Escherichia coli contamination of lettuce (*Lactuca sativa*) sold in Trinidad and Tobago

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The increasing consumption of fresh produce as part of a healthy diet has been mirrored by an increasing incidence of foodborne disease outbreaks. Contamination of fresh vegetable produce with foodborne pathogens is of particular concern since these products undergo minimal processing or are consumed raw. In addition to being a potential source of foodborne pathogens, antimicrobial resistant bacteria can be easily transmitted to humans when they consume or come in contact with contaminated vegetable produce. Using *Escherichia coli* as an indicator of faecal contamination, we evaluated lettuce (*Lactuca sativa*) as a potential source of foodborne disease and antimicrobial resistant bacteria. A cross-sectional study was carried out targeting six retail vegetable markets (A-F), and the *E. coli* counts (CFU/g lettuce) were assessed using standard laboratory techniques. Recovered *E. coli* isolates were then subjected to antimicrobial sensitivity testing to the four primary antibiotics used in livestock production in Trinidad and Tobago. Overall, the *E. coli* counts ranged from 0.8 to 800,000 CFU/gram of lettuce, with approximately 55% of samples exceeding 100 CFU/gram (2 log 10 CFU/g). The *E. coli* counts varied with market location ($P \leq 0.001$), with the highest average count being at market E (3.41 log 10 CFU/g), and the lowest at location D (1.47 log 10 CFU/g). Approximately, 22% of the *E. coli* isolates screened (4/18) exhibited resistance to amoxicillin-clavulanic acid, while 11% (2/18) exhibited resistance to tetracycline. None of the isolates exhibited resistance to more than one antibiotic type. These high *E. coli* counts are indicative of either preharvest or postharvest faecal contamination of lettuce. These results demonstrate that lettuce can potentially serve as a vehicle for the dissemination of both foodborne pathogens and antimicrobial resistant organisms.

Keywords: *Escherichia coli*, lettuce, food safety, antibiotic resistance, Trinidad

Annually, foodborne diseases are estimated to affect over 600 million persons globally resulting in greater than 420,000 deaths (Havelaar et al. 2015). Trinidad and Tobago is a country located in the southern Caribbean; and despite having a population of only 1.3 million persons, the annual foodborne disease burden is estimated to be over 130,000 cases (Lakhan et al. 2013). Similar to most other Caribbean countries, the etiology of most of these cases remain unidentified.

The increasing consumption of vegetable produce due to their perceived health benefits has placed greater focus on the microbial safety of vegetable produce (Callejón et al. 2015). Vegetable produce, especially leafy greens are of particular concern since they are consumed raw or undergo minimal processing with no deactivation or 'kill-step' (Holvoet et al. 2013; Berger et al. 2010). These plants are of further concern since they are grown close to the ground thus increasing the risk of transfer of microbial pathogens from the soil or the environment onto to the leaves of the produce. Like most countries, the consumption of raw fruits and vegetables is popular in Trinidad and Tobago, with 38% of the population reporting they regularly consumed raw mixed vegetable salads and a further 22% regularly ate raw tomatoes and lettuce (Ramdath et al. 2011). These dietary consumption patterns highlight the potential risk of contaminated vegetables being a source of foodborne diseases.

Historically the majority of foodborne diseases have been associated with animal products, but recent trends have demonstrated an increasing incidence of these diseases associated with vegetable produce consumption (Bennett et al. 2018) . While

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there is no published data available on the role of plant produce in the epidemiology of foodborne disease in Trinidad and Tobago, its role should not be ignored since plant produce has been implicated in foodborne disease outbreaks in many other geographic regions. For example, in the United States, from 2009 to 2015, foods of plant origin accounted for 26% of all foodborne disease outbreaks and 37% of all disease cases (Dewey-Mattia et al. 2018). Similarly, in China, approximately 48% of the foodborne disease outbreaks during 2003–2008 were attributed to plant commodities (Wu et al. 2018). In Europe the incidence of plant associated foodborne disease was lower than USA and China but still significant, with 6.1% of foodborne disease outbreaks being attributed to foods of plant origin (European Food Safety Authority 2018).

From the literature cited above, it is clear that plant commodities play an integral role in the epidemiology of foodborne diseases. However, despite a plethora of published research globally, there is a dearth of published research on its role in Trinidad and Tobago and the Caribbean. The objective of this study is to partially fill this critical void in the literature by determining the frequency of *Escherichia coli* contamination on locally produced vegetable produce and the potential of lettuce to act as a source of antimicrobial resistant organisms.

Methodology

Sample collection

A cross-sectional study was conducted targeting six vegetable retail markets (A-F) in Trinidad (Figure 1). Lettuce (*Lactuca sativa*) was sampled at each of these locations. For inclusion in this study, the persons selling the lettuce had to be the growers, and also lettuce had to be grown using a soil media, since most commercial lettuce is still grown using a soil media. At each market five farmers were selected and three heads of lettuce were purchased from each farmer and aseptically placed in a Ziploc ® bag. These bags were then placed in a cooler containing ice packs and stored at 4°C before being transferred to the laboratory for analysis. Farmers were also asked to complete a questionnaire to ascertain their production practices including type of fertilisers used, source of irrigation water and type and frequency of manure application.

Detection of E. coli

At the laboratory, 25g of lettuce leaves was weighed using an electronic balance (Scout Pro SP202, Ohaus Corporation, USA) and aseptically transferred into a Ziploc ® bag containing 225 mL of Buffered Peptone Water (BPW; Oxoid, UK). Contents were then manually agitated thoroughly for 1 minute to ensure any sediment from the lettuce leaves were in solution. Following agitation, a 1 mL aliquot of the lettuce wash solution was sequentially diluted up to the 10^{-3} dilution. Following this, 100µL of each dilution was then surface plated onto MacConkey agar (Oxoid, UK) plates using sterile glass loops and incubated for 24 hours at 37°C. The remainder of lettuce suspension was placed in the incubator at 37°C for 24 hours to allow for enrichment.

The following day, the MacConkey plates were examined, and the number of *E. coli* colonies was enumerated and up to five *E. coli* colonies from each sample were selected for confirmation. For confirmation, the colonies were streaked onto Eosin Methylene Blue (EMB, Oxoid, UK) and incubated at 37°C for 24 hours. The next day these colonies were observed to ensure they had the morphology consistent with *E. coli* (colonies with a green metallic sheen) and each colony was then re-streaked onto sheep blood agar. These plates were also incubated at 37°C for 24 hours. The next day colonies were subjected to standard biochemical assays to confirm their identity as *E. coli* (Feng et al. 2016). Positive *E. coli* colonies were then banked at -80°C for further antibiotic susceptibility testing.

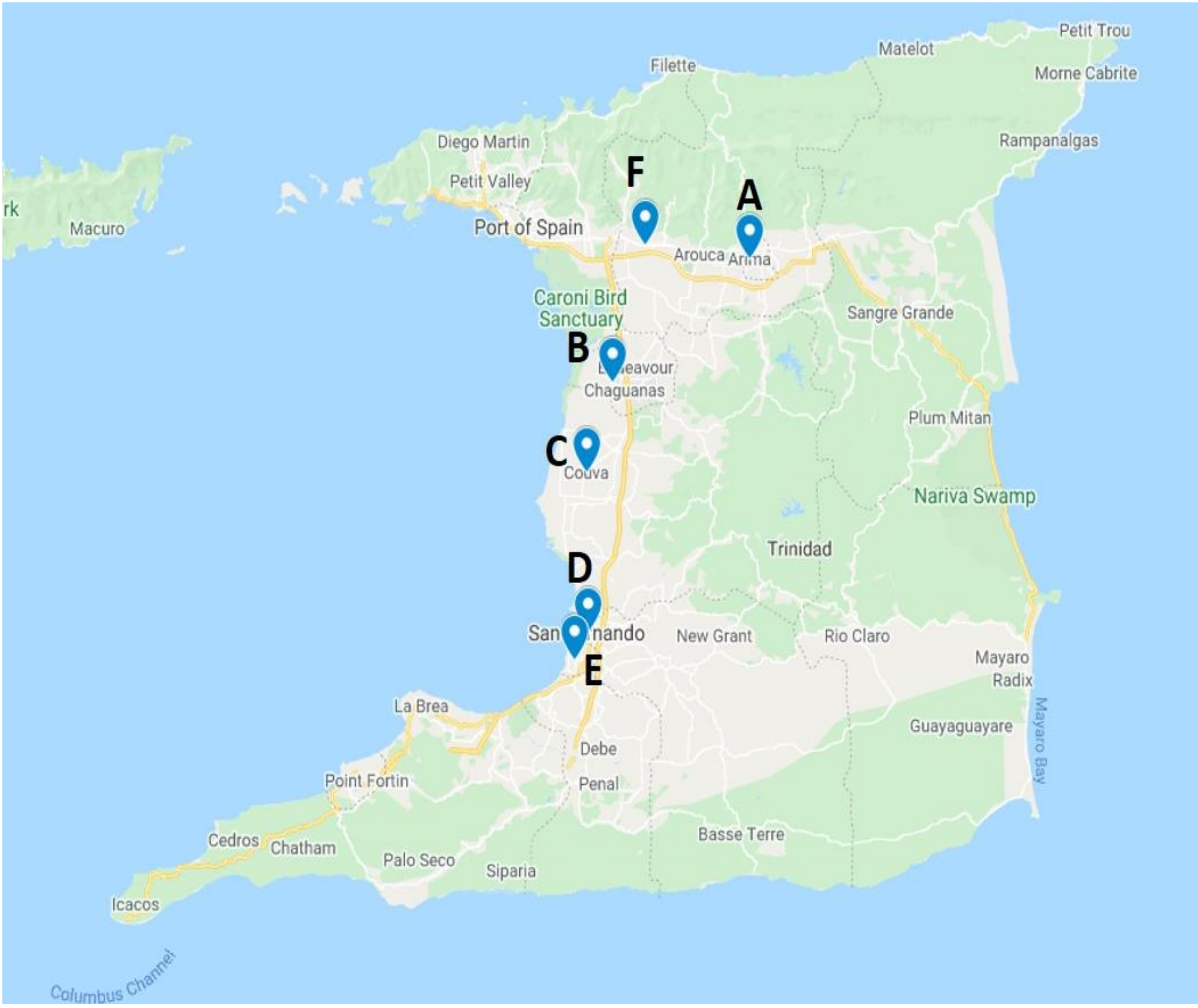

Figure 1: Map of Trinidad showing the location of vegetable markets sampled

Antibiotic sensitivity testing

Antimicrobial sensitivity testing was performed on 18 randomly selected isolates. These isolates were randomly selected ensuring isolates from each of the six market locations were selected. Antimicrobial sensitivity testing was done using the Kirby-Bauer disk diffusion method (Hudzicki 2009). The antimicrobial discs (Oxoid Ltd., Basingstoke, Hampshire, U.K.) and concentrations used in the study were: amoxicillin-clavulanic acid (30 µg), sulphamethoxazole/trimethoprim (25 µg), tetracycline (30 µg) and ceftiofur (30 µg). The sensitivity of each *E. coli* isolate to the antimicrobial agents was interpreted using guidelines established by the Clinical Laboratory Standards Institute (CLSI 2012).

Statistical analysis

Data were tabulated using Microsoft Excel. Univariate differences in the frequency of risk factors (manure use and water source) for exceeding the limit of 100CFU/g were compared using the Fisher Exact Test. To evaluate the effect of location, a one-way analysis of variance test was done followed by post hoc comparisons using Tukey's test. Data were analysed using Minitab 16.0® statistical software (Minitab Inc., State College, Pa., USA.). Differences were considered to be statistically significant at $P \leq 0.05$.

Results

Production practices

Most farmers (77%) reported using manure regularly on their production fields. The other farmers reportedly used only inorganic fertilisers to improve the soil fertility. The main types of animal manures used were of cattle or poultry origin. These farmers reported obtaining raw manure from livestock enterprises and applying it topically to the soil without subjecting the manure to any treatment (eg. Composting). There was no established time between manure application and planting or harvesting of lettuce. Another production practice investigated was their source of irrigation water. The majority of farmers (97%), indicated they used pipe-borne water to irrigate their fields while only one farmer used pond water to irrigate his fields.

Escherichia coli contamination

All lettuce samples tested positive for *Escherichia coli*. The level of contamination ranged from 0.8 Colony Forming Units/gram (CFU/g) of lettuce to 800,000 CFU/g of lettuce. The *E. coli* contamination of the samples tested varied with location ($P < 0.001$) with the highest average count being reported at location E (3.41 log 10 CFU/g), and the lowest at location D (1.47 log 10 CFU/g) (Figure 2). Interestingly, 55% of all the lettuce samples tested exceeded the recommended maximum *E. coli* limit of 100 CFU/g (2 log 10 CFU/g) for fresh produce (Institute of Medicine and National Research Council Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food 2003; Feng 2015). When evaluating production practices, lettuce obtained from fields where manure was used was more likely to exceed the recommended maximum *E. coli* limit of 100 CFU/g compared to fields where manure was not used ($P = 0.02$).

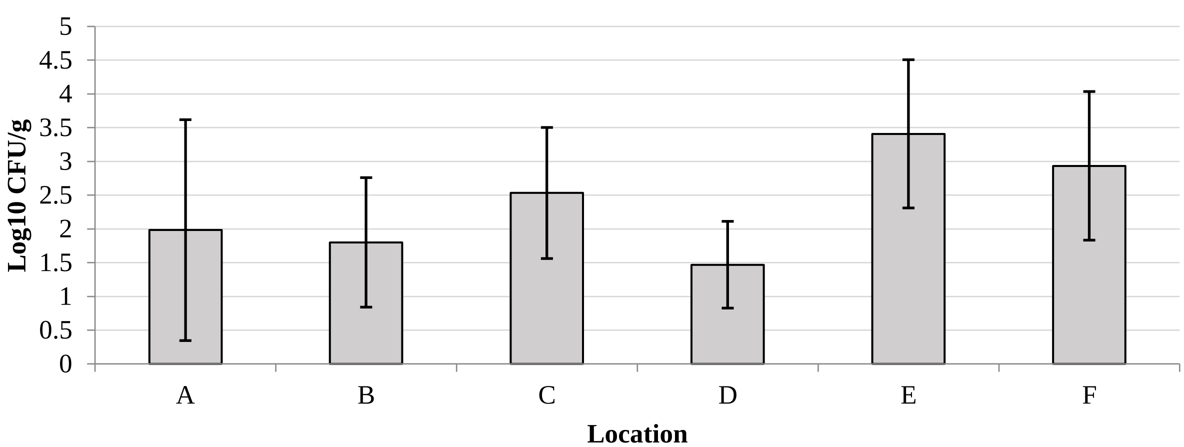

Figure 2: The average *E. coli* counts (Colony Forming Units/gram (CFU/g)) on lettuce sold at 6 markets (A – F). Counts represent the average of 15 samples per location. Error bars represent the standard deviation.

Antimicrobial resistance

All *E. coli* isolates (n = 18) tested were susceptible to sulphamethoxazole/ trimethoprim and ceftiofur (Table 1). While the overall resistance level was reportedly low, approximately 22% of the *E. coli* isolates screened exhibited resistance to amoxicillin-clavulanic acid, and only one isolate exhibited intermediate resistance. Only two of the *E. coli* isolates screened (11%) exhibited resistance to tetracycline. None of the *E. coli* isolates tested exhibited resistance to more than one antibiotic.

Table 1: Susceptibility of the 18 *E. coli* isolates tested to commonly used antibiotics

Antimicrobial	Susceptible	Intermediate	Resistant
Amoxicillin–clavulanic acid	13	1	4
Ceftiofur	18	ND	ND
Trimethoprim/sulphamethoxazole	18	ND	ND
Tetracycline	16	ND	2

* ND Not Detected

Discussion

We report for the first time that lettuce sold in the English-speaking Caribbean, specifically Trinidad and Tobago, is a source of *E. coli* and antimicrobial resistant organisms. *E. coli* is an indicator for faecal contamination and its presence on food increases the potential for other more pathogenic enteric foodborne to also be present. Previous studies in Trinidad have focused on already prepared ready to eat salads and the results have been inconclusive. For example, Hosein et al. (2008) reported 1.4% of ready to eat salads were contaminated with *E. coli*, whereas a similar study conducted by Singh and Badrie (2018) reported that 100% of salad samples (n = 14) tested were positive for *E. coli*.

In this study the lettuce sold at markets were contaminated with *E. coli*, with 55% of the samples testing having *E. coli* levels exceeding that allowed for human consumption. *E. coli* isolates were recovered from 100% of lettuce samples tested. The survival of *E. coli* on lettuce has been reported to be less than 7 days and thus high presence is indicative of recent faecal contamination (Fonseca et al. 2011). This contamination may have occurred during cultivation, harvesting or during postharvest handling. The origin of this contamination necessitates further investigation. This high level of *E. coli* contamination is similar to that reported for Romaine lettuce in the upper Midwest, USA (Greve et al. 2015). Similarly, in that study, all samples tested positive for *E. coli*, however most samples (98%) had *E. coli* counts of less than 10 CFU/g, while in this study 89% of the samples had *E. coli* counts greater than 10 CFU/g. The high frequency of detection of *E. coli* in lettuce reported in these two studies are in contrast to previously published data, which reported significantly lower lettuce *E. coli* contamination level with prevalences ranging from 0 to 13% (Gao et al. 2018; Wood et al. 2015).

Another alarming revelation is that 55% of lettuce samples exceeded the *E. coli* count limit of 100 CFU/g limit for minimally processed produce (Feng 2015; Institute of Medicine and National Research Council Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food 2003). This high contamination level coupled with the fact that household washing only reduces contamination levels by 3 to 5% (Uhlig et al. 2017) illustrates that lettuce can potentially be the missing link in the epidemiology of human diarrheal diseases in Trinidad and Tobago (Lakhan et al. 2013). These high contamination levels of lettuce sold at Trinidad markets may also account for the high contamination of ready to eat to salads as reported by Singh and Badrie (2018).

In this study lettuce being sold at markets was sampled, and thus *E. coli* contamination could have occurred during production (pre-harvest), harvest or postharvest handling. Possible sources of *E. coli* contamination during pre-harvest production include: poor worker hygiene, animal intrusion and defecating on the production fields; use of uncomposted or untreated manure; use of polluted irrigation water and use of contaminated equipment (Brackett 1999). Additionally, produce may be contaminated via aerosol deposition of pathogens of nearby animal facilities or deposition of pathogens as a result of flooding. Postharvest contamination may occur from either poor worker hygiene, contaminated harvest/packing equipment or cross-contamination with water used to wash produce. Many vegetable produce retailers in Trinidad can be seen routinely sprinkling their produce with water to prevent quilting and maintain its "fresh" appearance. In this study we evaluated two potential sources of produce contamination: manure use and water. Overall, 77% of farmers reported using organic manure to fertilise their production fields. In this study, lettuce samples obtained from fields using manure were more likely to exceed to the minimum safety level of 100 CFU/g compared to lettuce samples obtained where no manure was used (P = 0.02). Alarmingly, despite being recommended in numerous good agriculture practice documents, all of the farmers surveyed indicated they did not observe any no-harvest interval between manure application and lettuce cultivation or harvesting. With respect to irrigation water, 97% of farmers surveyed reported using pipe-borne or chlorine treated water for irrigation and produce washing purposes. Previously published studies investigating the microbial quality of pipe-borne water in Trinidad reported 67% water samples in urban areas and 66% of water samples in rural areas were contaminated with *Escherichia coli* (Agard et al. 2002; Welch et al. 2000). In addition to potential contamination via contaminated irrigation/wash water, it should be noted that many agriculture fields in Trinidad and Tobago are prone to flooding annually; thereby increasing the risk of field contamination with effluent from humans and livestock farms (Persad et al. 2007).

Although lettuce has been identified as being capable of harboring antimicrobial resistant bacteria (McGowan et al. 2006; Wang et al. 2015), this is the first study in the English-speaking Caribbean to identify vegetable produce as a potential source of antimicrobial resistant bacteria. There are currently no antibiotic preparations approved for horticulture use in Trinidad, however there are anecdotal reports of farmers using water soluble animal antibiotic preparations on their fields. Additionally, the use of uncomposted animal manure on fields increases the risk of transfer of enteric micro-organisms from animals to plants.

In this study we evaluated antimicrobial resistance (AMR) against antibiotics commonly used in livestock production: Amoxicillin-clavulanic acid, Sulphamethoxazole/ Trimethoprim, Tetracycline, and Ceftiofur. The high prevalence of tetracycline resistance (11%) observed in this study is consistent with Araujo et. al (2017) who reported 24% of vegetable *E. coli* isolates tested were also resistant to Tetracycline. However, unlike that study which reported 21% of isolates were multidrug resistant, none of the isolates in our study exhibited resistance to more than one of the antibiotics, but it should be noted, that in our study we only screened 18 *E. coli* isolated and these isolates were only screened against four antibiotics.

Previously published studies in Trinidad have reported the presence of AMR organisms in eggs, meat and milk (Adesiyun et al. 2007; Adesiyun et al. 2007; Khan et al. 2018). These animal products are usually subjected to cooking at high temperatures or pasteurisation which render the zoonotic risk of these organisms to be minimal. Conversely, lettuce is eaten raw with minimal processing and thus the presence of AMR organisms should be of far greater concern. These organisms can potentially colonise the gastrointestinal tract of humans or transfer their AMR genes to other pathogenic organisms. The net result of either of these scenarios is difficult to treat human infections.

There are a couple limitations to our study; firstly, we did not test for the presence of pathogenic organisms. Instead, we tested for *E. coli* and while *E. coli* is an indicator of faecal contamination, it is not always reflective of the presence or concentration of other pathogenic organisms (Miskimin et al. 1976). Another limitation is the limited number of E. coli isolates screened for antimicrobial resistance. Additionally, based on our study design, we could not exclude the possibility of postharvest contamination of lettuce occurring.

Nevertheless, the findings of high *E. coli* contamination as well as the presence of antimicrobial resistant bacteria on lettuce are quite significant public health risks. The high *E. coli* contamination levels necessitates further farm to fork studies to identify if the source(s) of contamination are preharvest, during harvest or postharvest.

In summary, this study highlights the potential food safety risks posed by fresh lettuce produce sold at the retail markets in Trinidad. These results provide critical data, which should be included in any risk assessments to better estimate the role that vegetables play in the epidemiology of foodborne diseases in Trinidad.

Conflict of interest

The authors declare no conflicts of interest.

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References

Adesiyun, A., N. Offiah, N. Seepersadsingh, S. Rodrigo, V. Lashley, and L. Musai. 2007. "Antimicrobial Resistance of *Salmonella* spp. and *Escherichia coli* isolated from table eggs." *Food Control* **18 (4):** 306–311.

Adesiyun, A.A., Simone Stoute, and B. David. 2007. "Pre-Processed Bovine Milk Quality in Trinidad: Prevalence and Characteristics of Bacterial Pathogens and Occurrence of Antimicrobial Residues in Milk from Collection Centres." *Food Control* **18 (4):** 312–320.

Agard, Lavaughn, Corlis Alexander, Simone Green, Michael Jackson, Sanjeev Patel, and Abiodun Adesiyun. 2002. "Microbial Quality of Water Supply to an Urban Community in Trinidad." *Journal of Food Protection* **65 (8)**: 1297–1303.

Araujo, S., A.T. Silva I, M. Tacao, C. Patinha, A. Alves, and I. Henriques. 2017. "Characterization of Antibiotic Resistant and Pathogenic Escherichia Coli in Irrigation Water and Vegetables in Household Farms." *Int J Food Microbiol* **257**:192–200. doi: 10.1016/j.ijfoodmicro. 2017.06.020.

Bennett, S.D., S.V. Sodha, T.L. Ayers, M.F. Lynch, L.H. Gould, and R.V. Tauxe. 2018. "Produce-Associated Foodborne Disease Outbreaks, USA, 1998-2013." *Epidemiol Infect* **146 (11):** 1397–1406. doi: 10.1017/ S0950268818001620.

Berger, Cedric N., Samir V. Sodha, Robert K. Shaw, Patricia M. Griffin, David Pink, Paul Hand, and Gad Frankel. 2010. "Fresh Fruit and Vegetables as Vehicles for the Transmission of Human Pathogens." *Environmental Microbiology* **12 (9):** 2385–2397.

Brackett, Robert E. 1999. "Incidence, Contributing Factors, and Control of Bacterial Pathogens in Producs." *Postharvest Biology and Technology* **15 (1999):** 305–311.

Callejón, Raquel M., M Isabel Rodríguez-Naranjo, Cristina Ubeda, Ruth Hornedo-Ortega, M. Carmen Garcia-Parrilla, and Ana M Troncoso. 2015. "Reported Foodborne Outbreaks Due to Fresh Produce in the United States and European Union: Trends and Causes." *Foodborne Pathogens and Disease* **12 (1):** 32–38.

CLSI. 2012. "Performance Standards for Antimicrobial Susceptibility Testing." *Clinical and Laboratory Standards Institute (M100eS22)* (s22nd Informational Supplement).

Dewey-Mattia, Daniel, Karunya Manikonda, Aron J. Hall, Matthew E. Wise, and

Samuel J. Crowe. 2018. "Surveillance for Foodborne Disease Outbreaks - United States, 2009–2015." *MMWR Surveillance Summaries* **67 (10):**1.

European Food Safety Authority. 2018. "The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Borne Outbreaks in 2017." *EFSa Journal* **16 (12):** e05500.

Feng, Peter. 2015. "Shiga Toxin-Producing *Escherichia coli* in Fresh Produce: A Food Safety Dilemma." In *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli*, 245–259. American Society of Microbiology.

Feng, P., S.D. Weagant, A. Michael, and W.B. Grant. 2016. *Enumeration of Escherichia coli and the Coliform Bacteria.* Bacteriological Analytical Manual, revision A, 1998.

Fonseca, J.M., S.D. Fallon, C.A. Sanchez, and K.D. Nolte. 2011. "*Escherichia coli* Survival in Lettuce Fields Following its Introduction Through Different Irrigation Systems." *J Appl Microbiol* **110 (4):** 893–902. doi: 10.1111/j.1365-2672.2011. 04942.x.

Gao, Xiuzhi, Yiqian Liu, Qing Chang, Zihao Zhang, Xu Guo, and Xinxin Yi. 2018. "Microbiological Survey of Field-Grown and Retail Lettuce in Beijing." *Journal of Food Safety* **38 (4)**: e12479.

Greve, J.D., M.S. Zietlow, K.M. Miller, and J.L. Ellingson. 2015. "Occurrence of Coliform and *Escherichia coli* Contamination and Absence of Escherichia coli O157:H7 on Romaine Lettuce from Retail Stores in the Upper Midwest." *J Food Prot* **78 (9):** 1729–32. doi: 10.4315/0362-028X.JFP-14-588.

Havelaar, Arie H., Martyn D. Kirk, Paul R. Torgerson, Herman J. Gibb, Tine Hald, Robin J. Lake, Nicolas Praet, David C. Bellinger, Nilanthi R. De Silva, and Neyla Gargouri. 2015. "World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010." *PLoS medicine* **12 (12):**e1001923.

Holvoet, Kevin, Imca Sampers, Benedicte Callens, Jeroen Dewulf, and Mieke Uyttendaele. 2013. "Moderate Prevalence of Antimicrobial Resistance in *Escherichia coli* Isolates from Lettuce, Irrigation Water, and Soil." *Appl. Environ. Microbiol.* **79 (21):** 6677–6683.

Hosein, A., K. Muñoz, K. Sawh, and A. Adesiyun. 2008. "Microbial Load and the Prevalence of *Escherichia coli, Salmonella spp.* and *Listeria spp.* in Ready-to-Eat Products in Trinidad." *The Open Food Science Journal* **2** (1).

Hudzicki, Jan. 2009. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol."

Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food. 2003. *Scientific Criteria to Ensure Safe Food.* Washington (DC): National Academies Press

Khan, Anisa S., Karla Georges, Saed Rahaman, Woubit Abdela, and Abiodun A. Adesiyun. 2018. "Antimicrobial Resistance of *Salmonella* Isolates Recovered from Chickens Sold at Retail Outlets in Trinidad." *Journal of Food Protection* **81 (11)**: 1880–1889.

Lakhan, Carelene, Neela Badrie, Adash Ramsubhag, Kumar Sundaraneedi, and Lisa Indar. 2013. "Burden and Impact of Acute Gastroenteritis and Foodborne Pathogens in Trinidad and Tobago." *Journal of Health, Population, and Nutrition* **31** (4 Suppl 1):S30.

McGowan, Lori L., Charlene R. Jackson, John B. Barrett, Lari M. Hiott, and Paula J. Fedorka-Cray. 2006. "Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Fruits, Vegetables, and Meats." *Journal of Food Protection* **69 (12)**: 2976–2982.

Miskimin, D.K., K.A. Berkowitz, M. Solberg, W.E. Riha Jr, W.C. Franke, R.L.

Buchanan, and V. O'leary. 1976. “Relationships Between Indicator Organisms and Specific Pathogens in Potentially Hazardous Foods.” *Journal of Food Science* **41 (5):** 1001–1006.

Persad, Seunarine., I. Ramnarine, and H. Wilson. 2007. “Soil and Water Constraints to Food Crop Production in Trinidad and Tobago - Challenges and Opportunities for Small Farms.” Caribbean Food Crops Society 43rd Annual Meeting, San Jose, Costa Rica, 16-22 September 2007.

Ramdath, Dan D., Debbie G. Hilaire, Kimlyn D. Cheong, and Sangita Sharma. 2011. “Dietary Intake Among Adults in Trinidad and Tobago and Development of a Quantitative Food Frequency Questionnaire to Highlight Nutritional Needs for Lifestyle Interventions.” *International Journal of Food Sciences and Nutrition* **62 (6)**: 636–641

Singh, Narita, and Neela Badrie. 2018. “A Preliminary Microbial Survey of Ready-To-Eat Salads at Popular Food Establishments in Trinidad.” *Farm and Business-The Journal of the Caribbean Agro-Economic Society* **10 (533–2018–3655):** 27–37.

Uhlig, E., C. Olsson, J. He, T. Stark, Z. Sadowska, G. Molin, S. Ahrne, B. Alsanius, and A. Hakansson. 2017. “Effects of Household Washing on Bacterial Load and Removal of *Escherichia coli* from Lettuce and "Ready-to-Eat" Salads.” *Food Sci Nutr* **5 (6):** 1215–1220. doi: 10.1002/fsn3.514.

Wang, Feng-Hua, Min Qiao, Zheng Chen, Jian-Qiang Su, and Yong-Guan Zhu. 2015. “Antibiotic Resistance Genes in Manure-Amended Soil and Vegetables at Harvest.” *Journal of Hazardous Materials* **299**:215–221.

Welch, Pedro, Joanna David, Wayne Clarke, Aaron Trinidade, Dana Penner, Sean Bernstein, Laura McDougall, and Abiodun A. Adesiyun. 2000. “Microbial Quality of Water in Rural Communities of Trinidad.” *Revista Panamericana de Salud Pública* **8:**172–180.

Wood, J.L., J.C. Chen, E. Friesen, P. Delaquis, and K.J. Allen. 2015. “Microbiological Survey of Locally Grown Lettuce Sold at Farmers' Markets in Vancouver, British Columbia.” *J Food Prot* **78 (1):** 203–8. doi: 10.4315/0362-028X.JFP-14-199.

Wu, Yong-ning, Xiu-mei Liu, Qian Chen, Hong Liu, Yue Dai, Yi-jing Zhou, Jian Wen, Zhen-zhu Tang, and Yan Chen. 2018. “Surveillance for Foodborne Disease Outbreaks in China, 2003 to 2008.” *Food Control* **84**:382–388.