*Original Article*

# Virulence and drug resistance of Aeromonas veronii isolated from shellfish

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***Abstract***

*Aeromonas veronii* (*A. veronii*) is one of the important zoonotic pathogens. The development of drug resistance and enrichment of resistant genes not only cause misery from bacterial diseases but also pose a threat to public health security. In this study, *A. veronii* was isolated and identified from 357 shellfish samples imported from 6 aquaculture bases in Jilin Province, China, and tested for virulence, tolerance and drug resistance genes. According to 16S rRNA and *gyrB* gene PCR result, 47 strains were identified as *Aeromonas veronii*. The distribution of 9 virulence factors including *fla* (55.3%), *Alt* (42.5%), *Act* (31.9%), *Aer* (31.9%), *Lip* (19.1%), *gcaT* (12.7%), *ahyB* (12.7%), *ascV* (10.6%) and *Ast* (8.5%) was determined exclusively in *Aeromonas veronii*. Nine virulence genes were detected in two isolates. In addition, the resistance rate of isolates to sulfamonomethoxine and florfenicol was 100%, while to tetracycline, aureomycin and doxycycline it was 34.0%（16/47）, 29.8%（14/47）and 25.5%（12/47）, respectively. Among all tested strains, thirty-five isolates were sensitive to enrofloxacin, and the majority of drug-resistant strains carried *strA*（95.7%）, *strB*（93.6%）, *qnrA*（40.4%）, *qnrD*（31.9%）, *tetQ* (31.9%）and *tetK* (29.8%) resistance genes. The results provide a basis for a risk assessment of the drug resistance of *Aeromonas veronii*.

**Keywords:** *Aeromonas veronii*, Drug resistance, shellfish, virulence genes

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Received July 25, 2020.
Accepted December 16, 2020.
doi: 10.14456/tjvm.2021.4

## Introduction

*A. veronii* is a group of Gram-negative facultative anaerobic bacteria, which has strong adaptability and widely exists in the environment, especially in fresh water and estuaries. At present, there are 27 species of *Aeromonas* such as *Aeromonas hydrophila* and *A. veronii*. *A. veronii* is extremely harmful to aquaculture, especially shellfish and some economically important fish species.

*A. veronii* cannot only infect fish, amphibians and reptiles, but also mammals including human beings, especially the elderly, children and people with low immunity(Janda and Abbott, 2010). It can cause huge economic loss to aquaculture, and seriously threatens human health. The number of reported domestic cases of *A. veronii* and those abroad is increasing year by year and prevalence is on the rise (Esteve *et al*., 2015). Dallal *et al*., (Soltan Dallal *et al*., 2016) analysed 391 stool samples from acute and chronic diarrhoea collected at a children's hospital in Iran between 2013 and 2014, and found that 12 of these were from *Aeromonas* of which 4 cases were caused by *A. veronii*, and all of them carried aerolysin gene *areA*. This shows that the damage caused by *A. veronii* disease is becoming more and more serious and great attention needs to be paid.

Low dose and long-term use of antibiotics in aquaculture will easily lead to the emergence and spread of drug-resistant bacterial strains (Nnadozie and Odume, 2019). Investigations have shown that *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *A. hydrophila* related to aquatic animal infection have a certain drug resistance to common antibiotics such as ampicillin, sulfamethoxazole compound, tetracycline, chloramphenicol and ciprofloxacin, that is increasing to different degrees year by year (Chandrarathna *et al*., 2018; Smyrli *et al*., 2019). Bacterial drug resistance not only reduces the curative effect of antibiotics, which is manifested in the increase of the drug dose, the prolonged course of treatment, an increased recurrence rate, but also causes complications and increased mortality(Laudy, 2018). The main purpose of using antibiotics in aquaculture is to treat bacterial infectious diseases, prevent the spread of bacterial infectious diseases and serve as a feed additive. Although antibiotics can reduce breeding costs, long-term use will lead to the emergence of drug-resistant bacteria, especially in animals (Shao *et al*., 2018). Once drug-resistant bacteria appear and spread among animals, then large-scale cultured animals will become a huge reservoir of drug-resistant genes (Roberts and Schwarz, 2016; Viswanathan, 2014).

In recent years, many Chinese researchers have carried out studies on the virulence and drug resistance of *A. veronii* (Jiang *et al*., 2019; Kong *et al*., 2019b; Ran *et al*., 2018; Weiss *et al*., 2019). Two million tons of shellfish are imported into China every year. Therefore, it is necessary to detect the drug-resistant level and the virulence gene and drug-resistance gene of A. veronii in shellfish. This study aims to isolate and identify A.Veronii in shellfish from 6 aquaculture bases in Jilin Province and test its drug-resistant level, virulence gene and drug resistance gene.

## Materials and Methods

***Sample collection:*** A total of 357 shellfish samples were purchased from 6 aquaculture bases in Jilin Province, China. The samples were packed in sterile ice boxes and then sent to our laboratory. The shellfish meat was cut with a sterile blade and a sterilized ring was inserted into the tissue and scribed on RS agar medium. All processes were conducted in bechtop. The shellfish meat was cultured at 37℃ for 24 hours. The colonies with different phenotypes were isolated from RS and re-streaked on LB agar plates. All isolates were sub -cultured and kept in nutrient broth with 80% glycerol and stored at -80 ℃ until further identification.

***Bacterial isolation and identification:*** Genomic DNA was extracted from presumptively identified *A. veronii* isolates using Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, Shanghai,China). Universal primers were used to amplify a 1465 bp 16S rRNA gene and a 1100bp gyrB gene segment (Table S1), polymerase chain reaction (PCR) amplification was performed in a total volume of 50 μL containing 25 μL of 2×Taq PCR MasterMix (Tiangen Biotech, Beijing, China), 2 μL (10 μM) of each forward and reverse primer, 19 μL dd$H_2O$ and 2 μL DNA as a template. The PCR conditions were as follow: 95℃ for 5 mins, 30 cycles of denaturation at 95 ℃ for 1 min, annealing at 55℃ for 45 s, and extension at 72℃ for 1 min and 30 s, with a final extension step at 72℃ for 10 mins. Aliquots from amplification reactions were analysed by 1.0% agarose gel electrophoresis and viewed under UV light. The PCR products were sent to Sangon Biotech (Shanghai,China) for sequencing. The sequencing results were compared with the NCBI database National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov)(Kong *et al*., 2019a).

**Table S1** 16s rRNA and M13 primer sequence

| Gene name | Primer Sequences(5`→3`) | Product sizes/bp |
|---|---|---|
| 16s rRna | F: AGAGTTTGATCCTGGCTCAG<br>R: GGTTACCTTGTTACGACTT | 1465 |
| gyrB | F: TCCGGCGGTCTGCACGGCGT<br>R: TTGTCCGGGTTGTACTCGTC | 1100 |

***Detection of virulence genes:*** All isolates were subjected to PCR assays to identify genes encoding virulence factors (Hossain *et al*., 2019), including Heat-labile cytotonic enterotoxin (*Alt*), Heat-stable cytotonic enterotoxin (*Ast*), Cytotoxic enterotoxin (*Act*), Elastase (*ahyB*), Serine protease (*Ser*), Flagellin (*fla*), Aerolysin(*Aer*), Lipase (*Lip*), Glycerophospholipid-cholesterol acyltransferase (*gcaT*) and Type III secretion system (*ascV*)( Table S2) using the primers described by Hossain (Hossain *et al*., 2019). *A. veronii*

*biovar veronii* ATCC 35624, was used as the positive control. The primers, target genes, temperature and amplicon sizes are listed in Table S2. The PCR products were analysed by 1.0% agarose gel electrophoresis (Zhang *et al*., 2019).

**Table S2** Primers used for the detection of virulence genes

| Target Gene | Primer Sequences(5`→3`) | Tm/℃ | Product sizes/bp | Function |
|---|---|---|---|---|
| Alt | F: TGACCCAGTCCTGGCACGGC<br>R: GGTGATCGATCACCACCAGC | 63 | 442 | Heat-labile cytotonic enterotoxin |
| Ast | F: TCTCCATGCTTCCCTTCCACT<br>R:GTGTAGGGATTGAAGAAGCCG | 63 | 331 | Heat-stable cytotonic enterotoxin |
| Act | F: AGAAGGTGACCACCACCAAGAACA<br>R: AACTGACATCGGCCTTGAACTC | 64 | 232 | Cytotoxic enterotoxin |
| aer | F: CCTATGGCCTGAGCGAGAAG<br>R: CCAGTTCCAGTCCCACCACT | 62 | 431 | Aerolysin |
| fla | F: TCCAACCGTYTGACCTC<br>R: GMYTGGTTGCGRATGGT | 55 | 608 | Flagellin |
| Ser | F: CACCGAAGTATTGGGTCAGG<br>R: GGCTCATGCGTAACTCTGGT | 56 | 350 | Serine protease |
| ahyB | F: ACACGGTCAAGGAGATCAAC<br>R: CGCTGGTGTTGGCCAGCAGG | 58 | 513 | Elastase |
| lip | F: ATCTTCTCCGACTGGTTCGG<br>R: CCGTGCCAGGACTGGGTCTT | 62 | 382 | Lipase |
| gcaT | F: CTCCTGGAATCCCAAGTATCAG<br>R: GGCAGGTTGAACAGCAGTATCT | 65 | 237 | Glycerophospholipid-cholesterol acyltransferase |
| ascV | F: AGCAGATGAGTATCGACGG<br>R: AGGCATTCTCCTGTACCAG | 58 | 891 | Type III secretion system |

***Antimicrobial susceptibility testing:*** The antimicrobial susceptibility was determined by broth microdilution and serial two-fold agar dilution using a standard procedure from the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018). The antimicrobial agents tested include kanamycin (KAN), enrofloxacin (ENR), ceftriaxone (CRO), sulfamonomethoxine (SMM), tetracycline(TE), gentamicin (GEN), florfenicol(FFC), chloramphenicol (CM), doxycycline (DOX) and aureomycin (AUR). Escherichia coli (ictalized) was used as the control.

***Detection of antimicrobial resistance genes:*** All isolates were tested by PCR assays to identify genes encoding drug resistance, including the tetracycline resistance gene (*tetA*, *tetM*, *tetO*, *tetK*, *tetS*), pan-drug resistance gene (*NDM*-1), Polymyxin resistance gene(*mcr*1, *mcr*2), quinolone resistance gene (*acrA*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qepA*, *oqxA*, *oqxB*), streptomycin resistance gene(*strA*, *strB*), chloramphenicol resistance gene(*CAT*) and vancomycin resistance gene(*vanX*). The primers, target genes, temperature, classification and amplicon sizes are listed in Table S3. The PCR products were analysed by 2.0% agarose gel electrophoresis.

## *Results*

***Bacterial isolation and identification:*** We tested the water from 6 markets with pH of 7.0 ~ 7.3 and water salinity of 0.5% ~ 0.8%. After 24 h incubation, the yellow round colony of the isolate was observed on RS agar plate. A total of 94 isolates were obtained. Molecular identification was performed on isolates using 16S rRNA gene and gyrB (Bsubunit of DNA gyrase, a type-II DNA topoisomerase) sequence analysis. A total of 47 from 94 isolates were confirmed as *A. veronii*.

***Detection of virulence genes:*** Previous observations demonstrated that haemolytic and cytotoxic activities correlated with the virulence genes in *Aeromonas* strains (Li *et al*., 2011). In this study, ten virulence genes (*Ale*, *Ase*, *Ace*, *ahyB*, *Ser*, *fla*, *Aer*, *Lip*, *gcaT*, *ascV*) were screened by PCR assay. Our results show that genes encoding *Ale*(20/47; 42.5 %), *Ase* (4/47; 8.5%), *Ace*(15/47;31.9%), *ahyB*(6/47;12.7%), *Ser*(0/47;0%), *fla*(26/47; 55.3%), *Aer*(15/47;31.9%), *Lip*(9/47;19.1%), *gcaT*(6/47;12.7%) and *ascV* (6/47; 10.6%) were highly prevalent among the isolates (Table 1)(Khor *et al*., 2018).

***Antimicrobial susceptibility testing:*** For some of the selected anti-biological agents, the reference strain used as quality control for the MIC determination assessment indicated the *A. veronii* strains in compliance with CLSI recommendations (CLSI, 2018). A 100% of the specialized *Aeromonas* strains displayed susceptibility to FFC and SMM antibiotics. Table 2 lists the resistance prole for isolates. All tested *A. veronii* strains showed resistance to two or more antibiotics. The number of sensitive, intermediate and resistant strains with reference to the CLSI breakpoints were the most resistant to SMM and FFC (47/47; 100%), the most intermediate to AUR (33/47; 70.2%) and the most sensitive to ENR (35/47; 74.4%) (Yang *et al*., 2019).

***Detection of antimicrobial resistance genes:*** In this study, 20 drug resistance genes (*tetQ*, *tetM*, *tetO*, *tetK*, *tetS*, *NDM*-1, *areA*, *mcr*1, *mcr*2, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qepA*, *oqxA*, *oqxB*, *strA*, *strB*, *CAT*, *vanX*) were screened by PCR assay. Our results showed that genes encoding *tetQ* (15/47;31.9%), *tetM*(2/47;4.3%), *tetO*(0/47;0%), *tetK*(14/47;29.8%), *tetS*(4/47;8.5%), *NDM*-1(2/47;4.3%), *areA*(0/47;0%), *mcr*1(0/47;0%),

*mcr*2(0/47;0%), *qnrA*(19/47;40.4%), *qnrB*(10/47;21.3%), *qnrC*(11/47;23.4%), *qnrD*(15/47;31.9%), *qepA*(13/47;27.7%), *oqxA*(11/47;23.4%), *oqxB*(13/47;27.7%), *strA*(45/47;95.7%), *strB*(44/47;93.6%), *CAT*(0/47;0%) and *vanX*(5/47;10.6%) (Table 3). According to the detection results of drug resistance genes, *strA*, *strB*, *tetQ* and *qnrA* genes were highly detected.

**Table S3** Primer sequence of drug resistance gene

| Antibiotic | Gene name | Primer Sequences(5`→3`) | Tm/℃ | Product sizes/bp |
|---|---|---|---|---|
| Tetracycline | tet（Q） | F:AGAATCTGCTGTTTGCCAGTG<br>R:CGGAGTGTCAATGATATTGCA | 52 | 169 |
| | tet（M） | F:CAATATCACCAGAGCAGGCT<br>R: CTAAGATATGGCTCTAACAA | 47 | 406 |
| | tet（O） | F:GATGGCATACAGGCACAGAC<br>R:CAATATCACCAGAGCAGGCT | 54 | 539 |
| | tet（K） | F: CTTGTTCGAGTTCCAATG<br>R: TCGATAGGAACAGCAGTA | 50 | 539 |
| | tet（S） | F: TGGAACGCCAGAGAGGTATT<br>R: ACATAGACAAGCCGTTGACC | 53 | 667 |
| - | NDM-1 | GGCGGAATGGCTCATCACGA<br>CGCAACACAGCCTGACTTTC | 57 | 287 |
| Polymyxin B | mcr1 | F:TGCTCCAAAATGCCCTACAGACC<br>R:TGCCCCAAGTCGGATAATCCAC | 56 | 141 |
| quinolone | acrA | CTCTCAGGCAGCTTAGCCCTAA<br>TGCAGAGGTTCAGTTTTGACTGTT | 55 | |
| | qnrA | F: ATTTCTCACGCCAGGATTTG<br>R: GATCGGCAAAGGTTAGGTCA | 52 | 516 |
| | qnrB | F: GATCGTGAAAGCCAGAAAGG<br>R: ACGATGCCTGGTAGTTGTCC | 53 | 469 |
| | qnrC | F: ATTTCTCACAGGCAAACT<br>R: CTGGAATAACAATCACCC | 46 | 666 |
| | qnrD | F: TTTTCGCTAACTAACTCGC<br>R: GAAAGGATAAACAGGCAAAT | 47 | 984 |
| | qepA | F: GCAGGTCCAGCAGCGGGTAG<br>R: CTTCCTGCCCGAGTATCGTG | 52 | 417 |
| | oqxA | F: GATCAGTCAGTGGGATAGTTT<br>R: TACTCGGCGTTAACTGATTA | 50 | 670 |
| | oqxB | F: CTCGGCCATTTTGGCGCGTA<br>R: TTCTCCCCCGGCGGGAAGTAC | 58 | 512 |
| streptomycin | strA | F:CTTGGTGATAACGGCAATTC<br>R:CCAATCGCAGATAGAAGGC | 57 | 548 |
| | strB | F:ATCGTCAAGGGATTGAAACC<br>R:GGATCGTAGAACATATTGGC | 56 | 509 |
| Chloramphenicol | CAT | F:ATAACGTTACTCTCCTATTTC<br>R:GGATATGAAATTTATCCCTC | 52 | 486 |
| Vancomycin | VanX | F:TCGCGGTAGTCCCACCATTCGTT<br>R:AAATCATCGTTGACCTGCGTTAT | 56 | 454 |

**Table 1** Virulence gene test results

| Virulence Gene | Frequency of the Isolates (%) | |
|---|---|---|
| | ***A. veronii*** **(n = 47)** | **Total (%)** |
| *Alt* | 20 | 42.5 |
| *Ast* | 4 | 8.5 |
| *Act* | 15 | 31.9 |
| *ahyB* | 6 | 12.7 |
| *Ser* | 0 | 0 |
| *fla* | 26 | 55.3 |
| *Aer* | 15 | 31.9 |
| *Lip* | 9 | 19.1 |
| *gcaT* | 6 | 12.7 |
| *ascV* | 5 | 10.6 |

**Table 2** Results of drug sensitivity test

| Antimicrobial agents | MIC (μg/mL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **≤0.03** | **0.06** | **0.0125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **≥512** |
| KAN | 2 | 2 | | 3 | 4 | 4 | | 12 | 4 | 4 | 1 | 1 | 6 | 3 | 1 |
| TE | | | | 3 | 2 | 2 | | 12 | 10 | 2 | 4 | 6 | 3 | 3 | |
| GEN | | | | | 5 | 17 | 6 | 5 | 3 | 2 | 2 | | 2 | 5 | |
| CM | | | | | 2 | 3 | 3 | 10 | 11 | 7 | 6 | 5 | | | |
| DOX | | | | | 2 | | 1 | 2 | 30 | | 10 | 2 | | | |
| AUR | | | | | | | | 10 | 4 | 19 | 8 | 2 | 2 | 2 | |
| ENR | 22 | 2 | 5 | 6 | 2 | 2 | | | 1 | 2 | 2 | 3 | | | |
| CRO | | | | 4 | 6 | 1 | 1 | 6 | 5 | 1 | 12 | | 2 | 7 | 2 |
| SMM | | | | | | | | | | | | | | | 47 |
| FFC | | | | | | | | | | | 9 | 4 | 3 | 27 | 4 |

**Table 3** Results of drug resistance genes test

| Resistance Genes | Frequency of the Isolates (%) | |
|---|---|---|
| | ***A. veronii* (n = 47)** | **Total (%)** |
| *tet（Q）* | 15 | 31.9 |
| *tet（M）* | 2 | 4.3 |
| *tet（O）* | 0 | 0 |
| *tet（K）* | 14 | 29.8 |
| *NDM-1* | 2 | 4.3 |
| *tet（S）* | 4 | 8.5 |
| *areA* | 0 | 0 |
| *mcr1* | 0 | 0 |
| *mcr2* | 0 | 0 |
| *qnrA* | 19 | 40.4 |
| *qnrB* | 10 | 21.3 |
| *qnrC* | 11 | 23.4 |
| *qnrD* | 15 | 31.9 |
| *qepA* | 13 | 27,7 |
| *oqxA* | 11 | 23.4 |
| *oqxB* | 13 | 27.7 |
| *strA* | 45 | 95.7 |
| *strB* | 44 | 93.6 |
| *CAT* | 0 | 0 |
| *VanX* | 5 | 10.6 |

## *Discussion*

*A. veronii* is a kind of gram-negative bacteria. *Aeromonas*, commonly found in fresh water, sewage, silt and soil; it is a common pathogen of aquatic animals and can also cause diseases in human beings and animals. In 2011, Aravena *et al*.tested 144 clinical samples (54 wound infection samples, 33 blood samples, 34 stool samples and 23 mixed samples), of which 49 strains were tested. *A.veronii*, accounted for a large proportion (Aravena-Roman *et al*., 2012). A previous study reported that *Aeromonas caviae*, *A. hydrophila* and *A. veronii* were found pathogens in a collection of 36 cases, of which *A. veronii* accounted for 13.9% (Kimura *et al*., 2013). In 2014, Esteve et al., collected river, fish and clinical samples from Valencia, Spain. A total of 185 strains of *Aeromonas* were analysed, of which *A. veronii* accounted for 19.5%. The isolated strains had moderate virulence in mice and increased drug resistance(Esteve *et al*., 2015). In 2016, Sin-Clair *et al*., analysed clinical samples collected by Queensland Clinical Microbiology Laboratory in Australia from 2012 to 2014, and obtained 100 strains of *Aeromonas*, of which 21 strains were *A.veronii*(Sinclair *et al*., 2016). In this study, 47 strains of *A. veronii* were isolated from 357 samples of shellfish, which accounted for a large proportion. The virulence gene of *A. veronii* isolated from loach in Nanjing, China was detected, and *Ser* gene was found to be the most widely distributed (Zhu *et al*., 2016).The virulence genes of *Aeromonas* isolated from Korean scallop were detected. The results showed that the detection rate of *ahyB, gcaT, aer* and *Ser* genes were 94%, 84%, 78% and 75%, respectively (Scarano *et al*., 2018). Differently (Prediger *et al*., 2019; Zhu *et al*., 2016), found a high distribution of virulence factors flagellin instead of serine protein in the genetic among the majority of *A. veronii* isolates in our study. *gcaT*, *ahyB* and *Ace* genes were also detected, indicating that these virulence genes are widely distributed in aquatic animals in many countries and regions. However, it was found that the detection rate of *fla* gene in our samples was the highest, reaching 55.2%, and the detection rate of *Alt* gene was also relatively high, reaching 42.5%. Meanwhile, *Ser* gene was not detected, indicating that the pathogenic mechanism of *A. veronii* in Jilin Province was different from that in others.

The detection of drug resistance and drug resistance genes of *A. veronii* has attracted great attention from researchers at home and abroad. In this study, the drug resistance of the isolated strains was detected and it was found that the drug resistance rate to SMM and FFC was 100%, while sensitivity was more

to ENR (74.4%). Qian Yang *et al*.,(Yang *et al*., 2017) tested the drug sensitivity of 42 strains of *A. veronii* isolated from channel catfish in China. Most of the isolated strains showed drug resistance to penicillin, oxacillin and doxycycline, and most of the strains were sensitive to ceftriaxone. Scarano *et al*., carried out drug sensitivity tests on 104 strains of *Aeromonas* isolated from Sparus italicus. The bacterial strains showed high resistance to sulfadiazine, ampicillin, amoxicillin, erythromycin, cephalothin, streptomycin and trimethoprim antibiotics. Conversely, many isolates were susceptible to oxolinic acid, gentamicin, florfenicol, flumequine, kanamycin and oxytetracycline antibiotics. Almost all *Aeromonas spp*. strains showed multiple antibiotic resistance (Scarano *et al*., 2018); (Yang *et al*., 2017). Considering the difference in drug resistance, we speculate that it may be related to antibiotics commonly used in local aquaculture or caused by drugs used in local aquaculture entering groundwater through various channels. According to the results of drug resistance, we selected some drug resistance genes to detect the isolated strains. Based on the results of drug resistance, some drug resistance genes were selected to detect the isolate. Our results showed that 45 strains carried streptomycin resistance genes strA, 44 strains carried streptomycin resistance genes strB and 19 strains carried quinolone resistance gene qnrA. It has been reported that quinolone and tetracycline resistance genes can be transmitted horizontally through plasmids (Lerminiaux and Cameron, 2019; Liu *et al*., 2019; Roberts and Schwarz, 2016; Shao *et al*., 2018; Wu *et al*., 2019) and the transmission of these genes is to animals and humans. It is worth noting that we found 2 strains carrying *NDM*-1 gene by PCR. The emergence of pan-drug resistant strains warned us to pay attention to the increasingly serious global drug resistance.

However, in this study, the environmental drug-resistant genes have not been analyzed. Next, the virulence island is to be studied for these drug-resistant genes in order to lay a foundation for the research of the drug resistance and drug resistance genes of *A. veronii*.

*A. veronii* were found in 47 out of 357 shellfish samples by 16s rRNA gene and *gyrB* gene sequencing. Virulence gene detection showed that 55.3% of the isolates contained *fla* gene and 31.9% contained *Act* and *Aer* genes. Drug susceptibility tests showed that the isolates were resistant to SMM and sensitive to ENR. According to drug resistance gene detection, *strA*, *strB*, *qnrA*, *qnrD*, *tetQ* and *tetK* are the most prevalent genes in the isolates.

***Conflict of interest :*** No authors have conflicts of interest.

***Compliance with ethical standards Statement of Animal Rights :*** All procedures performed with the animals in the present experiment were in accordance with the ethical standards established by the committee of Jilin Agriculture University

***Data Availability Statement:*** The data used to support the findings of this study is included within the article.

## *Acknowledgements*

We thank Hai-peng Zhang for their laboratory contributions.

***Funding information*** **:** This research was funded by National Natural Science Foundation (Grant number:31702293), Jilin Natural Science Foundation (Grant number: 20190201294JC), Theme Guidance Project of Jilin Provincial Science and Technology Department (Grant number: 20180101003JC).

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