

## Moleculartyping and antibiotic resistance pattern of *Aeromonas hydrophila* isolated from children with diarrhea in Qom, Iran

Pegah Shakib<sup>1</sup>, Mohammad Reza Zolfaghari<sup>2\*</sup>, Mina Hosseini<sup>2</sup>, Fatemeh Mosaei<sup>2</sup>, Seyed Soheil Aghaei<sup>2</sup>

<sup>1</sup>Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

<sup>2</sup>Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran

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### ABSTRACT

**Background and Objectives:** Today there are reports of the spread of multiple antibiotic resistance in *Aeromonas* spp., which can be opportunistic pathogens and can cause infection in the host. This study aimed to investigate ERIC-PCR and BOX-PCR typing, drug resistance pattern, and tetracycline, sulfonamide, and fluoroquinolone resistance genes in *Aeromonas hydrophila* collected from diarrhea samples of children in Qom City, Iran.

**Materials and Methods:** In our cross-sectional descriptive study, isolates of *A. hydrophila* isolated from children's diarrhea samples at Hazrat Masoumeh Hospital in Qom were evaluated between 2018 and 2020. Once isolates confirmed, the level of antimicrobial resistance of the strains was determined by the disk diffusion assay following the recommendations of Clinical and Laboratory Standards Institute (CLSI). The genes of *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *sul1*, *sul2*, *Edq1qc*, *qnrA*, *qnrB*, *qnrS* were studied using the PCR method. Then the genetic diversity of the isolates was investigated by ERIC-PCR and box-PCR. Finally, data analysis was done with SPSS version 16.

**Results:** From 100 *A. hydrophila* strains, the highest resistance, and sensitivity were related to ampicillin (92%) and azithromycin (96%), respectively. The frequency of *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *sul1*, *sul2*, *Edq1qc*, *qnrA*, *qnrB*, *qnrS* genes was 23, 24, 27, 23, 21, 26, 10, 22, 8, 11, 15%, respectively. With ERIC-PCR and BOX-PCR techniques, all isolates could be typed, so that 8 genetic patterns were determined in ERIC-PCR and 10 genetic patterns were determined in BOX-PCR. A significant relationship was found between cefotaxime and *tetA* ( $p = 0.012$ ), ciprofloxacin and *tetC* ( $p = 0.018$ ), cefotaxime and *qnrA* ( $p = 0.04$ ), and also between tetracycline resistance genes and both sulfonamide genes ( $p > 0.05$ ).

**Conclusion:** For the first time this study revealed resistance genes for tetracycline, sulfonamide, and fluoroquinolones in *A. hydrophila* isolated from children's diarrhea samples in Qom. Even though these antimicrobial resistance genes are present in our study and bacteria are resistant to different types of antimicrobials, it can be predicted that the abundance of these genes will increase in the future. We also observed that *A. hydrophila* isolates had high genetic diversity.

**Keywords:** *Aeromonas hydrophila*; Antibiotic sensitivity pattern; Polymerase chain reaction (PCR); Molecular typing

### INTRODUCTION

*Aeromonas hydrophila* is facultatively anaerobic, heterotrophic, sporeless, motile gram-negative ba-

cilli (1). They produce amylase, lipase, phosphatase, proteinase, and nuclease enzymes and several virulence factors are involved in the pathogenicity. *A. hydrophila* is the cause of diarrhea, infection of the

\*Corresponding author: Mohammad Reza Zolfaghari, Ph.D, Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran. Tel: +25-37780001 Email: mreza.zolfaghary@gmail.com

inner membrane of the heart, meningitis, soft tissue infection, and bacteremia (1, 2). Previously, *Aeromonas* spp was considered an organism with low virulence, but its role as a human pathogen is being recognized more and more (3). Studies have shown that *Aeromonas* spp is an important diarrheal pathogen in Iran (4, 5).

The prevalence of *A. hydrophila* and its clinical significance have been reported worldwide with considerable variation. In developing countries, especially in Asia and Africa, *Aeromonas* spp have been recognized as important enteric pathogens associated with diarrheal diseases, particularly in children. According to the investigations conducted in India and China, the isolation rate of *Aeromonas* spp has been reported to be 5% to 15% in diarrhea cases, while lower prevalence rates have been recorded in European countries (6). In Iran, *Aeromonas* spp have been identified as one of the bacterial agents in gastroenteritis. In addition, the emergence of multi-drug-resistant *A. hydrophila* strains has been reported globally, raising concerns about the limitations of treatment and its impact on public health (7). These results highlight the need for regular monitoring of its distribution and resistance in various geographical regions.

Antibiotics such as tetracycline, chloramphenicol, and sulfonamide are usually used to treat *A. hydrophila* infections. Today, there are reports about the occurrence of resistance to different families of antibiotics in *A. hydrophila*. The emergence of resistance in bacteria causes treatment problems such as lengthening the treatment period of patients, increasing costs, and increasing the death rate of patients (7). Tetracycline resistance genes are mainly located on plasmids or transposons and are transferred via conjugation. The resistance genes can be *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(D)*, *tet(G)*, *tet(H)*, and *tet(L)* current genes or *tet(M)*, *tet(O)*, *tet(S)* and *tet(W)* ribosomal protected genes (8). Bacterial resistance to sulfonamides is usually caused by *sulI*, *sul2*, and *sul3* genes. which is found in Gram-negative bacteria, e.g., *Salmonella* spp and *Escherichia coli* in both humans and animals. The *sul3* gene has been found more in chickens than in other animals (9, 10). The gene responsible for creating quinolone resistance was named *qnr*, comprising *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrVC*, and *qnrS* genes. The purified *qnr* protein protects the DNA gyrase and topoisomerase IV enzymes against the action of quinolone antibiotics,

giving rise to resistance (11).

Molecular methods like enterobacterial repetitive intergenic consensus sequence (ERIC), repetitive extragenic palindromic sequence (REP), and the BOX have been used for typing and differentiating bacterial strains for the last decade (12). These techniques are also used to determine the transmission factor and evolutionary relationship between isolates, which are very fast, sensitive, and inexpensive. So, by molecular typing of strains and finding closely related strains, researchers have given the possibility of transmission of a common strain between patients and clonal spread, and after that, more practical and precise solutions can be followed to distinguish closely related strains (13, 14). Thus, our study is designed to study ERIC-PCR and BOX-PCR typing, drug-resistance pattern, and resistance genes to tetracycline, sulfonamide, and fluoroquinolones in *A. hydrophila* isolated from diarrhea samples of children in Qom, Iran.

## MATERIALS AND METHODS

**Ethics approval.** The current research was approved by the Ethical Committee of Islamic Azad University, Qom branch (ethics code: IR.IAU.QOM.REC.1400.074).

**Collection of *A. hydrophila* isolates.** All *A. hydrophila* strains were isolated from feces samples taken from patients in Hazrat Masoumeh Children's Hospital in Qom Province from 2018 to 2020. In this study, sampling was conducted using convenience sampling method. The sample size (n=100) was determined based on the availability of confirmed isolates during the study period. To confirm the genus and species of the isolates collected after performing heat staining tests, motility test, oxidase test, catalase, indole, nitrate recovery, starch hydrolysis, DNase and gelatinase, citrate consumption, and MR-VP, the *rpoD* gene (15) was detected with F: 5'-CCGGCTTCATCGATCCCAAC-3' and R: 5'-GG-GCGATCGACAGACCGG-3' primer (designed in current study). The *rpoD* gene was amplified using the following program: initial denaturation at 95°C for 5 min; 35 cycles of denaturation, annealing, and extension at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, respectively, and a final extension at 72°C for 5 min.

**Antimicrobial resistance pattern of strains.** To measure the pattern of sensitivity and resistance to antibiotics, we accomplished the Kirby-Boer test by disk-diffusion assay on Muller-Hinton-Agar (MHA) medium (Merck, Germany) and based on the relevant CLSI (16). The discs used in this test consisted of cef-tazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), azithromycin(15 µg), imipen-em (10 µg), ampicillin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), cotrimoxazole (30 µg), sulfa-methoxazole (1.25/23.75 µg), and tetracycline (30 µg), all are from Rosco, Denmark.

**Tracking antibiotic resistance genes.** In this study, the genes *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *sul1*, *sul2*, *Edq1qc*, *qnrA*, *qnrB*, and *qnrS* were detected by specific primers arranged in our study (listed in Table 1 and by PCR technique. Next, the product of each reaction was assessed after electrophoresis on agarose gel and staining by DNA-safe stain.

**ERIC-PCR.** To perform ERIC-PCR, the final volume of PCR solution was considered equal to 25 µl. Therefore, 18 µl Master Mix, 25 pmol of from each

primer (Table 2), and 5 µl DNA extracted were used for each reaction. For the observation of ERIC bands, ERIC-PCR products were run on 2% agarose gel (17).

**BOX-PCR.** To perform BOX-PCR, the final volume of PCR solution was considered equal to 25 µl. Therefore, 18 µl Master Mix, 25 pmol primer, 2 units of Taq DNA polymerase enzyme, and 5 µl DNA were used for each reaction. To observe BOX bands, BOX-PCR products were electrophoresed on 2% agarose gel (18).

The analysis of the data obtained from ERIC-PCR and BOX-PCR bands was done by Gelcompare version 2 software. The photos taken from the gel doc were first converted to JPEG format, and then the Dice similarity coefficient was used to determine the similarity of the strains and the UPGEMA (Unweighted pair-group method with arithmetic mean) method was applied to draw the phylogeny tree (17, 18).

**Data analysis.** The data were analyzed using SPSS-16 program, Fisher's exact test, graphing in the program Excel and the chi-square statistical test. The significance level of gene groups in isolates were considered to be  $P \leq 0.05$ .

**Table 1.** Primers used in this study

Primers	Sequence of primers	TM (C°)	Size (bp)
tetA-F	GTAATTCTGAGCACTGTCCG	50°C	957
tetA-R	CTGCCTGGACAACATTGC	50°C	
tetB-F	CTCAGTATTCCAAGCCTTTG	46°C	436
tetB-R	CTAAGCACTTGTCTCCTGTT	46°C	
tetC-F	TCTAACAATGCGCTCATCG	50°C	587
tetC-R	TTGAAGGCTCTCAAGGGC	50°C	
tetD-F	ATTACTGCTGGACGCG	50°C	1124
tetD-R	CTGATCAGCAGACAGATTGC	50°C	
tetE-F	GTGATGATGGCACTGGTC	50°C	1199
tetE-R	CTCTGCTGTACATCGCTCT	50°C	
Edq1qc-F	GTGATGATGGCACTGGTC	49°C	224
Edq1qc-R	AAGCTTTTGCCCATGAAG	49°C	
qnrA-F	AGGATTTCTCACGCCAGG	50°C	576
qnrA-R	GCCAGGCACAGATCTTGA	50°C	
qnrB-F	GGMATHGAAATTCGCCACTG	52°C	650
qnrB-R	TTTGCYGYCCGAGTCGAA	52.5°C	
qnrS-F	GCAAGTTCATTGAACAGGGT	50°C	427
qnrS-R	CTAAACCGTCGAGTTCGG	50°C	
Sul1-F	TTCCTGACCCTGCGCTCTATCCC	63°C	308
Sul1-R	CGCAAGGCTCGCTGGACCC	62°C	
Sul2-F	GCGCGTAAGCTGATGGCCGAG	63.5°C	619
Sul2-R	CGGCGGCGCAAGCTCTG	64.5°C	

**Table 2.** The sequence of primers for ERIC/BOX- PCR

Primer	Primer sequence	TM (C°)	Size (bp)	Reference
ERIC PCR-F	ATGTAAGCTCCTGGGGATTAC	52	100-3500 bp	(17)
ERIC PCR-R	AAGTAAGTGACTGGGGTGAGCG			
BOX-PCR-P	TACGGCAAGGCGACGCTGACG	48	100-3500 bp	(18)

## RESULTS

During the sample collection period, 100 isolates of *A. hydrophila* were isolated. Identification of *A. hydrophila* was performed using standard biochemical tests. The isolates were Gram-negative, oxidase-positive and catalase-positive bacilli. They were capable of fermenting glucose, reducing nitrate to nitrite, motile and indole-positive. Among the 100 isolates, the highest frequency was related to male children (57%). Also, most of the children were under 3 years old (46%) (Table 3).

For the primary identification of the bacterium, a completely conserved gene called *rpoD* was used, which is conserved in this bacterium, and the results prove this bacterium. The results of electrophoresis of the samples in 1% agarose gel in the primary PCR reaction showed a band of 594 bp related to the amplification of the desired gene, and 100% of the samples showed a positive response by *rpoD* detection.

**Antibiotic sensitivity and resistance of *A. hydrophila* isolates.** The highest resistance value was associated with ampicillin (92%), while nalidixic acid (51%) was associated with the lowest resistance value according to the results of the frequencies. The highest sensitivity was related to azithromycin (96%), cotrimoxazole (95%) and gentamicin (94%), respectively (Table 4).

**Frequency of resistance genes and their relationship with antibiotic resistance pattern.** From the 100 strains, 23 isolates carried the *tetA*, 24 isolates carried the *tetB*, 27 isolates carried the *tetC*, 23 isolates carried the *tetD*, 21 isolates carried the *tetE*, 26 isolates carried the *sul1*, 10 isolates carried the *sul2*, 22 isolates carrying the *Edq1qc*, 8 isolates carrying the *qnrA*, 11 isolates carrying the *qnrB* and 15 isolates carrying the *qnrS* gene (Fig. 1).

Based on the results, a significant relationship exists among cefotaxime and *tetA* ( $P=0.012$ ), ciprofloxacin and *tetC* ( $P=0.018$ ). The positive samples were

**Table 3.** Frequency of age and gender distribution in the studied children

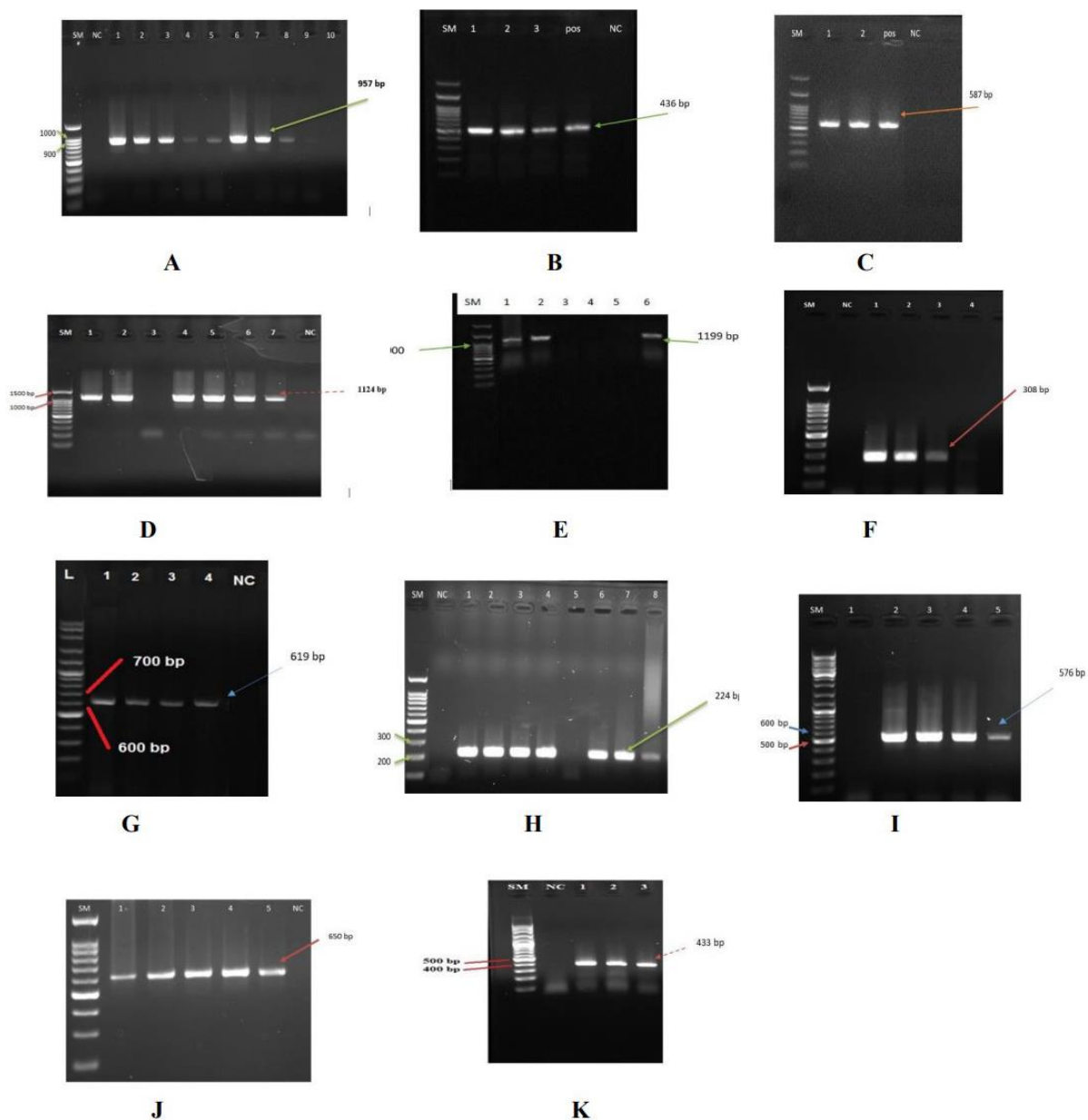
Variable		Frequency (%)
Gender	Female	43 (43)
	Male	57 (57)
Age	Less than 3 years	46 (46)
	3-6 years	26 (26)
	6-10 years	28 (28)

**Table 4.** Antibiotic sensitivity and resistance of *A. hydrophila* isolates

Antibiotics	Resistance Susceptible Intermediate		
	(N=%)	(N=%)	(N=%)
Ceftazidime (30 µg)	48	41	11
Cefotaxime (30 µg)	29	54	17
Ciprofloxacin (5 µg)	12	44	44
Cefepime (30 µg)	45	55	-
Azithromycin (15 µg)	4	96	-
Imipenem (10 µg)	10	86	4
Ampicillin (10 µg)	92	8	-
Gentamicin (10 µg)	4	94	2
Nalidixic Acid (30 µg)	51	49	-
SXT (1.25+23.75 µg)	5	95	-
Tetracycline (30 µg)	20	79	1

cefotaxime (13.6%) and ciprofloxacin (25.9%) resistance to *tetA* and *tetC*, respectively. Tetracycline was found to have a significant relationship with both sulfonamide genes ( $P<0.05$ ). The rate of resistance to tetracycline compared to *Sul1* (7.7%) and the rate of resistance to *Sul2* (60%) were positive samples. There was a significant correlation between *qnrA* and cefotaxime ( $P=0.04$ ). Cefotaxime resistance to this gene was (25%) in positive samples.

A high level of correlation was observed between the presence of specific antibiotic resistance genes and the antibiotic resistant pattern, as revealed by statistical analysis. Significant relationships were observed between cefotaxime resistance and *tetA* ( $p = 0.012$ ), ciprofloxacin resistance and *tetC* ( $p = 0.018$ ), and ce-



**Fig. 1.** PCR product of the studied genes. (A) *tetA*; lane 1: positive control, lanes 2-10: samples. (B) *tetB*; lanes 1-3: samples. (C) *tetC*; lanes 1-2: samples. (D) *tetD*; lane 7: Positive control; lanes 1-6: samples. (E) *tetE*; lane 5: negative control; lane 6: positive control, Llanes 1-4: samples. (F) *sulI*; lane 1: positive control, lanes 2-4: samples. (G) *Sul2*; lane 4: positive control, lanes 1-3: samples. (H) *Edq1qc*; lane 1: positive control; lanes 2-8: samples. (I) *qnrA*; lane 2: positive control; lanes 3-5: samples. (J) *qnrB*; lane 5: positive control; lanes 1-4: samples. (K) *qnrS*; lane 1: positive control; lanes 2 and 3: samples. SM: marker 100 bp; NC: negative control, Pos: positive control.

fotaxime resistance and *qnrA* ( $p = 0.04$ ). In addition, tetracycline resistance was significantly associated with sulfonamide resistance genes ( $p < 0.05$ ).

**ERIC-PCR results.** *A. hydrophila* ( $n = 100$ ) was analyzed for their genetic diversity by ERIC-PCR. In general, 8 different genetic patterns are observed from ER-

IC-PCR (Fig. 2). Most of them with a similar pattern were placed in category III, and the ATCC 7966(T) strain is also in this category and cluster. Three isolates in Category I, 7 isolates in Category II, 9 isolates in Category III with positive control, 1 isolate in Category IV, 4 isolates in Category V, 7 isolates in Category VI, 1 isolate in Category VII and VIII There

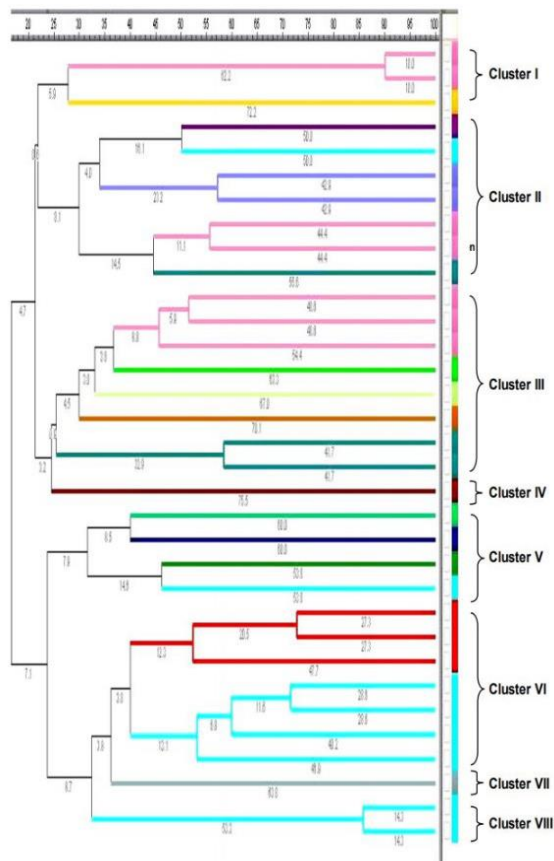


Fig. 2. Dendrogram of UPGMA for *A. hydrophila* strains based on ERIC-PCR

are 2 isolates. To classify the strains into branches or clusters, the similarity percentage of 50-100 was considered and the strains that did not have the minimum similarity were considered as single type. Also, strains with 100% similarity were considered as common type, and strains with similarity higher than 90% were considered as a clone. For example, category IV is the single type and category VIII is a common type.

**BOX-PCR results.** All samples were evaluated to check the genetic diversity by the BOX-PCR method. In general, 10 different genetic patterns were observed from BOX-PCR (Fig. 3). Most of them with a similar pattern were placed in category VI, and the ATCC 7966 (T) strain was also included in this category and cluster. 2 isolates in category I, 2 isolates in category II, 3 isolates in category III with positive control, 4 isolates in category IV, 4 isolates in category V, 12 isolates in category VI, 14 isolates in category VII. There were 21 isolates in VIII, 1 isolate in category IX and 1 isolate in category X. To classify the strains in this method also into branches or clusters, the similar-

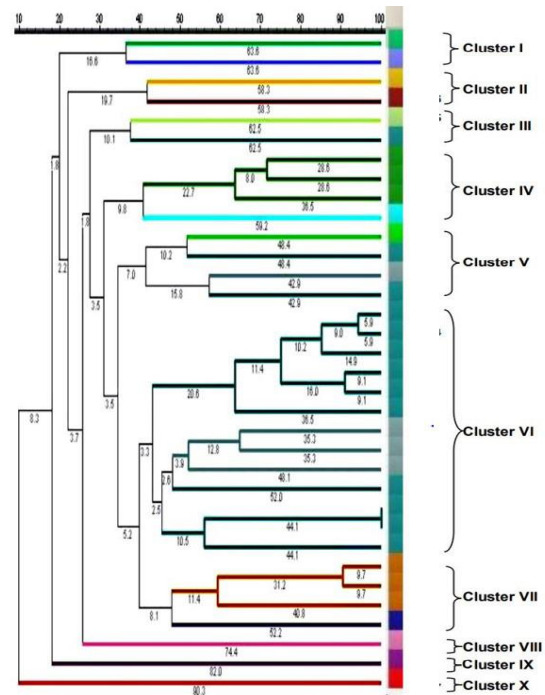


Fig. 3. BOX-PCR dendrogram in *A. hydrophila* strains by UPGMA

ity percentage of 50-100 was considered and the strains that did not have the minimum similarity were considered as single type. Also, strains with 100% similarity were considered as common type, and strains with similarity higher than 90% were considered as a clone. Category VIII was a single type and category III was the common type.

## DISCUSSION

Today, with the spread of antibiotic resistance in bacteria, finding an effective antibiotic to treat bacterial infections is important in reducing costs, reducing the treatment period, and controlling infection. Through different mechanisms, bacteria acquired resistance to different antibiotics, such as by mutations of their own chromosomal genes and by horizontal gene transfer (19, 20). In a study in Hamedan (2016), Iran, the investigation of the antibiotic sensitivity of *A. hydrophila* isolated from children with diarrhea (2005) showed that all the isolates were susceptible to gentamicin, cotrimoxazole, chloramphenicol, and amikacin, whereas all the isolates were resistant to erythromycin (21). Their outcome is consistent with our study and the isolates of the present study also

showed the best response to gentamicin and cotrimoxazole. In another study conducted in Iran, 100% of the strains showed resistance to ampicillin and all were sensitive to both gentamicin and ciprofloxacin (22). Therefore, in this study, ciprofloxacin and gentamicin were the most effective drugs against the examined isolates because all the isolates were sensitive to these antibiotics. The results of this report are consistent with our study and the isolates of the present study also showed the best response to gentamicin. In our study, the highest resistance was related to ampicillin (92%), followed by nalidixic acid (51%). The highest drug sensitivity was also related to azithromycin 96%, cotrimoxazole 95%, and gentamicin 94% respectively.

In a study on the prevalence and identification of quinolone resistance genes on plasmids of *Aeromonas* spp. isolated from freshwater fish in South African, Chenia (2016) reported that the release genes of *qnrB* and *qnrS* were found in 41% and 24% of the samples, respectively (23). Kosikowska et al. (2022) studied the plasmid-mediated fluoroquinolone resistance genes in quinolone-susceptible *Aeromonas* spp. (phenotypically) collected from the surface freshwater reservoir in Poland. Their results demonstrated the presence of *qnrS* and *qnrA* in 34.8% and 14.5% of the isolates, respectively (24). Wimalasena et al. (2017) investigated the incidence of quinolone resistance genes in *Aeromonas* spp. collected from domestic turtles. They observed that the prevalence of *qnrS* gene was 68% (17.25), while that of *qnrB* gene was 8% (2.25) (25). In our study, the prevalence of *qnrA*, *qnrB*, *qnrS*, and *Edq1qc* genes were 8%, 11%, 15%, and 22% respectively. Among the studied isolates, the most common gene was *Edq1qc*, a finding that is in agreement with previous studies.

Likewise, *sul1* was reported as the most abundant sulfonamide resistance gene in identification of aerobic bacteria carrying both tetracycline and sulfonamide-resistant genes isolated from a meat processing factory reported, followed by *sul2*. However, the *sul3* was not found in any of the strains (26). In Tekedar et al., (2020) study identified the antimicrobial resistance in *A. veroni* from catfish, they reported the prevalence of *sul1* and *sul2* genes as 7.5% and 1.8% (27). In our study, the gene frequency of *sul2* and *sul1* was 26% and 10%, respectively, with *sul1* being the most common. This observation is in line with the findings of some previous studies. The difference in

the results of the presence of the investigated genes in our study with other studies depends on the type of sample and the size of the sample. In addition, the frequency of studied genes in *A. hydrophila* has been less, and most of the studies have been conducted on common bacterial agents that cause diarrhea, including *Salmonella*, *Shigella*, and *Escherichia coli*.

The present study investigated the first-time resistance genes to tetracycline, quinolones, and sulfonamides in Qom, Iran, and the connection between the presence of genes and antibiotic resistance. Based on the results, tetracycline relates significantly to both of the sulfonamide genes, between cefotaxime and *tetA*, ciprofloxacin and *tetC*, and between cefotaxime and *qnrA* gene ( $P < 0.05$ ). It can be concluded that these isolates carry several resistance genes, which can be an alarm for health and treatment centers.

Knowing the principles of epidemiology of microbial populations is important in microbiology (28). The introduction of bacterial strains that have a lethal pathogenic phenotype or strains that are resistant to several antibiotics has prompted researchers to identify and investigate the origin and genetic diversity of strains within a species (29). Today, molecular genetic techniques are an important tool in the identification of bacteria. With molecular typing, it is possible to determine the reservoirs of contamination or the spread of bacterial strains, and these methods also give us more knowledge about the principles of epidemiology and the evolution and spread of many bacterial diseases (30-32). In this study, the genetic diversity was investigated using ERIC-PCR and BOX-PCR methods (33, 34). The results suggested that the isolates can be typed by BOX-PCR and ERIC-PCR, and that BOX-PCR and ERIC-PCR could be used to differentiate the strains into genotypic groups. One of the most important features of BOX-PCR and ERIC-PCR is that they are fast, have appropriate discrimination power, and are carried out in a research laboratory. Besides, the two methods are able to take a quick and simple check of the genetic characteristics of bacteria. Therefore, this method can be widely used for epidemiological studies (35, 36).

Singh et al. (2010) by examining the diversity among isolates of *A. hydrophila* with the BOX-PCR method, it was determined that all the studied isolates showed band patterns of 300-500 bp in this study. The 20 isolates studied in this method were divided into 6 groups (14). Sing et al., examined 120

samples isolated from feces and diarrhea samples of gastrointestinal patients and environmental samples with RAPD-PCR, REP-PCR, and ERIC-PCR techniques on *A. hydrophila*, *A. bestiarum*, *A. salmonicida*, and *A. caviae*. It was found that there was no genetic similarity between environmental and hospital samples (14). In a study on *A. hydrophila* isolates obtained from human stool samples, RAPD-PCR analysis revealed multiple distinct genetic profiles, indicating a high level of genetic diversity and low similarity among the strains (37).

In the present study, 8 different genetic patterns were observed from ERIC-PCR. Most of them with the same pattern were placed in category III, and the strain ATCC 7966(T) was also in this category and cluster. 10 different genetic patterns were observed from BOX-PCR. Most of them with a similar pattern were placed in category VI, and ATCC 7966(T) strain was also included in this category and cluster. Thus, the studied strains had genetic diversity. The existence of different clones allows the emergence of new strains and their rotation. Therefore, identification of the strains and origin of infection is necessary in terms of predicting hygiene methods to control and limit infection. The present study is important by providing information about the genetic diversity of common bacteria in the development of molecular studies and its use in the health management of hospital infections.

In addition to its strengths, this study had several limitations. Among the limitations of this study are the relatively small number of isolates and their geographic location, which may limit the generalizability of the findings. In addition, only a select set of resistance genes related to tetracyclines, quinolones, and sulfonamides was examined, and other resistance mechanisms were not identified. Although associations between resistance genes and antibiotic susceptibility patterns were observed, these associations were based on statistical correlations and do not imply causality. Finally, molecular typing was accomplished using ERIC-PCR and BOX-PCR techniques, which, although rapid and cost-effective, have lower discriminatory power compared to more advanced molecular approaches.

## CONCLUSION

The outcome of clustering analysis and dendro-

gram drawing according to genetic similarities have separated the studied samples into 8 different genetic patterns by ERIC-PCR and 10 different genetic patterns by BOX-PCR. Thus, the studied strains had genetic diversity. Also, the incidence of resistance genes in our study indicates the need to pay attention to antibiotic resistance in *A. hydrophila* isolates, which shows the importance of modifying the way antibiotics are used. It can be concluded that these isolates carry several resistance genes, which can be an alarm for health and treatment centers.

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