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# Prevalence and distribution of *E. coli* and its virulence genes from different water sources in Alborz province, Iran

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## Abstract

The event of pathogenic microorganisms in ecological waters is a progressing worry for general wellbeing authorities and those in the water administration territory around the world. *Escherichia coli* (*E. coli*) is considered as a standout amongst the most vital reasons for bacterial diseases transmits over foods and water. The pathogenecity relies upon the outflow of a variety of destructiveness elements created via *E. coli*. The point of this examination was to explore the prevalence of *E. coli* strains carrying virulence genes *eltB, PCVD, VT1, VT2, ial*, isolated from different water sources in Alborz province, Iran. The research was conducted from September 2013 to September 2014. The study included all *E. coli* strains isolated from different surface water sources (surface water, refinery inputs, water well) in Alborz province of Iran. *E. coli* isolates were detected and identified via biochemical tests and standard microbiological. The strains were evaluated for the occurrence of virulenc genes *eltB, PCVD, VT1, VT2, ial*, by PCR using specific primers. The PCR amplicons were visualized via electrophoresis and stained by ethidium bromide. One hundred *E. coli* strains were isolated and involved in the research. The frequency of virulence genes was: *VT2* (43%), *eltB* (19%), *Ial* (15%), *PCVD* (13%), *VT1* (3%). Our finding showed the prevalence rate of virulence genes *VT2* is very high among *E. coli* strains isolated from different surface water sources in Alborz province. Considering their plasmid borne nature, the risk of transmission of these genes between other bacterial species could pose a high threat for public health.

**Keywords**: Alborz province, *E. coli*, PCR, Virulence genes, Water.

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## Introduction

*Escherichia coli* (*E. coli*) is the leader of the great bacterial family, Enterobacteriaceae and a section of the normal gut flora in human, animals and birds. Though, few strains are pathogenic and origin gastrointestinal infections and extra-intestinal illness similar urinary tract infection. The pathogenecity depends on the appearance of an collection of virulence factors produced via *E. coli* **(**Rahimi et al. 2012; Helina and Manab 2014; Raissy et al. 2014; Hemmatinezhad et al. 2015; Tajbakhsh et al. 2015).

Surface water sources are measured to be at great danger for pathogen infection since they are open to numerous ways via which together human food-borne disease and plant infection-causing microorganisms can arrive. Human-pathogenic bacteria are thought to enter surface waters generally over infection since fecal material from livestock and wildlife indirectly or directly through dirty soil, water, or debris. Plant pathogens can arrive surface water sources done numerous ways, counting infested water, soil, and debris; cull piles; and field drainage tiles (Hong and Moorman 2005; Ranjbar et al. 2016 a; Ranjbar et al. 2016 b; Ranjbar et al. 2016 c; Ranjbar et al. 2017).

Quantification of *E. coli* in surface waters too attends to assess the routine of a sewage treatment plants for microbial reduction (Kaper 2005). The occurrence of fecal infection in lakes, creeks, and rivers, can principal to the dreadful conditions of water quality and subsequently effect in the water becoming unfit for potable/nonpotable routines, aquaculture, and recreational activities for instance fishing and swimming (Marsalek and Rochfort 2004; Ishii et al. 2007; Hamilton et al. 2010; Noble et al. 2006).

*E. coli*, an overall sign of water quality, can, though, moreover be pathogenic and several diarrheagenic pathotypes, such as enterotoxigenic *E. coli* or ETEC, whose greatest typical genes are the stable thermotoxin -*st*- and thermolabile -*lt*-; shiga-toxin *E. coli* or STEC, whose toxins are encoded in the *stx1* y *stx2* genes, enteroaggregative *E. coli* or EAEC, by the pCVD432 plasmid, for which the *aatA* gene is one of the greatest stable regions; and diffusely adherent *E. coli* or DAEC, whose virulence genes have yet to be fully reported

have been recognized based on the specific virulence genes present (Karper et al. 2004; Nishi et al. 2003) and implicated in many waterborne outbreaks (Jenkins et al. 2003; Hrudey et al. 2003; Hunter 2003).

Totally of the strains are related by watery diarrhea, nonetheless approximately strains are related by vomiting (ETEC), bloody diarrhea (EHEC), and fever (ETEC and EIEC) (Todar 2008; Tajbakhsh et al. 2016). Numerous studies have displayed that fecal material from many animals and humans covers *E. coli* carrying virulence genes related by pathogenic *E. coli* (Caprioli et al. 2005; Fairbrother and Nadeau 2006; Ishii et al. 2007; Chandran and Mazumder 2013; Kheiri et al. 2016) and can be a possible source of pathogenic *E. coli* in the surface waters. Amongst the diarrheagenic pathotypes of *E. coli*, STEC is additional commonly related by waterborne outbreaks global (Jenkins et al. 2003; Hrudey et al. 2003; Bruneau et al. 2004; Nwachuku and Gerba 2008).

Infection occurrences related to contact to contaminated freshwaters are well known (Olsen et al. 2002; Ackman et al. 1997; Shelton et al. 2006; Chalmers et al. 2000). The rate of pathogenic *E. coli* strains harbouring virulence genes (VGs) in ecological waters could be related to pollution through storm events, faeces from wild and domestic animals in addition to humans, extras from sewage overflows, agricultural lands, farm animals, birds and pets (Ishii et al. 2007; Parker et al. 2010; Sidhu et al. 2012; Brownell et al. 2007; Sauer et al. 2011).

However, single some studies have investigated the presence of *E. coli* strains carrying VGs in environmental waters (Martins et al. 1992; Lauber et al. 2003; Chern et al. 2004; Hamelin et al. 2006; Ahmed et al. 2005; Ram et al. 2007; Hamilton et al. 2010). To the best of our information, no study on *E. coli* virulence gene delivery has been carried out on the different water sources in Iran. Therefore, a well sympathetic of the prevalence of *E. coli* virulence genes in water sources used for potable, non-potable or recreation purposes could be a significant device in the improvement of public health risk mitigation plans. Therefore, the aim of the present research was to study the prevalence of *E. coli* strains carrying virulence genes *eltB, PCVD, VT1, VT2, ial,* isolated from different water sources in Alborz province, Iran.

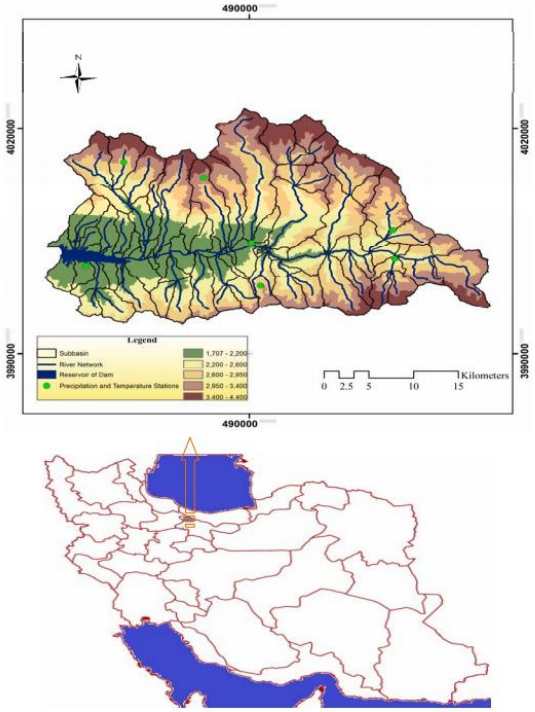


**Figure 2.** Study areas and the location where exactly is that refinery inputs in the Alborz province.

**Table 1:** Primers used in the PCR

| Target Gene | Primer sequence (5′→ 3′) | Size of product (bp) | Annealing temperature | Reference |
|---|---|---|---|---|
| *PCVD* | F: CTGGCGAAAGACTGTATCA<br>R: CAATGTATAGAAATCCGCTGTT | 630 | 57°C | Akter et al. 2013 |
| *VT1* | F: GAAGAGTCCGTGGGATTACG<br>R: AGCGATGCAGCTATTAATAA | 130 | 57°C | |
| *VT2* | F: TGTGGCTGGGTTCGTTAATACGGC<br>R: TCCGTTGTCATGGAAACCGTTGTC | 102 | 65°C | |
| *ial* | F: CTGGATGGTATGGTGAGG<br>R: GGAGGCCAACAATTATTTCC | 320 | 56 °C | |
| *eltB* | F: GGCGACAGATTATACCGTGC<br>R: CCGAATTCTGTTATATATGTC | 708 | 55°C | |

***PCVD*** - Representative plasmid of EAEC*;* ***VT1***- Verotoxin type 1; ***VT2*** - Verotoxin type 2; ***ial*** - Invasion-associated locus; ***eltB*** - Heat-labile enterotoxin subunit b gene.

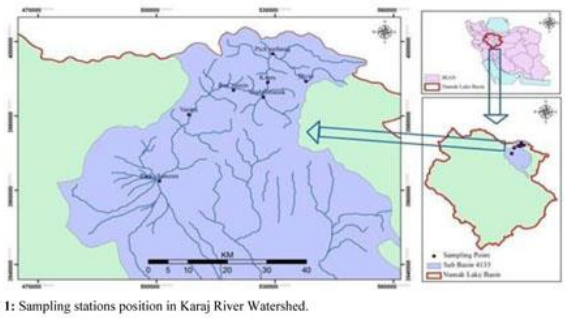


Fig. 1: Sampling stations position in Karaj River Watershed.

**Figure 1.** Study areas and the location where exactly is that surface water in the Alborz province.

## Materials and methods

This research was conducted from September 2013 to September 2014. Different water sources samples (surface water, refinery inputs, water well) were accidentally collected from the different parts of Alborz province, Iran. The Latitude and Longitude of Alborz Province is 35.8163 and 50.9354 respectively. Surface water samples were collected from the Karaj River with geographic coordinates of 35° 48′46.48″N; 51° 0′43.03″E (Figure 1). Refinery inputs samples were collected from the Rajai-shahr (Taleghan) water treatment plants. Taleghan with about 1300 square kilometers in area

**Table 2:** Virulence genes of *E. coli* isolated from different water sources in Alborz province of Iran

| Number of isolated | *ial* | *VT2* | *VT1* | *eltB* | *PCVD* | Number of isolated | *ial* | *VT2* | *VT1* | *eltB* | *PCVD* | Number of isolated | *ial* | *VT2* | *VT1* | *eltB* | *PCVD* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | - | - | - | - | - | **36** | - | - | - | - | + | **71** | - | - | - | - | - |
| **2** | - | - | + | - | - | **37** | - | + | - | - | - | **72** | - | - | - | - | - |
| **3** | - | + | - | - | + | **38** | - | + | - | - | - | **73** | - | - | - | - | - |
| **4** | + | + | - | - | - | **39** | - | + | - | - | - | **74** | - | - | - | + | - |
| **5** | + | + | - | - | + | **40** | - | - | - | - | - | **75** | - | - | - | + | - |
| **6** | - | - | - | - | - | **41** | - | + | - | - | + | **76** | - | - | - | + | - |
| **7** | + | + | - | - | - | **42** | | + | - | - | - | **77** | - | - | - | + | + |
| **8** | + | + | - | - | - | **43** | - | + | - | - | - | **78** | - | - | - | + | - |
| **9** | - | + | - | - | - | **44** | - | - | - | - | - | **79** | - | - | - | | + |
| **10** | + | + | - | - | - | **45** | - | - | - | - | - | **80** | - | - | - | + | - |
| **11** | + | + | - | - | - | **46** | - | - | - | - | + | **81** | - | - | - | + | - |
| **12** | - | - | - | - | + | **47** | + | - | - | - | - | **82** | - | - | - | + | - |
| **13** | + | + | - | - | - | **48** | - | + | - | - | - | **83** | + | - | - | + | - |
| **14** | + | + | - | - | - | **49** | - | - | - | - | - | **84** | + | - | - | + | - |
| **15** | + | + | - | - | - | **50** | - | - | - | - | + | **85** | - | - | - | + | - |
| **16** | + | + | - | - | - | **51** | - | + | - | - | - | **86** | - | - | - | + | - |
| **17** | - | + | - | - | + | **52** | - | - | - | - | - | **87** | - | - | - | - | - |
| **18** | - | - | - | - | - | **53** | - | + | - | - | - | **88** | - | - | - | - | - |
| **19** | - | - | - | - | - | **54** | - | - | - | - | - | **89** | - | - | - | + | - |
| **20** | - | - | - | - | - | **55** | - | - | - | - | - | **90** | - | - | - | -- | - |
| **21** | - | - | - | - | + | **56** | - | - | - | - | - | **91** | - | + | - | - | - |
| **22** | - | + | - | - | + | **57** | - | - | - | - | - | **92** | - | + | - | - | - |
| **23** | - | + | - | - | - | **58** | - | - | - | - | - | **93** | - | + | - | - | - |
| **24** | - | + | - | - | - | **59** | - | - | - | - | - | **94** | - | + | - | - | - |
| **25** | - | + | - | - | - | **60** | - | - | - | - | - | **95** | - | + | - | + | - |
| **26** | - | + | - | - | - | **61** | - | - | - | + | - | **96** | - | + | + | - | - |
| **27** | - | + | - | - | - | **62** | - | - | - | - | - | **97** | - | + | + | - | + |
| **28** | - | - | - | - | - | **63** | - | - | - | + | - | **98** | + | - | - | + | - |
| **29** | - | + | - | - | - | **64** | - | - | - | + | - | **99** | + | + | - | - | - |
| **30** | - | + | - | - | - | **65** | - | - | - | - | - | **100** | - | + | - | - | - |
| **31** | - | - | - | - | - | **66** | - | - | - | - | - | | | | | | |
| **32** | - | + | - | - | - | **67** | - | - | - | + | - | | | | | | |
| **33** | - | + | - | - | - | **68** | - | - | - | - | - | | | | | | |
| **34** | - | + | - | - | - | **69** | - | - | - | - | - | | | | | | |
| **35** | - | + | - | - | - | **70** | - | - | - | - | - | | | | | | |

36 degrees 12 minutes north latitude and 50 degrees is 47 minutes North West of Tehran and part of Alborz province (Figure 2). Also, some water wells of Karaj water supply system were collected. The sterile glass bottles comprising 0.5 g of sodium thiosulphate for dechlorination of the water were used for samples collection. To minimize the risks of infection, all samples were collected 30 seconds later opening of faucet. Water samples were immediately moved to the laboratory in cooler by ice-packs. All techniques were applied to deterrence of cross contamination within and between various parts of sampling.

Analysis of the water samples was finished in 2 hours later collection via Standard Total Coliform Multiple-Tube (MPN) fermentation methods. After determination of MPN the tubes showing growth were inoculated on 5% sheep blood and MacConkey agar (Merck, Germany) and incubated for 18 to 24 h at 37 °C. Colonies by the typical color and appearance of *E. coli* were picked and streaked again on blood agar plates and re-streaked on EMB agar (Merck, Germany). The green metallic sheen colonies were considered as *E.* coli. After 24 h incubation at 35°C ± 0.5°C for 24 h ±2 h gram-negative microorganisms were isolated from MacConkey agar and EMB agar and determined at the species level via cytochrome oxidase, triple sugar iron agar, urea and indole tests as putatively *E. coli.* All strains were coded by Molecular Biology Research Center (MBRC) (from MBRC1-MBR100) Purification of DNA directly from water samples filtered was achieved by a AccuPrep® Genomic DNA Extraction Kit (Bioneer, South Korea) as said by the manufacturer's guidelines. The stock was kept at –20°C until use. The DNA concentration has been determined through measuring absorbance of the sample at 260 nm using spectrophotometer (Sambrok 2001).

The colonies were confirmed by Polymerase Chain Reaction (PCR) based on the technique previously described (Woo et al. 2001). The 10 ml bacterial DNA extract and controls were amplified with 0.5 mM primers (Forward: 5'-AGTTTGATCCTGGCTCAG-3' and Reverse: 5'-AGGCCCGGGAACGTATTCAC-3') (1343 bp) (Woo et al. 2001), 200 mM of each dNTP (Fermentas, Germany), 2 mM MgCl2, 10 mM KCl PCR buffer and 1.0 U Taq polymerase (Fermentas, Germany). The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Germany) PCR device by the following protocol: 94 °C for 5 min, 40 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and final 72 °C for 5 min.

List of primers and the annealing temperature used for amplification of virulence genes of *E. coli* isolates are shown in Table 1 (Akter et al. 2013). The PCR amplification products (15 µl) were subjected to electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 80 V for 30 min, stained by ethidium bromide, and images were obtained in a UVIdoc gel documentation systems (UK). The PCR products were identified through 100 bp DNA size marker (Fermentas, Germany). For each PCR test, corresponding negative (sterile water) and positive controls were included.

## Results and Discussion

### *Prevalence of virulence genes from E. coli isolates*

Amongst one hundred confirmed *E. coli* isolates were evaluated for the various virulence genes. The strain numbers of 1-35, 36-77 and 78-100 *E. coli* were selected from the sources of surface water, refinery inputs, and water well, respectively. Ninety-seven percent of the isolates harbored at least 1 virulence gene while 3% isolates harboured none (Table 2). The most frequent virulence factor genes were *VT2*, each of which was observed in 43%, of the isolates. In contrast, four genes, including *eltB, ial*, *PCVD* and *VT1*, were each detected in less than 20% of isolates (Table 3). In the current research, the frequencies of virulence genes were: *VT2* (43%), *eltB* (19%), *Ial* (15%), *PCVD* (13%), *VT1* (3%). Our finding showed the prevalence rate of virulence genes *VT2* is very high among *E. coli* strains isolated from different surface water sources. Therefore, *E. coli* strains isolated from different water sources carried the virulence-associated *VT2* genes more frequently.

In addition, the result of the PCR test for Identification of *VT2*, *eltB*, *ial*, *PCVD* and *VT1* genes are shown in Figure 3 to Figure 7, respectively.

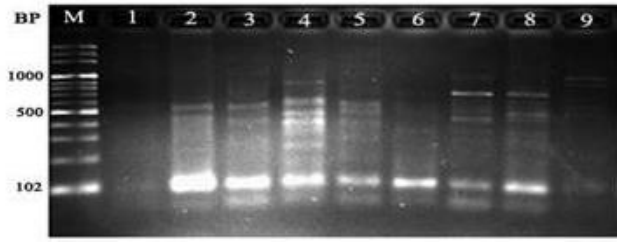


**Figure 3.** Result of the PCR Assay for Identification of *VT2* gene. M: DNA size ladder 100 bp (Fermentas); number 1: negative control; number 2-8: positive samples (102 bp); number 9: negative samples.

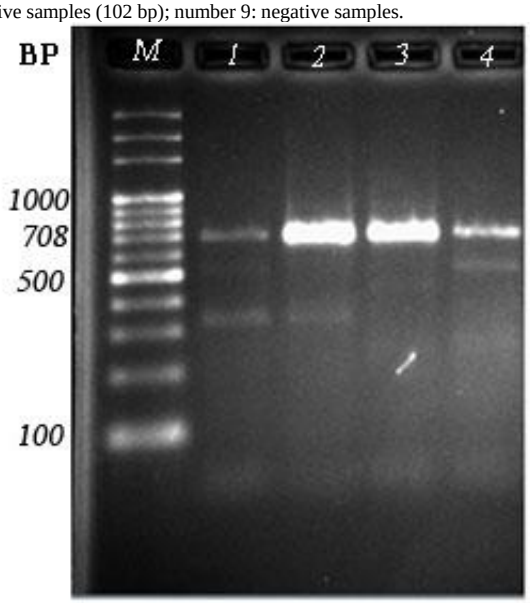


**Figure 4.** Result of the PCR Assay for Identification of *eltB* gene. M: DNA size ladder 100 bp (Fermentas); number 1: negative control; number 2: positive control; number 3: positive samples (708 bp).

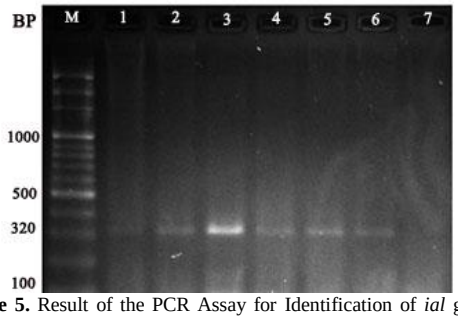


**Figure 5.** Result of the PCR Assay for Identification of *ial* gene. M: DNA size ladder 100 bp (Fermentas); number 1: negative control; number 2-6: positive samples (320 bp); number 7: negative samples.

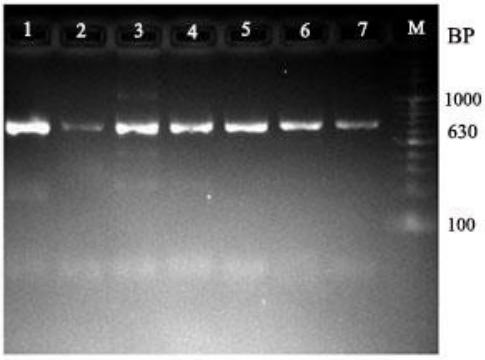


**Figure 6.** Result of the PCR Assay for Identification of *PCVD* gene. M: DNA size ladder 100 bp (Fermentas); number 1: positive control; number 2: negative control; number 3-7: positive samples (630 bp).

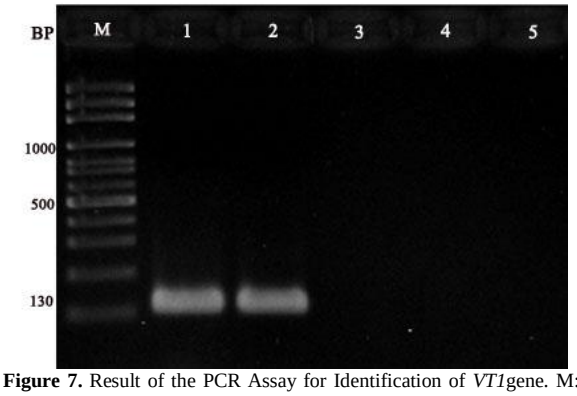


**Figure 7.** Result of the PCR Assay for Identification of *VT1*gene. M: DNA size ladder 100 bp (Fermentas); number 1: positive control; number 2: positive samples (130 bp); number 3: negative control; number 4 and 5: negative samples.

Though the incidence of *E. coli* in usual waters has long been used as an pointer of fecal contamination, there is a growing body of indication suggesting that there happens a specific subsection of *E. coli* strains that can make a replica and keep on minor environments rather than being obligate intestinal flora in both tropical (Bermudez and Hazen 1988; Solo-Gabriele et al. 2000) and temperate climates (Byappanahalli and Fujioka 1998; Gordon et al. 2002; Ishii et al. 2006; Jjemba et al. 2010; McLellan 2004). The present study investigated the prevalence of *E. coli* strains carrying virulence genes *eltB, PCVD, VT1, VT2, ial,* isolated from different water sources in Alborz province, Iran.

**Table 3:** Result of Virulence gene

| Virulence gene | Prevalence (%) |
|---|---|
| *VT2* | **43 (43%)** |
| *eltB* | **19 (19%)** |
| *ial* | **15 (15%)** |
| *PCVD* | **13 (13%)** |
| *VT1* | **3 (3%)** |

In our research, virulence genes were detected in the *E. coli* isolates telling the occurrence of pathogenic *E. coli* strains in different waters sources. Earlier studies have too informed the presence of great numbers of faecal indicator bacteria originating from defective septic systems and grazing animals in freshwater places and surface waters of developing countries (Frahm and Obst 2003; Juhna et al. 2007).

Our finding showed the prevalence rate of virulence genes *VT2* is very high among *E. coli* strains isolated from different surface water sources and four genes, including *eltB*, *ial*, *PCVD* and *VT1*, were each detected in less than 20% of isolates. Previous studies conducted on the detection of virulence genes in *E. coli* showed that, of 104 strains found from water samples, 10 (10%) moreover carried one or extra of the tested virulence genes involved eaeA, VT1, 2 and 2e, LT1, ST1 and 2, Einv gene, EAgg gene, CNF1 and 2, papC, O111 and O157 side chain LPS. This result indicate that the sources of clinically significant *E. coli* strains found in surface waters because of faecal infection can be predicted through the detection of virulence genes.

The occurrence of virulence markers in *E. coli* isolates from different water sources is indicative of increased risks of mortality, should they contract contaminations over the use of different water sources for ingesting or additional household associated purposes. In general, the results establish that the risk of contracting contamination, though, may increase over time if no appropriate preventive and controlling measures are ensured. Although the skill of *E. coli* isolates described in this study to source human diarrhoeal diseases was not established, a high proportion of isolates carrying a full set of virulence genes have been linked to defined pathotypes. Additional screening for other virulence genes along with serotype testing and other assays may offer further data on pathogenicity of these isolates.

## Conclusion

This study demonstrates the occurrence of *E. coli* strains carrying virulence genes isolated from different water sources in Alborz province, Iran. The results of the present study exposed the presence of potential pathogenic *E. coli* in water samples. In addition, this finding suggests that the prevalence rate of virulence genes *VT2* is very high amongst *E. coli* strains isolated from different surface water sources in Alborz province. This highlights the significance of safe water supply, good hygiene and sanitation practices both in urban and rural populations. Therefore, the emergence and dissemination of *E. coli* virulence genes from different water sources in Iran is a reason of worry and opinions to a need to identify their origins, reservoirs, and transmission pathways so that better prevention plans are considered. From the public health point of view this data will be of great status for estimating the risk related by public use of the catchment and also provides the need to develop a better understanding of public health implications of occurrence of *E. coli* carrying virulence genes in different water sources.

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