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Evaluation of the prevalence of *Vibrio cholerae* isolated from water and vegetables and the presence of virulence genes and antibiotic resistance pattern in Qom province

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ABSTRACT

Vibrio cholerae is one of the important human pathogens that is transmitted through contaminated water and food. In Qom province, due to special weather conditions, diseases caused by V. cholerae are endemic. The aim of this study was the prevalence of V. cholerae in water and vegetables of Qom province and the presence of virulence genes. During two years (2020-2021), 120 samples of agricultural water (70) and vegetables (50) in Qom province were collected. The samples were cultured on specific media. Suspicious colonies were evaluated by Gram staining and biochemical tests and the serotype V. cholerae was identified by serology test. Finally, Then, the presence of virulence genes was investigated by PCR method and also the antibiotic resistance pattern by disk diffusion method was evaluated in the isolates. V. cholerae bacteria were isolated from 17 samples (16.14%), all of which were non-O1. The rate of contamination of water and vegetables was 28.14% (10 cases) and 14.00% (7 cases), respectively. In molecular evaluation, the abundance of virulence genes including: toxR (88.32%), rtxA (58.82%), hlyA (47.05%), chxA (5.88%), and 100% of isolates did not have ctxA, ace and tcpA genes. The most antibiotic resistance is related to ampicillin and amoxicillin (34.29%), followed by cefuroxime (17.46%), imipenem (11.76%), and cefoxitin and trimethoprim-sulfamethoxazole (5.88%). The results of this study showed that V. cholerae non-O1 is present in water and vegetables of Qom province, and as an important source of disease for humans therefore, continuous health monitoring of water and vegetables and proper disinfection of these foods is very important.

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1. Introduction

Vibrio cholerae is a Gram-negative, curved, facultative anaerobic bacterium that exists primarily in aquatic ecosystems and could cause cholera through ingestion of contaminated food and water. To date, seven cholera epidemics have been recorded in the world [1]. According to reports published by the World Health Organization (WHO), about 3 to 5 million cholera cases and more than 140,000 deaths from this disease are annually recorded worldwide [1-3].

V. cholerae could be divided into more than 200 different serotypes based on the variation in the O antigen structure of the cell wall lipopolysaccharide. Serotype O1 is classified into two biotypes (classical and Eltor) and three serogroups (Inaba, Ogawa, and Hikojima). Strains belonging to other serogroups are classified as non-O1, non-O139 *V. cholerae* (NOVC). Initially, these strains were non-toxic and unimportant due to the absence of pathogenic toxin genes *ctx* and *tcpA* [4]. Non-toxigenic strains are unable to cause epidemic diseases, although they have caused small and sporadic epidemics in the past [5].

Bacteria and viruses are known to be the main causes of diarrhea, and many diarrhea cases caused by these pathogens have been reported so far [6-9]. During the last two decades, there have been reports about the presence of non-O1, non-O139 *V. cholerae* strains toxin-producing genes in patients with diarrhea and other invasive infections [10, 11]. Recent studies have shown that virulence genes and their homologues are distributed among *V. cholera* environmental strains in addition to clinical strains [12, 13]. Although the reason for the greater survival of environmental vibrios containing virulence genes compared to other environmental strains is not well known, these virulence gene-containing environmental strains may be the origin of pathogenic strains; in other words, they may be involved in the gene transfer process that leads to the emergence of pathogenic strains [14]. NOVCs often carry genes that cause mild to severe gastroenteritis [15]. There are pathogenic and non-pathogenic strains of non-O1, non-O139 *V. cholera* in aquatic ecosystems, but cholera endemicity in a region depends on the environmental resources of that region [16]. NOVC strains mainly lack cholera toxin (CT) and toxin-coregulated pilus (TCP) and cause disease by a mechanism different from those of *V. cholerae* O1 or O139. In addition to these two main virulence factors, other virulence factors in *V. cholerae* include: regulatory protein ToxR (activator of transcription of genes related to CT and TCP), accessory cholera enterotoxin (*ace*), repeats in toxins (*rtx* CABD), and hemolysin (*hlyA*), which lead to diarrheal disease. In non-O1, non-O139 *V. cholera*, extracellular pore-forming toxins (RtxA), hemolysin (hlyA), heat-stable enterotoxin (stn), and cholix toxin (chxA) play important roles in the pathogenesis of these strains [17].

Hydration therapy is the primary treatment for cholera, although antibiotics are recommended in the treatment of severe cholera cases and extra-intestinal infections. The emergence of antibiotic-resistant pathogens has become a serious global health issue. Although *V. cholera* antibiotic susceptibility testing (AST) was not suggested in the past due to the low resistance of *V. cholerae* to common antibiotics, today there are reports of resistance of non-O1, non-

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O139 *V. cholerae* to aminoglycosides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, ampicillin, and carbapenems, necessitating antibiotic sensitivity testing [18].

In order to detect and track the path of creation of pathogenic *V. cholerae* from non-pathogenic types, it is necessary to identify intermediate strains with less pathogenicity compared to epidemic strains. Since cholera is a water-borne disease, it is important to investigate environmental strains and their virulence genes and antibiotic resistance patterns in order to prevent cholera epidemics or sporadic gastroenteritis. Every year, several *V. cholerae* infected cases are reported in the southern and central regions of Iran, especially in Qom province and during hot seasons. For this reason, in this study, the presence of virulence and antibiotic resistance genes was investigated in *V. cholerae* environmental strains isolated from water and vegetables in Qom city and surrounding rural areas.

2. Materials and methods

This study was reviewed and approved by the Research Ethics Committee of Shahrekord University of Medical Sciences (code: IR.SKU.REC.1401.029).

2. 1. Sample collection

In this study, a total of 120 samples, including 70 samples of surface and agricultural water and 30 samples of vegetables, were collected from Qom city and surrounding rural areas in the spring and summer seasons of 2020 to 2021 (where a large number of *V. cholera*e non-O1 positive samples were previously collected). To collect the samples, 1 to 2 L of each water sample was collected in a sterile glass container, and 100 g of each vegetable sample was collected in a sterile polyethylene plastic. The samples were immediately transferred to the research center laboratory of Qom University of Medical Sciences.

2. 2. Isolation and identification of *V. cholerae*

In the next step, 500 mL of washed vegetable water and collected surface water were filtered through a membrane paper with a pore size of 0.45 μm (Advantec Toyo, Ltd., Tokyo). Then the membrane filters were placed in an alkaline saline peptone water (ASPW) environment with a pH of 6.8±0.2 and kept in a greenhouse at 37 °C for 6-8 hours. This solution was then centrifuged at 4000 rpm for 5 min. About 0.5 mL of the suspension was cultured on plates containing TCBS (thiosulfate–citrate–bile salts–sucrose agar) medium. After 18-24 hours of incubation at 37 °C, yellow single colonies that were morphologically similar (2-4 mm in diameter with a dark center and clear edges) to *V. cholerae* colonies were cultured on BHI (brain heart infusion) medium.

Bacterial culture on this medium was used for diagnostic biochemical tests and serotyping of the strains. The initial identification of the suspected bacteria was based on a series of biochemical tests, such as catalase, oxidase, triple sugar-iron (TSI) agar, Kligler iron agar (KIA), Voges-Proskauer (VP), bile esculin agar, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, citrate, and grow in different sodium chloride concentrations tests. All media were purchased from Ibresco, Iran.

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2. 3. Identification of isolates with specific antiserum

All colonies whose biochemical test results were consistent with *V. cholerae* were examined for serological grouping using polyvalent O1 antiserum (Bahar Afshan, Iran) by plate agglutination method. First, a drop of a specific antiserum was poured on a slide, then a colony from the fresh culture of the isolates was taken using a sterile ring anus and dispersed in the antiserum. The observation of agglutination was the proof of the positive reaction and led to the identification of the desired bacteria [19-20].

2. 4. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from *V. cholerae* strains using the boiling method and kept in a freezer at 20 °C until used in the PCR test [21]. PCR test was performed to confirm *V. cholerae* isolates by targeting the *ompW* gene and to check the presence of virulence genes *ctxA*, *hlyA*, t*oxR*, *ace*, *chxA*, *rtxA*, and *tcpA* using specific primer pairs for each gene (Table 1). The final volume of each PCR reaction was 25 µL, including 10 µL of master mix X1 (Amplicon, Denmark), 1 µL of each primer (10 pmol per µL), 3 µL of genomic DNA, and 10 µL of distilled water. PCR amplification was performed in a thermocycler (Eppendorf, Hamburg, Germany) under the following thermal cycling conditions: an initial denaturation step at 95 °C for 5 min; 25 cycles including denaturation at 95 °C for 30 s, annealing (temperature/time according to Table 1), and elongation at 72 °C for 1 min; and a final elongation step at 72 °C for 10 min. PCR products were then checked by agarose gel electrophoresis in terms of amplification or non-amplification of the desired gene. PCR products were electrophoresed on 1% (weight/volume) agarose gel in an electrophoresis machine with a voltage of 100 volts for one hour. DNA bands were observed and recorded using a gel documentation device (Thermo Fisher, USA) with ultraviolet (UV) radiation. In the last step, the size of the PCR product was estimated by comparing the position of the amplified DNA band with the size of the marker bands.

Table 1. Specific primer sequence used in PCR test

Target Gene(s)	Primer Sequence(5→ 3')	Product Size(bp)	Annealing Temperature (°C/s)	References
ompW	F: CACCAAGAAGGTGACTTTATTGTG R: GAACTTATAACCACCCGCG	588	55 /30	[22]
ctxA	F: CTCAGACGGGATTTGTTAGGCACG R: TCTATCTCTGTAGCCCCTATTACG	301	58 /60	[23]
toxR	F: CCTTCGATCCCCTAAGCAATAC R: AGGGTTAGCAACGATGCGTAAG	779	60/ 60	[7]
rtxA	F:CTGAATATGAGTGGGTGACTTACG R: GTGTATTGTTCGATATCCGCTACG	418	56 /30	[24]
tcpA	F: CACGATAAGAAAACCGGTCAAGAG R: TTACCAAATGCAACGCCGAATG	620 420	60 /60	[25]

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hlyA	F: AGATCAACTACGATCAAGCC R: AGAGGTTGCTATGCTTTCTAC	1677	40/55	[8]
chxA	F: GAAAGAGGGTTCACGCCATAA R: CGGGATGGTGAGTGACATAATC	554	58 /30	In study
ace	F: TAAGGATGTGCTTATGATGGACACCC R: CGTGATGAATAAAGATACTCATAGG	316	54 /30	[13]

2. 5. Antimicrobial sensitivity of isolates

Antibiotic susceptibility of *V. cholerae* isolates was determined by disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018b) [10, 26]. First, isolates were cultured in sterile saline for 24 hours to obtain a turbidity equivalent to 0.5 McFarland. Then the bacterial suspension was cultivated linearly and uniformly on Mueller-Hinton agar (MHA) plates using a sterile swab. Then antibiotic discs (Podten Teb Company, Iran) suggested to check the antibiotic sensitivity of *V. cholerae* isolates were placed on the inoculated MHA medium. These discs included: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), amikacin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (10 µg), imipenem (10 µg), meropenem (10 µg), piperacillin (100 µg), levofloxacin (5 µg), ofloxacin (5 µg), and tetracycline (30 µg). The plates were kept in a greenhouse at 37 °C for 48 hours. Then the growth inhibition zone diameter of each antibiotic was measured and according to the CLSI standard, *V. cholerae* isolates were classified into three categories: sensitive, semi-sensitive, and resistant to each antibiotic. The antibiogram of the isolates was performed in three replicates.

3. Results

Based on the results, a total of 17 (14.16%) *V. cholerae* isolates were identified, of which seven (14%, 7 of 50 samples) strains were isolated from vegetables, including four (57.15%) isolates from rural and three (42.58%) isolates from urban areas, and 10 (14.28%, 10 of 70 samples) strains were isolated from agricultural water, including eight (80%) isolates from rural and two (20%) isolates from urban areas. Serotyping of the isolates using polyvalent O1 antiserum showed that all the isolates belonged to non-O1 serotype.

Based on the results, 100% of the isolates harbored the *ompW* gene and were confirmed to be *V. cholerae*. Out of 17 isolates, 88.23% had the *toxR* gene, 58.82% had the *rtxA* gene, 47.05% had *hlyA* the gene, 5.88% had the *chxA* gene, and 100% lacked *ctxA*, *ace*, and *tcpA* genes. Also, in five isolates (29.41%), all three genes *rtxA*, *toxR*, and *hlyA* were present simultaneously, and both *rtxA* and *toxR* genes were present in six isolates (35.29%) (Figures 1 and 2).

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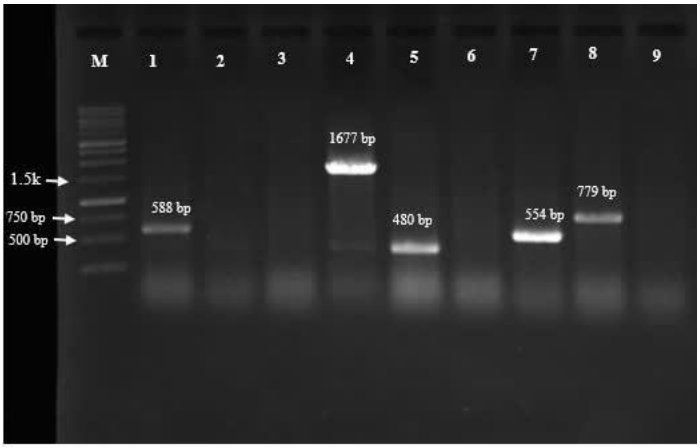

Fig. 1. Electrophoresis of PCR products of virulence genes in some environmental strains of *V. cholerae* non-O1: lane M: 1 kb DNA ladder, lane 1: positive sample for *ompW* gene (588 bp band), lanes 2, 3, 6: negative sample for *tcpA*, *ace*, and *ctxA* genes, lane 4: positive sample for *hlyA* gene (1677 bp band), lane 5: positive sample for *rtxA* gene (480 bp band), lane 7: positive sample for *chxA* gene (554 bp band), lane 8: positive sample for *toxR* gene (779 bp band), lane 9: negative control

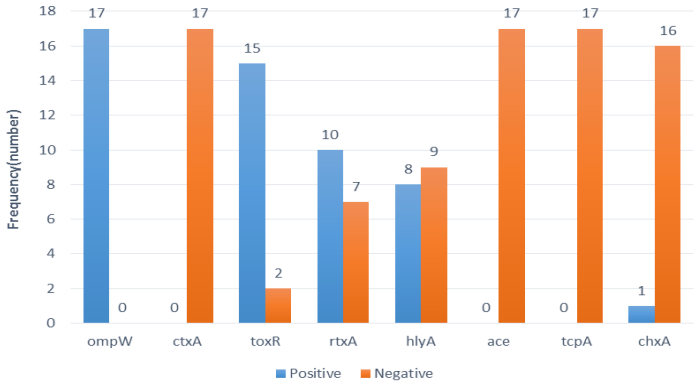

Fig. 2. Frequency of *V. cholerae* non-O1 virulence genes, isolated from water and vegetable samples

The antibiotic susceptibility testing results showed that 100% of *V. cholerae* non-O1 isolates were sensitive to amikacin, chloramphenicol, ciprofloxacin, cefepime, cefotaxime, gentamicin, levofloxacin, ofloxacin, and tetracycline antibiotics. The highest intermediate susceptibility was related to amoxicillin and meropenem antibiotics (47.05%), and the highest resistance was related to ampicillin and amoxicillin antibiotics (34.29%). Also, 17.46% of the isolates were resistant to cefuroxime, 11.76% to imipenem, and 5.88% to cefoxitin and trimethoprim-sulfamethoxazole antibiotics (Table 2). One isolate (5.88%) showed multiple resistance to four antibiotics: ampicillin, amoxicillin, imipenem, and cefoxitin.

Table 2. Antibiotic susceptibility of 17 *V. cholerae* non-O1 isolates from water and vegetable samples

Antibiotic	*Vibrio cholerae* N (%)		
	S	**I**	**R**
Ampicillin (AM)	7 (41.17)	4 (23.52)	6 (35.29)
Amoxicillin-clavulanic acid (AMC)	3 (17.64)	8 (47.05)	6 (35.29)
Amikacin (AN)	17 (100)	0 (00)	0 (00)

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Chloramphenicol (C)	17 (100)	0 (00)	0 (00)
Ciprofloxacin (CP)	17 (100)	0 (00)	0 (00)
Cephothaxim (CTX)	17 (100)	0 (00)	0 (00)
Ceftazidime (CAZ)	13 (76.47)	4 (23.52)	0 (00)
Cefoxitin (FOX)	15 (88.23)	1 (5.88)	1 (5.88)
Cefepim (PEP)	17 (100)	0 (00)	0 (00)
Gentamycin (G)	17 (100)	0 (00)	0 (00)
Trimethoprim-sulfamethoxazole (SXT)	16 (94.11)	0 (00)	1 (5.88)
Imipenem (IPM)	14 (82.35)	1 (5.88)	2 (11.76)
Piperacillin (PIP)	12 (70.58)	5 (29.41)	0 (00)
Levofloxacin (LEV)	17 (100)	0 (00)	0 (00)
Meropenem (MEN)	9 (52.94)	8 (47.05)	0 (00)
Ofloxacin (OFX)	17 (100)	0 (00)	0 (00)
Tetracycline (TE)	17 (100)	0 (00)	0 (00%)
Cefuroxime (X)	10 (58.82)	4 (23.52)	3 (17.64)

S: Susceptible, I: inter susceptible, R: resistant

4. Discussion and conclusion

Diarrhea caused by microorganisms is the most common infectious disease among people, especially among children. This disease is the second leading cause of death, accounting for about 10% of all deaths worldwide. Among infectious agents, bacteria and viruses are responsible for a significant proportion of acute diarrhea cases, and various reports have been published on the prevalence of diarrheal diseases in developing and developed countries [19, 27-30]. Therefore, in this study, the prevalence of *V. cholerae* bacteria in agricultural water and vegetables and their virulence genes and antibiotic resistance patterns were investigated for the first time in Qom province. Different methods are used to detect bacterial infections [31-33]. In this study, in addition to biochemical investigation, PCR method was also performed to confirm the isolated strains.

Diarrhea caused by *Vibrio* species has increased as an important disease in recent decades in developed and developing countries [8, 34, 35]. *V. cholerae* is known as a faecal-oral pathogen, and surface water and raw food are the most important habitats and sources of transmission of this bacterium to humans [36]. Considering that in Iran, sewage water is usually used to irrigate vegetables, consumers are potentially exposed to infections caused by this bacterium. The quality and quantity of drinking water, agricultural water, and environmental or recreational water could be affected by climate changes. Evidence shows that the prevalence rate of this infection is significantly higher in naturally hot areas with salty soils, and the presence of this bacterium is mainly reported in warmer months and areas with poor hygiene [37, 38]. Qom province is also one of the endemic areas of *V. cholerae* bacteria due to its hot climate and presence of salty waters [19].

V. cholerae O1 in cholera endemic areas is difficult to identify for two reasons: the larger number of *V. cholerae* non-O1 strains and the hypothesis that *V. cholerae* is viable but non-Culturable (VBNC). Therefore, *V. cholerae* non-O1 is easily isolated from environmental samples [39, 40]. In this study, the prevalence of this bacterium was 14.16%, and all the strains isolated from water and vegetable samples belonged to non-O1 serotype, which confirm the above hypotheses.

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Various studies have been conducted in Iran and the world to identify *V. cholerae* in different waters. In a study by Momtaz et al. (2013), the prevalence of *V. cholerae, Escherichia coli*, and *Salmonella* detected in mineral and tap water in Isfahan province was 0.66, 4.58, and 0.89%, respectively [41]. In another study by Fraga et al. (2007), the prevalence rate of *V. cholerae* non-O1, non-O139 in water and plankton isolated from freshwater and saltwater was 36.10% [42], which is much higher compared to the prevalence rate obtained in the present study for this bacterium in water samples (14.28%). There is little research in the world and Iran on the prevalence of *V. cholerae* non-O1 in vegetables. Azimi Rad et al. (2021) examined different types of raw and fresh vegetables collected from agricultural lands, supermarkets, hypermarkets, and greengrocers in different areas of Tehran for the presence of foodborne pathogens, including *V. cholerae*, and *V. cholerae* was found in none of the samples [43]. However, in the present study, seven (14%) vegetable samples were contaminated with this bacterium. In a study in 2022 in Indonesia, the presence of *V. cholerae* in fruits and vegetables offered in stores was investigated, and the prevalence of *V. cholerae* O1 and non-O1 among the investigated vegetables was found to be 11.84 and 0.00%, respectively [44]. Hounmanou et al. (2016) in Tanzania reported the prevalence of *V. cholerae* O1 in fish, sewage, and vegetables irrigated with sewage as 36.70, 21.70, and 23.30%, respectively [45].

Several studies in Iran have investigated the causes of cholera and its relationship with the consumption of water and vegetables. In a study by Khazaei et al. (2005), it was found that 89.10 and 14.90% of infected patients consumed surface and underground water, respectively [46]. During the cholera outbreak in Alborz city in 2016, it was found that 76.60% of cholera patients consumed vegetables in the three days before the disease [47]. Therefore, it is most likely that water and vegetables are the primary sources of these bacteria that cause cholera in people.

In general, environmental strains of *V. cholerae* non-O1, non-O139 are unable to cause any outbreaks and pseudo-epidemics, but there is evidence of the presence of virulence genes in these environmental strains, which has attracted attention. The emergence of non-O1 pathogenic strains and their transformation into O1 may pose major risks to public health [48]. Many studies have been conducted on the identification of virulence genes in *V. cholerae* non-O1 bacteria [49-51]. In the present study, the prevalence of essential toxin gene *toxR* and auxiliary gene *hlyA* in *V. cholerae* non-O1 isolates was 88.23 and 47.05%, respectively. In a study by Ceccarelli et al. (2015), among 389 non-O1, non-O139 strains isolated from the sediments and waters of Chesapeake Bay (Maryland, USA), the prevalence of *hlyA*, *toxR*, *ace*, *ctxA*, and *tcpA* genes was 89, 80, 1, 1, 0%, respectively [17]. In another study in South Africa, Mavhungu et al. (2023) identified *V. cholerae* bacteria in 27% of sewage effluents, and 78, 44, and 44% of these strains harbored *toxR*, *hlyA*, and *tcpA* genes, respectively [52]. These results regarding the prevalence of *toxR* and *hlyA* genes are very similar to the results obtained in the present study. In a study in China, the prevalence of *V. cholerae* non-O1 isolated from two freshwater rivers in Zhejiang province was 39.10 and 47.30%, respectively; 99 and 98% of these isolates harbored *toxR* and *hlyA* genes, and the

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rest of the virulence genes were present in a small number of strains [53]. Schwartz et al. (2019) isolated 100 *V. cholerae* non-O1, non-O139 strains from the Baltic Sea and the North Sea of Germany, all the strains harbored the *toxR* gene, and 97% harbored the *hlyA* gene [50]. Based on the results of several studies mentioned above and the present study, it seems that a high percentage of *V. cholerae* non-O1 strains found in open water and seas harbor *toxR*, *rtxA*, and *hlyA* genes, and finding out the cause of this problem requires more research.

Antibiotic resistance among different organisms, especially *V. cholerae*, is an important challenge in the field of health [54, 55]. Lue et al. (2021) investigated the antibiotic resistance of 115 isolates of *V. cholerae* non-O1, non-O139, and the highest resistance of these isolates was against ampicillin (47.83%) and cefazolin (67.07%) [53]. Also, Chen et al. (2021) showed that most of the isolates were resistant to moxafloxacin (74.90%), and 59.10% of them were resistant to ampicillin [56]. In this research, the highest antibiotic resistance was against ampicillin and amoxicillin (35.29%). Based on the results of this study and other research, it seems that resistance to ampicillin is more common among *V. cholerae* isolates. In general, due to the presence of virulence genes in *V. cholerae* non-O1 isolates, it could be said that these bacteria in water and vegetables are an important reservoir of virulence genes, and *V. cholerae* non-O1 is potentially pathogenic. These findings strengthen the link between environmental reservoirs and human infections. Therefore, continuous monitoring of the healthiness of agricultural water and vegetables by public health assessment teams is very important to prevent the spread of these pathogens.

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مقاله علمی–پژوهشی

ارزیابی فراوانی باکتری های *ویبریو کلرا* جدا شده از آب و سبزیجات و حضور ژن های حدّت و الگوی مقاومت آنتی بیوتیکی در استان قم

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چکیده

ویبریو کلرا یکی از پاتوژن های مهم انسانی است که از طریق آب و غذای آلوده منتقل می شود. بیماری های ناشی از *ویبریو کلرا* در استان قم به دلایل شرایط آب و هوایی خاص اندمیک است. این مطالعه با هدف بررسی میزان شیوع *ویبریو کلرا* در آب و سبزیجات استان قم، حضور ژن های حدّت و بررسی الگوی مقاومت آنتی بیوتیکی آن ها انجام شد. در یک دوره دو ساله (۱٤۰۰-۱۳۹۹) ، ۱۲۰نمونه آب های کشاورزی(۷۰ نمونه) و سبزیجات (۵۰ نمونه) تولید شده در استان قم جمع آوری شدند. نمونه ها روی محیط اختصاصی کشت داده شدند. کلنی های مشکوک رنگ آمیزی گرم شده و آزمون های بیوشیمیایی روی آنها انجام شد و با آزمایش سرولوژی، سروتیپ *ویبریو کلرا* شناسایی شد. سپس بررسی حضور ژن های حدّت با روش PCR و همچنین ارزیابی الگوی مقاومت آنتی بیوتیکی در جدایه ها با روش انتشار در آگار بررسی شد. بطور کلی از ۱۷ نمونه (۱٤/۱٦٪) باکتری *ویبریو کلرا* جدا شد که همه non-O1 بودند. میزان آلودگی آب و سبزیجات به این باکتری به ترتیب ۱٤/۲۸٪ (۱۰مورد) و ۱٤/۰۰٪ (۷ مورد) بود. در ارزیابی ملکولی، میزان فراوانی ژن های حدّت شامل *toxR* (۲۳ /۸۸٪)، *rtxA* (۵۸/۸۲٪)، *hlyA* (٤۷/۰۵٪)، *chxA* (۵/۸۸٪) بود، و ۱۰۰ ٪ جدایه ها فاقد ژن های *ctxA*، *ace* و *tcpA* بودند. بیشترین مقاومت آنتی بیوتیکی مربوط به آمپی سیلین و آموکسی سیلین (۳٤/۲۹ ٪) و سپس به ترتیب سفوروکسیم (۱۷٪/٤٦)، ایمی پنم (۱۱٪/۷٦)، سفوکسیتین و تری متوپریم–سولفومتوکسازول (۵٪/۸۸) بودند. نتایج این مطالعه نشان داد که *ویبریو کلرا* non-O1 در آب و سبزیجات استان قم وجود دارد. لذا به عنوان یک منشاء مهم ایجاد بیماری برای انسان، نظارت بهداشتی مستمر بر آب و سبزیجات و ضد عفونی مناسب این مواد غذایی دارای اهمیت زیادی است.

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