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Antibiotic Resistance of *Escherichia coli* Strains from Leafy Vegetables

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Abstract: Leafy vegetables habour microorganisms and may serve in vehicle transmission of diarrhoeal causing *Escherichia coli* strains. This study investigated the antibiotics susceptibility of *E. coli* strains from six (6) leafy vegetables retailed in the two major markets around Joseph Ayo Babalola University, Ikeji Arakeji, Osun State. The *E. coli* were isolated using defined substrate technique (DST) and characterized by morphological, biochemical and molecular techniques. Their antibiotic susceptibilities were tested using Kirby-Bauer's disc diffusion method. Multiple Antibiotic Resistance (MAR) index was determined for the isolates and the vegetables. Eighteen (18) strains of *Escherichia coli* were isolated, and their identities confirmed by molecular characterization (PCR technique). Eight (8) of them were on vegetables from Ipetu Ijesa market and ten (10) on vegetables from Owena Ijesa market. *E. coli* was found most frequently on *Amaranthus hybridus* and *Solanecio biafrae* (50% of samples) and least frequently on *Talium triangulare* (10% of samples). Vegetables from Owena Ijesa market generally haboured higher *E. coli* populations than vegetables from Ipetu Ijesa market. All *E. coli* strains isolated had MAR index greater than 0.2, and two of them had MAR index of 1.0. *Telfairia occidentalis* from Owena market had the highest MAR index (0.9) however, the two markets had similar MAR index (0.6). The presence of multidrug resistant *E. coli* strains on retailed vegetables portends a serious challenge in managing infections due to consumption of the fresh vegetables and highlights the need to properly decontaminate fresh leafy vegetables before consuming them.
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Introduction

Consumption of fresh vegetables has increased in the last two decades, primarily because they are recognized as important sources of vitamins, nutrients and fibre (Olaimat and Holley, 2012). A lot of leafy vegetables are eaten raw as salad with mild washing or in soups with mild heating. Vegetables can become contaminated with organisms at any of several stages in the value chain; from growing in fields to harvesting, during postharvest handling, during distribution (sales), and processing (Rai *et al*., 2007; Davis and Kendall, 2012; Haute *et al*., 2015; Luna-Guevara *et al*., 2019). Surfaces of leafy vegetables easily get colonized by microbes, and rinsing may not be enough to remove bacteria which adhere firmly to them. Therefore, fresh leafy vegetables can serve in vehicle transmission of foodborne pathogens such as pathogenic *E. coli* strains (Delaquis *et al*., 2007; Shaw *et al*., 2011).

Consumption of fresh vegetables has been associated with increased number of foodborne outbreaks and the pathogen most often implicated is *Escherichia coli* (Warriner *et al*., 2009; Luna-Guevara *et al*., 2019). In 2006, several cases of foodborne illness in US were attributed to *E. coli* contamination of lettuce and spinach (Sela *et al*., 2009). Warriner and Namvar (2010) reported that several cases of foodborne illness in Canada in 2008 were attributed to *E. coli* contamination of lettuce. Normally harmless *E. coli* can acquire specific virulence factors through mobile genetic elements and evolved into pathogenic *E. coli* strains (Waturangi *et al*., 2019). This can be complicated if the genetic elements also confer antibiotic resistance on the microorganism.

Drug resistance by pathogens is one of the problems in contemporary management of infectious diseases. To assess the potential risk associated with leafy vegetables retailed in the two major markets around Joseph Ayo Babalola University, *E. coli* strains were isolated from selected leafy vegetables retailed in the markets using defined substrate technique, they were characterized and identified using molecular technique and their antibiotic susceptibility determined.

Materials and Methods

Six (6) types of leafy vegetables: *Telfairia occidentalis* (Ugu), *Amaranthus hybridus* (Efo tete spinach), *Crassocephalum crepidioides* (Ebolo), *Talinum triangulare* (Gure), *Corchorus olitorius* (Ewedu) and *Solanecio biafrae* (Worowo) were purchased from five (5) different sellers in each of the two major markets: Owena-Ijesa market (7.51°N 4.88°E) and Ipetu-Ijesa market (7.43°N 4.91°E) around Joseph Ayo Babalola University, Ikeji Arakeji (7.36°N 5.10°E).

The leaves of the vegetables were rinsed with 50ml sterile saline to dislodge organisms on them. *E. coli* was isolated from the vegetables using defined substrate techniques (DST). The suspension was serially diluted (tenfold) using sterile distilled water and 1 mL of the 10^{-3} and 10^{-4} dilutions were plated out using EMB agar and Sorbitol- MacConkey agar which are selective for *E. coli*. The plates were incubated at the 37°C for 24 hours and inspected for colonies. Colonies with characteristic greenish metallic sheen were counted and enumerated as population of *E coli* on the vegetable.

Suspected *E coli* isolates were purified by subculturing on Nutrient agar and stored on Nutrient agar slant. The isolates were initially characterized on cultural morphology, cellular morphology and biochemical reactions (Cheesbrough, 2010 and Benjamin *et al.,* 2018). Their identities were subsequently confirmed using molecular technique (PCR and NCBI Blast). Suspensions of pure cultures of the isolates were prepared and standardized to 0.5 McFarland standard. The susceptibility of the *E. coli* isolates to various antibiotics were determined by the Kirby-Bauer disk diffusion method using Muller-Hinton agar (Lim *et al.,* 2007).

Results

Some of the vegetables haboured large populations of *Escherichia coli*, with one having counts as high as 5.2×10^4 cfu/g, while some did not carry any *E. coli*. Vegetables from one of the retailers in Owena were found to generally habour high populations of *E. coli* (Figure 1). *Escherichia coli* strains were isolated from 30% of the vegetables which included all types of the leafy vegetable. *E. coli* was most frequently found on *Solanecio biafrae* from Ipetu Ijesa market (60%), and *Amaranthus hybridus* from Owena Ijesa market (60%). *E. coli* was not isolated from *Talinum triangulare* from Ipetu Ijesa market and *Telfairia occidentalis* from Owena Ijesa market. *E. coli* was found on all the vegetables purchased from one of the retailers, while it was not found on vegetables purchased from three of the retailers (Table 1). Eighteen (18) strains of *E. coli* were obtained, eight (44%) of them were on vegetables from Ipetu Ijesa market and ten (56%) of them on vegetables from Owena Ijesa market. *Solanecio biafrae* and *Amaranthus hybridus* yielded five strains (28%) each, while *Talinum triangulare* yielded only one (3%) strain (Table 2).

All the *E. coli* strains showed resistance to at least two antibiotics, they varied in their susceptibility to the antibiotics with different multiple antibiotic resistances (MAR) pattern (Table 2). All of the *E. coli* strains were resistant to Cefuroxime, and none of the test antibiotics tested was active against all the strains of *E. coli*. The MAR index of the *E. coli* strains varied from 0.25 (6 strains) to 1.00 (2 strains) (Table 3). The MAR index of the vegetables also varied: *Talinum triangulare* had the lowest MAR index (0.25) and *Telfairia occidentalis* had the highest MAR index (0.88). The two markets had similar MAR index (0.57).

Discussion

The presence of *E. coli* on leafy vegetables as found in this study is consistent with previous works that reported *E. coli* on vegetables. Dutta *et al.* (2014) found *E. coli* in 43% of the vegetable samples they examined. Reuben and Makut (2014) found *Escherichia coli* O157:H7 in 17.5% of the samples they examined. Similarly, Abu-Duhier (2015) reported *E. coli* in 14.3% of the vegetables and fruits they examined from markets in a city of Saudi Arabia. Benjamin *et al.* (2018) also reported that presence of *E. coli* in vegetables from 5 different markets in Kaduna. The presence of *E. coli* on leafy vegetables as found in this study indicates possible fecal contamination and the potential presence of pathogens of fecal origin (Luna-Guevara *et al*. 2019).

The presence of large populations of *E. coli* on the leafy vegetables is likely due the raising of vegetables with irrigation water, which could have contaminated the vegetables as indicated by MacDonald *et al.* (2015) and Jongman and Korsten (2016). Luna-Guevara *et al.* (2019) reported that contaminated water is the most common sources of the shiga toxigenic *E. coli* (STEC) that green leafy vegetables are associated with. Vegetables can also get contaminated with pathogenic *E. coli* from the soil while growing in the field (Ingham *et al.*, 2004; Benjamin *et al.*, 2018). This will be influenced by the factors that affect the period for which enteric pathogens remain viable in the soil after exiting the gut of humans or animal, because the organisms encounter long-term stresses that vary in nature and complexity (Delaquis *et al*., 2007).

The handling of the vegetables during sales in the market could also contribute to contamination of the vegetables. During negotiations there is frequent transfer of the vegetables between the retailers and potential customers which involve handling of the vegetables without any sanitary protocols (hand washing). It is plausible that microflora from the hands of retailers and/ or customers could be transferred to the vegetables during these negotiations. Luna-Guevara *et al*. (2019) indicated that hands of persons involved in harvesting and postharvest handling of vegetables are potential source of contamination, especially where there is no access to latrines or handwashing facilities.

The vegetables could also have been contaminated while been displayed for sales. Most retailers display the vegetables on woolly sacks or nylons laid on the ground; Reuben and Makut (2014) indicated that this makes it possible for the vegetables to be contaminated by aerosols from the wet soil around where the vegetables are displaced. In this study, it was observed that *E. coli* was not found on vegetables from four retailers who displaced their vegetables on tables and overlaid them with nylon, while it was from vegetables purchased from other retailers. It is our reasoning that their wares were spared from contamination by aerosols from the soil of the sales area, which were wet, because of the elevated position, and covering of the wares, unlike the vegetables of other retailers which were laid on the ground, and left uncovered.

We also observed that vegetables from Owena Ijesa market generally had higher counts of *E. coli*. The market is bigger, busier and rowdier than Ipetu Ijesa market hence there is a higher chance of contamination of the displaced wares (vegetables). In addition, the retailers in Owena market stay close to the road where there is heavy human and vehicular traffic that can more easily result in contamination of the wares (vegetables). Another factor that may contribute to the higher count on vegetables from Owena market is the proximity of the market to the vegetable farms where the crops were raised. Reuben and Makut (2014) found in their study that samples of vegetables from farms were more densely contaminated than samples obtained from the markets. In view of the proximity of Owena market to the vegetable farms it plausible that more of the flora on the vegetables while in the farm were still viable by the time the vegetables were purchased in the market because of the shorter time lapse between harvesting and retailing compared to Ipetu Ijesa market, which is not close to any vegetable farm.

In this study, *E. coli* was not uniformly associated with the vegetables: *A. hybridus* and *S. biafrae* (50%) were the most contaminated while least contaminated vegetable was *T. triangulare*. Dutta *et al.* (2014), Reuben and Makut (2014), and Benjamin *et al.* (2018) similarly observed variation in occurrence of *E coli* on the different types of vegetables they examined. The factor responsible for the variation in *E. coli* contamination of the vegetables is not clear. Delaquis *et al*. (2007) identified this uncertainty as parts of the critical gaps in knowledge of *E. coli* contamination of leafy vegetables. However, Harapas *et al*. (2010) indicated that *E coli* persisted in injured vegetables than on uninjured ones. Luna-Guevara *et al.* (2019) reported that various strains of *E. coli* colonize leafy vegetables by different mechanisms, including molecular mechanisms of adherence and fitness to the vegetable biosphere (entrapped by parts of the plant) and attributed thriving of some enteric pathogens in plant tissues with mechanical damage to availability of nutrients. They opined that the bacteria survive on and penetrate the plant interior where they remain with low metabolic activities, and thus survive the drastic changes in temperature, pH, osmolality, and nutrient deprivation.

All the *Escherichia coli* strains were resistant to more than two antibiotics, which is similar to the result obtained by Adzitey (2018), who found in his study that all *E. coli* isolated from cabbage and lettuce samples in Tamale metropolis of Ghana were resistant to at least 3 antibiotics. Abu-Duhier (2015) found in his study that most of the *E. coli* strains isolated from vegetables exhibited resistance to all the antibiotics they tested. Jongman and Korsten (2016) found that 70.7% of the *E. coli* isolated from irrigation water and leafy vegetables were multidrug resistant. Waturangi *et al.* (2019) also reported that most of the *E. coli* recovered from salad vegetables and fruits samples in Jakarta were multidrug resistant. It therefore appears that vegetables commonly habour multidrug resistant organisms.

Krumperman (1983) had indicated in his work that multiple antibiotics resistance (MAR) indexing can provide information about the origin of contamination. High MAR index suggests the strain originated from an environment where antibiotics were often used (Paul *et al.,* 1996). MAR index value greater than 0.2 suggest high-risk source of contamination where antibiotics are often used (Joseph *et al.*, 2017). The MAR index obtained in this study (which are greater than 0.2) suggest there is widespread use of antibiotics in Ipetu Ijesa and Owena Ijesa areas, hence it is plausible that the isolates found on the vegetables had been exposed to antibiotics or interacted with organisms exposed to antibiotics. This highlights the need for concerted efforts in antibiotic stewardship to mitigate the indiscriminate use of antibiotics. This could be achieved through community outreach and regulation of drug access.

Development of resistance due to acquisition of genetic elements through horizontal transfer among organisms or from the environment is a very high possibilities under the conditions in which vegetables are grown. Development of resistance due to acquisition of genetic element (plasmids) by *E. coli* was alluded to by Krumperman (1983) in explaining the difference in antibiotic resistance of isolates. This portends a grave danger if acquired or transferred genetic element also confer pathogenic traits. Krumperman (1983) also reported that multiple-antibiotic-resistant *E. coli* exist in large numbers within the major reservoirs of enteric diseases for humans. Therefore, the presence of multiple-antibiotic-resistant *E. coli* suggests that the vegetables are potential reservoirs of enteric pathogen. Hence, there is need for careful handling of the vegetables before consumption, especially those consumed raw.

Conclusion

Contamination of raw vegetables with *E. coli* is of serious public health importance because vegetables are used for fresh food preparations, and low doses of infection by the pathogen are sufficient to cause intestinal disease. There have been various interventions recommending ways by which vegetables should be handled to guarantee that food borne illnesses do not occur. The findings from this study further highlight the importance of observing basic protocols such as rinsing and blenching of vegetables before consumption. This study found that practices involved in retailing of vegetables should be improved, and retailers should be encouraged to rinse their wares when brought to the market, and consumers should rinse the vegetables when preparing them for consumption. During sales, the vegetables should be placed on elevated platforms and covered. Hand rinsing can be introduced as routine practice, in view of the fact that transfer of vegetables between retailers and customers cannot be avoided. The need for antibiotic stewardship to reduce the exposure of isolates to antibiotics is also identified. Further studies to establish the source of contamination of the vegetables is desirable, as this will inform remedial actions that can be taken to mitigate contamination of food. In addition, it is desirable to ascertain the involvement, or otherwise, of plasmid in the antibiotic resistance, and whether or not there was transfer of the genetic element (plasmid) between organisms that contaminate vegetables.

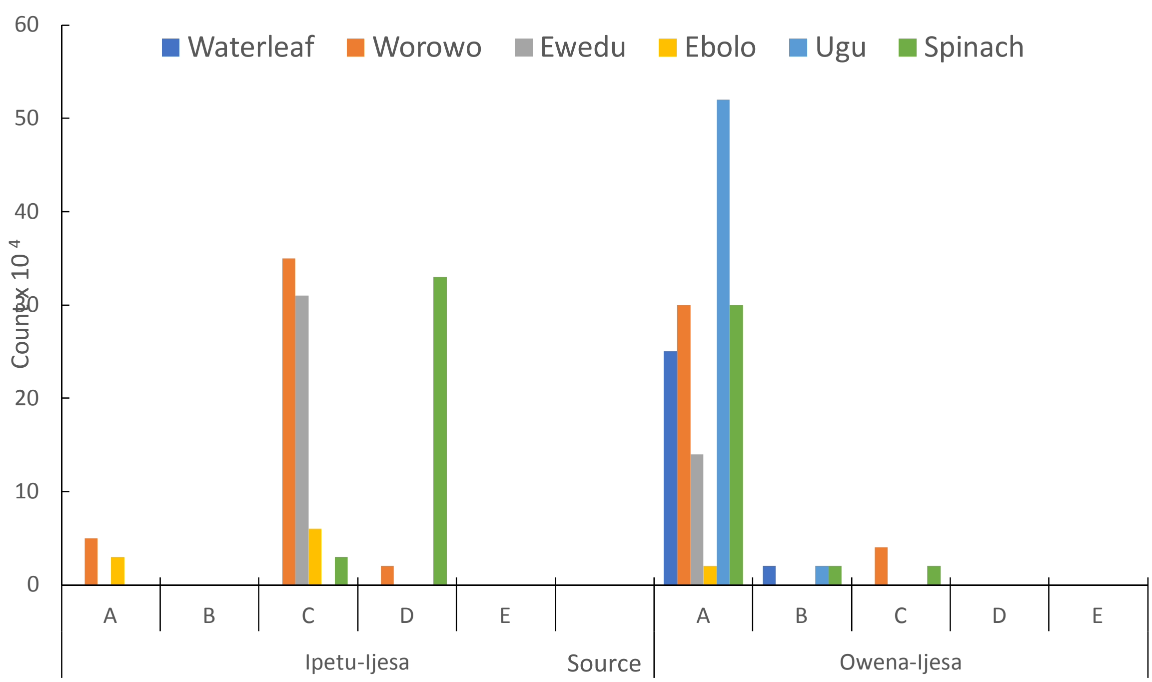

Figure 1. Mean Population of *Escherichia coli* on leafy vegetables retailed in the markets

Table 1: Occurrence of *Escherichia coli* strains on Vegetables Examined

Source of Vegetables		Presence of *Escherichia coli* on Vegetables					
		T. triangulare	***S. biafrae***	***Co. olitorius***	***Cr. crepidioides***	***T. occidentalis***	***A. hybridus***
Ipetu-Ijesa	A	-	+	-	+	-	-
	B	-	-	-	-	-	+
	C	-	+	+	+	-	+
	D	-	+	-	-	-	-
	E	-	-	-	-	-	-
Owena-Ijesa	A	+	+	+	+	+	+
	B	-	-	-	-	+	+
	C	-	+	-	-	-	+
	D	-	-	-	-	-	-
	E	-	-	-	-	-	-

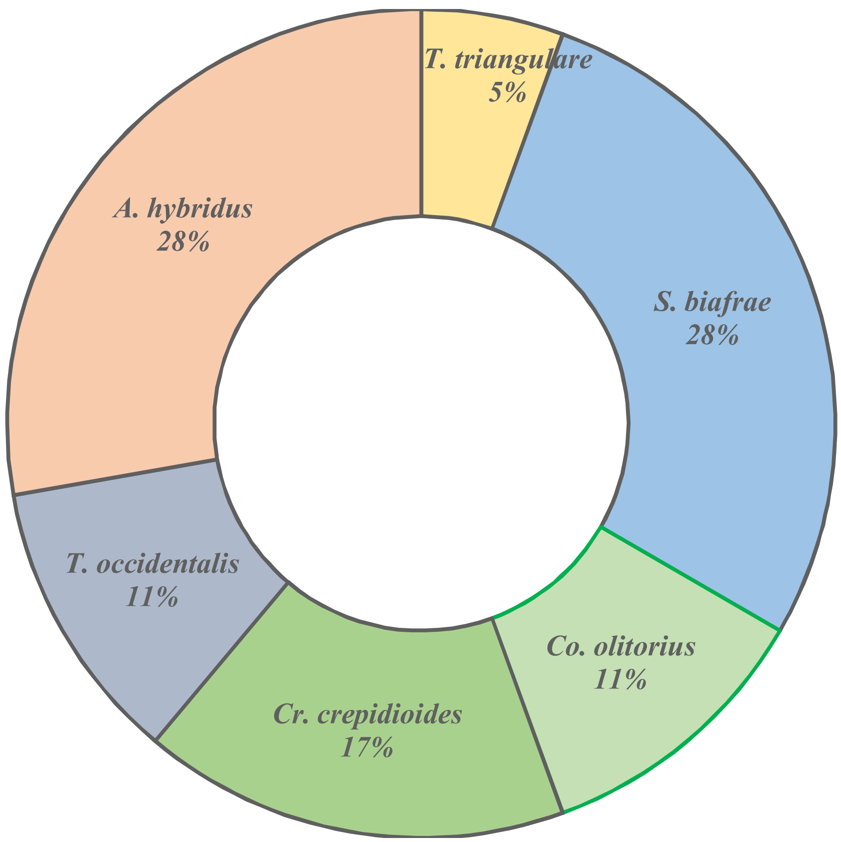

Figure 2: Prevalence of *Escherichia coli* strains on the Vegetables

Table 2: Antibiotics susceptibility of isolates from vegetables and Their MAR Index

	Source of E. coli strain	***Antibiotics Susceptibility (MAR Index)***
Ipetu-Ijesa	*Crassocephalum crepidioides* A	0.50
	Crassocephalum crepidioides C	0.25
	Corchorus olitorius C	0.63
	Amaranthus hybridus B	0.63
	Amaranthus hybridus C	1.00
	Solanecio biafrae A	0.75
	Solanecio biafrae C	0.25
	Solanecio biafrae D	0.63
Owena-Ijesa	*Talinum triangulare* A	0.25
	Crassocephalum crepidioides A	0.25
	Corchorus olitorius A	0.75
	Amaranthus hybridus A	0.63
	Amaranthus hybridus B	0.25
	Amaranthus hybridus C	0.88
	Telfairia occidentalis A	1.00
	Telfairia occidentalis B	0.75
	Solanecio biafrae A	0.25
	Solanecio biafrae C	0.63

Table 3. MAR Index of the Vegetables

	Type of Vegetable					
	T. triangulare	***S. biafrae***	***Co. olitorius***	***Cr. crepidioides***	***T. occidentalis***	***A. hybridus***
MAR Index	0.25	0.50	0.69	0.33	0.88	0.68

References

[1]. Abu-Duhier, F. M. (2015). *Escherichia coli* contamination of selected vegetables and fruits from markets of Tabuk city, Saudi Arabia: An anticipatory surveillance using real-time PCR for the presence of pathogenic strain *E. coli* O104:H4. *International Journal of Healthcare and Biomedical Research*. 4.1. 126-134

[2]. Adzitey F. (2018). Antibiotic resistance of Escherichia coli and Salmonella enterica isolated from cabbage and lettuce samples in Tamale metropolis of Ghana. *International Journal of Food Contamination* 5:7, 1-7. https://doi.org/10.1186/s40550-018-0068-z

[3]. Benjamin, B., Uba, A., Yusha'u, M., Maikaje, D. B. Nyakaat, N. N. and Daniel, A. M. (2018). Isolation of *Escherichia coli* from Fruits and Vegetables in Kaduna Metropolis. *International Journal of Engineering Science and Computing*. 8:7 18598- 18601

[4]. Cheesbrough, M. (2010): *District Laboratory Practice in Tropical Countries* part 2, Second edition update. 187-195.

[5]. Davis J. G. and Kendall, P. (2020). Preventing *E. coli* From Garden to Plate. *Food and Nutrition Series*, Fact Sheet No. 9.369. Food Safety. Accessed on 28.03.2020

[6]. Delaquis, P., Bach, S., and Dinu, L. (2007) Behavior of *Escherichia coli* O157:H7 in Leafy Vegetables. *Journal of Food Protection*. 70:8. 1966–1974

[7]. Dutta, A., Joshi, N., Joshi, R. K. and Kamal, A. (2014). Molecular Characterization of *E. coli* Isolated from Raw Vegetable. *Advances in Animal and Veterinary Sciences*. 2:1. 42–45. http://dx.doi.org/10.14737/journal.aavs/2014.2.1.42.45

[8]. Harapas D, Premier R, Tomkins B, Franz P. Ajlouni S. (2010). Persistence of *Escherichia coli* on injured vegetable plants. *Internal Journal Food Microbiology*. 138: 232–237.

[9]. Haute S.V, Tryland I., Veys A, Sampers I. (2015). Wash water disinfection of a full-scale leafy vegetables washing process with hydrogen peroxide and the use of a commercial metal ion mixture to improve

disinfection efficiency. *Food Control* 50:173–183

[10]. Ingham, S. C. Losinski, J. A., Andrews, M. P., Breuer, J. E., Breuer, J. R., Wood, T. M. and Wright, T. H. (2004). *Escherichia coli* Contamination of Vegetables Grown in Soils Fertilized with Noncomposted Bovine Manure: Garden-Scale Studies. *Applied and Environmental Microbiology* 70:11 6420–6427.

[11]. Jongman and Korsten, (2016). Genetic Diversity and Antibiotic Resistance of Escherichia coli Isolates from Different Leafy Green Production Systems *Journal of Food Protection* 79(11):1846-1853

[12]. Joseph N., Ryu, D. Malano, H. George, B., Sudheer K. (2017) Investigation of sustainable national water resources management of India in a changing climate, 22nd International Congress on Modelling and Simulation

[13]. Krumperman, P. H. (1983). Multiple Antibiotic Resistance Indexing of Escherichia coli to Identify High-Risk Sources of Fecal Contamination of Food. *Applied and Environmental Microbiology*, 46.1, 165-170.

[14]. Lim P.O, Kim H.J, Nam H.G. (2007). Leaf senescence. *Annual Review of Plant Biology*. 58, 115–136

[15]. Luna-Guevara, J. J. Arenas-Hernandez, M. M. P. Martinez de la Peña, C. Silva, J. L. and Luna-Guevara, M. L. (2019). The Role of Pathogenic E. coli in Fresh Vegetables: Behavior, Contamination Factors, and Preventive Measures. *International Journal of Microbiology*, 1- 10 https://doi.org/10.1155/2019/2894328

[16]. MacDonald, E., Moller, K.E., Wester, A.L., Dahle, U.R., Hermansen, N.O., Jenum, P.A. (2015). An outbreak of enterotoxigenic Escherichia coli (ETEC) infection in Norway, 2012: a reminder to consider uncommon pathogens in outbreaks involving imported products. *Epidemiology Infections* 143: 486–493.

[17]. Olaimat N.A., and Holley R.A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, 32(1). 1-19.

[18]. S. Paul, R.L. Bezbaruah, M.K. Roy and A.C. Ghosh (1997). Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. *Letters in Applied Microbiology* 24: 169–171

[19]. Rai P.K and Tripathi B.D. (2007) Microbial contamination in vegetables due to irrigation with partially treated municipal wastewater in a tropical city. *International Journal of Environmental Health Research,* 17:389–395.

[20]. Reuben, C. R. and Makut, M. D. (2014). Occurrence of Escherichia coli O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria. *World Journal of Microbiology*, 1(3): 017-021.

[21]. Sela S, Fallik E, Wojciech J.F, Robert L.S, Bernhard B, Stanley E.P and Florkowski R.L.S. (2009). Microbial Quality and Safety of Fresh Produce Postharvest Handling In *Postharvest Handling* Florkowski W. J., Shewfelt R. L., Brueckner, B. and Prussia, S. E. (Eds) 2nd edition, San Diego: Academic Press. 351-398.

[22]. Shaw, R.K., Berger, C.N., Pallen, M.J., Sjoling, A., and Frankel, G. (2011). Flagella mediate attachment of enterotoxigenic *Escherichia coli* to fresh salad leaves. *Environmental Microbiology Report* 3: 112–117.

[23]. Warriner K., Huber, A. Namvar, A. Fan W. and Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advanced Food Nutrition Research* 57. 155- 208

[24]. Warriner K and Namvar A. (2010). The tricks learnt by human enteric pathogens to persist within the plant environment. *Current Opinion in Biotechnology*. 21:131-136.

[25]. Waturangi, D. E., Hudiono, F. and Aliwarga, E. (2019). Prevalence of pathogenic *Escherichia coli* from salad vegetable and fruits sold in Jakarta. *BMC Research Notes* 12:247. 1- 9. https://doi.org/10.1186/s13104-019-4284-2.

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