Virulence Genes and Phenotypic Evaluation of the Antibiotic Resistance of Vero Toxin Producing *Escherichia coli* Recovered From Milk, Meat, and Vegetables

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Abstract

Background: Resistant and virulent strains of Shiga toxin producing *Escherichia coli* (STEC) are one of the main prevalence sources of food infection due to consumption of meat, milk, and vegetables.

Objectives: This study was conducted to assess the frequency of virulence factors and phenotypes of antibiotic- resistant STEC bacteria recovered from meat, milk, and vegetable samples.

Methods: In this study, 280 samples were assessed. *Escherichia coli* isolates were assessed by PCR and disk diffusion.

Results: Out of 280 samples, 59 (21.07%) were contaminated with *E. coli*. Vegetables had the highest prevalence (31.25%), while raw meat (14%) had the lowest. *Stx1*, *eae*, and *ehly* genes were found in all enterohemorrhagic *Escherichia coli* (EHEC) bacteria. Moreover, 21 out of 59 *E. coli* strains were STEC (35.59%). Shiga toxin producing *E. coli* strains showed the most resistance against ampicillin (100%), gentamycin (90.47%), tetracycline (85.71%), and ciprofloxacin (71.42%). Shiga toxin producing *E. coli* strains harbored resistance to at least 2 antibiotics (100%).

Conclusions: Judicious prescription of imipenem and cotrimoxazole based on the results of disk diffusion and also proper washing of the vegetables, cooking of the meat, and boiling of the milk before consumption can control and reduce the risk of incidence of food poisoning due to the virulent and resistant STEC strains.

Keywords: Shiga Toxin, Antibiotic Resistance, Meat, Milk, Vegetable, *Escherichia coli*

1. Background

Access to hygienic foods is an important factor in ensuring health. Unconfident food containing pathogenic agents cause more than 300 diseases worldwide (1, 2). Foodborne diseases root around 80 million sicknesses, 330,000 hospitalizations, and 6000 deaths in the United States annually (3, 4). *Escherichia coli* is a member of the huge family of Enterobacteriaceae and mostly causes food poisoning. Shiga (vero) toxin (Stx)-producing *E. coli* (STEC) is a sector of a substantial virulent group of *E. coli* entitled, enterohemorrhagic *E. coli* (EHEC) (4-6). Shiga toxin producing *E. coli* bacteria are causative agents for concentrated diseases, such as diarrhea (bloody and non-bloody), hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC), and thrombotic thrombocytopenic purpura (TTP) (4-6).

Shiga toxin producing *E. coli* strains harbored several types of virulence factors and especially Shiga toxins (*stx1* and *stx2*), intimin (*eaeA*), and hemolysin (*hlyA*). These genes are causative factors of settlement, adhesion, and attack of STEC bacteria to gastrointestinal mucosa (4-6). Shiga toxin producing *E. coli* bacteria represents an extensive prevalence of resistance to numerous clusters of antibiotics, especially quinolone, fluoroquinolone, cephalosporin, macrolide, aminoglycoside, sulfonamide, and tetracycline (4-12).

2. Objectives

Considering the indeterminate impact of STEC bacteria in milk, meat, and vegetable samples, this study aimed at assessing the frequency of virulence genes and phenotypic characterization of antibiotic- resistant STEC bacteria recovered from raw meat, milk, and vegetable samples in Iran.

3. Methods

3.1. Ethical Considerations

This study was approved by the ethical commission of enquiry adjutancy of the Islamic Azad University, Karaj Branch, Karaj, Iran (Agreement Ref Number 95-20208). This enquiry and the certificates associated with sampling were approved by Professor Afshin Akhondzadeh Basti and Dr. Valiaollah Koohdar (Endorsement Ref Number Vet-95-33711).

3.2. Samples Collection

From March to May 2015, a total of 280 samples including raw meat (n = 100), raw milk (n = 100), and vegetable (n = 80) samples were collected from the supermarkets and butcheries of Tehran province, Iran. All samples were directly moved to the laboratory using ice packs. All food samples showed normal physical properties.

3.3. Escherichia coli Isolation

A total of 10 grams of samples were standardized for 2 minutes in 90 mL of peptone water (Merck, Germany). *Escherichia coli* isolation and identification were done using the method described previously by Dehkordi et al. (2014) (6).

3.4. PCR Approval of Escherichia coli Strains

Escherichia coli isolates were approved again using polymerase chain reaction (PCR), according to the technique labelled by Woo et al. (2001) (13).

3.5. PCR Amplification of STEC Virulence Genes

Table 1 demonstrates the sequence of primers and PCR programs applied for identifying virulence factors (6, 14, 15). Eppendorf Flexrcycler2 (Eppendorf, Germany) was also applied. Electrophoresis was done using agarose gel (1.5%) and 1X TBE buffer strained using SYBR Green (Fermentas, Germany). Electrophoresis was done at 80 - 90 V for at least 30 minutes. Positive DNA samples taken from our previous works were applied as positive controls.

3.6. Antimicrobial Analysis

Susceptibility of STEC bacteria was assessed according to the principles of Clinical and Laboratory Standards Institute guidelines (16). Tetracycline (30 u/disk), ampicillin (10 u/disk), cefotaxime (30 μg/disk), gentamycin (10 μg/disk), ciprofloxacin (5 μg/disk), amikacin (30 u/disk), imipenem (30 u/disk), cotrimoxazole (30 μg/disk), enrofloxacin (5 μg/disk), sulfamethoxazole (25 μg/disk), trimethoprim (5 μg/disk), streptomycin (10 μg/disk), and chloramphenicol (30 μg/disk) antibiotic agents (Oxoid, UK) were used for this purpose.

3.7. Statistical Analysis

Chi-square test and SPSS/16.0 software were used for statistical analysis. Statistical significance was set at < 0.05.

4. Results

Table 2 characterizes the prevalence of *E. coli* in numerous kinds of samples. In this study, 59 out of 280 (21.07%) food samples were contaminated with *E. coli* strains. Vegetables (31.25%) had the highest prevalence of *E. coli*, while raw meat (14%) had the lowest. Statistically significant differences were observed in the frequency of *E. coli* in numerous samples (P < 0.05). Table 3 demonstrates the frequency of virulence genes in *E. coli* subtypes. Frequency of AEEC and EHEC cluster in the raw meat, raw milk, and vegetable samples was 25% and 37.50%, 16.66% and 33.33%, and 33.33% and 22.22%, respectively. Positive EHEC bacteria for *stx1*, *eae*, and *ehly* genes were determined as O157 serogroup.
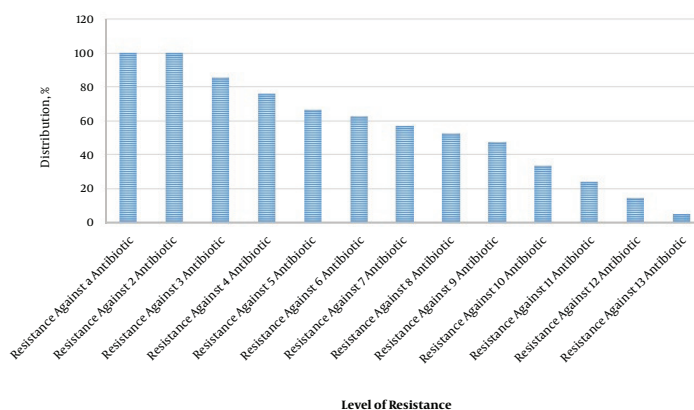
Table 4 presents the antibiotic resistance pattern of the STEC bacteria recovered from numerous types of samples. STEC bacteria revealed the vastest prevalence of resistance against ampicillin (100%), gentamycin (90.47%), tetracycline (85.71%), and ciprofloxacin (71.42%). Statistically substantial differences were observed in the incidence of resistance among different types of samples (P < 0.05). Figure 1 demonstrates the occurrence of multidrug resistance in the STEC bacteria. Shiga toxin producing *E. coli* bacteria were resistant to at least 2 antibiotics (100%), however, the incidence of resistance against 10, 11, 12, and 13 antibiotics was 33.33%, 23.80%, 14.28%, and 4.76%, respectively.

5. Discussion

Results of the present study revealed that virulent STEC strains of meat, milk, and vegetable samples had a considerable prevalence rate (21.07%) and high levels of antibiotic resistance against more than one antibiotic used mainly for treatment and control of food poisoning. A conceivable description for the higher frequency of STEC strains in vegetables is due to the close contact of vegetables with polluted soil, water, and animal and/or human-based mature. Another reason for this finding may be the structure of vegetables, which can facilitate the survival of bacteria. Therefore, bacteria can easily grow and increase in vegetables away from any disturbing factors such as sunlight, acidic PH, high and low temperature, low moisture, activated water, and even presence of competitor flora. Previous investigations showed that vegetables, especially lettuce, are the main reservoir of STEC strains (17-20).

Table 1. Primer Sequence and PCR Conditions Applied for Detection of the Virulence Genes of *Escherichia coli* Isolates of Food Samples

Target Gene	Primer Sequence (5' - 3')	PCR Product, bp	PCR Programs	PCR Volume, 50 μL
stx1	F: AAATCGCCATTCGTTGAC-TACTTCT	366	1 cycle: 95°C - 3 min	5 μL PCR buffer 10X; 2 mM Mgcl$_2$; 150 μM dNTP (Fermentas); 0.75 μM of each primers F and R; 1.5 U Taq DNA polymerase (Fermentas); 3 μL DNA template
	R: TGCCATTCTGGCAACTCGC-GATGCA		34 cycle: 94°C - 60 s	
stx2	F: CGATCGTCACTCACTG-GTTTCATCA	282	56 °C - 45 s	
	R: GGATATTCTCCCCACTCTGA-CACC			
eaeA	F: TGCGGCACAACAGGCGGCGA	629	72 °C -60 s	
	R: CGGTCGCCGCACCAGGATTC			
ehly	F: CAATGCAGATGCAGATACCG	432	1 cycle: 72°C -10 min	
	R: CAGAGATGTCGTTGCAGCAG			

Figure 1. Prevalence of multi-drug resistant STEC strains isolated from food samples

Table 2. Distribution of *Escherichia coli* in Studied Samples[a]

Types of Samples	No. Samples Collected	Positive Strains	PCR Confirmation
Raw meat	100	14 (14)	14 (14)
Raw milk	100	20 (20)	20 (20)
Vegetable	80	25 (31.25)	25 (31.25)
Total	280	59 (21.07)	59 (21.07)

[a]Values are expressed as No. (%).

The most important reasons for the higher prevalence of STEC strains in milk than meat is the fact that raw milk is mainly taken from the udder of the cows by workers' hands. Maintaining milk in cool temperature (below 4°C) for proper safety. STEC strains are one of the most important causes of mastitis in dairy cattle. Many cases of mas-titis are subclinical, and workers of milking halls are able to identify the subclinical mastitic milk. Therefore, sub-clinical mastitic milk samples will be collected instead of healthy milk. Momtaz et al. (2012) found that out of 268 milk samples, 73 (27.23%) were positive for *E. coli*, which was similar to our finding. They showed that raw milk had a high potential for survival of STEC strains (5, 21). We also found that the type of sample is a determining factor in statistical analysis of data collected from each part of the study. On the other hand, we observed a statistically significant difference in the frequency of *E. coli* and numerous samples (P < 0.05) and also in the incidence of antibiotic resistance among different types of samples (P < 0.05). Additionally, differences in the prevalence of *E. coli* were significant among milk, meat, and vegetable samples. Similarly, there were differences in the prevalence of antibiotic resistance among the *E. coli* strains recovered from milk,

Table 3. Frequency of Virulence Genes Amongst the *Escherichia coli* Isolates of Studied Samples

Samples (No. Positive)	Subtypes	No. Positive Samples	Virulence Genes
Raw meat (14)	Non detected	3 (37.50)	-
	EHEC	2 (25)	*stx1*, *eae*, *ehly*: 2 (100)
	AEEC	3 (37.50)	*stx1*: 3 (100)
			stx2: 1 (33.33)
			eaeA: 2 (66.66)
			stx1, *eaeA*: 1 (33.33)
			stx2, *eaeA*: 1 (33.33)
			stx1, *stx2*, *eaeA*: 1 (33.33)
	Total	8 (57.14)	-
Raw milk (20)	Non detected	6 (50)	-
	EHEC	2 (16.66)	*stx1*, *eae*, *ehly*: 2 (100)
	AEEC	4 (33.33)	*stx1*: 4 (100)
			stx2: 2 (50)
			eaeA: 3 (75)
			stx1, *eaeA*: 2 (50)
			stx2, *eaeA*: 1 (25)
			stx1, *stx2*, *eaeA*: 1 (25)
	Total	12 (60)	-
Vegetable (25)	Non detected	8 (44.44)	-
	EHEC	6 (33.33)	*stx2*: 2 (50)
			eaeA: 3 (75)
			stx1, *eaeA*: 2 (50)
			stx2, *eaeA*: 1 (25)
			stx1, *stx2*, *eaeA*: 1 (25) *stx1*, *eae*, *ehly*: 6 (100)
	AEEC	4 (22.22)	*stx1*: 4 (100)
	Total	18 (72)	-

meat, and vegetable samples.

We found that 14% of raw meat samples were contaminated with *E. coli*. Possible explanations for this finding may be the transmission of *E. coli* from offal discharge, blood, floor of slaughter house, other contaminated carcasses, and also hands of meat inspectors and butchers during the process of slaughter in slaughterhouses and also transmission of bacteria during storage, transport, and maintenance of the meat. Momtaz et al. (2013) in-

dicated that 29.02% of various types of raw meat samples were contaminated with *E. coli*, which was higher than our findings (4). They showed that meat, especially bovine, ovine, and caprine samples, are reservoir of virulent and resistant STEC strains.

In this study, we also focused on the virulence gene profile of the STEC strains isolated from meat, milk, and vegetable samples. We found that stx1 and eaeA were the most commonly detected virulence factors. In addition, the number of strains, which harbored all these genes (*stx1*, *stx2*, *eaeA*, and *hly*) together, was high. High presence of *stx1*, *stx2*, *eaeA*, and *ehly* genes in subtypes guarantees their considerable pathogenicity. Concurrent attendance of different virulence genes in *E. coli* strains specified a significant public health issue. Concurrent occurrence of Shiga toxins and intimin has been described before (22-26). In a survey accompanied by Momtaz et al. (2012), the incidence of AEEC and EHEC clusters was 49.31% and 15.06%, respectively (21). Higher frequency of AEEC subtypes was described by numerous surveys (22-26). STEC bacteria exhibited severe prevalence of resistance to tetracycline, ampicillin, gentamycin, and ciprofloxacin. Also, the level of resistance against multiple antibiotics was very high in STEC strains in our study. High prevalence of resistance against human-based antimicrobial agents in tested food samples in our study has indirectly confirmed the transmission of STEC strains from the meat inspectors and butchers, staffs of the milking halls, and human manure to meat, milk, and vegetable samples.

Frequency of resistance to chloramphenicol in the STEC bacteria of our investigation was 9.52%. There were no positive results in raw meat and milk samples. Chloramphenicol is a forbidden antibiotic. The high presence of resistance against chloramphenicol displayed its lopsided and illegal use in veterinary and, particularly, poultry science. Using poultry-based manure for growth and reinforcement of agricultural soils used for planting vegetables is the main factor that facilitates the transmission of chloramphenicol-resistant strains of *E. coli* to the vegetables. Highly irregular prescription of antibiotics in medicine and veterinary fields causes high prevalence of antibiotics resistance. Therefore, antibiotic resistance will appear in a very short period of time. Comparable conclusions have been described by Momtaz et al. (2012), Momtaz et al. (2013), Dehkordi et al. (2014), Rasheed et al. (2014), Colello et al. (2015), and Miles et al. (2006) (5, 6, 21, 27-29).

Momtaz et al. (2013) indicated that the maximum resistance level of STEC bacteria of meat samples was to penicillin and that resistance against ciprofloxacin and nitrofurantoin was low (4). Momtaz et al. (2012a) showed that the STEC bacteria of raw milk samples displayed severe resistance to penicillin (100%) and tetracycline (57.44%),

Table 4. Antibiotic Resistance Pattern of STEC Strains Isolated from Studied Samples[a]

Types of Samples (No. STEC Strains)	Antibiotic Resistance Pattern												
	Tet30	**Am10**	**Cef30**	**Gen10**	**Cip5**	**Amk30**	**Imp30**	**Cot30**	**En5**	**Sul25**	**Tri5**	**S10**	**C30**
Raw meat (5)	4 (80)	5 (100)	2 (40)	5 (100)	4 (80)	3 (60)	-	2 (40)	4 (80)	3 (60)	3 (60)	2 (40)	-
Raw milk (6)	5 (83.33)	6 (100)	3 (50)	5 (83.33)	5 (83.33)	4 (66.66)	1 (16.66)	3 (50)	3 (50)	4 (66.66)	4 (66.66)	3 (50)	-
Vegetable (10)	9 (90)	10 (100)	4 (40)	9 (90)	6 (60)	4 (40)	1 (10)	3 (30)	6 (60)	7 (70)	5 (50)	4 (40)	2 (20)
Total (21)	18 (85.71)	21 (100)	9 (42.85)	19 (90.47)	15 (71.42)	11 (52.38)	2 (9.52)	8 (38.09)	13 (61.90)	14 (66.66)	12 (57.14)	9 (42.85)	2 (9.52)

Abbreviations: amk30, amikacin (30 u/disk); am10, ampicillin (10 u/disk); cef30, cefotaxime (30 μg/disk); c30, chloramphenicol (30 μg/disk); cip5, ciprofloxacin (5 μg/disk); cot30, cotrimoxazole (30 μg/disk); en5, enrofloxacin (5 μg/disk); gen10, gentamycin (10 μg/disk); imp30, imipenem (30 u/disk); s10, streptomycin (10 μg/disk); sul25, sulfamethoxazole (25 μg/disk); tet30, tetracycline (30 u/disk); tri5, trimethoprim (5 μg/disk).
[a]Values are expressed as No. (%).

while resistance to cephalothin (6.38%) was rare (5). Hemmatinezhad et al. (2015) described that the highest level of antibiotic resistance in the STEC bacteria of poultry meat samples belonged to gentamicin, tetracycline, and ampicillin (15). Kim and Woo (2014) found that *E. coli* strains, which were recovered from vegetables, exhibited rare resistance, except for cephalothin (71%). They showed that prohibiting the use of human and animal manure is the main factor for the low prevalence of antibiotic resistance of *E. coli* strains isolated from organic vegetables (30). High prevalence of foodborne pathogens and their considerable levels of antibiotic resistance had also been described previously from different parts of Iran (31-37).

Significant differences in the prevalence of STEC strains and the pattern of antibiotic resistance between our results and those of other investigations may be due to difference in types of samples, number of samples collected, method of sampling, method of experiment, place of sampling, weather and climate of geographical zone of sampling, opinion of medical and veterinary practitioners in antibiotic prescription, costs of antibiotics and their availability. Differences in the levels of health care and hygiene, detailed control of antibiotic prescription, accuracy of meat inspection at the slaughterhouse, hygiene of the milking halls, application of animal and human manure for fertilization of the agricultural soils used for planting vegetables, and finally the levels of personal hygiene were other factors which might have affected the prevalence of bacteria and antibiotic resistance. Moreover, considerable prevalence of *E. coli* and other pathogenic bacteria and their high antibiotic resistance have been described previously (38-49).

6. Conclusions

Virulent and multidrug- resistant STEC strains had a considerable prevalence in meat, milk, and vegetable samples of Iranian markets. Judicious prescription of antibiotics and accurate monitoring of meat inspection in slaughterhouses, milking in milking halls, and washing vegetables can reduce the risk of virulent and resistant STEC strains. Careful prescription of imipenem and cotrimoxazole, according to the results of disk diffusion, can help treat cases of STEC infections, especially in patients who have consumed contaminated meat, milk, and vegetables. Properly washing vegetables, cooking meat, and boiling milk, or using pasteurized milk can reduce the risk of STEC food poisoning.

Footnotes

Authors' Contribution: Zohreh Mashak: study design, samples collection, and culture-based identification, PCR genetic alignment, statistical analysis, writing and drafting of the manuscript.

Conflicts of Interests: The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Gould LH, Walsh KA, Vieira AR, Herman K, Williams IT, Hall AJ, et al. Surveillance for foodborne disease outbreaks—United States, 1998–2008. *MMWR Surveill Summ.* 2013 Jun 28;**62**(2):1–34. [PubMed: 23804024].

2. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, et al. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Med.* 2015;**12**(12). e1001923. doi: 10.1371/journal.pmed.1001923. [PubMed: 26633896].

3. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne Illness Acquired in the United States—Major Pathogens. *Emerg Infect Dis.* 2011;**17**(1):7–15. doi: 10.3201/eid1701.P11101.

4. Momtaz H, Karimian A, Madani M, Safarpoor Dehkordi F, Ranjbar R, Sarshar M, et al. Uropathogenic Escherichia coli in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. *Ann Clin Microbiol Antimicrob.* 2013;**12**(1):8. doi: 10.1186/1476-0711-12-8.

5. Momtaz H, Farzan R, Rahimi E, Safarpoor Dehkordi F, Souod N. Molecular Characterization of Shiga Toxin-ProducingEscherichia coliIsolated from Ruminant and Donkey Raw Milk Samples and Traditional Dairy Products in Iran. *Sci World J.* 2012;**2012**:1–13. doi: 10.1100/2012/231342.

6. Dehkordi F, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. *BMC Res Notes.* 2014;**7**(1):217. doi: 10.1186/1756-0500-7-217.

7. Bai X, Zhang W, Tang X, Xin Y, Xu Y, Sun H, et al. Shiga Toxin-Producing Escherichia coli in Plateau Pika (Ochotona curzoniae) on the Qinghai-Tibetan Plateau, China. *Front Microbiol.* 2016;**7**. doi: 10.3389/fmicb.2016.00375.

8. Dhaka P, Vijay D, Vergis J, Negi M, Kumar M, Mohan V, et al. Genetic diversity and antibiogram profile of diarrhoeagenic Escherichia coli pathotypes isolated from human, animal, foods and associated environmental sources. *Infect Ecol Epidemiol.* 2016;**6**:31055. doi: 10.3402/iee.v6.31055. [PubMed: 27197617].

9. Wang J, Stanford K, McAllister TA, Johnson RP, Chen J, Hou H, et al. Biofilm Formation, Virulence Gene Profiles, and Antimicrobial Resistance of Nine Serogroups of Non-O157 Shiga Toxin-Producing Escherichia coli. *Foodborne Pathog Dis.* 2016;**13**(6):316–24. doi: 10.1089/fpd.2015.2099. [PubMed: 27023165].

10. Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohammadzadegan R. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. *Arch Iran Med.* 2012 May;**15**(5):312–6. doi: 012155/AIM.0013.

11. Amezquita-Lopez BA, Quinones B, Soto-Beltran M, Lee BG, Yambao JC, Lugo-Melchor OY, et al. Antimicrobial resistance profiles of Shiga toxin-producing Escherichia coli O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. *Antimicrob Resist Infect Control.* 2016;**5**:1. doi: 10.1186/s13756-015-0100-5. [PubMed: 26734130].

12. Abdi S. Frequency of bla TEM, bla SHV, bla CTX-M, and qnrA among Escherichia coli isolated from urinary tract infection. *Arch Clin Infect Dis.* 2014;**9**(1):1–5.

13. Woo PC, Cheung EY, Leung K, Yuen K. Identification by 16S ribosomal RNA gene sequencing of an Enterobacteriaceae species with ambiguous biochemical profile from a renal transplant recipient. *Diagn Microbiol Infect Dis.* 2001;**39**(2):85–93. [PubMed: 11248520].

14. Momtaz H, Dehkordi F, Hosseini M, Sarshar M, Heidari M. Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. *Gut Pathogens.* 2013;**5**(1):39. doi: 10.1186/1757-4749-5-39.

15. Hemmatinezhad B, Khamesipour F, Mohammadi M, Safarpoor Dehkordi F, Mashak Z. Microbiological Investigation of O-Serogroups,

16. CLSI. *Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests approved standard.* 2012.

17. Yahaghi E, Khamesipour F, Mashayekhi F, Safarpoor Dehkordi F, Sakhaei MH, Masoudimanesh M, et al. Helicobacter pyloriin Vegetables and Salads: Genotyping and Antimicrobial Resistance Properties. *BioMed Res Int.* 2014;**2014**:1–11. doi: 10.1155/2014/757941.

18. Wachtel MR, Whitehand LC, Mandrell RE. Association of Escherichia coli O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J Food Prot.* 2002;**65**(1):18–25. [PubMed: 11808792].

19. Solomon EB, Yaron S, Matthews KR. Transmission of Escherichia coli O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl Environ Microbiol.* 2002 Jan;**68**(1):397–400. [PubMed: 11772650].

20. Solomon EB, Pang HJ, Matthews KR. Persistence of Escherichia coli O157:H7 on lettuce plants following spray irrigation with contaminated water. *J Food Prot.* 2003;**66**(12):2198–202. [PubMed: 14672213].

21. Momtaz H, Safarpoor Dehkordi F, Taktaz T, Rezvani A, Yarali S. Shiga Toxin-ProducingEscherichia coliIsolated from Bovine Mastitic Milk: Serogroups, Virulence Factors, and Antibiotic Resistance Properties. *Sci World J.* 2012;**2012**:1–9. doi: 10.1100/2012/618709.

22. Franz E, Klerks MM, De Vos OJ, Termorshuizen AJ, van Bruggen AHC. Prevalence of Shiga Toxin-Producing Escherichia coli stx1, stx2, eaeA, and rfbE Genes and Survival of E. coli O157:H7 in Manure from Organic and Low-Input Conventional Dairy Farms. *Appl Environ Microbiol.* 2007;**73**(7):2180–90. doi: 10.1128/aem.01950-06.

23. Zhou D, Jay-Russell MT, Hake AF, Bengson Y, Thiptara A, Nguyen T. Prevalence and Characterization of Escherichia coli and Salmonella Strains Isolated from Stray Dog and Coyote Feces in a Major Leafy Greens Production Region at the United States-Mexico Border. *PLoS ONE.* 2014;**9**(11). e113433. doi: 10.1371/journal.pone.0113433.

24. Kabiru L, Bello M, Kabir J, Grande L, Morabito S. Detection of Pathogenic Escherichia coli in Samples Collected at an Abattoir in Zaria, Nigeria and at Different Points in the Surrounding Environment. *Int J Environ Res Public Health.* 2015;**12**(1):679–91. doi: 10.3390/ijerph120100679.

25. Dormanesh B, Siroosbakhat S, Karimi Goudarzi P, Afsharkhas L. RETRACTED ARTICLE: Shiga toxigenic Escherichia coli in Iranian pediatric patients with and without diarrhea: O-serogroups, virulence factors and antimicrobial resistance properties. *Iran Red Crescent Med J.* 2015;**17**(10). doi: 10.5812/ircmj.29706.

26. Dormanesh B, Safarpoor Dehkordi F, Momtaz H, Mirnejad R, Hoseini MJ, Yahaghi E, et al. Virulence Factors and O-Serogroups Profiles of Uropathogenic Escherichia Coli Isolated from Iranian Pediatric Patients. *Iran Red Crescent Med J.* 2014;**16**(2). doi: 10.5812/ircmj.14627.

27. Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K. Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. *Revista do Instituto de Medicina Tropical de Sao Paulo.* 2014;**56**(4):341–6. doi: 10.1590/s0036-46652014000400012.

28. Colello R, Etcheverria AI, Di Conza JA, Gutkind GO, Padola NL. Antibiotic resistance and integrons in Shiga toxin-producing Escherichia coli (STEC). *Braz J Microbiol.* 2015;**46**(1):1–5. doi: 10.1590/S1517-838246120130698. [PubMed: 26221083].

29. Miles TD, McLaughlin W, Brown PD. Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. *BMC Vet Res.* 2006;**2**:7. doi: 10.1186/1746-6148-2-7. [PubMed: 16460561].

30. Kim S, Woo GJ. Prevalence and characterization of antimicrobial-resistant Escherichia coli isolated from conventional and organic vegetables. *Foodborne Pathog Dis.* 2014;**11**(10):815–21. doi: 10.1089/fpd.2014.1771. [PubMed: 25140978].

31. Dehkordi Hasanpour A, Khaji L, Shahreza MH, Mashak Z, Dehkordi FS,

Safaee Y, et al. One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus recovered from raw meat. *Trop Biomed*. 2017;**34**(2):396–404.

32. Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E. Shiga (Vero)-toxin producing Escherichia coli isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. *Antimicrob Resist Infect Control*. 2017;**6**:4. doi: 10.1186/s13756-016-0163-y. [PubMed: 28074125].

33. Safarpoor Dehkordi F, Parsaei P, Saberian S, Moshkelani S, Hajshafiei P, Hoseini SR, et al. Prevalence study of theileria annulata by comparison of four diagnostic techniques in Southwest Iran. *Bulgarian J Vet Med*. 2012;**15**(2).

34. Atapoor S, Safarpoor Dehkordi F, Rahimi E. Detection of Helicobacter pylori in Various Types of Vegetables and Salads. *Jundishapur J Microbiol*. 2014;**7**(5). e10013. doi: 10.5812/jjm.10013. [PubMed: 25147709].

35. Safarpoor Dehkordi F, Barati S, Momtaz H, Hosseini Ahari SN, Nejat Dehkordi S. Comparison of Shedding, and Antibiotic Resistance Properties of Listeria monocytogenes Isolated From Milk, Feces, Urine, and Vaginal Secretion of Bovine, Ovine, Caprine, Buffalo, and Camel Species in Iran. *Jundishapur J Microbiol*. 2013. doi: 10.5812/jjm.6616.

36. Rahimi E, Sepehri S, Safarpoor Dehkordi F, Shaygan S, Momtaz H. Prevalence of Yersinia Species in Traditional and Commercial Dairy Products in Isfahan Province, Iran. *Jundishapur J Microbiol*. 2014;**7**(4). doi: 10.5812/jjm.9249.

37. Momtaz H, Davood Rahimian M, Safarpoor Dehkordi F. Identification and characterization of Yersinia enterocolitica isolated from raw chicken meat based on molecular and biological techniques. *J Appl Poult Res*. 2013;**22**(1):137–45. doi: 10.3382/japr.2012-00549.

38. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A. Detection of Escherichia coli, Salmonella species, and Vibrio cholerae in tap water and bottled drinking water in Isfahan, Iran. *BMC Public Health*. 2013;**13**:556. doi: 10.1186/1471-2458-13-556. [PubMed: 23742181].

39. Momtaz H, Safarpoor Dehkordi F, Rahimi E, Ezadi H, Arab R. Incidence of Shiga toxin-producing Escherichia coli serogroups in ruminant's meat. *Meat Sci*. 2013;**95**(2):381–8. doi: 10.1016/j.meatsci.2013.04.051. [PubMed: 23747633].

40. Shahrani M, Dehkordi FS, Momtaz H. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biol Res*. 2014;**47**:28. doi: 10.1186/0717-6287-47-28. [PubMed: 25052999].

41. Safarpoor Dehkordi F, Haghighi N, Momtaz H, Rafsanjani M, Momeni M. Conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. *Bulgarian J Vet Med*. 2013;**16**(2):102–11.

42. Mousavi S, Dehkordi F, Rahimi E. Virulence factors and antibiotic resistance of Helicobacter pylori isolated from raw milk and unpasteurized dairy products in Iran. *J Venomous Animals Toxins Trop Dis*. 2014;**20**(1):51. doi: 10.1186/1678-9199-20-51.

43. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. *J Appl Poult Res*. 2013;**22**(4):913–21. doi: 10.3382/japr.2012-00673.

44. Safarpoor Dehkordi F, Tirgir F, Valizadeh Y. Effects of Guajol((R)) ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. *Vet Res Forum*. 2017;**8**(3):215–21. [PubMed: 29085609].

45. Madahi H, Rostami F, Rahimi E, Safarpoor Dehkordi F. Prevalence of Enterotoxigenic Staphylococcus aureus Isolated From Chicken Nugget in Iran. *Jundishapur J Microbiol*. 2014;**7**(7). doi: 10.5812/jjm.10237.

46. Safarpoor Dehkordi F, Khamesipour F, Momeni M. Brucella abortus and Brucella melitensis in Iranian Bovine and Buffalo Semen Samples: The First Clinical Trial on Seasonal, Senile and Geographical Distribution Using Culture, Conventional and Real-time Polymerase Chain Reaction Assays. *Kafkas Universitesi Veteriner Fakultesi Dergisi*. 2014;**20**(6):821–8. doi: 10.9775/kvfd.2014.10827.

47. Safarpoor Dehkordi F, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. *Antimicrob Resist Infect Control*. 2017;**6**:104. doi: 10.1186/s13756-017-0257-1. [PubMed: 29034091].

48. Ghorbani F, Gheisari E, Safarpoor Dehkordi F. Genotyping of vacA alleles of Helicobacter pylori</i>strains recovered from some Iranian food items. *Trop J Pharm Res*. 2016;**15**(8):1631. doi: 10.4314/tjpr.v15i8.5.

49. Dehkordi FS, Borujeni MR, Rahimi E, Abdizadeh R. Detection of Toxoplasma gondii in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran. *Foodborne Pathog Dis*. 2013;**10**(2):120–5. doi: 10.1089/fpd.2012.1311. [PubMed: 23441913].