زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

Resistance Profile of *Escherichia coli* Isolated from Leafy Vegetables in Erbil Province

Research Project

Submitted to the Department of (Biology) in partial fulfillment of the requirements for the degree of **BSc.** in **Biology**

By

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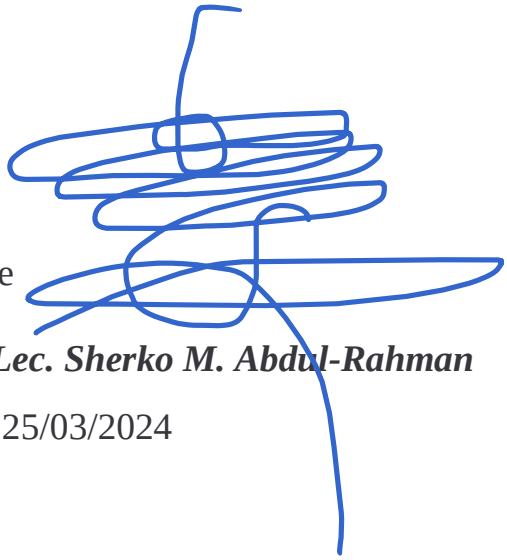
APRIL – 2024

CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc.** degree in **Biology** with my approval as a supervisor.

Signature 

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Date: 25/03/2024

DEDICATION

- To our supervisor **Lec. Sherko M. Abdul-Rahman** for his support.
- To our friends, colleagues, and all whom we love.
- We dedicate this research to our families who always supported and helped us, also we dedicate it to those people who helped us. all our friends whom contributed more or less in completing this research.

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ACKNOWLEDGMENTS

"In the name of God most merciful, most compassionate" First of all, thanks to God for giving me the strength, health and facilitating the ways to complete ours work.

We wish to express our sincere gratitude to our supervisor, **Lec. Sherko M. Abdul-Rahman** for his wise guidance in planning this research work, and for his valuable suggestions.

Also, we would like to grateful thanks and great respect to **Dr. Muayad A. Mahmood** in Medical Laboratory Technique Department, Shaqllawa Technical College, Erbil Polytechnic University, Erbil, Iraq for giving laboratory facilities.

We also indebted to all who helped me during in the process of this study.

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ABSTRACT

Leafy vegetables, including lettuce and spinach, have been implicated in several outbreaks of foodborne disease caused by *Escherichia coli* O157:H7, a pathogen of increasing public health significance because of the severity of the gastrointestinal illness and long-term, chronic sequelae that can result from infection. A definitive association between the consumption of leafy vegetables and human disease provides implicit evidence of transfer from animal sources to field crops and retail commodities, including minimally processed or fresh-cut products. Understanding the behavior of *E. coli* O157:H7 in leafy vegetables during production, after harvest, in storage, during processing, and in packaged fresh-cut products is essential for the development of effective control measures. To this end, previous research on the fate of the species at each step in the production of market-ready leafy vegetables is reviewed in this study. Several critical gaps in knowledge are identified, notably uncertainty about the location of contaminating cells on or in plant tissues, behavior in packaged products stored at low temperatures, and the influence of environmental stresses on growth and infectivity.

Keywords: *Escherichia coli*; Food safety; Leafy green vegetables.

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1. INTRODUCTION

Escherichia coli is a bacterium with a special place in the microbiological world since it can cause severe infections in humans and animals but also represents a significant part of the autochthonous microbiota of the different hosts. of major concern is a possible transmission of virulent and/or resistant e. coli between animals and humans through numerous pathways, such as direct contact, contact with animal excretions, or via the food chain (Poirel et al., 2018, Johannessen et al., 2005)

E. coli also represents a major reservoir of resistance genes that may be responsible for treatment failures in both human and veterinary medicine. An increasing number of resistance genes has been identified in E. coli isolates during the last decades, and many of these resistance genes were acquired by horizontal gene transfer. In the enterobacterial gene pool, E. coli acts as a donor and as a recipient of resistance genes and thereby can acquire resistance genes from other bacteria but can also pass on its resistance genes to other bacteria. In general, antimicrobial resistance in E. coli is considered one of the major challenges in both humans and animals at a worldwide scale and needs to be considered as a real public health concern.(Poirel et al., 2018, Faour-Klingbeil et al., 2016).

Foodborne illnesses caused by various microorganisms including bacteria are major concern to public health that led to morbidity and mortality in both developed and developing countries (Van et al., 2008). Foodborne diseases not only affect people's well-being, but also cause hospitalization and economical loss (Sadiq et al., 2015). Generally, food contributes as an important part for transfer of antibiotic resistance in terms of antibiotic residues or resistant genes from food microflora to pathogenic bacteria. In order to improve and implement food safety system, monitoring the prevalence of pathogenic bacteria along with antibiotic resistant foodborne pathogens in food chain is one of the major tasks. Fresh produces are normally colonized by a wide variety of spoilage and pathogenic microorganisms (Akbar and Anal, 2013, Faour-Klingbeil et al., 2016).

Pathogenic Escherichia coli is generally regarded as part of the normal flora of the human intestinal tract and animals. Most of Escherichia coli strain are nonpathogenic, however some of them are major food borne pathogen of public health importance and responsible for watery and bloody diarrhea, infantile diarrhea, traveler's diarrhea, hemolytic uremic syndrome (Alzubaidy, 2019). E. coli divided into diarrheal pathogens causing diarrhea (DPEC) and extra intestinal pathogenic E. coli. (Alzubaidy, 2019, Benjamin et al., 2018). Based on their pathogenic phenotypes and the diseases that they cause, diarrhoeagenic E. coli have been classified into 6 groups: enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteroaggregative E. coli (EAEC), diffusely adherent E. coli (DAEC), and Shiga toxin producing E. coli (STEC)/enter hemorrhagic E. coli (EHEC)/ verocytotoxin-producing E. coli (VTEC) (Kaper et al., 2004). Epidemiology, treatment, pathogenesis, and clinical manifestations can be used to preliminary identify each pathotype (Huang et al., 2006, Johannessen et al., 2005).

Due to the importance of E. coli as the primary causal agent of traveler's diarrhea with an easy transmission with food in addition to their high drug resistance pattern, this study aimed to find out the exact effluent level of E. coli, particularly toxins in food samples especially leafy vegetables collected from in Erbil province, to find out genetic diversity and clonal relatedness of the isolated strains and to determine the resistant profile against different antibiotics that most commonly used for therapeutic purposes in animal and human being.

2. LITRATURE REVIEW

2.1. Typical and atypical enteropathogenic E. coli

The term enteropathogenic E. coli (EPEC) was first used to describe a number of E. coli strains epidemiologically related to a series of outbreaks of infantile diarrhea in the 1940s and 1950s. Originally identified by serotype, EPEC are now defined as those E. coli strains having the ability to cause diarrhea, to produce a histopathology on the intestinal epithelium known as the attaching and effacing (AE) lesion, and the inability to produce Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins.(Taylor et al., 1995, Jablasone et al., 2005).

Improvements in techniques allowing a better understanding of the genome and virulence mechanisms among EPEC strains over the years have led to the sub-classification of EPEC into typical EPEC (tEPEC) and atypical EPEC (aEPEC). Typical EPEC strains causing human infectious diarrhea possess a large virulence plasmid known as the EPEC adherence factor (EAF) plasmid (pEAF), which encodes the type IV fimbriae called the bundle-forming pilus (BFP), while aEPEC do not possess this plasmid (Faour-Klingbeil et al., 2016, Delaquis et al., 2007).

2.2. Behavior of E. coli O157:H7 on leafy vegetables during production. Microbiological examination of commercially grown fresh produce suggests that contamination with pathogenic E. coli is a rare occurrence. E. coli O157:H7 was not detected in 1,000 samples of U.S. domestic and imported produce (including 230 lettuce samples) collected in 1999 or 2000. A similar result was reported for 466 produce (175 leafy green lettuce samples) and environmental swabs collected in eight packing sheds in the southern United States. Leaf lettuce (179 samples) collected directly from Norwegian farms with organic production practices that included the use of manure fertilizers was also free of E. coli O157: H7. Analysis of approximately 4,000 green leaf and romaine lettuce samples as part of a large U.S. Department of Agriculture survey composed of more than 10,000 samples collected in 2002 indicated that lettuce was more likely to be contaminated with generic E. coli than other produce. Isolates bearing virulence factors indicative of pathogenic potential were found more frequently in lettuce than in celery, tomatoes, or cantaloupe, although the frequency of their isolation was well below 1%, and none belonged to the O157:H7 serotype. In contrast, neither serotype O157:H7 nor virulence genes (stx1 and stx2) were detected among E. coli isolates recovered from organically or conventionally grown produce from Minnesota. Similar findings were reported for an expanded investigation that included farms with varied agronomic practices in both Minnesota and Wisconsin. It should be noted that two non-O157:H7 strains isolated from cilantro and cantaloupe during the course of the 2002 U.S. Department of Agriculture survey carried the stx1 and stable toxin genes that are associated with Shiga-toxigenic E. coli and enterotoxigenic E. coli, respectively, a finding that illustrates the genetic diversity and variability in pathogenic potential within E. coli isolates recovered from fresh produce(Riley et al., 1983, Faour-Klingbeil et al., 2016, Delaquis et al., 2007).

2.3. Fate of E. coli O157:H7 during harvest and postharvest handling.

Field practices at harvest and immediately postharvest vary according to commodity and intended use. Loose leaf and head lettuces destined for the whole market are usually hand cut, trimmed, and boxed in the field before postharvest handling(Riley et al., 1983, Takeuchi et al., 2000). Cellophane wrapping of head lettuce may also be done at this time. The boxes are generally vacuum cooled, although air cooling is not uncommon in smaller operations. Lettuce destined for fresh cut processing is typically hand cut, occasionally field cored, and transported to the processing facility in bulk containers. Specialty leaf lettuces are normally harvested by hand, although mechanical harvesting is possible. Spinach destined for the fresh market is harvested by hand, tied in bunches, and boxed in the field. Processed spinach is harvested by machine and transported in bulk to the packinghouse. Cooling is generally achieved under forced air, but more gradual reduction in temperature in passive storage is still common. All produce intended for processing may be stored for a time before processing(Sapers et al., 2005, Delaquis et al., 2007, Benjamin et al., 2018).

2.4. Fate of E. coli O157:H7 during processing.

The production of fresh-cut leafy vegetables involves the application of several unit operations, including trimming, cutting, and washing. Disruption of the tissues and their conversion into bite-size pieces are known to affect both the quality and microbiology of the final product. Exposure to sanitizers, low temperatures commonly employed in wash water, and mechanical forces encountered in flumes, cutting equipment, or centrifuges constitutes stresses that have a significant bearing on the fate of contaminating microorganisms(Takeuchi et al., 2000, Benjamin et al., 2018). Lettuces are typically sliced or shredded prior to packaging. The resulting damage irrevocably alters biochemical processes and provides ample opportunity for microbial invasion of tissues. Microscopic techniques, notably confocal scanning laser microscopy, have been used to examine the interaction between E. coli O157:H7 and cut lettuce surfaces. Immersion of cut leaves in active cell suspensions results in attachment to sites on the leaf surface and trichomes, and cell densities tend to be highest in stomata and along cut edges. Penetration into the tissues adjacent to cut edges can occur, particularly at low temperature (Faour-Klingbeil et al., 2016). Stomata have long been suspected to serve as passive ports of bacterial entry into plant tissues, although, to our knowledge, there is no evidence that human pathogens do so in intact plants. Interestingly, a recent report describes stomatal closure activated by guard cells in Arabidopsis, which restricts bacterial passage unless the invading bacterium possesses specific virulence factors that cause stomatal reopening. Whether such closure mechanisms exist in leafy vegetables and whether they remain active after harvest are not known.(Takeuchi et al., 2000, Delaquis et al., 2007)

2.5. Fate of E. coli O157:H7 in packaged leafy vegetables.

Native or introduced microorganisms associated with entire plants are presented with highly altered physical and chemical environments in packaged leafy vegetables. Although nutritional conditions at the leaf surface are growth limiting the release of cell sap from bruised, punctured, or cut tissues provides a supply of nutrients that can support extensive microbial growth (Sapers et al., 2005). The composition and growth of spoilage microflorae in packaged vegetables are primarily influenced by the ability of individual species to compete for these resources at refrigeration temperatures. Additional selective pressures, including competition between species, the gaseous composition of the atmosphere, and the presence of antimicrobial compounds, also contribute to these events. The fate of E. coli O157:H7 cells. Consistent with research conducted in growing plants, experimental conditions varied widely between individual investigations. Growth of E. coli O157:H7 was clearly stimulated at temperatures considered abusive for chilled foods, and population increases of 3 log CFU g1 were reported after 1 week at 21 to 22C. Storage at temperatures 8C led to different outcomes, ranging from slight growth to no change or to measurable losses in viability. Francis and O'Beirne reported an increase of 1 log CFU g1 after 12 days at 8C. The lower limits of growth are generally held to be 5C for generic E. coli and 8C for E. coli O157:H7, although reference to growth in synthetic media and milk at 5.5 to 6.5C (38) or at 6C in synthetic media is found in the scientific literature. An attempt has been made to model the growth of E. coli O157:H7 in cut lettuce. Comparisons between observed and predicted responses showed that the model adequately predicts growth at temperatures above 5C, although maximum population density was severely overestimated. The model failed to predict behavior at 5C(Sapers et al., 2005, Delaquis et al., 2007, Benjamin et al., 2018).

3. METHODOLOGY & RESEARCH DESIGN

3.1. Sampling

All equipment (conical flask, prudish, contender, loop, etc.) used for the culturing bacteria was disinfected with %95 ethanol and a short period of flaming. A total of 20 lettuce orchard samples collected on a random basis from Shaqllaua, hujran, masif, korey, Hawler The samples were immediately transferred to appropriate containers and labeled with an identification mark. The samples were carefully handled, kept in refrigerator (4℃) and immediately transported to our laboratory facilities for analysis. The isolates were performed in the Microbiology Laboratory of at the department of Medical Laboratory Technology/ Shaqlawa Technical College/EPU. Briefly, 1g of each sample was homogenized in a sterile universal tube, which contained 9 ml BHI broth supplemented with 0.05mg/L Cefixime to prevent the growth of other unwanted lactose fermentators. After overnight incubation at 37°C, a portion of the mixture was cultivated on MacConkey agar plate and further incubated at 37°C for overnight.

3.2. Analysis of the E. coli samples

After storing the samples in the special plastic bag, the plates were prepared according to the instruction. first, near a Bunsen burner was spread the necessary amount of MAC agar on the surface tenfold of the plates, after a few minutes the plates were labeled and stored with contrast in the refrigerator. similar tenfold of containers were labeled and amount 7.5ml of BHI was transferred to the containers and a small piece of the samples was taken with a sterile scissor and mixed with BHI , then containers were stored for 24 hours in the incubator , The next day،receiving a drop of BHI and streak on the agars with sterile loop , the plates were kept in an incubator at 37c for 24 hours .The culture plates are then incubated at 37oC for 24 hours and the bacterial colonies inspected for the presence of lactose fermenter pink to dark pink color on MAC plates

4. RESULTS AND DISCUSSION

A total of 10 samples of lettuce and 10 samples of chard were collected from 5 selected markets in Erbil cities, during a time-period from a February 2024 to March 2024. During each of the visits to the markets, the sampler behaved like an average Kurdish consumer and shopped lettuce and chard, all samples were collected aseptically was stored in clean bags and transferred to the micro laboratory within 15 hours.

LOCATION	LETTUCE	CHARD
Shaqlawa	3	1
Hujran	1	3
Kore	3	3
Masif	1	1
Erbil-kuran	2	2


Mac
6

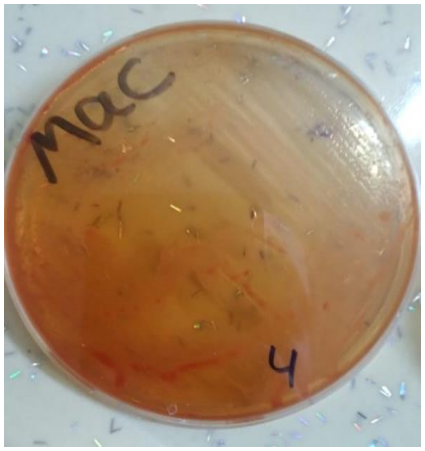

Mac
5


Mac
1


Mac
8


Mac
3


Mac
10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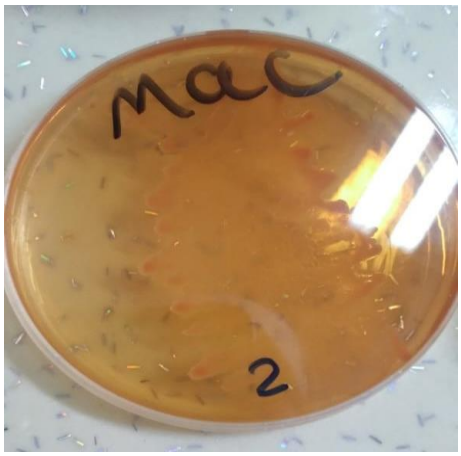
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4. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our data suggests that the fresh raw green leaves should be washed thoroughly prior to eating. This might also be true for fruiting vegetables to remove bacteria and chemicals, this research is using to raise awareness of the potential for bacterial contamination in raw vegetables as well as especially E. coli, and how it resists on vegetables and the risk of adverse health effects if these vegetables are irrigated with untreated wastewater.

Conclusions and future prospects. The prevalent view in classical food microbiology is that individual foods provide vast habitats where microorganisms compete under constant stress. Pioneering studies in dairy and meat microbiology have defined the major stresses (temperature, pH, water activity, etc.) that must be overcome for successful colonization by spoilage microorganisms or human pathogens. These food systems generally have a fairly constant chemical composition, provide a rich supply of nutrients for microbial growth, tend to offer almost unlimited sites for colonization, and are usually contaminated by direct means (i.e., contamination of meat during slaughter). Interest in the behavior of human pathogens in horticultural products, including leafy vegetables, is comparatively recent. Although valuable insights were gained from original research that employed assumptions, approaches, and techniques widely applied in food microbiology, accumulating evidence indicates that a new paradigm is necessary to elucidate their behavior in fresh horticultural products.

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پوختە

سەوزەی گەڵادار، لەوانەش کاهوو و سپێناخ، بەشدارن لە چەندین بڵاوبوونەوەی نەخۆشی گواستراو بە خۆراک کە بەهۆی ئێشێریچیا کۆلای**O157:H7** هوە دروست دەبێت، کە مادەیەکی نەخۆشخوازە کە گرنگی تەندروستی گشتی زیاد دەکات بەهۆی توندی نەخۆشی گەدە و ڕیخۆڵە و لێکەوتەی درێژخایەن و درێژخایەن کە دەتوانێت لە ئەنجامدا بێت لە هەوکردنەوە. پەیوەندییەکی یەکلاکەرەوە لە نێوان خواردنی سەوزەی گەڵادار و نەخۆشی مرۆڤ بەڵگەی ناڕاستەوخۆی گواستنەوە لە سەرچاوە ئاژەڵییەکانەوە بۆ بەرهەمە کێڵگەییەکان و کاڵاکانی تاکەکەسی، لەوانەش بەرهەمە کەمترین پرۆسێس کراوەکان یان تازە بڕاوەکان، دەخاتە ڕوو. تێگەیشتن لە هەڵسوکەوتی ئی.کۆلای **O157:H7**لە سەوزە گەڵادارەکاندا لە کاتی بەرهەمهێنان، دوای دروێنەکردن، لە هەڵگرتن، لە کاتی پرۆسێسکردن و لە بەرهەمە تازە بڕاوەکانی پاکەتکراودا زۆر گرنگە بۆ پەرەپێدانی ڕێوشوێنی کۆنترۆڵکردنی کاریگەر. بۆ ئەم مەبەستەش لێکۆڵینەوەکانی پێشوو لەسەر چارەنووسی جۆرەکان لە هەر هەنگاوێکی بەرهەمهێنانی سەوزەی گەڵادار کە ئامادەن بۆ بازاڕ لەم لێکۆڵینەوەیەدا پێداچوونەوەیان بۆ کراوە. چەندین بۆشایی گرنگ لە زانیاریدا دەستنیشان دەکرێن، بەتایبەتی نادڵنیایی سەبارەت بە شوێنی خانە پیسکەرەکان لەسەر یان لە شانەکانی ڕووەک، ڕەفتار لە بەرهەمە پاکەتکراوەکاندا کە لە پلەی گەرمی نزمدا هەڵگیراون، و کاریگەری فشارە ژینگەییەکان لەسەر گەشەکردن و تووشبوون.

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پرۆفایلی بەرگری بەکتریای (*Escherichia coli*) جیاکراوەتەوە لە سەوزەی گەڵادار لە پارێزگای هەولێر

ئەم پرۆژەی دەرچوونە پێشکەشە بە بەشی بایۆلۆجی وەك بەشێك لە پێداویستیەکان بۆ بەدەستهێنانی بڕوانامەی بەکالۆریۆس لە زانستی بایۆلۆجی

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