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PREVALENCE OF GRAM-NEGATIVE BACTERIA ISOLATED FROM FRUIT AND VEGETABLES RETAILED IN ADANA PROVINCE

ADANA İLİNDE PERAKENDE SATILAN MEYVE VE SEBZELERDEN İZOLE EDİLEN GRAM-NEGATİF BAKTERİLERİN YAYGINLIĞI

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ABSTRACT

In this study, 65 samples of fresh products (leafy vegetables, ready-to-eat mixed salads, and fruits) retailed in Adana province of Turkiye were investigated for presence of Gram-negative bacteria. *Salmonella* spp. was undetected in any samples. However, *Klebsiella pneumoniae* (22%), *Stenotrophomonas maltophilia* (15%), *Citrobacter freundii* (11%), *E. coli* (11%), *Klebsiella oxytoca* (11%), *Acinetobacter* spp. [*A. baumannii*, *A. pittii*, (7%)], *Pseudomonas putida* (7%), were the predominant microorganisms and were detected in (19/65) 29.2% of samples by MALDI-TOF system. Their bacterial counts were between 3.6 and 8.3 log CFU/g. Strains of *Enterobacter ludwigii* (4%), *Delftia lacustris* (4%), *Burkholderia cenocepacia* (4%), and *Burkholderia vietnamiensis* (4%) were less detected. Our results revealed the fruits and vegetables produced in Adana province generally comply with microbiological criteria of the Turkish Food Codex interms of *Salmonella* spp., but higher bacterial counst of *E. coli* (between 5.8 and 6.3 log CFU/g) was found. Besides, *Escherichia coli*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Stenotrophomonas maltophilia* species could be risky to human health.

Keywords: Contamination, Gram-negative bacteria, fresh produce, foodsafety.

ÖZET

Bu çalışmada, Türkiye'nin Adana ilinde perakende satışı yapılan 65 adet taze ürün örneğinde (yapraklı sebzeler, hazır karışık salatalar ve meyveler), Gram- negatif bakteri varlığı araştırılmıştır. *Salmonella* spp. hiç bir örnekte bulunmamıştır. Ancak, *Klebsiella pneumoniae* (%22), *Stenotrophomonas maltophilia* (%15), *Citrobacter freundii* (%11), *E. coli* (%11), *Klebsiella oxytoca* (%11), *Acinetobacter* spp. [*A. baumannii*, *A. pittii*, (%7)], *Pseudomonas putida* (%7) baskın mikroorganizmalar olup, örneklerin (19/65) %29.2'sinde MALDI-TOF sistemi ile tespit edilmiştir. Bakteri sayıları 3.6 ile 8.3 log CFU/g arasında bulunmuştur. *Enterobacter ludwigii* (%4), *Delftia lacustris* (%4), *Burkholderia cenocepacia* (%4) ve *Burkholderia vietnamiensis* (%4) suşları ise daha az oranda tespit edilmiştir. Sonuçlarımız Adana ilinde üretilen meyve ve sebzelerin, *Salmonella* spp. açısından Türk Gıda Kodeksi mikrobiyolojik kriterlerine genel olarak uygun olduğunu, ancak *E. coli* sayısının daha yüksek olduğunu (5.8 ile 6.3 log CFU/g arasında) ortaya koymuştur. Ayrıca, *Escherichia coli*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Citrobacter freundii* ve *Stenotrophomonas maltophilia* türleri, insan sağlığı için riskli olabilir.

Anahtar kelimeler: Kontaminasyon, Gram-negatif bakteriler, taze ürünler, gıda güvenliği

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INTRODUCTION

In the last decade, foodborne outbreaks associated with the consumption of fresh vegetables and fruits have been increasing worldwide (Lynch et al., 2009; Carvalheira et al., 2017). In the United States, the proportion of all foodborne outbreaks reported with a defined food source associated with consumption of fresh fruit and vegetables was 0.7% in the 1970s; In the 1990s, this rate increased to 6%, and in 2000s this rate reached 13%. Similarly, an outbreak of *Listeria monocytogenes* in European countries that affected Austria, Denmark, Finland, Sweden, and the United Kingdom between 2015 and 2018 was reported to be caused by contaminated frozen vegetables (EFSA, 2018). Recently (June 2020), it was reported that the *Salmonella* Newport epidemic (1127 cases, 167 hospitalizations) in the USA, which affected approximately 48 States, was caused by the consumption of red onions (Sivapalasingam et al., 2004; EFSA, 2018; Colosi et al., 2020). Numerous foodborne bacteria cause bacterial intoxication amongst these the most important and most frequently encountered are *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium* spp., *Escherichia coli* O157:H7, *Shigella* spp., *Yersinia enterocolitica*, *Brucella* spp., and *Aeromona*s spp. (Abebe et al., 2020; Sun et al., 2021). Among these bacteria, *Salmonella enteritidis* and *Campylobacter* spp. takes the first place in terms of disease potential. The crucial pathogens of them; *E. coli* serotype O157:H7 (included in the Enterohemorrhagic *E. coli* (EHEC) group) is serious in humans and most often the cause lethal infections (HUS; Hemolytic Uremic Syndrome), pathogens transmitted by food all over the world in recent years are one of the most important major public health hazards (Luna Guevara et al., 2019).

The majority of gastrointestinal infections are linked to the fresh produce contaminated with pathogenic bacteria in particular the *Enterobacteriaceae* spp. (Ehuwa et al., 2021). In general, fruits and vegetables carry non-pathogenic/opportunistic microorganisms; during many production practices, such as growing, harvesting, transport, storage, and subsequent processing, these products can be contaminated with pathogens of animal and human origin. These products, which were considered seasonal until recently, have increased rapidly in recent years because they are considered healthy foods. Besides, these foods are consumed raw; their microbial content poses a risk to consumer health (Carvalheira et al., 2017; Ehuwa et al., 2021). The presence of pathogenic and non-pathogenic bacteria carrying antibiotic resistance genes in fresh vegetables and fruits, and the spread of antibiotic resistance genes through possible horizontal gene transfer among these bacteria may be in question (Falomir et al., 2010a; Ehuva et al., 2021). In the last few years, antibiotic-resistant Gram-negative bacteria have become a serious problem worldwide (Carvalheira et al., 2017; Ehuva et al., 2021).

The coliform group bacteria that show fecal characteristics spread to the environment through feces, since they are found in the intestines of humans, animals, and birds. Therefore, *E. coli* an important member of coliforms is an indicator species of fecal contamination in foods. *Acinetobacter* spp. (which are opportunistic bacteria), and important *Enterobacter* species: *Klebsiella* spp., *Citrobacter freundy*, *and Salmonella* spp. are usually found in soil, water, and fresh produce (Heaton and Jones, 2008). Besides, both *Enterobacter* and *Klebsiella* species, primarily *E. cloacae* and *K. pneumoniae*, and to a minor extent other species, have a crucial clinical impact because of the emergence of antibiotic resistance around the world (Dunn et al., 2019). The endophytes, such as *Streptomyces* species, *Klebsiella pneumonia*, *Morganella morganii*, and *Pantoea agglomerans* do not usually cause symptoms in plants, but they are clinically important (Kirzinger et al., 2011). Although most vegetable processors and consumers assume that washing fresh vegetables will decrease the microbial load on their surface, studies have shown that washing with water alone is not effective in declining the microbial population in fresh vegetables (Carvalheira et al., 2017).

As can be seen in studies conducted in several countries in the world, it is known that there are significant microbial contamination problems in fresh produce. However, it is of great importance to determine what kinds of health risks are caused by these detected microbiological problems (Ehuva et al., 2021; Kirzinger et al., 2011). Besides, the emergence of antibiotic resistance among Gram-negative *Enterobacter* species threatens the effective prevention and treatment of an increasing number of infections all over the world.

Therefore, in this study, raw or unprocessed fruits and vegetables, which are one of the ways of transmission of Gram-negative bacteria to humans, were evaluated to determine the safety of fruits and vegetables sold commercially in the South region of Türkiye.

MATERIAL AND METHOD

Material

In the present study, 65 food samples [n=25 fruit; apples, pears, strawberries, bananas, n=25 vegetables; lettuces, parsleys, purple cabbages, and n=15 packet salads; mixed ready to eat salads] were purchased from 3 local markets and 1 bazaar located in Adana province (Southern part of Türkiye) from 2019 March to September 2021. All food samples originated from Turkish food production and were processed in Southern Türkiye. All samples were transported to the microbiology laboratory (University of Çukurova, Department of Food Engineering) in a sterile container as soon as possible.

Method

Bacterial Strains and Growth Conditions

The reference methods: EN/ISO 6579 for *Salmonella* spp. and EN/ISO 16649-2 for *E. coli* were applied respectively to the isolation and counting of pathogenic/Gram- negative bacteria in fresh products (*Table 2*) (Turkish Food Codex, 2011). Sample preparation and homogenization were performed under aseptic conditions; 25 g of samples were added into sterile filter-stomaching bags (Isolab) and 225 mL of buffered peptone water was added into the stomaching bags and homogeneously mixed in a stomacher (Bagmixer-Interscience, France) twice for 1 min. Each sample was serially diluted with buffered peptone water (10^{-3}). Then 0.1 mL each of the 10^{-2} and 10^{-3} dilution fractions was separately spread plated onto a selective agar [for *Enterobacteriaceae* spp., Violet Red Bile Glucose (VRBD) Agar (Merck 1.10275, Deutschland); for *E. coli*, Tryptone Bile X-Glucuronide (TBX) Agar (LAB-M HAL003); for *Acinetobacter* spp. CHROM agar™ Acinetobacter (CHROM agar, Paris, France)] and Mac Conkey agar plates (Oxoid), and incubated at 30-37 °C for 24 h. Isolation and counting of the bacteria took part in two parallel processes.

The isolation of *Enterobacteriaceae* spp.; further bacterial species-level identification of suspicious isolates took place on selective agar plates mentioned above. The presumptive colonies (1-2 mm in diameter, red and ring-shaped were considered as *Enterobacteriaceae*; colonies with opaque bluish-green color were considered as *E. coli*). Suspected colonies were inoculated on Tryptone Soy Agar (TSA) plates containing 5% sheep blood (Oxoid) media for pure culture. Than following biochemical tests were performed; catalase production, Gram staining, motility, and oxidase tests in line with Bergey’s Manual of Determinative Bacteriology. The colonies (non-motile, Gram-negative, catalase-positive, oxidase-negative) were identified by MALDI-TOF (Bruker, Germany) system (Carvalheira et al., 2017; Lupo et al., 2014; Espinal et al., 2012; Turkish Food Codex, 2011; Holt et al., 1994).

Statistical Analysis

Statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, Illinois, USA) version, expressing the values in mean and standard deviations shown by descriptive statistics (Özdamar, 1999).

RESUTS AND DISCUSSION

A total of 27 Gram-negative bacteria were identified from 65 fruit and vegetable samples. The highest numbers of bacterial species were obtained from lettuce samples. The majority of the isolates identified; were *Klebsiella pneumoniae* (6, 22%), and *Stenotrophomonas maltophilia* (4, 15%), respectively (Figure 1). The viable bacterial counts of the identified species and their sources were shown in (Table 1). In this study, *E. coli* (3, 11%) was isolated from lettuce and purple cabbage samples (Table 1 and Figure 1), while *Salmonella* spp. was not isolated from any of the samples.

Different types of freshly consumed vegetables contain many types of microorganisms inside and on the surface of the plant tissue. Fresh fruits and vegetables have shown increasing levels of bacterial contamination worldwide. Similarly, it is observed in this study that most of the fresh produce consumed raw/undercooked in Southern Türkiye are contaminated with *Enterobacteriaceae* species. Various factors affect the being of *Enterobacteriaceae* spp. in fruits and vegetables. These contamination factors are respectively the types of fruits and/or vegetables, soil contact, and seasonal differences (Ruimy et al., 2010; Mesbah Zekaret al., 2017). Fruits and vegetables can be contaminated indirectly through fecal bacteria from animals during fertilization or through direct contact with humans during harvest, processing, and packaging. The production stages: harvesting, transporting, processing, packaging, washing, and selling of fruits and vegetables by untrained persons can cause bacterial contamination and pose a risk to public health (Lynch et al., 2009; Ruimy et al., 2010; Igiehon et al., 2020). Direct contact with these products usually occurs between sellers and consumers. Schwaiger et al. (2011b) confirmed this situation and reported that the contamination of fruits and vegetables is higher at the cultivation stage. In a similar study conducted in Bangladesh by Alam et al. (2015), it was determined that almost all vegetable samples were highly contaminated with pathogenic bacteria. Pathogens including *Klebsiella* spp., *Staphylococcus* spp., and *Pseudomonas* spp. were found to be dominant. In addition, *Klebsiella* spp., *Bacillus* spp., *Listeria* spp, *Escherichia coli*, and *Vibrio* spp., were also detected. Their results are in line with our study in terms of Gram-negative microorganism species.

Table 1. Distribution of the Isolates and Viable Bacterial Counts in Different Fruit and Vegetable Samples and Results of MALDI- TOF MS

No	Isolate No	Fruits/ vegetables	Bacterial species	MALDI-TOF MS Score	Origin	Bacterial count range (log CFU/g Min-Max)	Bacterial count range Mean (log CFU/g ±SD)
1	F-45	Apple (red)	*A. pittii*	1.890	Market-1	4.1-5.8	5.07±0.76
2	F-53	Spinach	*Klebsiella pneumoniae*	2.206	Market-1	4.1-5.8	5.00±0.73
3	F-46	Apple (green)	*Klebsiella pneumoniae*	2.097	Market-1	4.1-4.8	4.37±0.14
4	F-4	Pear	*Citrobacter freundii*	2.255	Market-1	4.5-5.3	5.03±0.21
5	F-5	Lettuce	*E. coli*	1.911	Market-1	5.8-6.3	6.20±0.13
6	F-1	Parsley	*Klebsiella oxytoca*	2.276	Market-1	6.1-6.6	6.40±0.07
7	F-7	Banana	*Klebsiella pneumoniae*	2.322	Market-1	3.6-4.8	4.40±0.48
8	F-48	Purple cabbage	*Citrobacter freundii*	2.299	Market-2	5.8-6.6	6.30±0.19
9	F-52	Spinach	*Klebsiella pneumoniae*	2.163	Market-2	4.1-4.8	4.37±0.14
10	F-7	Lettuce	*E. coli*	1.911	Market-2	5.8-6.2	6.07±0.07
11	F-47	Purple cabbage	*E. coli*	1.943	Market-2	5.8-6.3	6.07±0.06
12	F-34	Strawberry	*Bulkholderia vietnamiensis*	1.997	Market-2	4.5-6.3	5.70±1.08
13	F-36	Banana	*Delftia lacustris*	1.786	Market-2	4.5-5.3	5.00±0.19
14	F-37	Banana	*Stenotrophomonas maltophilia*	1.770	Market-2	4.5-5.3	5.03±0.21
15	F-53	Spinach	*Stenotrophomonas maltophilia*	1.717	Bazaar	5.8-6.3	6.07±0.06
16	F-4	Pear	*Pseudomonas putida*	1.876	Bazaar	5.1-5.6	5.43±0.08
17	F-55	Lettuce	*Stenotrophomonas maltophilia*	1.720	Bazaar	4.2-4.8	4.43±0.10
18	F-50	Parsley	*Pseudomonas putida*	1.897	Bazaar	4.5-6.3	5.70±1.08
19	F-34	Strawberry	*Burkholderia cenocepacia*	2.023	Market-3	5.8-6.3	6.13±0.08
20	F-47	Purple cabbage	*Stenotrophomonas maltophilia*	1.768	Market-3	3.7-5.8	5.03±1.54
21	F-55	Lettuce	*Enterobacter ludwigii*	2.229	Market-3	4.5-6.3	5.70±1.08
22	F-54	Purple lettuce	*Citrobacter freundii*	2.219	Market-3	6.1-6.6	6.33±0.06
23	F-36	Lettuce	*Klebsiella pneumoniae*	2.040	Market-3	6.1-8.3	7.50±1.48
24	F-34	Strawberry	*Klebsiella oxytoca*	2.228	Market-3	4.5-6.3	5.67±1.02
25	F-38	Lettuce	*Klebsiella pneumoniae*	2.307	Market-3	6.8-8.3	7.77±0.70
26	F-2	Dill	*Klebsiella oxytoca*	2.245	Market-3	6.1-6.6	6.30±0.07
27	F-65	Packet salad	*A. baumannii*	2.062	Market-3	3.8-5.8	5.00±1.12

Min: Minumum, Max: Maximum, SD: Standard Deviation

E. coli is a normal part of the intestinal microflora of many healthy animals, including humans. However, some strains can cause diseases, these pathogenic species are increasingly associated with contamination of vegetables such as fresh spinach and lettuce (Papadopoulou et al., 2019; Abebe et al., 2020). Bacterial colonization of freshly grown plants can occur at high levels in outer tissue, although some studies have reported bacteria detected in plant tissue as well. The rate of *E. coli* detected in the total isolates and samples was found to be (3/27) 11% and (3/65) 4.6% respectively in the present work. The lowest total counting results of *E. coli* in the samples were found at 5.8 log CFU/g and the highest count was 6.3 log CFU/g (*Table 1*). Our result was lower than a similar study conducted to determine the microbiological quality of salads sampled in the food service units of a military hospital in Ankara (Türkiye); 70 salads were investigated, and *E. coli* was detected in 11.4% of these samples and was subsequently compared to the food samples. It has been observed that there is a high microbial load (Ayçiçek et al., 2004).

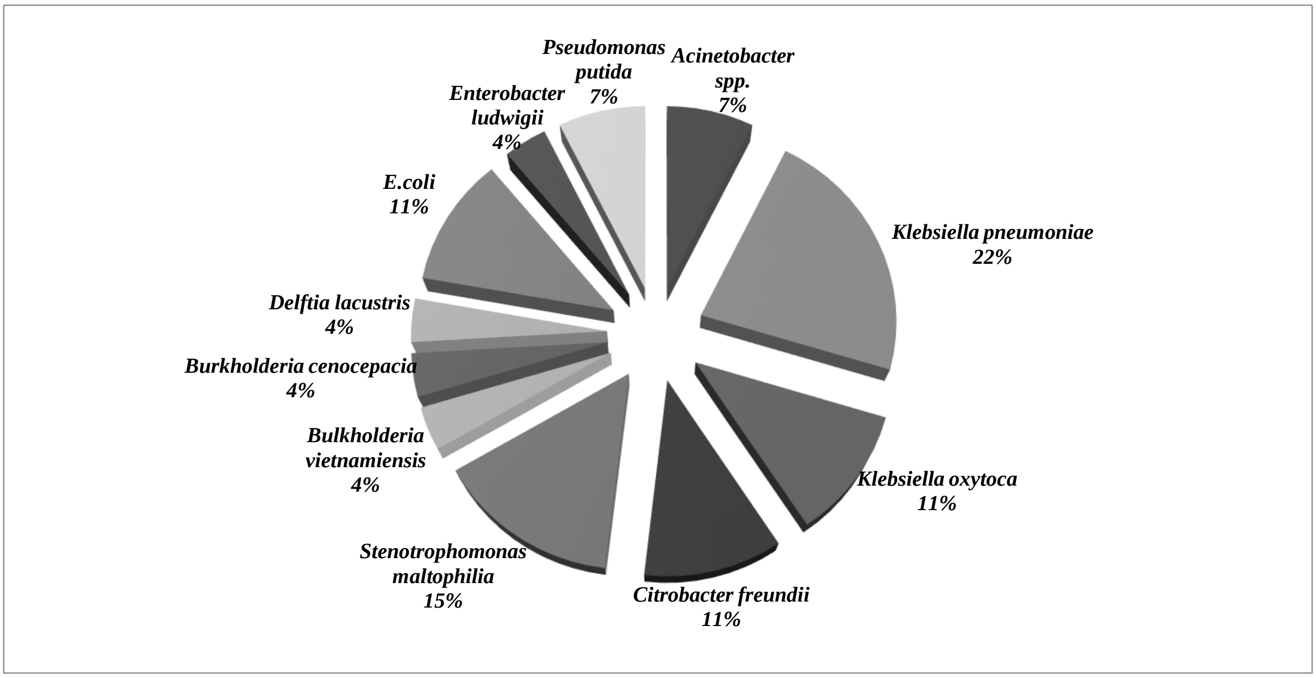

Figure1. The Distribution Rate of Gram-Negative Bacteria Isolated from Fresh Produce

Similarly, our results (11%) were lower than in another study in Kars city of Türkiye, in the analysis of 40 salad samples collected from 4 different restaurants; *E. coli* was detected higher level than the limits of the Turkish Food Codex in 8 (20%) of the samples (Gülmez et al., 2005). By contrast, in a study conducted in Brazil about Ready–to–eat of minimally processed leafy green vegetables, in which 162 samples of salads were collected, *E. coli* was detected in 86 (53.1%) of these samples was higher than our results (De Oliveira et al., 2011). In another study conducted in England, in line with our study, 4.7% of 1213 vegetable salad samples were found to be contaminated with *E. coli* (Meldrum et al., 2009). In a recent study conducted in Bangladesh, 60 ready-to-eat mixed vegetable salad samples were collected, and *E. coli* was found to be 4.98±0.20 to 6.66±0.80 log CFU/g higher than our results (Younus et al., 2020). The main root cause for these varying may depend on geographical differences, sample collection /isolation /identification methods as well as contamination level of the bacteria. In the Turkish Food Codex Microbiological Criteria Regulation, "Fruits and vegetables and their processed products, and other mentioned products.etc., considering the pathogenic bacteria limits for these products; the highest *E. coli* value allowed in these products is 10^3 CFU/g-mL; *L. monocytogenes, E. coli* O157, and *Salmonella* spp. should not be found in 25 g. of the sample'' (*Table 2*). Accordingly, in our study, it was determined that 11% of the *E. coli* positive samples (between 5.8 and 6.3 log CFU/g) were higher than the level of legislation.

In international regulations, the presence of bacteria belonging to *Enterobacteriaceae* spp. in foods is used to evaluate their general hygiene levels. According to guidelines of the health protection agency (United Kingdom of Great Britain), the microbiological safety of ready-to-eat foods, classifying *Enterobacteriaceae* count into 3 levels: (1) unsatisfactory (>4 log CFU/g), (2) borderline (2–4 log CFU/g), and (3) satisfactory (<2 log CFU/g) (Colosi et al., 2020; Bolton, 2009). Although the presence of these bacteria in fruits and vegetables is not generally considered dangerous, their high number indicates that hygiene practices are not followed during pre-during-post/ production processes (Bolton et al., 2009), The *Enterobacteriaceae* viable count levels in the present study ranged from 3.6 log CFU/g (banana) to 8.3 log CFU/g (lettuce), results which are higher than previous reports that have evaluated the microbial load of fresh fruits and vegetables (Xylia et al., 2019; Uhlig et al., 2017). The highest bacterial load was found in lettuce (8.3 log CFU/g), parsley, purple cabbage, purple lettuce, dill (6.6 log CFU/g), spinach, and strawberry (6.3 log CFU/g) (Table 1). The lowest bacterial count was found in the banana sample (F7), (3.6 log CFU/g). Leafy and bulbous vegetables showed the highest microbial diversity associated with higher CFU counts. Leafy vegetables are more vulnerable to micro-damage caused by environmental factors (heavy rain, wind, packaging/transportation conditions, etc.) because of their natural structure, this makes further possible contamination with various bacteria found in the soil more likely. These lesions may also act as an entry portal/site for the bacteria that preferentially adhere easily to cut edges. Bacteria counting level was less in bananas can be attributed to their growth above ground and they are not coming to contact with fertilizers and wastewater, thus preventing contamination with fecal *Enterobacteriaceae*. However, human contamination may have occurred in these products (Bekele et al., 2020).

In this study, *Salmonella* spp. was not detected in any of the samples. This result may depend on geographical differences or selected methods, sample collection, contamination level, etc. However, Gram-negative various bacteria were detected in the present work. Besides, *Acinetobacter* spp. (especially *Acinetobacter baumannii*, *A. pittii* species were nosocomial infection agents worldwide with higher antibiotic-resistant species), *Citrobacter* spp., *Klebsiella pneumonia*, *Pseudomonas* spp. as well as *E. coli* may play a role in antibiotic- resistant-bacterial development by acting as a permanent source of antibiotic resistance and virulence genes. They can accumulate resistance markers that can be transferred to more virulence bacteria it can also enable the transfer of other resistance and virulence genes, such as plasmids, transposons, and integrons, in addition to specific resistance and virulence genes (Falomir et al., 2010a; Usui et al., 2019). If both virulence markers and antibiotic resistance are located on the same plasmid or if naturally virulent organisms acquire a resistance plasmid and spread between hosts, this can also cause an epidemic (Usui et al., 2019). Therefore, monitoring these bacteria in fresh produce is necessary to eliminate the spreading of antibiotic-resistant bacteria.

Our results confirmed the hypothesis about microbial contamination can be occurred in fresh produce with opportunistic pathogens including *Acinetobacter* spp., *Enterobacteriaceae*, *K. pneumonia*, *P. putida*, and other species, which should be considered a food safety concern. Besides, this contamination can be the cause of crucial health problems in immunocompromised/children/elderly individuals. In addition, fresh produce can act as a carrier of antibiotic-resistant bacteria from the farm to the consumer (Falomir et al., 2010a; Falomir et al., 2010b; Schwaiger et al., 2011a; Schwaiger et al., 2011b).

Table 2. Microbiological Criteria of Pathogenic Bacteria in Fruit and Vegetables (Turkish Food Codex, 2011)

Food	**Microorganisms /Toxins/Metabolites**	**Sampling Plan (1)**		**Limits (2)**		**Reference Method(3)**
Fruits and Vegetables and their processed products		n	c	n	M	
Washed, chopped, and packed raw vegetables, individually or mixed, as well as frozen and dehydrated vegetables	*Salmonella* spp.	5	0	0/25g-mL		EN/ISO6579
	Listeria monocytogenes	5	0	0/25g-mL		RN/ISO11290-1
	E. coli O157	5	0	0/25g-mL		ISO16654
Ready to eat and (unpasteurized) fruit and vegetables	*E. coli*	5	2	10^2 CFU/g	10^3 CFU/g	ISO16649-1 or 2

(1); n: The number of samples, c: number of samples allowed having values between n and M limit, (2): Unless stated otherwise, the limit is evaluated as CFU/g-mL. CFU: Colony Forming Unit (in solid medium), (3): The latest published versions of the standards used in this regulation are used.

CONCLUSION

This study analyzed the presence of Gram-negative bacteria in retail fresh products in Adana province of Türkiye. The obtained results show that it is necessary to further investigate different types of vegetables and fruits from other regions of Türkiye as well as Middle-East Countries. These findings suggest that products that are generally consumed raw/undercooked, because they are thought to be more beneficial to the human body this way may pose serious health risks under certain conditions. In the food industry, for the elimination/limitation of Gram-negative bacteria in fruits and vegetables; with systems such as HACCP (Hazard Analyses Critical Control Point) and good hygiene practices, all stages of "farm to fork" food processing need to be controlled. Disinfection of fruit and vegetables is an important critical control point in the HACCP system. Proper hygiene practices such as raising food workers' awareness of hygiene, hand washing, and thorough washing of fruits and vegetables before consumption can reduce the risk of infection by these opportunistic pathogens. The elimination/reducing the dissemination of these bacteria may also play a role in preventing the spreading of antibiotic resistance. Therefore, the limitation of Gram- negative-bacterial contamination/proliferation in fresh produce is needed some new policies and regulations; environmental management, manure management practice, application of education programs for food handlers as well as effective handling application from the farms to the markets in the national and international area.

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REFERENCES

Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of Tropical Medicine*, 2020.https://doi.org/10.1155/2020/4674235

Alam, M.S., Feroz, F., Rahman, H., Das, K.K., Noor, R. (2015). Microbiological Contamination Sources of Freshly Cultivated Vegetables. *Nutrition & Food Science,* 45(4), 646-658.https://doi.org/10.1108/NFS-04-2015-0032

Ayçiçek, H., Sarimehmetoğlu, B., & Çakiroğlu, S. (2004). Assessment of the microbiological quality of meals sampled at the meal serving units of a military hospital in Ankara, Turkey. *Food Control*, 15, 379-384.https://doi.org/10.1016/S0956-7135(03)00101-4

Bekele, F.,Shumbej, T., Dendir, A., Mesfin, D., Solomon, A., Jemal, A., & Alemayheu, M. (2020). Contamination rate of commonly consumed fresh vegetables and fruits with parasites of medically importance in Wolkite and Butajira Towns of Gurage Zone, Southern Ethiopia. *International Journal of Public Health Science (IJPHS)*, 9(3), 211.https://doi.org/10.11591/ijphs.v9i3.20395

Bolton, E. (2009). Guidelines for assessing the microbiological safety of ready-to-eat, foods placed on the market; *Health Protection Agency: London UK*, p, 33.

Carvalheira, A., Silva, J., & Teixeira, P. (2017). Lettuce and fruits as a source of multidrug resistant *Acinetobacter* spp. *Food Microbiology,* 64,119-125.https://doi.org/10.1016/j.fm.2016.12.005

Colosi, I. A.,Baciu, A. M., Opriș, R. V., Peca, L., Gudat, T., Simon, L. M.,& Costache, C. (2020). Prevalence of ESBL, AmpC and carbapenemase-producing Enterobacterales isolated from raw vegetables retailed in Romania. *Foods,* 9(12), 1726. https://doi.org/10.3390/foods9121726

De Oliveira, M. A., De Souza, V. M., Bergamini, A., M. M., & De Martinis, E., C. P. (2011). Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control,* 22(8),1400-1403.https://doi.org/10.1016/j.foodcont.2011.02.020

Dunn, S. J., Connor, C., & McNally, A. (2019). The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids. *Current Opinion in Microbiology,* 51, 51-56.https://doi.org/10.1016/j.mib.2019.06.004

Ehuwa, O., Jaiswal, A. K., & Jaiswal, S. (2021). *Salmonella*, food safety, and food handling practices. *Foods*, 10(5), 907.https://doi.org/10.3390/foods10050907

Espinal, P., Seifert, H., Dijkshoorn, L., Vila, J., & Roca, I. (2012). Rapid and accurate identification of genomic species from the *Acinetobacter baumannii* (Ab) group by MALDI-TOF MS. *Clinical Microbiology and Infection*, 18(11), 1097-1103.https://doi.org/10.1111/j.1469-0691.2011.03696.x

European Food Safety Authority (EFSA) (2018). European Centre for Disease Prevention and Control multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables. First update, *EFSA Supporting Publ*, 15.

Falomir, M.P., Gozalbo, D., Sebastia C., & Rico, H. (2010a). Detection and antibiotic susceptibility of coliform bacteria in fresh vegetables. In: Microorganisms in industry and environment, from scientific and industrial research to consumer products. *Mendez-Vilas A (ed,), Hackensack, NJ: World Scientific Publishing Co*, 372–376.https://doi.org/10.1142/9789814322119_0080

Falomir, M. P., Gozalbo, D., & Rico, H. (2010b). Coliform bacteria in fresh vegetables: from cultivated lands to consumers. Current Research, Technology and Education TopicsIn *Applied Microbiology and Microbial Biotechnology,* 2,1175-1181.

Gülmez, M., Sezer, Ç., Duman, B., Vatansever, L., Oral, N., & Baz, E. (2005). Lokantalarda tüketime sunulan bazı gıdaların ve içme sularının mikrobiyolojik kaliteleri. *Kafkas Üniversitesi Veterinerlik Fakültesi Dergisi*, 11(1),5-10.

Heaton, J. C.,& Jones, K. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Journal of AppliedMicrobiology*, 104(3), 613-626.https://doi.org/10.1111/j.1365-2672.2007.03587.x

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. & William, S.T. (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore, 587-685.

Igiehon, O. O.,Adekoya, A. E., Idowu, A. T. (2020). A review on the consumption of vended fruits: microbial assessment, risk, and its control. *Food Quality and Safety*, 4(2), 77-81.https://doi.org/10.1093/fqsafe/fyaa014

Kirzinger, M. W., Nadarasah, G., & Stavrinides, J. (2011). Insights into cross-kingdom plant pathogenic bacteria. *Genes*, 2(4), 980-997.https://doi.org/10.3390/genes2040980

Luna-Guevara, J. J., Arenas-Hernandez, M. M., Martínez de la Peña, C., Silva, J. L., Luna-Guevara, M. L. (2019). The role of pathogenic *E. coli* in fresh vegetables: behavior, contamination factors, and preventive measures. *International Journal of Microbiology*, 2019. https://doi.org/10.1155/2019/2894328

Lupo, A., Vogt., D., Seiffert, S.N., Endimiani, A., & Perreten, V. (2014). Antibiotic resistance and phylogenetic characterization of *Acinetobacter baumannii* strains isolated from commercial raw meat in Switzerland. *Journal of Food Protection*, 77, 1976-1981.https://doi.org/10.4315/0362-028X.JFP-14-073

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology & Infection*, 137(3), 307-315.https://doi.org/10.1017/S0950268808001969

Meldrum R.J., Little C.L., Sagoo, S., Mithani, V., Mc Lauchlin J., & De Pinna E. (2009). Assessment of the microbiological safety of salad vegetables and sauces from kebab take-away restaurants in the United Kingdom. *Food Microbiology*, 26(6), 573-7.https://doi.org/10.1016/j.fm.2009.03.013

MesbahZekar, F., Granier, S. A., Marault, M., Yaici, L., Gassilloud, B., Manceau, C., & Millemann, Y. (2017). From farms to markets: Gram-negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of North Africa. *Frontiers in Microbiology*, 8, 1569.https://doi.org/10.3389/fmicb.2017.01569

Özdamar, K. (1999). Paket Programlar ile İstatistiksel Veri Analizi. Kaan Kitabevi, Eskişehir, Türkiye, 535s.

Papadopoulou, E., Haug, L. S., Sakhi, A. K., Andrusaityte, S., Basagaña, X., Brantsaeter, A. L., & Chatzi, L. (2019). Diet as a source of exposure to environmental contaminants for pregnant women and children from six European countries. *Environmental Health Perspectives*, 127(10),107005.https://doi.org/10.1289/EHP5324

Ruimy, R., Brisabois, A., Bernede, C., Skurnik, D., Barnat, S., Arlet, G., & Andremont, A. (2010). Organic and conventional fruits and vegetables contain equivalent counts of Gram-negative bacteria expressing resistance to antibacterial agents. *Environmental Microbiology*, 12(3), 608-615.https://doi.org/10.1111/j.1462-2920.2009.02100.x

Schwaiger, K., Helmke, K., Hölzel, C. S., & Bauer, J. (2011a). Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs, supermarket). *International Journal of Food Microbiology*, 148(3), 191-196.https://doi.org/10.1016/j.ijfoodmicro.2011.06.001

Schwaiger, K., Helmke, K., Hölzel, C. S., & Bauer, J. (2011b). Comparative analysis of the bacterial flora of vegetables collected directly from farms and from supermarkets in Germany. *International Journal of Environmental Health Research*, 21(3), 161-172.https://doi.org/10.1080/09603123.2010.515672

Sivapalasingam, S, Friedman, C, R, Cohen, L, & Tauxe, R., V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997.*Journal of Food Protection*, 67(10), 2342-2353.https://doi.org/10.4315/0362-028X-67.10.2342

Sun, L., Zhang, H., Chen, J., Chen, L., Qi, X., & Zhang, R. (2021). Epidemiology of foodborne disease outbreaks caused by *Nontyphoidal Salmonella* in Zhejiang Province, China, 2010–2019. *Foodborne Pathogens and Disease*, 18(12), 880-886.http://doi.org/10.1089/fpd.2021.0006

Turkish Food Codex (2011). Official newspaper. Turkish Food Codex Regulation on Microbiological Criteria. (29 December 2011) Official Gazette. 2011:28157.

Uhlig, E, Olsson, C, He, J, Stark, T, Sadowska, Z, Molin, G., & Håkansson, Å., (2017). Effects of household washing on bacterial load and removal of *Escherichia coli* from lettuce and "ready-to-eat" salads. *Food Science &Nutrition*, 5(6), 1215-1220.https://doi.org/10.1002/fsn3.514

Usui, M, Ozeki K, Komatsu, T, &Fukuda, A, (2019), Tamura, Y, Prevalence of extended-spectrum b-lactamase–producing bacteria on fresh vegetables in Japan. *Journal of Food Protection,* 82, 1663–1666.https://doi.org/10.4315/0362-028X.JFP-19-138

Xylia, P., Botsaris, G, Chrysargyris, A, Skandamis, P., & Tzortzakis, N, (2019). Variation of microbial load and biochemical activity of ready-to-eat salads in Cyprus as affected by vegetable type, season, and producer. *Food Microbiology*, 83, 200-210.https://doi.org/10.1016/j.fm.2019.05.013

Younus, M.I., Sabuj, A.A.M., Haque, Z.F., Sayem, S. M., Majumder, S., Parvin, M. S., & Saha, S. (2020). Microbial risk assessment of ready-to-eat mixed vegetable salads from different restaurants of Bangladesh Agricultural University campus. *Journal of Advanced Veterinary Animal Research*, 7(1), 34-41. https://doi.org/10.5455/javar.2020.g390