Prevalence and Antimicrobial Susceptibility Patterns of *Escherichia coli*, *Salmonella* spp. and *Citrobacter freundii* Isolated from Ready-to-Eat Fresh Vegetables

Panjaphorn Nimmanee*, Porntip Paungmoung, Chatnapa Simarub, Nattiya Phromrungsawat, Pojnicha Yimnongpho, Marisa Phuttatate, Suthinan Arnamwong, Phatsaraphon Lumlong, Chalinee Boonchamchoo, Poochisa Sangchan, Thanapron Champhong and Tanaporn Teethong

Faculty of Medical Technology, Huachiew Chalermprakiet University, Samut Prakan Province, 10540, Thailand

* Corresponding author. E-mail address: pnimmanee@gmail.com

Received: 24 September 2023; Revised: 8 March 2024; Accepted: 11 March 2024; Available online: 13 March 2024

Abstract

Thai farmers rely on manure-based fertilizers and irrigation water for plant cultivation, which poses a potential risk of bacterial contamination in their vegetables for sale. The objective of this study was to detect the contamination and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella* spp. and *Citrobacter freundii* in 80 samples of ready-to-eat vegetables from various food vendors in Bang Phli district, Samut Prakan Province. *E. coli* was found in 5 (6.25%) of the vegetable samples and *C. freundii* was found in 15 (18.75%) of the samples, while *Salmonella* spp. was not detected in any of the vegetable samples. Most *E. coli* isolates were found in basil samples whereas most of *C. freundii* were isolated from sprouts. The results of antimicrobial susceptibility tests of both *E. coli* and *C. freundii* against 8 antimicrobial agents using the disk diffusion method revealed that the isolates were susceptible to all antimicrobial agents tested. This study highlights the presence of bacterial contamination in ready-to-eat fresh vegetables, which stems from the use of manure fertilizers. This practice could be a significant source of important enteric pathogens, posing substantial health risks to consumers.

Keywords: Ready-to-Eat Fresh Vegetables, Antimicrobial susceptibility, *Escherichia coli*, *Salmonella* spp., *Citrobacter freundii*

Introduction

The consumption of fresh vegetables has increased due to the recognition of their high nutritional value and fiber content (Rahman et al., 2022; Alabi et al., 2022). Fresh vegetables, however, can be contaminated with enteric bacteria and bacterial pathogens in various ways, including from irrigation water, soil, and animal manure used as fertilizer. Previous studies have revealed the presence of *Escherichia coli*, *E. coli* O157:H7, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Providencia* spp., *Proteus* spp., *Citrobacter* spp., *Pseudomonas* spp., *Salmonella* spp. and *Listeria monocytogenes* in salad and fresh vegetables (Sapkota et al., 2019; Liu et al., 2017), indicating the potential exposure of consumers to the commensals and food-borne pathogens of mammalian gastrointestinal tract. The Enterobacterales such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. are usually considered non-pathogens, but they can cause serious infections in immunocompromised patients including urinary tract infections (Rahman et al., 2022). Poor personal hygiene of the food handlers can result in consumers being infected with these microorganisms (Liu et al., 2017). *E. coli* can cause diarrhea in children and is a major cause of morbidity and mortality in infants and young children in developing countries (Alabi et al., 2022). *Salmonella* spp. is one of the most common foodborne pathogens responsible for human gastroenteritis (Niyomdecha et al., 2016). Some *C. freundii* isolates produce toxins that can cause food poisoning and diarrhea in humans (Liu et al., 2017)

The use of antimicrobial agents in animal farming and agriculture to prevent infectious diseases and support growth is prevalent but their overuse promotes the spread of multidrug-resistant bacteria (Eibach et al., 2018). The presence of antimicrobial-resistant bacteria (ARB) and plasmid-containing antimicrobial-resistant genes in agriculture soil, animal manure and wastewater is of concern in that the uses of these materials for growing plants might contribute to the dissemination of ARB to the consumers (Marti et al., 2013). Extended-spectrum beta-lactamases (ESBLs) are the enzymes that hydrolyze expanded-spectrum cephalosporin antimicrobial agents such as ceftazidime and cefotaxime. These enzymes are inhibited by beta-lactamase inhibitors, especially clavulanate (Castanheira et al., 2021). In the past few decades, ESBL-producing bacteria including Enterobacterales and non-fermentative bacteria have emerged as contributors to increased human morbidity and mortality and have become a global concern. The majority of previous studies have focused on the detection of ESBL-producing bacteria in meats such as chicken, beef or pork. However, the contamination of fresh vegetables by these bacteria is not sufficiently monitored. It has been shown that ARB can colonize the surface of plants and could be transmitted to humans and animals through the consumption of uncooked vegetables (Janalikova et al., 2018). Most of these bacteria carry antibiotic-resistant genes and can transfer and spread the resistant genes to other bacterial species with the help of mobile elements in their genomes. Therefore, the consumption of raw fresh vegetables and fruits contaminated with bacteria that contain antimicrobial-resistant genes could be an important source of resistant bacteria in consumer intestinal flora (Schill et al., 2017; Rahman et al., 2022).

Previous studies have reported the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* from vegetables found in Southern Thailand. ESBL-producing bacteria were isolated from 7 out of 10 types of vegetables and were most resistant to beta-lactam antimicrobial agents (Romyasamit et al., 2021). Literature data reported that the most commonly observed ARB in the fresh produce were *E. coli* followed by *Klebsiella* spp. and *Salmonella* spp. (Rahman et al., 2022). *Citrobacter* spp. has variable culture, biochemical and antigenic characteristics that resemble those of *E. coli* and *Salmonella*. A study in Nigeria revealed that 25 *Citrobacter* spp. were isolated from fruit and vegetables and most of them were *C. freundii*. All isolates were 100% resistant to ceftriaxone and amoxicillin (Adegun et al., 2019). Samut Prakan is a small province located close to Bangkok with many factories, communities and educational institutions located in the province, and are usually served by vendors of food which often includes raw fresh vegetables that are usually consumed without washing.

The objective of the current study was, therefore, to detect the contamination and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella* spp. and *Citrobacter freundii* in samples of ready-to-eat vegetables from various food vendors in Bang Phli district, Samut Prakan Province, and to examine the prevalence and characteristics of *E. coli*, *Salmonella* spp. and *Citrobacter freundii* in those samples. The results from this study may be used to identify potential health risks for consumers.

Materials and Methods

Sample collection

Eighty ready-to-eat fresh vegetable samples from different food vendors in Bang Phli district, Samut Prakan Province, Thailand were collected from June to August 2022. The samples were separately kept in a zip-locked plastic bag and transported aseptically to the laboratory within 24 hr for further analysis.

Isolation of *E. coli*, *Salmonella* spp. and *C. freundii*

The isolation method was adapted from Sapkota et al. (2019). Twenty-five grams of each vegetable sample was mixed with 225 ml sterile water and left covered at room temperature for 30 min. One milliliter of each sample was then pre-enriched in buffer peptone water (BPW) and Gram-negative (GN) broth and was incubated at 37 °C for 24 hr. One loopful of the culture was streaked on Eosin Methylene Blue (EMB) agar for identification of *E. coli*, and Xylene Lysine Deoxycholate (XLD) agar for *Salmonella* spp. and *C. freundii* identification. The plates were then incubated at 37°C for 24 hr. Each suspected colony growing on EMB (black center and metallic luster for *E. coli*) and XLD (colorless and colorless with a black center for *Salmonella* spp. and *Citrobacter freundii*) were sub-cultured on MacConkey agar and further identified based on their morphological and biochemical characteristics (motility, indole, lysine deaminase, lysine decarboxylase, citrate and urea tests). The isolates that were suspected to be *Salmonella* spp. were confirmed by serotyping using *Salmonella* O polyvalent and Vi antisera (Serosystem). *Escherichia coli* ATCC 25922 was used as a reference strain for biochemical test control.

Antimicrobial susceptibility test

An antimicrobial susceptibility test was performed using modified Kirby Bauer's disk-diffusion method (CLSI 2021). Briefly, the pure culture of isolated bacteria was grown overnight in Tryptic soy broth at 37 °C for 16 to 18 hr and the concentration was adjusted until a 0.5 McFarland turbidity was attained using sterile normal saline. The culture was then swabbed onto Mueller Hinton agar using a sterile cotton swab and antibiotic disks were placed on the surface of the agar plates. After incubation at 35±2 °C for 16 to 18 hr, the diameters of the inhibition zones were measured and the results were interpreted as susceptible (S), intermediate (I) and resistance (R) according to CLSI guidelines. Antimicrobial agents, including cefixime (5 µg), ceftaroline (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) are cephalosporins used for ESBL screening. Another different class of antimicrobial agents are composed of carbapenem [ertapenem (10 µg) and meropenem (10 µg)], aminoglycosides [gentamicin (10 µg)], and tetracycline [tigecycline (15 µg)]. These antimicrobial agents were chosen based on an antimicrobial susceptibility test for Enterobacterlales (CLSI 2021). Organisms demonstrating resistance to three or more antibiotics from different structural classes were classified as multidrug-resistant (MDR). *Escherichia coli* ATCC 25922 was used as a reference strain for quality control of antimicrobial susceptibility test.

Screening and confirmation of ESBL production

Screening and confirmation of ESBL production were performed using methods recommended by CLSI (CLSI, 2021). Bacterial isolates that were resistant to cefixime, certaroline, ceftazidime or ceftriaxone were considered to indicate ESBL production. For confirmation, the confirmatory method was done using a combination disk method. The standard disk diffusion procedure was performed using two pairs of antibiotics including ceftazidime pairs with ceftazidime-clavulanate and cefotaxime pairs with cefotaxime-clavulanate. The plates were incubated at 35±2 °C for 16 to 18 hr. A ≥5-mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate and the zone diameter of the antimicrobial agent tested alone confirmed the production of ESBL.

Results

Distribution of *E. coli*, *Salmonella* spp. and *C. freundii* in fresh vegetables

In this study, ready-to-eat fresh vegetables from vendors of various kinds of food including meatballs/Isaan sausages, Isaan food/grilled fish, noodles and Thai rice noodle, were collected and analyzed for the presence of *E. coli*, *Salmonella* spp. and *C. freundii*. A total of 80 vegetable samples were analyzed, with *E. coli* being isolated from 5 samples (6.25%) and *C. freundii* from 15 samples (18.75%) (Table 1). There was no *Salmonella* spp. detected in any vegetable samples.

Table 1 Prevalence of *E. coli*, *Salmonella* spp. and *C. freundii* isolated from various kinds of food shops

Food shops	No. of samples	No. of *E. coli*	No. of *Salmonella* spp.	No. of *C. freundii*
Papaya salad/grilled fish	24	1 (4.17)	0 (0.00)	4 (16.67)
Thai rice noodle	21	0 (0.00)	0 (0.00)	3 (14.28)
Noodles	21	2 (9.52)	0 (0.00)	5 (23.80)
Meatball/ Isaan sausage	14	2 (14.29)	0 (0.00)	3 (21.43)
Total	80	5 (6.25)	0 (0.00)	15 (18.75)

Ten kinds of fresh vegetables were collected, including cabbages, basil, bean sprouts, cucumbers, Thai yardlong beans, bitter gourds, lettuces, Chinese cabbages, dill and parsley. *E. coli* was isolated from the cabbages (16.67%), basil (18.18%) and bitter gourd (14.29%) (Fig. 1), whereas *C. freundii* were detected from most vegetables except cabbage, Thai yardlong bean and Chinese cabbage (Table 2). The highest occurrence of *C. freundii* was found in bean sprouts (36.36%), parsley (33.34%), bitter gourds (28.57%) and lettuces (28.57%) (Fig. 2).

Table 2 Prevalence of *E. coli*, *Salmonella* spp. and *C. freundii* isolated from various kinds of ready-to-eat vegetables

Vegetable samples	No. of samples	No. of *E. coli*	No. of *Salmonella* spp.	No. of *C. freundii*
Cabbage	12	2 (16.67)	0 (0.00)	0 (0.00)
Basil	11	2 (18.18)	0 (0.00)	3 (27.27)
Bean sprout	11	0 (0.00)	0 (0.00)	4 (36.36)
Cucumber	11	0 (0.00)	0 (0.00)	2 (18.18)
Thai yardlong bean	7	0 (0.00)	0 (0.00)	0 (0.00)
Bitter gourd	7	1 (14.29)	0 (0.00)	2 (28.57)
Lettuce	7	0 (0.00)	0 (0.00)	2 (28.57)
Chinese cabbage	7	0 (0.00)	0 (0.00)	0 (0.00)
Dill	4	0 (0.00)	0 (0.00)	1 (25.00)
Parsley	3	0 (0.00)	0 (0.00)	1 (33.34)
Total	80	5 (6.25)	0 (0.00)	15 (18.75)

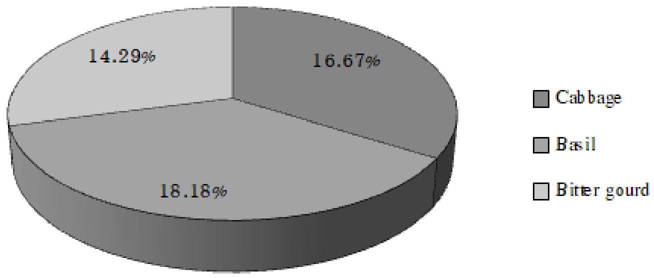

Figure 1 Prevalence of *E. coli* isolated from various kinds of ready-to-eat vegetables

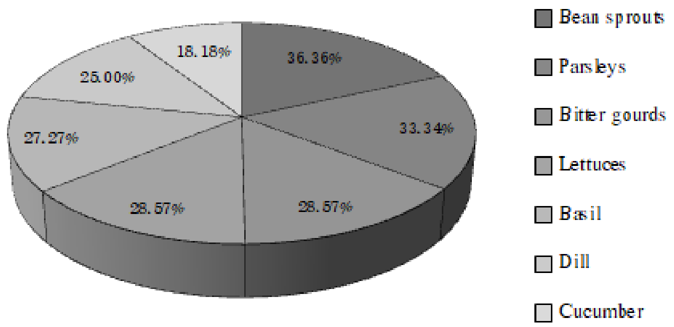

Figure 2 Prevalence of *C. freundii* isolated from various kinds of ready-to-eat vegetables

E. coli was found in basil samples from vendors of Isaan food/grilled fish and noodle shops, bitter gourd samples from noodle shops, and cabbages from meatball/Isaan sausage shops. No *E. coli* was identified in vegetables from Thai rice noodle shops. For *C. freundii*, the isolates were detected in dill, lettuces, basils, parsley, bean sprouts, bitter gourds and cucumbers from all kinds of food shops (Table 3).

Table 3 Prevalence of *E. coli* and *C. freundii* isolated from ready-to-eat vegetables from different food shops in Bang Phi district, Samut Prakan Province, Thailand

Food shops	Vegetable samples	No. of *E. coli*	No. of *C. freundii*
Isaan food/grilled fish			
1	Dill	-	1
2	Lettuce	-	1
3	Basil	-	1
4	Parsley	-	1
5	Basil	1	-
Thai rice noodle			
1	Bean sprout	-	1
2	Bean sprout	-	1
3	Bean sprout	-	1

Table 3 (Cont.)

Food shops	Vegetable samples	No. of *E. coli*	No. of *C. freundii*
Noodles			
1	Basil	1	-
	Bitter gourd	1	-
2	Bitter gourd	-	1
3	Lettuce	-	1
4	Bean sprout	-	1
5	Basil	-	1
6	Bitter gourd	-	1
Meatball/ Isaan sausage			
1	Basil	-	1
	Cucumber	-	1
2	Cabbage	1	-
3	Cucumber	-	1
4	Cabbage	1	-
Total		**5**	**15**

Antimicrobial susceptibility patterns

Antimicrobial susceptibility tests (AST) were performed for all *E. coli* and *C. freundii* isolates against 8 different antibiotics. The results revealed that all 5 isolates of *E. coli* and 15 isolates of *C. freundii* were susceptible to the antibiotics tested (Table 4).

Table 4 Antimicrobial susceptibility patterns of *E. coli* and *C. freundii* isolates

Antibiotics	**Susceptibility patterns***					
	***E. coli* isolates**			***C. freundii* isolates**		
	S	**I**	**R**	**S**	**I**	**R**
Cefixime	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Ceftaroline	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Ceftazidime	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Ceftriazone	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Ertapenem	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Gentamycin	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Meropenem	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)
Tigecycline**	5 (100%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)

*Results from two independent experiments

** US FDA The U.S. Food and Drug Administration

S, susceptible; I, intermediate; R, resistant

Discussion

The popularity of ready-to-eat vegetables can be attributed to their convenience and their high mineral content, dietary fiber and low fat and calorie content (Sapkota et al., 2019). In Thailand, fresh vegetables are commonly served as side dishes for various kinds of food such as meatballs, noodles or Isaan food. These vegetables are typically consumed raw and sometimes without washing, which can result in consumer exposure to enteric bacteria or food-borne pathogens contaminated by water, soil or manure (Rahman et al., 2022). Previous studies have reported the presence of antimicrobial-resistant bacteria and antibiotic-resistant genes on fresh produce. These include enteric bacteria such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. and pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., *Bacillus cereus* and *L. monocytogenes* (Rahman et al., 2022; Sapkota et al., 2019; Janalíková et al., 2018; Alabi et al., 2022). Consequently, the consumption of contaminated fresh vegetables may have adverse effects on human health and contribute to the spread of food-borne disease outbreaks.

E. coli is a common intestinal flora found in humans and animals and is often used as an indicator of fecal contamination in food (Janalíková et al., 2018). This bacterium can cause extraintestinal tract infections such as urinary tract infections and its pathogenic strain can cause poisoning in the gastrointestinal tract. Pathogenic strains of *E. coli* have been detected in fresh produce such as lettuces, cabbages, cucumbers and tomatoes (Rahman et al., 2022). A study conducted in Nepal reported the presence of *E. coli* in 13.45% of salad samples (Sapkota et al., 2019). Similarly, in a study conducted in Nigeria, *E. coli* was isolated from 37.7% of irrigated fresh vegetables (Alabi et al., 2022). In this study, 80 ready-to-eat fresh vegetable samples were analyzed, and it was found that 6.25% of samples were contaminated by *E. coli*. Specifically, *E. coli* was detected in cabbage, basil and bitter gourd samples. Two isolates from basil and bitter gourd were detected in the same noodle shop. This might indicate poor food vendor hygiene such as inadequate hand or vegetable washing. Romyasamit et al. (2021) examined the prevalence and characteristics of ESBL-producing *E. coli* and *K. pneumoniae* from raw vegetables retailed in Southern Thailand. The results revealed that the highest frequencies of ESBL-producing isolates were found in Thai yardlong beans (8.8%) and Thai basil (8.3%) followed by winged beans, tomatoes and cucumbers. The lower isolates of *E. coli* in this study compared with those of other studies may be attributed to differences in sample size and types of vegetables tested.

Citrobacter species are commensal bacteria in human and animal intestinal tracts and have been recovered from sewage, and contaminated water and soil. *C. freundii* is the most common *Citrobacter* species that can cause food poisoning and diarrhea in humans (Liu et al., 2017). It has been shown that biofilm is an important virulence factor for the establishment of C. *freundii* infection, enhanced pathogenesis and multidrug resistance (Bunyan, 2020). A Nigerian study by Adegun et al. (2019) investigated *Citrobacter* spp. contamination in fruit and vegetables and revealed that *Citrobacter* spp. was present in 44% of vegetable samples, with *C. freundii* being the most common isolates (81.8%). In this present study, 18.75% of fresh vegetable samples were contaminated with *C. freundii*. The isolates were cultured from almost all kinds of vegetables tested especially in bean sprouts and basil. Three of four isolates were found in three different Thai rice noodle food shops. Bean sprout production involves a usual seed germination process that can support the growth of microorganisms. Microbial pathogen contamination of bean sprouts is a major concern to the industry. Many treatment methods for seed disinfection have been recommended with the concern for environmental and consumer safety including chemical treatment with calcium hypochlorite or physical method using high pressure treatment (Ding et al., 2013).

Salmonella spp. is a food-borne pathogen widespread in Thailand (Niyomdecha et al., 2016). The genus *Salmonella* causes a disease referred to as salmonellosis. The symptoms are usually headache, abdominal pain, diarrhea and vomiting (Kowalska, 2023). Humans are infected via the food supply chain from food and materials of animal origin. However, consumption of fresh vegetables can also pose a risk of *Salmonella* infection due to the use of animal manure as fertilizer in agriculture. In this present study, no *Salmonella* spp. was isolated from the fresh vegetables tested. In contrast, a study by Niyomdecha et al. (2016) determined serotypes and antimicrobial resistance of *Salmonella enterica* isolated from pork, chicken meat and lettuce from Thailand and their results showed that *Salmonella* was found in 20% of lettuce samples. Out of 39 multi-drug resistant (MDR) isolates, 25% were from lettuce. It has been shown that *Salmonella* can tolerate various environmental stresses such as low pH, hyperosmolarity and heat (Keerthirathne et al., 2016). The absence of *Salmonella* in vegetable samples in our study can be ascribed to the sample size, prevalence and the amount of the organism in the samples.

Leafy vegetables such as lettuce, basil, dill and parsley possess a large surface area that can easily be colonized by enteric bacteria from soil, fertilizer or irrigation water. Nevertheless, the results from this present study demonstrated that *E. coli* and *C. freundii* could be isolated from both leafy and non-leafy vegetables. *E. coli* was detected in three kinds of fresh vegetables including basil, bitter gourd and cabbage, whereas *C. freundii* was found in seven kinds of vegetables but not in cabbage. This indicates that the dissemination of enteric bacteria is varied and contamination of enteric bacteria in ready-to-eat vegetables not only occurs during cultivation and harvesting but also during washing, cutting, packaging and transporting (Kowalska, 2023).

In this study, all bacterial isolates were still susceptible to the antibiotics used. This might be due to the low number of bacterial isolates found in fresh vegetables. Nonetheless, despite the absence of antibiotic-resistant isolate, there is still a concern about the contamination of the enteric bacteria in fresh vegetables that are commonly eaten raw.

Conclusion and Suggestions

The results from this study indicate the presence of bacterial contamination in ready-to-eat fresh vegetable samples. Consumption of contaminated fresh produce without further cleaning can pose a serious risk to human health and can contribute to the spread of antibiotic-resistant bacteria. It is crucial to prioritize personal hygiene practices among both sellers and consumers, including thorough handwashing and washing fresh produce before consumption. These measures are essential in reducing the infections caused by food-borne pathogens and opportunistic organisms.

Acknowledgements

We appreciatively acknowledge financial support from Faculty of Medical Technology, Huachiew Chalermprakiet University. We would also like to thank Miss Norapat Pinaisullakakij, Bachelor of Engineering - Aerospace, Aeronautical and Astronautical Engineering from the University of Manchester for manuscript correction. Many thanks also to Mr Roy I. Morien of the Naresuan University Graduate School for his editing of the grammar, syntax and general English expression in this manuscript.

Author Contributions

Author 1 (Panjaphorn Nimmanee) : Conceptualization of the research, development of methodology, formulate the topic of the research, data analysis, interpretation, manuscript writing, manuscript review and editing.

Author 2 (Porntip Paungmoung) : Conceptualization of the research, development of methodology, data analysis, interpretation and corrected manuscript.

Author 3 (Chatnapa Simarub) : Investigattion and collection of data.

Author 4 (Nattiya Phromrungsawat) : Investigattion and collection of data.

Author 5 (Pojnicha Yimnongpho) : Investigattion and collection of data.

Author 6 (Marisa Phuttatate) : Investigattion and collection of data.

Author 7 (Suthinan Arnamwong) : Investigattion and collection of data.

Author 8 (Patsaraphon Lumlong) : Investigattion and collection of data.

Author 9 (Chalinee Boonchamchoo) : Investigattion and collection of data.

Author 10 (Poochisa Sangchan) : Investigattion and collection of data.

Author 11 (Thanapron Champhong) : Investigattion and collection of data.

Author 12 (Tanaporn Teethong) : Investigattion and collection of data.

Conflict of Interests

The authors declare no conflict of interest.

Funding

This work was supported by Huachiew Chalermprakiet University.

References

Adegun, B. R., Oluduro, A. O., & Aregbesola, O. A. (2019). Isolation and molecular characterization of *Citrobacter* species in fruits and vegetables sold for consumption in ILE-IFE, Nigeria. *Scientific African, 6*, e00173. https://doi.org/10.1016/j.sciaf.2019.e00173

Alabi, O. H., Obiekezie, S. O., & Ekeleme, K. (2022). Antibiotic resistance profiling and molecular detection of *Escherichia coli* pathotypes isolated from irrigated fresh vegetables. *AROC in Pharmaceutical and Biotechnology*, *2*(1), 27-35. https://doi.org/10.53858/arocpb02012735

Bunyan, I. A. (2020). Molecular study of some virulence factors and antimicrobial susceptibility pattern of *Citrobacter freundii* isolated from human diarrhea. *Systematic Review Pharmacy*, *11*(4), 195-201. https://doi.org/10.31838/srp.2020.4.28

Castanheira, M., Simmer, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristic, epidemiology and detection. *JAC Antimicrobial Resistance*, *3*(3), 1-21. https://doi.org/10.1093/jacamr/dlab092

CLSI, (2021). Performance standard for antimicrobial disk susceptibility tests, *CLSI supplement M100* (30th ed.). Clinical and Laboratory Standards Institute.

Ding, H., Fu, T. J., & Smith, M. A. (2013). Microbial contamination in sprouts: how effective is seed disinfection treatment. *Journal of Food Science, 78*(4), R495-501. https://doi.org/10.1111/1750-3841.12064

Eibach, D., Dekker, D., Boahem, K. G., Akenten, C. W., Sarpong, N., Campos, C. B., Berneking, L., Aepfelbacher, M., Krumkamp, R., Owusu-Dabo, E. & May, J. (2018). Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana. *Verinary Microiology, 217*, 7-12. https://doi.org/10.1016/j.vetmic.2018.02.023

Janalíková, M., Pleva, P., Pavlicková, S., Lecomte, M., Godillon, T., & Holko, I. (2018). Characterization of *Escherichia c*oli strains isolated from raw vegetables. *Potravinarstvo Slovak Journal of Food Sciences, 12*(1), 304-312. https://doi.org/10.5219/897

Keerthirathne, T. P., Ross, K., Fallofield, H., & Whiley, H. (2016). A review of temperature, pH and other factors that influence the survival of Salmonella in mayonnaise and other raw egg products. Pathogens, 5(4), 63. https://doi.org/10.3390/pathogens5040063

Kowalska, B. (2023). Fresh vegetables and fruit as a source of *Salmonella* bacteria. *Annuals of agricultural and environmental medicine, 30*(1), 9–14. https://doi.org/10.26444/aaem/156765

Liu, L., Lan, R., Liu, L., Wang, Y., Zhang, Y., Wang, Y., & Xu, J. (2017). Antimicrobial resistance and cytotoxicity of *Citrobacter* spp. in Maanshan Anhui Province, China. *Frontiers in Microbiology, 20*(8), 1357. https://doi.org/10.3389/fmicb.2017.01357

Marti, R., Scott, A., Tien, Y. C., Murray, R., Sabourin, L, Zhang, Y., & Topp, E. (2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Applied and Environmental Microbiology, 79*(18), 5701-5709. https://doi.org/10.1128/aem.01682-13

Niyomdecha, N., Mungkorndaew, N., & Samosornsuk, W. (2016). Serotypes and antimicrobial resistance of *Salmonella enterica* isolated from pork, chicken meat and lettuce, Bangkok and central Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health, 47*(1), 31-39.

Rahman, M., Alam, M. U., Luies, S. K., Kamal, A., Ferdous, S., Lin, A., Sharior, F., Khan, R., Rahman, Z., Parvez, S. M., Amin, N., Hasan, R., Tadesse, B. T., Taneja, N., Islam, M. A. & Ercumen, A. (2022). Contamination of fresh produce with antibiotic-resistant bacteria and associated risks to human health : a scoping review. *International Journal of Environmental Research and Public Health, 360*(19), 1-15. https://doi.org/10.3390/ijerph19010360

Romyasamit C., Sornsenee, P., Chimplee, S., Yuwalaksanakun, S., Wongprot, D., & Saengsuwan, P. (2021). Prevalence and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from raw vegetabels retailed in Southern Thailand. *PeerJ, 9*, e:11787. https://doi.org/10.7717/peerj.11787

Sapkota, S., Adhikari, S., Pandey, A., Khadka, S., Adhikari, M., Kandel, H., Pathak, S. & Pandey, A. (2019). Multi-drug resistant extended-spectrum beta-lactamase producing *E. coli* and *Salmonella* on raw vegetable salads served at hotels and restaurants in Bharatpur, Nepal. *BMC Research Notes, 12*(1), 516. https://doi.org/10.1186/s13104-019-4557-9

Schill, R., Abdulmawjood, A., Klein, G., & Reich, F. (2017). Prevalence and characterization of extended-spectru β-lactamase (ESBL) and AmpC β-lactamase producing Enterobacteraceae in fresh pork meat at processing level in Germany. *International Journal of Food Microbiology*, *257*(18), 58-66. https://doi.org/10.1016/j.ijfoodmicro.2017.06.010