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Original Article

Prevalence of antimicrobial-resistant *Escherichia coli* from raw vegetables in Lebanon

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Abstract

Introduction: Fresh produce has been implicated in a number of documented outbreaks of foodborne illness caused by bacteria, viruses, and parasites. Shiga toxin-producing *Escherichia coli* (STEC) have been detected on vegetables, raising concerns about the prevalence of *E. coli* contamination in produce, which can take place at various points from farm to fork. This study aimed to detect the presence of STEC and multidrug-resistant (MDR) *E. coli* on fresh vegetables and water from different sources along the fresh produce supply chain in Lebanon.

Methodology: *E. coli* isolates (n = 60) were group serotyped using trivalent antisera (trivalent 1 [O111+O55+O26], trivalent 2 [O86+O119+O127], trivalent 3 [O125; O126; O128], and trivalent 4 [O114+O124+O142]) and tested for *stx1* and *stx2* genes by polymerase chain reaction (PCR) assay. Resistance to antimicrobial agents was determined using the disk diffusion method.

Results: The virulence genes *stx1* and *stx2* were not detected in any of the isolates. However, 60% of the isolates were MDR and predominantly observed in trivalent 2 (32%). It is postulated that the inadequate post-harvest washing contributed to transmission of antimicrobial-resistant *E. coli* at wholesale and retail levels. Fresh vegetables harbor MDR *E. coli* and their consumption poses risks of increasing the reservoir of antimicrobial resistance in the intestines of the Lebanese population.

Conclusions: Greater emphasis should be placed on vigilant sanitation measures at the consumption level, and effective national risk mitigation strategies are crucial to minimize fecal contamination in the early stages of production, particularly in the post-harvest washing processes.

Key words: *Escherichia coli*; Shiga toxins; antimicrobial resistance; multi-drug resistance; serogroup; raw vegetables.

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Introduction

Foodborne outbreaks have been linked to pathogen contamination of raw vegetables and leafy greens over different stages along the farm-to-fork continuum, including enterohemorrhagic *Escherichia coli* (EHEC) among the causative agents [1]. According to Painter *et al*. [2], leafy greens are the commodity most frequently implicated in foodborne illness in the United States. Moreover, emerging hazards in the food chain now include EHEC infections caused by virulent non-O157 serotypes, commonly including O26:H11, O103:H2, O111:NM, and O113:H21 [3] and Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 implicated in the major outbreak in Germany in 2011 [4], confirming the importance of investigating STEC presence in fresh vegetables. STEC is an important group of zoonotic human diarrheal pathogens and is associated with hemorrhagic colitis and hemolytic uremic syndrome. STEC strains are able to adhere to the epithelial cell of the gastrointestinal tract and cause bloody diarrhea [5] and their pathogenicity is mainly attributed to the production of Shiga toxins encoded by *stx1* and *stx2* genes, which exist in several variants [6]. More concerning is that these microorganisms have been isolated from bovine feces and cattle, suggesting a reservoir and a vector of STEC in the vegetable production environment [7,8]. In addition to prevalence concerns, the global increase of antibiotic resistance in Gram-negative bacteria inflicts critical challenges to healthcare systems to treat infection by multidrug-resistant bacteria such as *E. coli* O157:H7 with conventional antibiotics, opening the need to investigate new therapeutic approaches [9].

The prevalence of antimicrobial-resistant *E. coli* and potential transmission from soils and in the animal production environment to fresh produce at harvest has been documented [10], as has the further flow of resistance from fresh produce bacteria via gene transfer to enterobacterial strains in humans [11]. However, few studies have investigated the variations of

antimicrobial-resistant *E. coli* strains across the fresh vegetable supply chain from farms to consumers.

The most common route for fresh produce contamination is at the pre-harvest stage, when microorganisms from animal feces, contaminated irrigation water, and wild and domestic animals can be deposited in crops [12]. In Lebanon, most of the water resources are polluted by untreated sewage, domestic and industrial solid waste, and effluents [13,14]. An example of this are the high levels of chemical and bacterial contamination in the Litani river, which irrigates one of Lebanon's most extensive farming area, the Bekaa Valley [15]. As a result of irrigation with untreated wastewater or improper composting of manure, Halablab *et al*. [16] demonstrated that the health risks of leafy green and other vegetables from several regions of the Bekaa Valley typically eaten raw in the Lebanese diet pose a high risk to consumers' health. Considering this situation, further insight into the impact of farming and post-harvest practices along the food chain on the microbiological quality of fresh leafy vegetables was obtained in a separate study by one of the authors [17]. There, the prevalence rate of *E. coli* > 2 log cfu/g was 32%, and mean indicator levels ranged from < 0.7 to 7 log CFU/g, whereas coliforms levels ranged from 1.7 log CFU/g to 8.2 log cfu/g and *Salmonella* was detected in one sample (lettuce) obtained from a washing facility. Even though foodborne disease outbreaks due to contamination of fruit and leafy green vegetables with pathogens have been rarely documented in Lebanon or the whole Middle East Region, they undoubtedly occur based on surveillance data from other regions [2].

Therefore, the aim of this study was to complement earlier work by investigating the presence of the virulence factors (*stx1*, *stx2*), the prevalent serogroup, and the resistance phenotypes of *E. coli* isolates from ready-to-eat salad vegetables from restaurants and fresh produce traced from farms in the Bekaa Valley of Lebanon to the wholesale market, the major supply hub for the Beirut district.

Methodology

Sources of E. coli isolates

A total of 60 *E. coli* isolates obtained from the above-mentioned field work were included in this study. They were originally isolated from water samples collected from crop washing ponds and from salad vegetables and fresh produce sourced from small and medium food service establishments and from farms and wholesale market stalls, respectively. The provisions for sampling ensured selection of water and vegetables from locations and market stalls traced back to their origin, *i.e.*, farms in Bekaa Valley.

The source and origin of isolated *E. coli* are shown in Table 1.

Produce sampling and source features

Samples of fresh produce were sourced from 10 major horticulture farms on the Bekaa Valley, the most extensive farming and cultivated region in Lebanon, widely known as the area most affected by water pollution [13], and from crop-washing facilities in close proximity to the polluted source of water used for irrigation and washing ponds, the Litani and Berdawni rivers. Target commodities included leafy greens and radishes.

The sampling of salad vegetables (n = 90) was part of a separate study [17]. Briefly, fresh produce samples were collected in July–August 2013 and in July 2014. The sampling of each type was done from different points of the same field, and the samples were placed in sterile bags. Water samples were collected in 250 mL portions from different points of the crop-washing ponds or in 1-liter bulk from the wells in polystyrene sterile bottles. Samples were placed in insulated coolers

Table 1. Distribution of *E. coli* isolated from agri-food environment in Lebanon, by source and origin.

Source (Location, sample type)	Label[1]	N
Restaurants		
Salad vegetables	R-SV	28
Knife & board swabs	R-KB	4
Wholesale market		
Fresh produce	WM	14
Farm land		
Fresh produce	FL	7
Crop washing area		
Water	CW-W	4
Fresh produce	CW-FP	3
Total		**60**

[1] The labels indicating source and sample type are used for all following tables.

with ice packs and transported to the laboratory the same day.

The sampling locations fell short of good agricultural practices; sanitation and control measures to minimize risk of contamination of agricultural water and soil from other farms or animal operations were deficient. In some cases, the farming fields were located approximately 2 km away from landfills and chicken farms. Sprinkle irrigation was a general applied mode of water irrigation on farms; in the summer season, when water sources declined, raw sewage was an alternative source of irrigation in two of the surveyed farms, while other farmers used private wells. Water from the river is usually pumped into crop-washing ponds where parsley and radish are dip-washed, while lettuces were spray-washed in open crates whilst stacked in trucks prior to being distributed to the wholesale market. Generally, operations took place in unprotected areas where fresh produce was kept in plastic baskets appearing unwashed, exposed until the next consignment.

Sampling took place also in food service establishments (n = 50) where risks of cross-contamination were recorded [18]. A total of 118 samples of of salad vegetables (cucumber, parsley, tomato, mint, and lettuce) were sampled after washing and preparation, *i.e.*, chopping for display or serving. Samples were placed in sterile bags and precautious were taken to avoid touching the inner surface. Later, they were coded, dated, and transported to the food microbiology laboratory at the American University of Beirut in a cooled box.

E. coli isolation, biochemical tests, and serotyping

Isolation and identification of *E. coli* was performed after aseptically homogenizing 10 g of samples in 90 mL of buffered peptone water (Oxoid, Basingstoke, UK) followed by serial dilutions. An aliquot of 1 mL of the homogenate was used for the pour plating technique with selective agar, RAPID'*E. coli* 2 (Bio-Rad Laboratories, Hemel Hempstead, UK), while selective enrichment was used to test for the presence of *E. coli* in water samples with the filtration method following EN ISO 9308-1:2000. Samples were incubated for 24–48 hours at 37°C. *E. coli* isolates produce purple colonies that can be easily distinguished from the blue colonies of other coliforms. From each positive sample, one to three suspected *E. coli* isolates were used for confirmation with API 20E strips (bioMérieux, Marcy l'Etoile, France).

Isolates biochemically identified as *E. coli* were group serotyped with the slide agglutination test using four different trivalent O antisera (trivalent 1, trivalent 2, trivalent 3, and trivalent 4) (Bio-Rad Laboratories Ltd, Marnes-la-Coquette, France) following the manufacturers' instructions.

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates in according to the Clinical and Laboratory Standards Institute's guidelines [19] using paper rings with eight antibiotic disks (M14 Mastring-S, Mast Group, Bootle, UK). Briefly, an overnight *E. coli* isolate culture was adjusted to McFarland 0.5 opacity standard before the inoculation was spread onto Mueller-Hinton agar plates (Oxoid, Thermo Scientific, Basingstoke, UK). After 3–5 minutes, rings were applied to the plates and incubated at 37°C for 18–24 hours. The diameter of the inhibition zones was measured. The strains were tested for their resistance to the following antimicrobials (µg per disk): ampicillin (10), cephalothin (5), colistin sulphate (25), gentamicin (10), streptomycin (10), sulphatriad (200), tetracycline (25), and cotrimoxazole (25). Isolates that were resistant to to at least one agent in three or more antimicrobials were considered to be multidrug resistant (MDR) [20]. For straightforward result interpretations, intermediate resistant isolates were counted as resistant.

Genomic DNA extraction

DNA was extracted from samples using the InstaGene matrix (Bio-Rad Laboratories, Hercules, USA) according to the manufacturer's protocol. Briefly, each strain was suspended in an Eppendorf tube containing 200 µL InstaGene matrix. Suspensions were heated at 56°C for 30 minutes and vortex mixed at high speed for 10 seconds before being heated in a block at 100°C for 8 minutes and then centrifuged at 12,000 rpm for 3 minutes. The supernatant was stored at -20°C for later use as DNA.

Polymerase chain reaction (PCR) targeting STEC stx1 and stx2 genes

Genes encoding for selected virulence factors, *stx1* and *stx2*, were detected with PCR based on the work of Jinneman *et al*. [21]. A 25 µL volume of a PCR mix (Fermentas, Thermoscientific, Glen Burnie, MD, USA) containing 2.5 µL 10x Taq DNA polymerase buffer with $(NH4)_2SO_4$, 2.5 µL 2 mM dNTPs, 2 µL 25 mM MgCl2, 0.125 µL 5 U/µL Taq DNA polymerase, on 14.175 µL nuclease-free water (Amresco, Solon, USA), 2.5 µL extracted DNA, 0.6 µL of each primer

(Fermentas, Thermoscientific, Glen Burnie, MD, USA), *stx1* (forward 5'-GTG GCA TTA ATA CTG AAT TGT CAT CA-3'and reverse 5'-GCG TAA TCC CAC GGA CTC TTC-3'), and 0.6 µL of *stx2* primers (forward 5'-GAT GTT TAT GGC GGT TTT ATT TGC-3' and reverse 5'-TGG AAA ACT CAA TTT TAC CTT TAG CA-3') was prepared. The thermocycler program consisted of an initial denaturation at 95°C for 10 minutes followed by 35 cycles of 95°C for 40 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, and a final extension of 72°C for 10 minutes. PCR was performed in a C1000 thermal cycler (Bio-Rad Laboratories Munich, Germany).

The PCR products were separated in a 1.5% agarose gel (Invitrogen, Carlsbad, CA, USA) using electrophoresis at 100V for 45 minutes in Tris-Borate-EDTA buffer (TBE), with a 50 bp DNA ladder used as a standard. The gels were stained using a 0.5 mg/mL ethidium bromide solution (Bio-Rad Laboratories, Hercules, USA) and visualized in an UV transilluminator (Universal Hood II, Bio-Rad Laboratories, Hercules, USA).

Statistical analysis

Statistical analysis was carried out using SPSS version 21 software. The results were analyzed using descriptive statistics, and Chi-square and Fisher's exact tests were applied for testing associations between isolates' resistograms and sources. The homogeneity of variances was assessed using Levene's test. When normal assumption and equal variance were not tenable, the non-parametric Kruskal-Wallis test was used for group comparisons instead of one-way analysis of variance (ANOVA).

Results

Results presented in Table 2 confirm the trivalent group matches of all *E. coli* isolates. The frequency of prevalence was almost identical across the four trivalent serogroups, with a slightly higher presence of trivalent 2 (O86+O119+O127) (32%) isolates. Chi-square analysis showed no significant relationship between the isolate serogroups and the sample sources, possibly due to a variety of environmental factors and to sample size.

The virulence genes *stx1* and *stx2* were not detected in any of the isolates; nevertheless, there was a varied antimicrobial resistance profile of the *E. coli* (Table 3). In general, resistance to streptomycin and a first-generation cephalosporin (represented by cephalothin) was observed among all the 60 isolates (100%). The second-largest prevalence of resistant isolates was observed with ampicillin (78%). More than a third of the study isolates were resistant to tetracycline (42%), whereas the lowest prevalence was recorded for

Table 2. Distribution of *E. coli* isolated from agri-food environment in Lebanon, and by serotype groups.

		E. coli isolates in trivalent groups, N (%)[1]			
Location-Sample type	**N**	**Trivalent 1**	**Trivalent 2**	**Trivalent 3**	**Trivalent 4**
R-SV	28	6 (54.5)	9 (47.3)	6 (46.2)	7 (41.2)
R-KB	4	0 (0.0)	0 (0.0)	1 (7.7)	3 (17.6)
WM	14	2 (18.2)	4 (21.0)	2 (15.4)	6 (35.2)
FL	7	1 (9.1)	4 (21.0)	2 (15.4)	0 (0.0)
CW-FP	3	0 (0.0)	1 (5.3)	1 (7.7)	1 (5.8)
CW-W	4	2 (18.2)	1 (5.3)	1 (7.7)	0 (0.0)
Total count (%)[2]	**60**	**11 (18%)**	**19 (32%)**	**13 (22%)**	**17 (28%)**

[1] Percentage of isolates in each trivalent group; [2] Percentage from the total number of isolates (n = 60).

Table 3. Antimicrobial resistance profile of *E. coli* isolates from different sources of the fresh produce supply chain.

	N (%[1]) of resistant isolates from						
	CW-W (N = 4)	**R-SV (N = 28)**	**R-KB (N = 4)**	**WM (N = 14)**	**FL (N = 7)**	**CW-FP (N = 3)**	**Total N (%)[2]**
TS (25)	2 (9.1)	16 (72.7)	0 (0.0)	3 (13.6)	0 (0.0)	1 (4.5)	22 (36.7)
AP (10)	4 (8.5)	19 (40.4)	3 (6.38)	12 (25.5)	6 (12.8)	3 (6.4)	47 (78.3)
KF (5)	4 (6.7)	28 (46.7)	4 (6.7)	14 (23.3)	7 (11.7)	3 (5.0)	60 (100)
CO (25)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (3.3)
GM (10)	0 (0.0)	9 (69.0)	0 (0.0)	3 (23.0)	0 (0.0)	1 (7.7)	13 (22.0)
S (10)	4 (6.7)	28 (46.7)	4 (6.7)	14 (23.3)	7 (11.7)	3 (5.0)	60 (100)
ST (200)	1 (7.1)	4 (28.6)	2 (14.3)	6 (42.9)	1 (7.1)	0 (0.0)	14 (23.3)
T (25)	2 (8.0)	20 (80.0)	0 (0.0)	2 (8.0)	0 (0.0)	1 (4.0)	25 (41.7)

TS: cotrimoxazole; AP: ampicillin; KF: cephalothin; CO: colistin sulphate; GM: gentamicin; S: streptomycin; ST: sulphatriad; T: tetracycline; [1] Percentage from the total number of the resistant isolates for each antimicrobial; [2] Percentage from the total number of isolates (n = 60).

Table 4. Distribution of multidrug-resistant (MDR) *E. coli* isolated from different points on the fresh salad and vegetable supply chain.

Source	Label		*E. coli* isolates, count (%)	
(Location, sample type)		**N**	**MDR**	**Non-MDR**
Restaurants				
Salad vegetables	R-SV	28	22 (75)	6 (25)
Knife & board swabs	R-KB	4	2 (25)	2 (75)
Wholesale market	WM	14	6 (43)	8 (57)
Farm land	FL	7	2 (14)	5 (86)
Crop washing area				
Water	CW-W	4	2 (75)	2 (25)
Fresh produce	CW-FP	3	2 (67)	1 (33)
Total N (%)		**60**	**36 (60)**	**24 (40)**

gentamicin (22%) and colistin sulphate (3%). The obtained frequency of resistance to cotrimoxazole and sulphatriad were 36.7% and 23.3%, respectively, whereas cephalothin was not effective against all isolates. The present study found resistance to three or more antimicrobials in 60% of the isolates (Table 4). More than two-thirds of MDR *E. coli* (69%) showed resistance to ampicillin and tetracycline (Table 5), while a majority exhibited resistance to streptomycin and cephalothin (100%).

Concurrent resistance to cotrimoxazole, cephalothin, streptomycin, ampicillin, tetracycline was the most common co-resistance phenotype (16.6%), followed by resistance to tetracycline, streptomycin, cephalothin, cotrimoxazole (13.8%) and to ampicillin, cephalothin, streptomycin, sulphatriad as well as cotrimoxazole, ampicillin, cephalothin, gentamicin, streptomycin, tetracycline (11.1%), in each (Table 6).

Table 5. Multidrug resistance (MDR) prevalence[1] among *E. coli* isolates from agri-food environments tested against a range of clinically significant antimicrobials.

Antimicrobials (µg/disk)	MDR *E. coli* (n = 36)	
	Count	**%**
TS (25)	22	61
AP (10)	25	69
KF (5)	36	100
GM (10)	11	30
ST (200)	14	38
CO (25)	2	6
S (10)	36	100
T (25)	25	69

TS: cotrimoxazole; AP: ampicillin; KF: cephalothin; GM: gentamicin; ST: sulphatriad; CO: colistin sulphate; S: streptomycin; T: tetracycline;
[1] Percentage from the total number of MDR *E. coli* (n = 36).

Table 6. Co-resistance patterns of multidrug-resistant (MDR) *E. coli*

Co-resistance pattern	Total N (%)[1]
AP, KF, S	1 (2.7)
KF, S, ST	2 (5.5)
AP, KF, S, ST	4 (11.1)
AP, KF, GM, S	3 (8.3)
AP, KF, S, T	1 (2.7)
TS, KF, S, T	5 (13.8)
KF, S, ST, T	2 (5.5)
AP, KF, GM, S, T	1 (2.7)
TS, AP, KF, S, ST	1 (2.7)
TS, KF, S, ST, T	2 (5.5)
TS, AP, KF, S, T	6 (16.6)
TS, AP, KF, GEN, S, T	4 (11.1)
TS, AP, KF, S, ST, T	1 (2.7)
All except ST	1 (2.7)
All except CO	1 (2.7)
All antimicrobials	1 (2.7)

TS: cotrimoxazole; AP: ampicillin; KF: cephalothin; GM: gentamicin; ST: sulphatriad; CO: colistin sulphate; S: streptomycin; T: tetracycline;
[1] Percentage from the total number of MDR *E. coli* (n = 36).

Discussion

Serotyping

E. coli isolates belonging to the 12 O serogroups O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 are categorized as enteropathogenic *E. coli* (EPEC) strains by the World Health Organization (WHO) [22] and have been demonstrated to be frequently associated with Shiga toxin (verocytotoxin) production [23]. From this information, although we expected that some of our isolates could be toxin producers, in fact, none of the isolates tested in the current study were positive for the presence for *stx* genes. These non-O157 pathogenic EPEC on raw vegetables have been detected in few studies. Mazaheri *et al*. [24] reported a prevalence rate of 4% in lettuce in Iran, while in the Czech Republic, enteropathogenic *E.coli* were suspected in 11.1% of the isolates from imported vegetables that were found to have *eae* gene [25]. A variety of animals, such as cattle, goats, sheep, chickens, and pigeons are known to be

vehicles of EPEC strains [26]; these, as well as human sources, could likely be the origin of the serogroups we found in our samples.

Interestingly, our results were comparable to reported prevalence of EPEC in dairy products where O111, O86, O125, O119, and O111, O126, O128, O26, O25, O125 were commonly isolated in Iraq [27] and Egypt [28], respectively. Such observations indicate the likelihood of the agricultural production system as a primary source of contamination of raw vegetables.

Little is known about the prevalence of EPEC in food, specifically in fresh vegetables in Lebanon and the region. EPEC strains have been identified as the main causative agents of foodborne illnesses and infantile diarrhea in developing countries [3]. In a recent annual surveillance report in Lebanon, *E. coli* was found in 34% and 25% of the clinical specimens collected in 2011 and 2012, respectively, of which 18% were classified in each of O111 and O125 [29]. While it is difficult to pinpoint specific contamination pathways, it could be assumed, based on our field survey, that the recorded unhygienic post-harvest practices, particularly washing with contaminated water, promote cross-contamination in addition to the improper transportation and handling practices after purchase, as has been reported elsewhere [30]. It is also likely that transfer of *E. coli* to raw vegetables from contaminated fields irrigated with polluted water occurs due to mishandling of manure, grazing animals, and flooding from adjacent contaminated cattle farms. Trabulsi *et al*. [22] demonstrated that serotyping of isolates of known *E. coli* pathogenic serogroups does not always correlate with the pathogenic factors, and that the possession of EPEC-related O and K antigens does not necessarily indicate that those strains would cause illness. Accordingly, it may be more appropriate to conduct supplementary studies to define the *E. coli* isolates as EPEC based on their characteristic virulence determinants and adhesive properties. We consider the isolates in our study to be indicators of pathogenic EPEC that could easily be present in future produce samples.

Detection of stx1 and stx2 genes

The *stx1* and *stx2* genes encoding Shiga toxins were not detected in the 60 study isolates. In their work, Osterblad *et al*. [31] did not detect *stx1* or *stx2* genes in *E. coli* isolated from raw vegetables. However, the potential risk that raw vegetables can pose to consumers could be manifested in the detection of other possibly existing virulent factors. In Lebanon, out of 145 produce items, 6 were positive to *stx1* and to *stx2*, and two were found positive to *eaeA* [32]. Previous research indicated that the Shiga toxin genes and the adhesive factor *EAF* genes were found in a limited range of EPEC strains [33]. Hence, looking for Shiga toxin genes in a number of isolates would not reflect the pathogenic risks empirically. The investigation for the prevalence of EPEC and the *eaeA*, *bfpA*, and *EAF* DNA sequences remains of great relevance but could not be addressed in this study due to limited research funding.

Resistance to antimicrobials

The antimicrobial resistance of *E. coli* is increasingly becoming a public health concern in developing and developed countries, resulting from antibiotic selection pressure due to wide application of antibiotics on farms, a practice considered as the most important factor that contributes to the emergence of antimicrobial-resistant microorganisms in the environment. The occurrence of antibiotic-resistant enterobacteria in raw vegetables is increasingly gaining the attention of researchers [10]. However, studies on the occurrence of resistant bacteria, particularly in fresh vegetables in developing countries and mainly in our region are still very limited and lag behind the increasing pace of its threat. The resistance rate of isolates to ampicillin (78%) was similar to results reported by Hassan *et al*. [34] (76.5%) on *E. coli* isolated from fresh vegetables in Saudi Arabia. The obtained frequency of resistance to cotrimoxazole and sulphatriad was interestingly comparable to regional studies on *E. coli* isolates from fresh vegetables in Saudi Arabia and from leafy greens in Jordan [35]. It is widely recognized that tetracyclines are the most frequently used antibiotics on chicken farms, and sulfonamide is among the most commonly used antibiotic in animal production systems. The resistance trend to sulfonamide and tetracycline among *E. coli* from cattle and chicken has been documented, and cattle were suggested as potential harbors of antimicrobial-resistant *E. coli* strains [10].

Cephalothin was not effective against all isolates in this study; however, it is recognized to be the least effective as a first-generation antibiotic, and comparison to other generations is not valid. Yet, it is worth noting that the high prevalence level of resistant *E. coli* to more effective cephalosporins (third-generation antibiotics) in the Near East Mediterranean region compared to the European region is well documented. Such prevalence was remarkably higher in Pakistan (62%–94%) and Morocco (78%), followed by Oman (68%) [36]; this is assumed to be due to applications of higher hygienic standards, stricter

policies and state regulation over farming practices, antibiotic use, and water quality in developed countries. Locally, it was also suggested that antibiotic use in the Bekaa is probably higher than in other agricultural regions, which in turn contributed to a notably high level of resistant *Yersinia enterocolitica* isolated from dairy products in Bekaa Valley [37]. Although data of this study might not be sufficient to verify the precise causative factors for the overall high resistance to cephalothin and streptomycin, it is likely that the high use of antibiotics plays a critical role and provides an explanation for the results found in this work. Regionally, our findings were consistent with the resistance rate of MDR *E. coli* to cephalosporins (70%) in Saudi Arabia [34] and to the first-generation cephalosporin cefazoline (81.4%), in Iran [38]. It was suggested that food-producing animals are carriers of extended-spectrum beta-lactamases (ESBLs). ESBL-producing *E. coli* can be transmitted into the environment, and indiscriminate use of antibiotics during animal husbandry contributes to the emergence of resistant strains in the agri-environment.

Overall, a lower resistance was recorded to gentamicin (22%), which is in line with various studies [10,39], highlighting the promising treatment of *E. coli* by gentamicin as demonstrated by Fadlallah *et al*. [40].

Chi-square analysis showed a significant association between the antimicrobial resistance profile of *E. coli* for tetracycline, cotrimoxazole, ampicillin, sulphatriad, and the sampling sources ($p < 0.05$). The highest frequency of resistance to former drugs was particularly observed in isolates obtained from the restaurant group. As for sulphatriad, the highest rate of resistance was shown in the wholesale market isolates (Table 3).

Multiple drug resistance

The multi-resistance phenotypes of bacteria recovered from raw vegetables have been documented in different countries [31]. The prevalence rate of MDR isolates recorded in this study is higher compared to the rate detected in different regions of Lebanon (23%), namely in the south and the north [32], and to a recent study in Jordan (28%) [35]. On the other hand, a comparable level of MDR *E. coli* to cephalosporins was reported by Bezanson *et al*. [11].

Researchers substantiated that plasmid-borne sulfonamide resistance genes play a role in mediating multiple antimicrobial drug resistance genes and that streptomycin and ampicillin are the most common co-transferred resistance phenotypes among sulfonamide-resistant *E. coli* [41]. Of major concern is the largest proportion of MDR isolates detected in restaurants and in water and fresh produce samples from the washing facilities (Table 4). Presumably, the washing processes should have produced a lower prevalence rate. However, our results contrasted with those of Schwaiger *et al*. [42], who showed a higher resistance rate of bacteria from farms than from the retail markets. Comparisons might not be relevant in our case given the recorded deficits in risk reduction practices on farms and improper post-harvest washing practices. In fact, fresh produce was washed in ponds filled with unsafe and turbid water and crop areas were adjacent to landfills or chicken farms, lacking sewage treatment plants and sanitary infrastructure. Cultivated areas were accessible to grazing and domestic animals, irrigated in some cases with untreated sewage. More concerning, in Lebanon, 60%–70% of the natural sources of water are affected by bacterial contamination and untreated effluents of industries, chicken farms, and slaughterhouses that leach to the sea and into rivers used as water sources for irrigation and for crop washing [15]. Consequently, antimicrobial-resistant bacteria might have been transmitted to fresh produce during the use of water polluted with human and/or animal feces by sewage discharges or run-off from manure. However, further investigations are needed to validate our assertion and identify the source along the chain.

Conclusions

This study provides valuable information as baseline data on the prevalence of MDR *E. coli* and the common serotype groups found in raw salad vegetables in Lebanon. Despite the high levels of *E. coli*, no STEC strains were detected in the study isolates, but the isolates indicate the potential for pathogens. The results demonstrated that raw vegetables present a great risk to public health as mediators for the dissemination of antimicrobial-resistant bacteria from the environment to pathogens and commensal gut microflora in humans subsequent to ingestion [11]. The high prevalence of MDR *E. coli* to antibiotics commonly used in intensive animal production indicates a likelihood of excessive use of antibiotic on farms and presents a risk to human health. Our data suggest that the agricultural system is the primary reservoir of the antimicrobial-resistant bacteria transmitted to fresh produce and indicate the plausibility of the inadequate post-harvest washing practices, among other discussed possibilities, as a potential source of cross-contamination and antimicrobial resistant isolates observed at wholesale and consumer levels. In view of our findings, great emphasis should be placed on developing strict policies

to monitor and control application of polluted water in growing areas, and most importantly in the post-harvest washing stage. Equally important, vigilant sanitary measures for raw vegetables, particularly when not subjected to further treatment, are essentials to mitigate the risk of contamination and transmission of MDR *E. coli* to humans.

This study was conducted with limited research funding. Hence, we restricted our methodology to target only *stx1* and *stx2* genes and to the use of trivalent serotyping versus individual O and K sera or other typing methods such as pulsed-field gel electrophoresis for further characterization of isolates. Yet, this study is intended to direct supplementary research work at a larger scale towards the analysis of the risks associated with the consumption of raw vegetables in Lebanon by investigating the adhesive properties of the isolates and the characterization of the genetic background of the resistance.

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