisciplinary Studies, 2(2), [Online]. Available at: https://pdfs.semanticscholar.org/60c4/cebb6a85335e1307a8fd9bfe2789c5fd3db9.pdf (Accessed: 22 August 2019).

Rawat, S. (2015) 'Food Spoilage: Microorganisms and their prevention', Asian Journal of Plant Science and Research, 5(4), pp.47-56, [Online]. Available at: http://www.imedpub.com/articles/food-spoilage-microorganisms-and-their-prevention.pdf (Accessed: 23 August 2019).

Roberts, J. L.and Moreau, R. (2016) 'Functional properties of spinach (Spinacia oleracea L.) phytochemicals and bioactives', Food & Function, [Online]. Available at: https://pubs.rsc.org/en/content/articlelanding/2016/FO/C6FO00051G#!divAbstract (Accessed: 21 August 2019).

Smith, J., Fratamico, P. and Gunther, N. (2014) 'Shiga Toxin-Producing Escherichia coli', Advances in Applied Microbiology, 86, pp.145-197, Science Direct [Online]. Available at: https://www.sciencedirect.com/science/article/pii/B9780128002629000032 (Accessed: 21 August 2019).

Subramanya, S.H., Pai, V., Bairy, I., Nayak, N., Gokhale, S. and Sathian, B. (2018) 'Potassium permanganate cleansing is an effective sanitary method for the reduction of bacterial bioload on raw Coriandrum sativum', BMC Research Notes, 11, pp.124, National Center for Biotechnology Information [Online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5812110/ (Accessed: 22 August 2019).

Sudershan, R., Naveen Kumar, R., Kashinath, L., Bhaskar, V. and Polasa, K. (2019)

'Microbiological Hazard Identification and Exposure Assessment of Poultry Products Sold in Various Localities of Hyderabad, India', The Scientific World Journal, 2012, pp.1-7, [Online]. Available at: https://www.hindawi.com/journals/tswj/2012/736040/ (Accessed: 23 August 2019).

Yanamala, S., Miller, M., Loneragan, G., Gragg, S. and Brashears, M. (2011) 'Potential for microbial contamination of spinach through feedyard air/dust growing in close proximity to cattle feedyard operations', Journal of Food Safety, 31(4), pp.525-529, [Online]. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1745-4565.2011.00330.x (Accessed: 21 August 2019).