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Microbiological quality of ready–to–eat vegetables salads served at meat restaurants under the COVID-19 in Turkey

Calidad microbiológica de ensaladas de verduras listas para comer servidas en restaurantes de carne durante la pandemia de COVID-19 en Turquia

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ABSTRACT

In Turkish cuisine, ready–to–eat vegetable salads (REVS) served with pide/lahmacun, kebab types, and tantuni from animal source in meat restaurants were evaluated since they have the potential to carry risks in terms of Public Health. The microbiological properties of REVS were investigated using agar plate method. Antimicrobial resistance of foodborne pathogens including *Escherichia coli* and *Staphylococcus aureus* was tested using Kirby–Bauer disc diffusion method. Moreover, the presence of important enteric viruses was detected by Polymerase Chain Reaction (PCR). The number of total aerobic bacteria, coliform bacteria, yeast and molds and, *Staphylococcus* and *Micrococcus* spp. ranged from less than 1 to 6.40, 1 to 6.26, less than 1–5.82 and less than 1–5.66 $\log_{10}$ colony forming units·grams^{-1} (CFU·g^{-1}) in REVS samples, respectively. None of the REVS tested in this study contained *Salmonella* spp., whereas *E. coli* and *S. aureus* were isolated in 38.1% (16/42) and 2.4% (1/42), respectively. *S. aureus* was resistant to gentamicin, kanamycin, aztreonam, and ciprofloxacin in the disc diffusion assay, however, it was not harboring the *mecA* gene. *E. coli* strains (n=16) were resistant (100%) to aminoglycoside antibiotics and 35.7% (6/16) of the isolates were extended spectrum beta lactamase (ESBL) producing. bla_{TEM} and $bla_{CTXM8/25}$ were detected in two isolates, whereas one isolate carried bla_{CTXM-1} and bla_{TEM} together by PCR. Of the REVS, two were evaluated as positive for rotavirus (4.8%), six for hepatitis A (14%), and hepatitis E virus (14%). These results indicate the high microorganism load, presence of ESBL *E. coli*, and viral enteric pathogens in REVS, hence it is important to perform routine hygiene practices.

Key words: Microbial; ESBL; *E. coli*; viruses; ready–to–eat salad

RESUMEN

Ensaladas de verduras listas–para–comer (EVLC) que se sirven con pita/lahmacun, tipos de kebab y tantuni de origen animal en los asadores de la cocina turca, ya que tienen el potencial de conllevar riesgos en términos de salud pública fueron evaluadas. Se investigaron las propiedades microbiológicas de REVS utilizando el método de placa de agar. La resistencia a los antimicrobianos de los patógenos transmitidos por los alimentos, incluidos *Escherichia coli* y *Staphylococcus aureus*, se probó mediante método de difusión por disco de Kirby–Bauer. Además, se detectó la presencia de importantes virus entéricos por Reacción en Cadena de la Polimerasa (RCP). El número de bacterias aeróbicas totales, bacterias coliformes, levaduras y mohos y *Staphylococcus* y *Micrococcus* spp. varió de menos de 1 a 6,40; 1 a 6,26; menos de 1 a 5,82 y de menos de 1 a 5,66 $\log_{10}$ Unidades Formadoras de Colonias·gramos^{-1} (UFC·g^{-1}) en muestras EVLC, respectivamente. Ninguna muestra de EVLC analizadas en este estudio contenía *Salmonella* spp., mientras que *E. coli* y *S. aureus* se aislaron en el 38,1 % (16/42) y el 2,4 % (1/42), respectivamente. *S. aureus* fue resistente a la gentamicina, la kanamicina, el aztreonam y la ciprofloxacina en el ensayo de difusión en disco; sin embargo, no albergaba el gen *mecA*. Las cepas de *E. coli* (n=16) fueron resistentes (100 %) a los antibióticos aminoglucósidos y el 35,7 % (6/16) de los aislamientos produjeron beta lactamasa de espectro extendido (BLEE). bla_{TEM} y $bla_{CTXM8/25}$ se detectaron en dos aislados, mientras que un aislado portaba bla_{CTXM-1} y bla_{TEM} juntos mediante RCP. De los EVLC, dos fueron evaluados como positivos para rotavirus (5 %), seis para hepatitis A (14 %), y virus de la hepatitis E (14 %). Estos resultados indican la alta carga de microorganismos, presencia de ESBL *E. coli* y patógenos virales entéricos en REVS, por lo que es importante realizar prácticas de higiene de rutina.

Palabras clave: Microbiano; BLEE; *E. coli*; virus; ensalada lista para comer

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INTRODUCTION

Ready–to–eat (RTE) foods are those consumed without any additional processing or preparation, which may be industrially and/or conventionally processed, packaged or unpackaged [37]. Dietary preferences of individuals due to rapid urbanization and socio–economic transformations of their current lifestyles have shifted towards ready–made meals [53].

Research and Markets [30] data showed that the demand for RTE foods has increased rapidly, especially among consumers who do not like to cook during the COVID–19 pandemic. In addition, it was stated that, in connection with the increasing coronavirus cases, restaurants forced consumers to cook at home, causing an increase in the consumption of ready–made meals in the medium term.

On the other hand, the increasing consumption of RTE foods globally has been significantly associated with various outbreaks of foodborne infections and poisoning. Especially RTE vegetable salads (REVS) can be primarily contaminated by animals, soil, irrigation water, fertilizers, and others. Moreover, cutting and slicing raw vegetables in restaurants can cause the nutrients in the plants to be released and thus accelerate microbial development [39]. Also, cross–contamination from staff working in restaurants, tools/equipment used, contaminated water, as well as storage in inappropriate conditions are important risk factors affecting the final microbiological quality of the product.

Therefore, REVS may carry microbial risks under the influence of more than one unsuitable factors [13, 33]. This situation can lead to the emergence of pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, *Clostridium perfringens*, and *Listeria monocytogenes* among others, as well as an increased in total aerobic and spoilage bacteria, yeasts, and molds in REVS [2].

On the other hand, a high microorganism load can be contained multi–resistant bacteria of global health concern nowadays [48]. The increasing presence of extended spectrum beta lactamase (ESBL) producing bacteria from RTE foods, which is among the challenges of antibiotic resistance, is another important risk for Human Health due to its epidemiological importance [26]. In addition, it has been reported that REVS are reservoirs not only for bacterial pathogens, but also for enteric viruses such as norovirus (NoV), hepatitis A (HAV), hepatitis E (HEV), and rotavirus (RV) that may lead to an epidemic [48].

The increasing interest in the demand for RTE foods in Turkey has been in parallel with the developments in the World, especially during the COVID 19 pandemic. RTE foods in which meat is used as raw material such as pide/lahmacun, kebab types, and tantuni are notably in high demand [30]. The demand to reach hygienic foods by consumers is also for RTE foods, just like all other foods.

In some studies, conducted in Turkey, the microbiological quality of RTE foods was investigated and it was reported the presence of some foodborne pathogens including *L. monocytogenes*, *Salmonella* spp. and Norovirus [3, 28, 51, 58, 67]. However, it is noteworthy that in these studies, there was no comprehensive perspective on REVS in meat restaurants. Therefore, it is thought that ignoring the microbiological quality of the REVS may be an important risk in terms of Public Health. Moreover, to the best of the knowledge, there is no adequate data on the some microbiological in REVS from Eastern Turkey. Thus, this study aimed to investigate some microbiological properties, antimicrobial resistance of foodborne pathogens including *E. coli* and *S. aureus* and the presence of enteric viruses in REVS in Turkey.

MATERIALS AND METHODS

Samples

In this study, REVS samples were taken aseptically from 42 meat restaurants operating in Erzurum Region in Eastern Turkey. One sample of REVS served with RTE foods was collected from each restaurant. The samples were transferred to the on–ice packs in an insulated cooler. Samples were analyzed much less than 2 hours (h) after collection.

TABLE I presents information about the characteristics of REVS and restaurants. Information on the conditions under which most of the samples used in the study were stored was not given by the restaurant staff. REVS samples, which did not contain any additives, dressing and gravy were placed directly on a suitable packaging material and made ready for service. Since all REVS collected as a sample were washed, grated, and ready for direct consumption, it was named RTE.

TABLE I
Characteristics of collected REVS and restaurants

Symbol	Ingredients of Vegetable Origin	Packaging Method	Restaurant type
E1	Carrot and lettuce	Polyethylene	Döner kebab (Chicken)
E2	Parsley, salad, and tomatoes	Polystyrene foam	Pide–Lahmacun
E3	Carrot, parsley, and tomatoes	Polyethylene	Döner kebab (Chicken and beef)
E4	Carrot and lettuce	Polystyrene foam	Döner kebab (Chicken and beef)
E5	Carrot, lettuce, and purple cabbage	Polyethylene	Döner kebab (Chicken and beef)
E6	Carrot, lettuce, purple cabbage, and tomatoes	Polystyrene foam	Shish kebab
E7	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken)
E8	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken and beef)
E9	Carrot, lettuce, and purple cabbage	Polystyrene foam	Pide–Lahmacun and Döner kebab (Beef)
E10	Lettuce	Polyethylene	Tantuni
E11	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun

***TABLE I* (cont...)**
Characteristics of collected REVS and restaurants

Symbol	Ingredients of Vegetable Origin	Packaging Method	Restaurant type
E12	Carrot, lettuce, and purple cabbage	Polyethylene	Döner kebab (Chicken)
E13	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken)
E14	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun
E15	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun, Shish kebab, Döner kebab (Beef)
E16	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun, Shish kebab
E17	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun
E18	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken)
E19	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken)
E20	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun, Shish kebab, Döner kebab (Beef)
E21	Carrot and lettuce	Plastic bag	Döner kebab (Chicken)
E22	Carrot and lettuce	Polyethylene	Döner kebab (Chicken)
E23	Carrot	Polyethylene	Döner kebab (Chicken)
E24	Carrot, lettuce, and purple cabbage	Polyethylene	Döner kebab (Chicken and beef)
E25	Carrot, lettuce, and tomatoes	Polyethylene	Döner kebab (Chicken and beef)
E26	Carrot and lettuce	Polyethylene	Döner kebab (Chicken)
E27	Carrot and lettuce	Polyethylene	Döner kebab (Chicken)
E28	Carrot, lettuce, and tomatoes	Polystyrene foam	Shish kebab
E29	Carrot and lettuce	Polystyrene foam	Döner kebab (Chicken)
E30	Lettuce	Polyethylene	Döner kebab (Chicken)
E31	Lettuce	Polystyrene foam	Döner kebab (Chicken and beef)
E32	Lettuce	Polyethylene	Döner kebab (Chicken)
E33	Lettuce	Plastic bag	Döner kebab (Chicken)
E34	Carrot, lettuce, and purple cabbage	Polyethylene	Pide–Lahmacun, Shish kebab
E35	Carrot, lettuce, and purple cabbage	Cardboard bag	Pide–Lahmacun, Shish kebab, Döner kebab (Beef)
E36	Carrot, lettuce, and purple cabbage	Polyethylene	Döner kebab (Chicken)
E37	Lettuce, salad, and tomatoes	Polystyrene foam	Giblet shish kebab
E38	Carrot, lettuce, purple cabbage, and tomatoes	Polyethylene	Tantuni
E39	Carrot, lettuce, purple cabbage, and tomatoes	Polyethylene	Pide–Lahmacun, Shish kebab, Döner kebab (Beef)
E40	Lettuce and tomatoes	Plastic bag	Döner kebab (Chicken and beef)
E41	Lettuce and tomatoes	Polyethylene	Döner kebab (Chicken and beef)
E42	Carrot, parsley, and purple cabbage	Polystyrene foam	Döner kebab (Chicken)

Microbiological analysis

A 10 grams (g) sample was transferred to a filtered stomacher bag and 90 milliliters (mL) of sterile physiological saline solution (0.85% NaCl) was added. The mixture was homogenized in a stomacher (Masticator® Mixer, Basic, Neutec Group Inc., USA) for one minute (min). From this homogenate, 10–fold serial dilutions were prepared with sterile physiological saline solution, and 0.1 mL various dilution levels were spread–plated onto appropriate media and incubated in a laboratory oven (BINDER, Series ED, Binder GmbH, Germany) at appropriate condition (TABLE II). The bacterial colonies were counted and converted in colony forming units (CFU). CFU was calculated following formula:

$$CFU \cdot g^{-1} = Number\ of\ colonies \times \frac{Total\ dilution}{Volume\ actually\ plated}$$

All counts were reported as $\log_{10}$ CFU·g^{-1} [11].

The samples were analyzed for the presence of foodborne pathogens commonly isolated from REVS, i.e., *E. coli*, *Salmonella* spp., and *S. aureus*. To isolate *E. coli*, *Salmonella* spp., and *S. aureus*, 25 g of each sample were aseptically weighed (Shimadzu, ATX 224, Shimadzu Scientific Instruments, Japan) transferred to sterile filtered plastic bags, and homogenized using masticator with 225 mL buffered peptone water (BPW) (Merck, Germany) for 2 minutes (min). Then this homogenate was kept for 60 min at room temperature [7]. For *Salmonella* spp. isolation, the homogenate was incubated (BINDER,

TABLE II
Media and incubation conditions used for the enumeration of microorganisms

Microorganisms	Incubation		Culture media
	Time (h)	Temp (°C)	
Total aerobic mesophilic bacteria	72	35	Plate Count Agar (PCA)
Coliforms	24	35	Violet Red Bile Agar (VRBA)
Staphylococcus / *Micrococcus* spp.	48	37	Baird Parker Agar (BPA) (supplemented with egg yolk tellurite emulsion)
Yeast and Mold	168	25	Rose Bengal Chloramphenicol (RBC) Agar

Series ED, Binder GmbH, Germany) for 24 ± 2 h at 35°C followed by a 0.1 mL mixture transferred to 10 mL Rappaport–Vassiliadis (RV) medium and another 1 mL mixture to 10 mL Muller–Kauffmann Tetrathionate–Novobiocin Broth (MKTTN) and they were incubated (BINDER, Series ED, Binder GmbH, Germany) at 37°C for 24 h.

A loop–full culture of each broth was streaked onto Xylose Lysine Desoxycholate (XLD) agar and Xylose Lysine Tergitol 4 (XLT4) agar and incubated at 37°C for 24 h. Two or more colonies of showing typical *Salmonella* colony morphology were picked from each selective agar plate after incubation [11]. To *E. coli* isolation, a loop–full of pre–enriched sample was streaked onto Tryptone Bile X–glucuronide (TBX) agar and incubated at 37°C for 24 h. The blue–green colored suspicious *E. coli* colonies grown in TBX were subcultured on the Mueller Hinton agar (MHA) (HiMedia, Mumbai, India) plate for biochemical and molecular analysis [10]. A loop–full of homogenate was passaged on BP Agar supplemented with egg yolk tellurite emulsion to isolate *S. aureus* and incubated at 37°C for 24 h. The typical transparent zone around the colony as a result of lipolysis/proteolysis, black colonies due to the reduction of tellurite to tellurium were evaluated as suspicious *S. aureus*, and subcultured for further analysis as mentioned for *E. coli* above [27].

Antimicrobial susceptibility testing

Kirby–Bauer disk diffusion technique on MHA (HiMedia) was used for antimicrobial susceptibility testing against to a set of 12 different commercially available antibiotic disks (HiMedia, Mumbai, India) including Meropenem (10 micrograms – µg-), Chloramphenicol (30 µg), Gentamicin (10 µg), Kanamycin (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Sulfamethoxazole/Trimethoprim (25 µg), Cefepime (30 µg), Cefpodoxime (10 µg), Cefoxitin (30 µg), and Aztreonam (30 µg). The growth inhibition zones were measured and interpreted as sensitive or resistant as recommended by the guidelines of Clinical & Laboratory Standards Institute (CLSI) [64]. Resistance to at least one agent in three or more antimicrobial groups was defined as multi–drug resistance. *E. coli* ATCC 25922 was used as control strain.

Phenotypic and genotypic detection of ESBL *E. coli* and Methicillin–resistant *S. aureus*

The presence of ESBL phenotype was determined by the double–disc synergy test (DDST) using Cefotaxime (CTX) and Ceftazidime (CAZ) alone and in combination with Clavulanic acid as recommended by the CLSI [64]. For this purpose, CAZ (30 µg), CTX (30 µg), CAZ–Clavulanic acid (30/10 µg), and CTX–Clavulanic acid (30/10 µg) discs were placed on MHA. After 16–18 h of incubation at 35°C, ≥ 5 millimeters (mm) increase in zone diameter of CAZ/Clavulanic acid disc and CAZ disc alone, and/or ≥ 5 mm increase in the zone diameter of CTX/Clavulanic acid disc and CTX disc alone were considered as ESBL positive.

The genotypic assay was made by using genomic deoxyribonucleic acid (DNA) obtained from phenotypic ESBL positive isolates by boiling method as a template. Briefly, 100 microliters (µL) of Tris–Ethylenediaminetetraacetic acid (EDTA) buffer solution (pH 8.0) containing a few colonies were boiled in a dry heating block (TDB–100, Biosan, Latvia) for 10 min. At the end of the heating, the samples were cooled on ice and centrifuged (MIKRO 220R, Hettich, Germany) at 10.000 x *G* force – *G* – for 15 seconds (s). The supernatant was used as template in polymerase chain reaction (PCR) [10], components were provided by Vivantis Technologies (Subang Jaya, Malaysia).

Primers used in this study were obtained from Metabion International AG (Planegg-Martinsried, Germany). The isolates that were ESBL–positive by double disc synergy test (DDST) were subjected to amplification by PCR method using TEM, SHV, CTX–M–1, CTX–M–2, CTX–M–8/25, and CTX–M–9 group primers, as previously reported by Le *et al*. [35]. The PCR amplifications were performed in a total volume of 15 µL solution containing 2 µL of template DNA, 1X PCR buffer (Sigma), 0.25 milimolar – mM – $MgCl_2$ (Sigma), 200 micromole – µM – (each) dNTP (Sigma), 10 picomoles of each primer, 1.25 Units – U – of Taq polymerase (Sigma). The reaction conditions for amplification of DNA were 95°C for 5 min, 25 cycles of 95°C for 30 s, 60°C for 90 s and 72°C for 90 s, and 68°C for 10 min.

Oxacillin disc (1µg; Oxoid, Cambridge, United Kingdom–UK) diffusion assay was performed for evaluation of Methicillin–resistant *S. aureus* (MRSA) following CLSI guidelines for interpretation of the results and using *S. aureus* ATCC 29213 as a CLSI negative control. PCR was performed for the confirmation of *S. aureus* isolates with *femA* gene and presence of Methicillin–resistant gene *mecA* [23]. PCR reaction mixture was prepared the same mentioned above and the reaction condition was initial denaturation at 95°C for 5 min, 35 cycles of amplification (denaturation at 95°C for 2 min, annealing at 58°C for 1 min, extension at 72°C for 1 min), and final extension at 72°C for 10 min in a thermal cycler.

Phylo–typing analysis

Phylo–typing groups were determined using the quadruplex phylogroup assignment method for *E. coli* isolates, previously described by Clermont [18]. PCRs were carried out using thermal cycler (BioRad, USA) in a total volume of 25 µL containing 10 pmol of each three pair of primers (Sigma, USA), 25 µM of dNTPs, 5 µL of template DNA, 2.5 µL of 10X Taq buffer [50 mM KCl, 10 mM Tris–HCl (pH 8.3)], 2 mM $MgCl_2$, and 2.5 U of Taq polymerase (Fermentas, USA). The PCR products were separated by electrophoresis through 1.2% agarose gel in 1X TAE buffer. DNA fragments were visualized by ethidium bromide staining and photographed under ultraviolet light illumination (gelDoc™ XR+ Gel Documentation System, BioRad,USA) [11].

Viral ribonucleic acid (RNA) detection

PCR analysis was performed to investigate rotavirus (RV), hepatitis E virus (HEV), hepatitis A virus (HAV) , and Norovirus (NoV), that could be transmitted by the fecal–oral route. Primer sets used for each virus are shown in TABLE III [9, 29, 46, 60]. Each of the REVS samples were put 2 g in the tube and diluted with 1.5 mL of phosphate–buffered saline (PBS). After vortexing, it was centrifuged at 770 *G* at 4°C for 5 min and 500 µL of supernatant from this suspension was used for isolation of viral nucleic acid (VNA). VNA was extracted from RTE salad samples by using the GF–1 VNA extraction kit (Vivantis, Malaysia) according to the manufacturer's instructions. A NA concentration was measured using a NanoDrop spectrophotometer (NanoDrop 1000, Thermo Scientific, USA). The amplicons obtained from PCR were visualized by gel–electrophoresis and positive results on the gel were recorded for each virus. Positive control samples were viruses that have been previously confirmed by sequence analysis [5, 9].

TABLE III
Rotavirus, Hepatitis E virus, Hepatitis A virus and Norovirus PCR primer sequence used in this study

Primer	Sequence 5'–3'	Length (bp)
Rotavirus		
VP6 F	GACGGVGCRACTACATGGT	379
VP6 R	GTCCAATTCATNCCTGGTGG	
Hepatitis A Virus		
VP1/P2B F1	GACAGATTCTACATTTGGATTGGT	512
VP1/P2B R1	CCATTTCAAGAGTCCACACACT	
VP1/P2B F2	CTATTCAGATTGCAAATACAAT	394
VP1/P2B R2	AACTTCATTATTTCATGCTCCT	
Hepatitis E Virus		
3156 F	AATTATGCC(T)CAGTAC(T)CGG(A)GTTG	731
3157 R	CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC	
Norovirus		
JV12 F	ATACCACTATGATGCAGATTA	327
JV13 R	TCATCATCACCATAGAAAGAG	

Statistical analysis

The descriptive statistics were calculated for each parameter using SPSS Software (IBM SPSS statistics 20, USA).

RESULTS AND DISCUSSION

Microbiological quality of REVS

Of a total of forty–two REVS samples were collected from meat restaurants operating in the Erzurum Region in Eastern Turkey to investigate the foodborne pathogens including bacteria and viruses for their microbiological properties and antimicrobial resistance. The total aerobic bacteria (TAB) in the tested REVS samples ranged from less than 1 to 6.40 $\log_{10}$ CFU·g^{-1} (with an average of 4.72 $\log_{10}$ CFU·g^{-1}). Although the lowest TAB count was detected in the samples #E14 and #E16, the highest value was in the #E24 (TABLE IV).

In addition, the highest yeasts and molds (5.00 $\log_{10}$ CFU·g^{-1}), coliform (5.79 $\log_{10}$ CFU·g^{-1}), and *Staphylococcus* and *Micrococcus* bacteria (5.66 $\log_{10}$ CFU·g^{-1}) have been also detected in the sample #24. The count of bacteria in the salad samples tested in this study results showed an overall similar trend to each other. For example, when the TAB count was detected as high, other parameters showed a high trend as much as TAB (TABLE IV). A very few of the samples 3 out of 42 (7.14%) had a TAB count greatest than 6 $\log_{10}$ CFU·g^{-1}, which categorizes them as borderline, according to Health Protection Agency (HPA) guidelines [25].

It has been reported that TAB was ranged from 3.0 $\log_{10}$ CFU·g^{-1} to 6.7 $\log_{10}$ CFU·g^{-1}in RTE salads in Mexico city [15], however, it was between 5.12 and 9.75 $\log_{10}$ CFU·g^{-1} (with an average of 7.73 $\log_{10}$ CFU·g^{-1} in REVS in Cyprus [65]. In addition, a high level of TAB (6.43 $\log_{10}$ CFU·g^{-1} –mean {3.50–8.39}) was reported in raw salad vegetables sold in Lebanon [22]. Of note, these findings were found to be considerably higher than the present study. On the other hand, the high level of yeast and molds (ranged between less than 1 to 6.26 log CFU g^{-1}) were detected in the REVS samples. In the previous studies, which were consistent with the current study results, the yeast and mold were reported 10^4–10^7 $\log_{10}$ CFU·g^{-1}and 1.63 and 6.68 $\log_{10}$ CFU·g^{-1} from India [44] and Mexico [27], respectively.

TABLE IV
Microbiological quality of REVS

	Microbial Count ($\log_{10}$ CFU·g^{-1})				Presence (+/–)						
Symbol	TAB	YM	CO	STA/MICR	STA	SAL	EC	HAV	RV	HEA	NoV
E1	4.60	3.30	3.83	1.30	–	–	+	–	–	–	–
E2	4.48	3.53	2.48	1.85	–	–	–	–	–	–	–
E3	5.51	6.26	5.06	1.30	–	–	–	–	–	–	–
E4	4.78	4.23	4.65	1.48	–	–	+	–	–	–	–
E5	4.90	5.06	4.40	2.20	–	–	+	–	–	–	–
E6	5.20	5.43	5.25	1.00	–	–	+	–	–	–	–
E7	5.04	4.46	4.74	1.30	–	–	–	–	–	–	–
E8	5.97	5.45	4.88	1.30	–	–	–	–	–	–	–
E9	5.45	5.08	5.03	1.00	–	–	–	–	–	+	–
E10	5.00	4.88	4.83	1.48	–	–	–	–	–	–	–

***TABLE IV* (cont...)**
Microbiological quality of REVS

	Microbial Count (log_{10} CFU·g^{-1})				Presence (+/–)						
Symbol	**TAB**	**YM**	**CO**	**STA/MICR**	**STA**	**SAL**	**EC**	**HAV**	**RV**	**HEA**	**NoV**
E11	4.81	3.18	4.46	<1.00	–	–	–	+	–	–	–
E12	5.81	5.59	5.46	1.70	–	–	–	–	–	–	–
E13	4.90	4.86	4.89	1.30	–	–	–	–	–	–	–
E14	<1.00	3.48	3.70	<1.00	–	–	+	–	–	–	–
E15	5.00	5.09	5.06	1.48	–	–	–	–	–	–	–
E16	<1.00	3.00	<1.00	<1.00	–	–	+	–	–	–	–
E17	6.34	5.34	5.82	2.81	–	–	–	–	–	–	–
E18	4.00	4.82	4.11	1.48	–	–	+	–	–	–	–
E19	4.00	<1.00	3.30	<1.00	–	–	–	–	–	–	–
E20	4.48	3.70	3.60	2.64	–	–	–	–	–	–	–
E21	4.60	5.05	4.00	2.51	–	–	+	–	+	+	–
E22	5.81	5.09	4.58	2.82	–	–	+	–	–	–	–
E23	4.00	3.48	3.78	<1.00	–	–	–	–	–	–	–
E24	6.40	5.00	5.79	5.66	–	–	+	–	–	+	–
E25	4.30	4.40	3.78	<1.00	–	–	–	–	–	–	–
E26	4.60	3.90	4.78	2.04	–	–	–	–	–	–	–
E27	5.20	3.60	4.98	2.04	–	–	–	–	–	–	–
E28	5.32	5.00	4.68	1.90	–	–	–	–	–	–	–
E29	4.30	4.00	4.20	<1.00	–	–	–	–	–	–	–
E30	4.70	3.48	4.38	2.92	–	–	–	–	–	+	–
E31	4.30	4.58	3.78	1.70	–	–	+	–	–	–	–
E32	5.76	4.54	4.68	<1.00	–	–	–	–	–	–	–
E33	4.00	3.30	3.60	1.00	–	–	+	–	–	–	–
E34	4.00	4.11	5.10	1.78	–	–	+	–	–	–	–
E35	5.15	3.60	4.93	2.00	–	–	–	–	–	+	–
E36	5.04	4.61	4.67	1.60	–	–	–	–	–	–	–
E37	5.11	4.57	4.80	2.92	–	–	–	+	–	–	–
E38	4.78	3.48	4.40	2.53	–	–	–	–	–	–	–
E39	6.39	4.11	4.00	1.70	–	–	+	+	–	+	–
E40	4.95	4.08	4.78	2.38	+	–	–	+	–	–	–
E41	4.95	4.30	4.80	2.23	–	–	+	+	–	–	–
E42	4.30	4.23	4.08	2.70	–	–	+	+	+	–	–
MinV	<1.00	<1.00	<1.00	<1.00							
MaxV	6.40	6.26	5.82	5.66							
Variance	1.56	1.04	0.93	1.25							
SD	1.25	1.02	0.96	1.12							
SE	0.19	0.16	0.15	0.17							
Mean	4.72	4.27	4.38	1.62							

*TAB: Total aerobic bacteria, STA/MICR: *Staphylococcus*/*Micrococcus*, CO: Coliform bacteria, YM: yeasts and molds, SAL: *Salmonella* spp., STA: *S. aureus*, EC: *E. coli*, HEV: Hepatitis E, RV: Rotavirus, HAV: Hepatitis A, NoV: Norovirus; <1.00: below the detection level. The results are shown as log_{10} CFU·g^{-1}; (+) presence of bacteria and viruses in 25 g and 2 g of the product, respectively, (–) absence of bacteria and viruses in 25 g and 2 g of the product, respectively

In this context, coliform bacteria (CO) were detected at levels ranging from less than 1 to 5.82 log_{10} CFU·g^{-1} in all REVS samples. Regardless of the sources, the number of CO was detected in all salads served in Mexican restaurants with limits ranging from 5.4×10^3 to 1.7×10^8 log_{10} CFU·g^{-1} [27]. The high CO count identified in the current study may be associated with poor hygiene practices during the preparation of REVS. Apart from CO, the *Staphylococcus* and *Micrococcus* spp. count (STA/MICR) was determined between less than 1 and 5.66 log_{10} CFU·g^{-1} in the current study, indicating a possible transmission from the food handlers. Risk factors that play a role in the contamination of vegetables, such as unsafe water sources for irrigation, inappropriate fertilizers or manures, access to livestock wild animals in the field, and unhygienic post-harvest handling (unhygienic utensil, labor, handling, packaging material, and improper/inadequate storage conditions) were indicated by investigators in the previous studies [16, 38, 39, 47]. These results showed a relatively high contamination rate detected in the salad samples tested in this study, indicating that RTE salad serving in the meat restaurant is a risk factor for the transmission of food-borne diseases in humans [12].

Presence of *E. coli*, *S. aureus* and *Salmonella* spp. in REVS

Although the HPA guidelines indicated greater than or equal to 10^2 for *E. coli*, greater than or equal to 10^4 for *S. aureus*, and presence in 25 g for *Salmonella* spp. These are evaluated as an unsatisfactory product [25], Turkish Food Codex [59] has ruled that RTE foods should not contain *E. coli* (less than 10^1), *Salmonella* spp., *L. monocytogenes*, and staphylococcal enterotoxins. None of the REVS tested in this study contained *Salmonella* spp., whereas *E. coli* and *S. aureus* were isolated in 38.1% (16/42) and 2.4% (1/42), respectively, however, not using the colony counting method. Hence, it is impossible to evaluate the current study results according to HPA guidelines and/or the Turkish Food Codex (TFC) [59]. In contrast to current study result, it has been reported that a higher level of S. aureus (12 and 13.02%) was detected in ready-to-eat salads in Turkey [8, 41].

Considered by food vendors as an indicator of fecal contamination and improper hygiene practices, *E. coli* can cause gastroenteritis and diarrhea in humans when taken with contaminated food [2]. The prevalence of *E. coli* was found to be 38.10% (16/32) in REVS samples analyzed in this study. This result was lower than in studies done in other Countries: 96.7% in Ghana [2]; 94% in Cote d'ivoire [19]; 83.2% in Mexico [27]; 64% in Argentina [43]. But it was higher than some Countries: 20% in United Arab Emirates [6] and 16.7% in Spain [1].

The prevalence of *E. coli* contamination has displayed a significant variation between developed and developing countries [47]. For example, studies in low-income Countries such as Pakistan, Bangladesh, and India reported the prevalence of *E. coli* in raw vegetable and ready-to-eat salad samples sold in retail markets ranged from 34 to 60% [4, 49, 55] whereas it was 3.1 and 4.0% in the USA and Turkey, respectively [28, 36].

Phylo-typing of *E. coli* strains using quadruplex PCR displayed six isolates in group A, four isolates in groups B1 and C, and one isolate in groups E and F (TABLE V). Although most of *E. coli* isolates were detected in the group with commensal strains (group A and B1) in the current study, only one isolate was detected in the virulent group (F).

Despite the high STA/MICR count, *S. aureus* could only be detected in one sample in the current study. As it is known, *S. aureus* is one of the most important foodborne pathogens worldwide, some strains of which can produce one or more toxins, mainly enterotoxin [64]. The prevalence of *S. aureus* in REVS observed in the present study is comparable to similar studies [22, 40, 54].

TABLE V
Phylogenetic groups and ESBL presence of *E. coli* isolates from RTE vegetable salad samples

	Isolate ID	Phylogeny	ESBL	ESBL Genotype
	E1	B1	–	
	E4	B1	–	
	E5	A	–	
	E6	B1	–	
	E14	A	–	
	E16	B1	+	CTX–M–1, TEM
	E18	C	–	
	E21	A	–	
	E22	A	–	
	E24	C	+	–
	E31	C	–	
	E33	A	–	
	E34	A	+	CTXM8/25
	E39	E	+	–
	E41	C	+	TEM
	E42	F	+	–
Total	16/42		6/16	

The sources of contamination of *Salmonella* could be animal feces as fertilizer, cultivation of the plants with wastewater, and personal hygiene [40, 50, 52, 57]. *Salmonella* spp. was also isolated in fresh vegetables by investigators in the previous studies [21, 52, 66] in contrast to the present study findings. Similarly, it has been reported none of *Salmonella* spp. was detected from 45 REVS in Portugal [14]. This result suggests it might be no cross-contamination with *Salmonella* spp. during the sample collection in the current study.

Antimicrobial resistance of the isolates from REVS

Only one *S. aureus* was isolated and confirmed by PCR (with *femA* gene) in the current study, and the isolate was resistant to gentamicin, kanamycin, aztreonam, and ciprofloxacin in the disc diffusion assay. In addition, the PCR analysis conducted for detection of the *mecA* gene showed that the *S. aureus* strain isolated in the current study was not harboring the *mecA* gene. On the other hand, all *E. coli* strains (n=16) were resistant (100%) against aminoglycoside antibiotics (gentamicin and kanamycin) tested in this study, even though they were susceptible to meropenem in the disc diffusion assay (TABLE VI). The isolates displayed a low level of resistance against chloramphenicol (6.25%), tetracycline (18.75%), and ciprofloxacin (25.00%). These data indicated that antimicrobial-resistant *E. coli* strains from REVS salad samples in the current study still remain moderately low-level resistant to anti-Gram-negative drugs of importance for Human Medicine, suggesting that these antibiotics could be non-effective in the future due to the rising antimicrobial resistance. The double-disc synergy test used for the detection of the phenotypic resistant ESBL producing

TABLE VI
Antibiotic susceptibility pattern (Sensitive and Resistance) of *E. coli* isolates

Antibiotic	Antibiotic susceptibility pattern	
	Sensitive	Resistance
Meropenem (10 µg)	16 (100%)	0
Chloramphenicol (30 µg)	15 (93.75%)	1 (6.25%)
Gentamicin (10 µg)	0	16 (100%)
Kanamycin (30 µg)	0	16 (100%)
Tetracycline (30 µg)	13 (81.25%)	3 (18.75%)
Ciprofloxacin (5 µg)	12 (75.00%)	4 (25.00%)
Sulfamethoxazole/Trimethoprim (25 µg)	11 (68.75%)	5 (31.25%)
Cefepime (30 µg)	14 (87.5%)	2 (12.50%)
Cefpodoxime (10 µg)	9 (56.25%)	7 (43.75%)
Cefoxitin (30 µg)	12 (75.00%)	4 (25.00%)
Aztreonam (30 µg)	15 (93.75%)	1 (6.25)

strains in the current study displayed that 35.7% (6/16) of the isolates were ESBL producing. Molecular analysis of ESBL producing strains showed that one strain harbored two different genes (bla_{CTXM-1} and bla_{TEM}), whereas two isolates carried one gene (bla_{TEM} and $bla_{CTXM8/25}$). To the best of this knowledge, this is the first report of the presence of ESBL producing *E. coli* in REVS in Turkey. The outbreaks due to the multi-drug resistant bacteria on fresh vegetable products have been reported around the world by researchers in previous studies [24, 31, 36, 52]. A study in Japan indicated that fresh vegetables served as an important route of transmission of ESBL producer bacteria to humans [61].

In terms of Public Health, antimicrobial resistant zoonotic pathogens in foods pose a direct risk. Foods can be contaminated with bacteria harboring antimicrobial resistance genes, antibiotics using agricultural production, resistance genes of microorganisms used as starters during food processing, and cross contamination. Since raw foods are consumed without undergoing any other processing, they carry a significant risk of transferring antimicrobial resistance to humans. Ultimately, transfer of antimicrobial resistance genes between bacteria can also occur after ingestion by humans [63]. Moreover, poor processing and preservation conditions lead to the continued presence of damaged or stressed cells in food, increasing the risk of bacteria carrying antimicrobial resistance genes transmission [34].

Viral foodborne pathogens from REVS

The presence of HEV, RV, HAV and NoV was investigated in the current study to reveal important viral infections to Public Health in the REVS samples. VNA was detected in 14/42 salad samples tested in the current study by PCR. Of these, two were evaluated as positive for RV (5%), and six for HAV (14%) and HEV (14%). NoV could not be found in any of the samples in the current study. Gel-electrophorese images of VNA and control groups determined positive by PCR analysis were given in FIG. 1. HEV, HAV, RV, and NoV are transmitted to humans by food and environmental routes depending on the virus genotype, environmental conditions, hygienic conditions, and the types of food consumed [62].

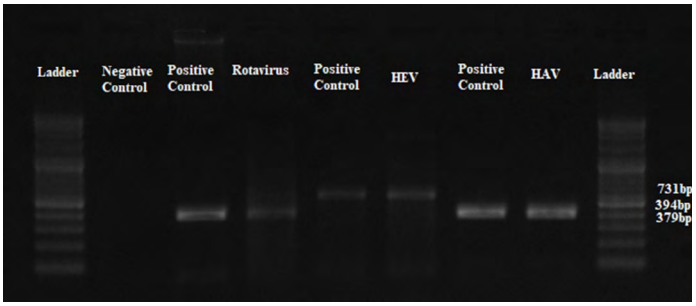

FIGURE 1. Rotavirus, Hepatitis E virus, and Hepatitis A virus positive PCR amplicons

In a study showed that in the total of 911 REVS samples from supermarkets in Italy, the total prevalence of HAV and HEV was 1.9% (18/911) and 0.6% (6/911), respectively, even though NoV could not be detected in any of the samples [58]. The prevalence of HAV and HEV was high in the samples tested in this study whereas NoV was not detected. In contrast to the obtained study data, a low level of NoV (2.90–3.75%) was reported in vegetables and fruits [42], indicating the less frequent detection of NoV in REVS products. Hence, salads are less frequently involved in foodborne viral outbreaks than other foods; however, they may be contaminated with unsanitary food workers or raw materials that have been contaminated [17]. Khan *et al*. [32] reported that 29 vegetable samples collected from 13 different locations of District Mardan in Pakistan, one was positive for HAV. Shin *et al*. [56] reported that one sample was positive for HAV in fresh vegetables and fruits from supermarkets in Mexico, 7/80 were positive for HAV [42]. In another study, of the 70 vegetable samples including 51 first range raw vegetables and one fourth range REVS from markets in Sicily,Italy, 1.4% for HEV [45]. The prevalence of RV was found variable in vegetables: 13.75% (11/80) in Mexico [42]; 22% (23/101) in Argentina [20]. To the best of the present knowledge, this is the first report from Turkey for HEV, HAV, and RV positivity in REVS, suggesting REVS can be a reservoir for the important viral pathogens and to be considered before consumption.

Viral contamination can occur at several points in the food production chain. Because they do not have a chance to growth outside of living cells, their presence in food can be explained by pre-harvest contamination of vegetables or post-harvest contamination from food processors. On the other hand, the fact that the food handlers in the field where the vegetables are harvested and the water quality used in agricultural irrigation can affect the microbiological properties of vegetables can also explain the high level of viral contamination [58].

CONCLUSIONS

The assessment of microbiological contamination status in REVS contributes to the identification of risks for government authorities as well as to the assessment of consumer exposure. This is the first report of the presence of ESBL producing *E. coli*, HEV, HAV and RV for REVS from Turkey. On the other hand, it can be predicted that the high microorganism load detected in REVS samples and the antimicrobial resistance of isolates may pose a threat to Public Health. Considering that the lack of an effective surveillance system led to the inability to identify the possible source of the epidemic, it was thought that REVS could be a reservoir in this sense. Elimination or at least mitigation of this current potential risk requires increasing the good agricultural and manufacturing practices throughout the vegetable production process from the farm to the retailer and restaurants. Moreover, hazard analysis and critical control point (HACCP) strategies need to be implemented and effectively supervised, especially at the restaurant level.

Conflict of Interest

The authors declare that they have no conflicts of interest in the research

BIBLIOGRAPHIC REFERENCES

[1] ABADIAS, M.; USALL, J.; ANGUERA, M.; SOLSONA, C.; VIÑAS, I. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. **Int. J. Food Microbiol.** 123: 121–129. 2008.

[2] ABAKARI, G.; COBBINA, S.J.; YELELIERE, E. Microbial quality of ready-to-eat vegetable salads vended in the central business district of Tamale, Ghana. **Int. J. Food Contam.** 5: 3. 2018.

[3] ABAY, S.; IRKIN, R.; AYDIN, F.; MÜŞTAK, H.K.; DIKER, K.S. The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates. **LWT–Food Sci. Technol.** 81: 202–209. 2017.

[4] AHMED, S.; SIDDIQUE, M.A.; RAHMAN, M.; BARI, M.L.; FERDOUSI, S. A study on the prevalence of heavy metals, pesticides, and microbial contaminants and antibiotics resistance pathogens in raw salad vegetables sold in Dhaka, Bangladesh. **Heliyon.** 5: e01205. 2019.

[5] AKTAŞ, O.; AYDIN, H.; TIMURKAN, M.O. A molecular study on the prevalence and coinfections of Rotavirus, Norovirus, Astrovirus and Adenovirus in children with gastroenteritis. **Minerva Pediatr.** 71: 431–437. 2019.

[6] ALMUALLA, N.A.; LALEYE, L.C.; ABUSHELAIBI, A.A.; AL-QASSEMI, R.A.; WASESA, A.A.; BABOUCARR, J. Aspects of the microbiological quality and safety of ready-to-eat foods in Sharjah supermarkets in the United Arab Emirates. **J. Food Prot.** 73: 1328–1331. 2010.

[7] ANDREWS, W.; HAMMACK, T. Chapter 5: Salmonella. 2007. US Food and Drug Administration (FDA) **Bacteriological Analytical Manual (BAM)**. Online: https://bit.ly/3MjbSdi. 02-12-2021

[8] AYCICEK, H.; CAKIROGLU, S.; STEVENSON, T.H. Incidence of Staphylococcus aureus in ready-to-eat meals from military cafeterias in Ankara, Turkey. **Food Control.** 16: 531–534. 2005.

[9] AYDIN, H.; UYANIK, M.H.; KARAMESE, M.; TIMURKAN, M.O. Seroprevalence of hepatitis e virus in animal workers in nonporcine consumption region of Turkey. **Future Virol.** 11: 691–697. 2016.

[10] BARAN, A.; ADıGÜZEL, M.C.; YÜKSEL, M. Prevalence of antibiotic-resistant and extended-spectrum beta-lactamase-producing *Escherichia coli* in chicken meat from eastern Turkey. **Pak. Vet. J.** 40: 355–359. 2020.

[11] BARAN, A.; ERDOĞAN, A.; KAVAZ, A.; ADIGÜZEL, M.C. Some specific microbiological parameters and prevalence of *Salmonella* spp. in retail chicken meat from Erzurum Province, Turkey and characterization of antibiotic resistance of isolates. **Biosci. J.** 35: 878–891. 2019.

[12] BAUTISTA-DE LEÓN, H.; GÓMEZ-ALDAPA, C.; RANGEL-VARGAS, E.; VÁZQUEZ-BARRIOS, E.; CASTRO-ROSAS, J. Frequency of indicator bacteria, *Salmonella* and diarrhoeagenic *Escherichia coli* pathotypes on ready-to-eat cooked vegetable salads from M exican restaurants. **Lett. Appl. Microbiol.** 56: 414–420. 2013.

[13] BENCARDINO, D.; VITALI, L.A.; PETRELLI, D. Microbiological evaluation of ready-to-eat iceberg lettuce during shelf-life and effectiveness of household washing methods. **Ital. J. Food Saf.** 7: 50–54. 2018.

[14] CAMPOS, J.; MOURÃO, J.; PESTANA, N.; PEIXE, L.; NOVAIS, C.; ANTUNES, P. Microbiological quality of ready-to-eat salads: An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. **Int. J. Food Microbiol.** 166: 464–470. 2013.

[15] CERNA-CORTES, J.F.; LEON-MONTES, N.; CORTES-CUETO, A.L.; SALAS-RANGEL, L.P.; HELGUERA-REPETTO, A.C.; LOPEZ-HERNANDEZ, D.; RIVERA-GUTIERREZ, S.; FERNANDEZ-RENDON, E.; GONZALEZ-Y-MERCHAND, J.A. Microbiological quality of ready-to-eat vegetables collected in Mexico City: occurrence of aerobic-mesophilic bacteria, fecal coliforms, and potentially pathogenic nontuberculous mycobacteria. **Biomed Res. Int.** 2015: 789508. 2015.

[16] CHAU, M.L.; AUNG, K.T.; HAPUARACHCHI, H.C.; LEE, P.S.V.; LIM, P.Y.; KANG, J.S.L.; NG, Y.; YAP, H.M.; YUK, H.-G.; GUTIÉRREZ, R.A. Microbial survey of ready-to-eat salad ingredients sold at retail reveals the occurrence and the persistence of *Listeria monocytogenes* Sequence Types 2 and 87 in pre-packed smoked salmon. **BMC Microbiol.** 17: 1–13. 2017.

[17] CHENG, D.; ZOU, S.; LIAO, N.; SHI, X.; CHEN, J.; ZHANG, Y.; SUN, L.; ZHANG, R. Evaluation of an Extraction Method for the Detection of GI and GII Noroviruses in Fruit and Vegetable Salads. **J. Food Sci.** 83: 393–400. 2018.

[18] CLERMONT, O.; BONACORSI, S.; BINGEN, E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. **Am. Soc. Mirobiol.** 66: 4555–4558. 2000.

[19] COULIBALY-KALPY, J.; AGBO, E.A.; DADIE, T.A.; DOSSO, M. Microbiological quality of raw vegetables and ready to eat products sold in Abidjan (Côte d'Ivoire) markets. **Afr. J. Microbiol. Res.** 11: 204–210. 2017.

[20] EMILSE, P.V.; MATÍAS, V.; CECILIA, M.L.; OSCAR, G.M.; GISELA, M.; GUADALUPE, D.; ELIZABETH, R.V.; VICTORIO, P.J.; RODNEY, C.; VIVIANA, N.S.; ANGÉLICA, B.P. Enteric virus presence in

green vegetables and associated irrigation waters in a rural area from Argentina. A quantitative microbial risk assessment. **LWT–Food Sci. Technol.** 144: 111201. 2021.

[21] EVELYNE, T.; ATTIEN, P.; MOROH, A.J. L.; HAZIZ, S.; KOUAME, D.N.; KAMBIRE, O.; BABA-MOUSSA, L.; GUESSENND, N.; DAKO, E.; DADIE, A.T. Prevalence and characterization of Salmonella isolated from vegetables salads and ready to eat raw mixed vegetable salads in Abidjan, Cte dIvoire. **J. Microbiol. Antimicrob.** 14: 15–25. 2022.

[22] FAOUR-KLINGBEIL, D.; MURTADA, M.; KURI, V.; TODD, E.C.D. Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. **Food Control.** 62: 125–133. 2016.

[23] FINDIK, A.; AKAN, N.; ONUK, E.E.; ÇAKIROĞLU, D.; CIFTCI, A. Methicillin resistance profile and molecular typing of *Staphylococcus aureus* strains isolated from noses of the healthy dogs. **Kafkas Univ. Vet. Fak. Derg.** 15: 925–930. 2009.

[24] FREITAG, C.; MICHAEL, G.; LI, J.; KADLEC, K.; WANG, Y.; HASSEL, M.; SCHWARZ, S. Occurrence and characterisation of ESBL-encoding plasmids among *Escherichia coli* isolates from fresh vegetables. **Vet. Microbiol.** 219: 63–69. 2018.

[25] GILBERT, R.; DE LOUVOIS, J.; DONOVAN, T.; LITTLE, C.; NYE, K.; RIBEIRO, C.; RICHARDS, J.; ROBERTS, D.; BOLTON, F. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. **Commun. Dis. Pub. Healt.** 3: 163–167. 2000.

[26] GIRI, S.; KUDVA, V.; SHETTY, K.; SHETTY, V. Prevalence and Characterization of Extended-Spectrum β-Lactamase-Producing Antibiotic-Resistant *Escherichia coli* and *Klebsiella pneumoniae* in Ready-to-Eat Street Foods. **Antibiotics.** 10: 850–850. 2021.

[27] GÓMEZ-ALDAPA, C.A.; RANGEL-VARGAS, E.; CASTRO-ROSAS, J. Frequency and Correlation of Some Enteric Indicator Bacteria and Salmonella in Ready-to-Eat Raw Vegetable Salads from Mexican Restaurants. **J. Food Sci.** 78: M1201–M1207. 2013.

[28] GURLER, Z.; PAMUK, S.; YILDIRIM, Y.; ERTAS, N. The microbiological quality of ready-to-eat salads in Turkey: A focus on *Salmonella* spp. and *Listeria monocytogenes*. **Int. J. Food Microbiol.** 196: 79–83. 2015.

[29] HUANG, F.F.; HAQSHENAS, G.; GUENETTE, D.K.; HALBUR, P.G.; SCHOMMER, S.K.; PIERSON, F.W.; TOTH, T.E.; MENG, X.J. Detection by reverse transcription–PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. **J. Clin. Microbiol.** 40: 1326–1332. 2002.

[30] GLOBAL MARKET RESEARCH AND PUBLIC OPINION SPECIALIST (IPSOS). Beverage products drew attention in the purchase of fast moving consumer goods in the household. 2020. Online: https://bit.ly/3TcnS2w. 02-02-2022.

[31] JANALÍKOVÁ, M.; PLEVA, P.; PAVLÍČKOVÁ, S.; LECOMTE, M.; GODILLON, T.; HOLKO, I. Characterization of *Escherichia coli* strains isolated from raw vegetables. **Potr. S. J. F. Sci.** 12: 304–312. 2018.

[32] KHAN, W.; IRSHAD, M.; REHMAN, G.; HUSSAIN, A. HAV in fresh vegetables: a hidden health risk in district Mardan, Pakistan. **SpringerPlus.** 3: 675. 2014.

[33] KIM, J.S.; BANG, O.K.; CHANG, H.C. Examination of microbiological contamination of ready-to-eat vegetable salad. **J. Food Hyg. Saf.** 19: 60–65. 2004.

[34] LAMMIE, S.L.; HUGHES, J.M. Antimicrobial resistance, food safety, and one health: the need for convergence. **Annu. Rev. Food Sci. Technol.** 7: 287–312. 2016.

[35] LE, Q.P.; UEDA, S.; NGUYEN, T.N.H.; DAO, T.V.K.; VAN HOANG, T.A.; TRAN, T.T.N.; HIRAI, I.; NAKAYAMA, T.; KAWAHARA, R.; DO, T.H. Characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* in retail meats and shrimp at a local market in Vietnam. **Foodborne Pathog. Dis.** 12: 719–725. 2015.

[36] LIU, S.; KILONZO-NTHENGE, A. Prevalence of multidrug-resistant bacteria from US-grown and imported fresh produce retailed in chain supermarkets and ethnic stores of Davidson County, Tennessee. **J. Food Prot.** 80: 506–514. 2017.

[37] MAKINDE, O.M.; AYENI, K.I.; SULYOK, M.; KRSKA, R.; ADELEKE, R.A.; EZEKIEL, C.N. Microbiological safety of ready-to-eat foods in low-and middle-income countries: A comprehensive 10-year (2009 to 2018) review. **Compr. Rev. Food Sci.** 19: 703–732. 2020.

[38] MERCANOGLU-TABAN, B.; HALKMAN, A.K. Do leafy green vegetables and their ready-to-eat [RTE] salads carry a risk of foodborne pathogens? **Anaerobe.** 17: 286–287. 2011.

[39] MIR, S.A.; SHAH, M.A.; MIR, M.M.; DAR, B.N.; GREINER, R.; ROOHINEJAD, S. Microbiological contamination of ready-to-eat vegetable salads in Developing Countries and potential solutions in the supply chain to control microbial pathogens. **Food Control.** 85: 235–244. 2018.

[40] NAJAFI, H.; MOHAMMAD, B.; BAHREINI, M. Microbiological quality of mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs in Mashhad, Iran. **Intern. Conf. Nutr. Food Sci.** 39: 62–66. 2012.

[41] PAMUK, Ş.; GÜRLER, Z.; YILDIRIM, Y.; ERTAŞ, N.J.K.U.V.F.D. The microbiological quality of ready to eat salads sold in Afyonkarahisar, Turkey. **Kafkas Univ. Vet. Fakult. Dergisi** 19: 1001–1006. 2013.

[42] PARADA-FABIÁN, J.C.; JUÁREZ-GARCÍA, P.; NATIVIDAD-BONIFACIO, I.; VÁZQUEZ-SALINAS, C.; QUIÑONES-RAMÍREZ, E.I. Identification of Enteric Viruses in Foods from Mexico City. **Food Environ. Virol.** 8: 215–220. 2016.

[43] PELLICER, K.; COPES, J.; MALVESTITI, L.; ECHEVERRÍA, M.G.; NOSETTO, E.O.; STANCHI, N.O. Ready to eat salads: an analysis of health and safety conditions. **Analecta Vet.** 22: 4–6. 2002.

[44] PINGULKAR, K.; KAMAT, A.; BONGIRWAR, D. Microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads: an evidence of inhibition of *Listeria monocytogenes* in tomatoes. **Int. J. Food Sci. Nutr.** 52: 15–23. 2001.

[45] PURPARI, G.; MACALUSO, G.; DI BELLA, S.; GUCCIARDI, F.; MIRA, F.; DI MARCO, P.; LASTRA, A.; PETERSEN, E.; LA ROSA, G.; GUERCIO, A. Molecular characterization of human enteric viruses in food, water samples, and surface swabs in Sicily. **Int. J. Infect. Dis.** 80: 66–72. 2019.

[46] RACHIDA, S.; TAYLOR, M.B. Potentially Infectious Novel Hepatitis A Virus Strains Detected in Selected Treated Wastewater Discharge Sources, South Africa. **Viruses.** 12: 1468. 2020.

[47] RAHMAN, M.; ALAM, M.-U.; LUIES, S.K.; KAMAL, A.; FERDOUS, S.; LIN, A.; SHARIOR, F.; KHAN, R.; RAHMAN, Z.; PARVEZ, S.M. Contamination of fresh produce with antibiotic-resistant bacteria and associated risks to human health: A scoping review. **Int. J. Environ. Res. Public Health.** 19: 360. 2021.

[48] RODRÍGUEZ-LÁZARO, D.; ŁEPECKA, A.; ZIELÍNSKA, D.; SZYMÁNSKI, P.; BURAS, I.; KOŁO, D. Assessment of the Microbiological Quality of Ready-to-Eat Salads-Are There Any Reasons for Concern about Public Health? **Int. J. Environ. Res. Public Health.** 19: 1582–1582. 2022.

[49] SAKSENA, R.; MALIK, M.; GAIND, R. Bacterial contamination and prevalence of antimicrobial resistance phenotypes in raw fruits and vegetables sold in Delhi, India. **J. Food Saf.** 40: e12739. 2020.

[50] SALLEH, N.A.; RUSUL, G.; HASSAN, Z.; REEZAL, A.; ISA, S.H.; NISHIBUCHI, M.; RADU, S. Incidence of Salmonella spp. in raw vegetables in Selangor, Malaysia. **Food Control.** 14: 475–479. 2003.

[51] ŞANLıBABA, P.; TEZEL, B.U.; ÇAKMAK, G.A. Prevalence and antibiotic resistance of *Listeria monocytogenes* isolated from ready-to-eat foods in Turkey. **J. Food Qual.** 2018: 7693782. 2018.

[52] SAPKOTA, S.; ADHIKARI, S.; PANDEY, A.; KHADKA, S.; ADHIKARI, M.; KANDEL, H.; PATHAK, S. Multi-drug resistant extended-spectrum beta-lactamase producing *E. coli* and *Salmonella* spp. on raw vegetable salads served at hotels and restaurants in Bharatpur, Nepal. **BMC Res. Notes.** 12: 1–6. 2019.

[53] SARKAR, C.; WEBSTER, C.; GALLACHER, J. Are exposures to ready-to-eat food environments associated with type 2 diabetes? A cross-sectional study of 347 551 UK Biobank adult participants. **Lancet Planet. Health.** 2: e438–e450. 2018.

[54] SEO, Y. H.; JANG, J. H.; MOON, K. D.J.F.S. Occurrence and characterization of enterotoxigenic *Staphylococcus aureus* isolated from minimally processed vegetables and sprouts in Korea. **Food Sci. Biotechnol.** 19: 313–319. 2010.

[55] SHAH, M.S.; EPPINGER, M.; AHMED, S.; SHAH, A.A.; HAMEED, A.; HASAN, F. Multidrug-resistant diarrheagenic *E. coli* pathotypes are associated with ready-to-eat salad and vegetables in Pakistan. **J. Appl. Biol. Chem.** 58: 267–273. 2015.

[56] SHIN, H.; PARK, H.; SEO, D.J.; JUNG, S.; YEO, D.; WANG, Z.; PARK, K.H.; CHOI, C. Foodborne Viruses Detected Sporadically in the Fresh Produce and Its Production Environment in South Korea. **Foodborne Pathog. Dis.** 16: 411–420. 2019.

[57] TABAN, B.M.; AYTAC, S.A.; AKKOC, N.; AKCELIK, M. Characterization of antibiotic resistance in *Salmonella enterica* isolates determined from ready-to-eat (RTE) salad vegetables. **Braz. J. Microbiol.** 44: 385–391. 2013.

[58] TERIO, V.; BOTTARO, M.; PAVONI, E.; LOSIO, M.N.; SERRAINO, A.; GIACOMETTI, F.; MARTELLA, V.; MOTTOLA, A.; DI PINTO, A.; TANTILLO, G. Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. **Int. J. Food Microbiol.** 249: 61–65. 2017.

[59] TURKISH FOOD CODEX (TFC). Microbiological Criteria Notification. **T.C. Resmi Gazete.** 2009: 62001. 2009.

[60] TIMURKAN, M.; AYDIN, H.; AKTAŞ, O. Frequency and molecular characterization of human norovirus in Erzurum, Turkey. **Turk. J. Med. Sci.** 47: 960–966. 2017.

[61] USUI, M.; OZEKI, K.; KOMATSU, T.; FUKUDA, A.; TAMURA, Y. Prevalence of extended-spectrum β-lactamase-producing bacteria on fresh vegetables in Japan. **J. Food Prot.** 82: 1663–1666. 2019.

[62] VAN DER POEL, W.H.M. Food and environmental routes of Hepatitis E virus transmission. **Curr. Opin. Virol.** 4: 91–96. 2014.

[63] VERRAES, C.; VAN BOXSTAEL, S.; VAN MEERVENNE, E.; VAN COILLIE, E.; BUTAYE, P.; CATRY, B.; DE SCHAETZEN, M. A.; VAN HUFFEL, X.; IMBERECHTS, H.; DIERICK, K. Antimicrobial resistance in the food chain: a review. **Int. J. Environ. Res. Public Health.** 10: 2643–2669. 2013.

[64] WAYNE, P. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; CLSI supplement M100. Pp. 362. 2019.

[65] XYLIA, P.; BOTSARIS, G.; CHRYSARGYRIS, A.; SKANDAMIS, P.; TZORTZAKIS, N. Variation of microbial load and biochemical activity of ready-to-eat salads in Cyprus as affected by vegetable type, season, and producer. **Food Microbiol.** 83: 200–210. 2019.

[66] YANG, J.; ZHANG, Z.; ZHOU, X.; CUI, Y.; SHI, C.; SHI, X. Prevalence and characterization of antimicrobial resistance in *Salmonella enterica* isolates from retail foods in Shanghai, China. **Foodborne Pathog. Dis.** 17: 35–43. 2020.

[67] YILMAZ, A.; BOSTAN, K.; ALTAN, E.; MURATOGLU, K.; TURAN, N.; TAN, D.; HELPS, C.; YILMAZ, H. Investigations on the frequency of norovirus contamination of ready-to-eat food items in Istanbul, Turkey, by using real-time reverse transcription PCR. **J. Food Prot.** 75: 840–843. 2011.