

Novobiocin: The Most Sensitive Antibiotic against *Vibrionaceae* Isolated from *Pinctada margaritifera* (Black-Lipped Pearl Oyster) in the South of Iran

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ABSTRACT

Pinctada margaritifera (black-lipped pearl oyster) is one of the most important species of mollusks used for the production of breeding pearls. The fatality of *Pinctada margaritifera* has been observed in all stages of growth, which the reasons were not discussed before. This study aims to isolate, identify, and determine antibiotic susceptibility of *vibrionaceae*, one of the most pathogenic factors in the *Pinctada margaritifera*. The process of detecting and identifying bacteria was done based on the culture in TSA and TCBS media, and then biochemical tests. Also, the antibiotic susceptibility of the bacteria was studied using the diffusion disc method. As a result, Four species of *Vibrionaceae*, including *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio splendidus*, and *Vibrio anguillarum*, have been identified in the gills of *Pinctada margaritifera*. All of the species were resistant to ampicillin, erythromycin, streptomycin, and oxacillin, sensitive to novobiocin, and semi-sensitive to the oxytetracycline.

Keywords: *Pinctada margaritifera*, *Vibrionaceae*, Black-Lipped Oyster, Novobiocin, Pearl

INTRODUCTION

Fishery and aquaculture-related industries, especially their reproduction and breeding, are essential not only for the economic aspects and supplying the country's protein but also in social and employment dimensions, especially in the coastal strip. In this sense, mollusks are a large group of invertebrates that have a variety of uses, especially the *Pinctada margaritifera*, which are economically and commercially important. The pearl shell used in wood carving arts, dress button ornaments, decoration, and livestock-poultry feeds, and it's the large muscle used for human feed. The most important purpose of its cultivation is the harvesting of natural and breeding pearls [1].

Oysters fishing and harvesting of natural pearls have long been prevalent in the Persian Gulf and especially in western Hormozgan in the Lavan, Hendurabi and Kish Islands, which are considered as the primary habitats of this oyster species, and is one of the best and most profitable natural resources of the Persian Gulf [2].

The influx of pathogens has been observed in breeding tanks and even in the natural environment of the *Pinctada margaritifera*. Disease-causing factors are classified according to the type of illness, and the most important of these factors are primarily bacterial pathogens. Among pathogenic bacteria in breeding oysters, *Vibrionaceae* cause a bacterial disease called Vibriosis and it has also been reported as the most important pathogenic bacteria in the mollusks [3]. Vibriosis has different mortality rates for aquatic animals in Iran at various spots and accounts for between 10% and 15% of casualties. This mortality rate in the world is estimated to be between 15% and 20% due to the type of breeding system. *Vibrio* species that can be found in the aquaculture breeding system include *Vibrio harveyi*, *Vibrio splendidus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio campbelli*, *Vibrio fischeri*, *Vibrio plagiocus*, *Vibrio orientalis*, *Vibrio logei*, *Vibrio penaeicida*, *Vibrio cholerae* [4].

In recent years, the widespread use of antibiotics has led to the emergence of resistant strains of microorganisms and antibiotic

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resistance in the world [2]. Therefore, determining antibiotic susceptibility will play an important role in controlling bacterial diseases.

This research was conducted to investigate one of the pathogenic factors in the spat and *Pinctada margaritifera* breeders and also to determine the antibiotic susceptibility of *vibrionaceae* isolates. Therefore, the present study, aims to isolate, identify, and determine antibiotic susceptibility of *vibrionaceae*, one of the most pathogenic factors in the black-lipped pearl oyster.

MATERIALS AND METHODS

Sampling

For Sampling, diving was carried out at a depth of 10 to 12 meters in the sea and the habitat of oysters in the islands of Hindurabi and Lavan, which 30 samples include 20 Spats and 10 black-lipped pearl oyster breeders were collected (due to lack of breeders). Then, the specimens were transferred to a breeding workshop at the Bandar Lengeh Fishery Mollusk's Research Center. Internal organs (gill, hepatopancreas, and intestines) were used for bacterial examination [5,6].

Culture

First, the specimens homogenized and serial-diluted in sterilized seawater (2.5% salts) [7,8]. Afterward, diluted suspensions were cultured in sterilized Trypticase Soy Agar (TSA) and Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS) media and incubated at 37°C for 48 h.

Biochemical diagnosis

In addition to gram staining, biochemical tests such as Oxidase, Catalase, Urease, Indole, Gelatinase, Methyl red, Citrate hydrolysis, etc. examined for identifying the isolates [8].

Antibiotic susceptibility test

To investigate the antibiotic susceptibility, the disk diffusion method was used according to the CLSI protocol. First, a 0.5 McFarland suspension of each bacterial isolate was prepared and inoculated on Muller Hinton agar medium. Afterward, the

antibiotic disks such as ampicillin, erythromycin, oxytetracycline, novobiocin, oxacillin, and streptomycin were placed on the surface of the cultured medium [9] and the plates incubated at 37°C for 24 h. The sensitivity to the antibiotics was detected by measuring the growth inhibition zone in diameter, finally.

RESULTS

Culture and biochemical tests

Gram-negative curved bacilli detected in TCBS and TSA media. Due to biochemical test results (Table 1), 50 isolates of *Vibrionaceae* were identified, which 22, 14, 9, 10 isolates belonged to *V. harveyi*, *V. alginolyticus*, *V. splendidus*, and *V. anguillarum* species, respectively (Figure 1).

Notably, all these isolated species were from the gill of *Pinctada margaritifera*, while any species from hepatopancreas and intestinal organs did not isolate (Figure 2).

Disc diffusion

The antibiotic susceptibility test results indicated that all isolates are sensitive to novobiocin and intermediate to oxytetracycline. Also, the data assured that all isolates are resistant to ampicillin, erythromycin, oxacillin, and streptomycin (Figure 3 and Table 2).

DISCUSSION

Given the important role of the aquaculture industry in the sustainable economy of a society and job creation in local communities, continuous study of factors affecting aquaculture, especially the breeding of *Pinctada margaritifera*, which in addition to pearl extraction It has various applications in industry, is important.

Bacteria are one of the factors that cause disease and mortality of aquatic organisms, especially *Pinctada margaritifera*. There have been reports of some species of *Vibrio* being the primary cause of oyster disease in some areas, including *Vibrio tapetis* (*Vibrio* P1), which is found in the Manihiki region in the Pacific Ocean, has caused high losses in *P. margaritifera* [10].

Also, *Vibrio* bacteria cause Brong ring disease (BRD) in oysters, which is characterized by thinning of the shells of oysters. According

Table 1: Biochemical and physiological characteristics of *Vibrio* isolates.

Tests	Isolated bacteria			
	<i>V. harveyi</i>	<i>V. alginolyticus</i>	<i>V. splendidus</i>	<i>V. anguillarum</i>
Motility	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Indol	+	+	+	+
Methyl red	-	+	-	-
Voges-proskauer	-	+	-	+
O/F	+	+	+	+
Citrate	-	+	-	+
Gelatinase	+	+	+	+
Urease	+	-	-	-
Growth at 0% NaCl	-	-	-	-
Growth at 3% NaCl	+	+	+	+

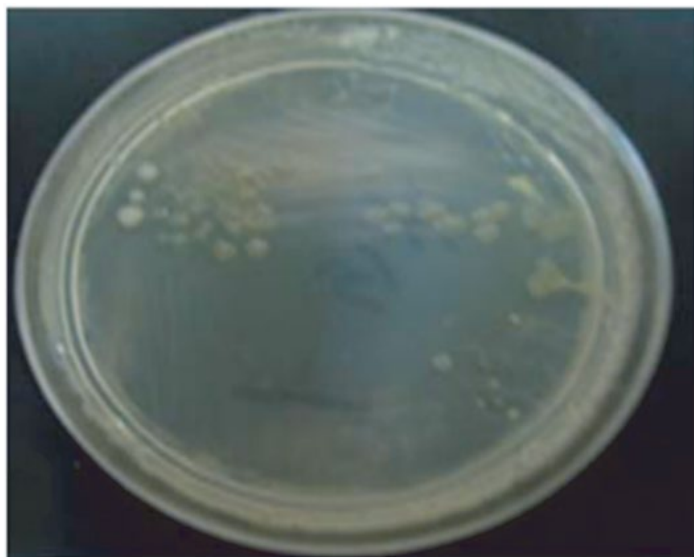


Figure 1: Colonies of cultured on TSA medium.



Figure 2: Positive motility test.



Figure 3: Disc diffusion test.

to Perkins in 1996, this condition was caused in Australia by the bacterium *Vibrio harveyi* in oysters [11]. Examination of isolated

Table 2: Results of the antibiotic susceptibility test.

Antibiotics	Isolated bacteria			
	<i>V. harveyi</i>	<i>V. alginolyticus</i>	<i>V. splendidus</i>	<i>V. anguillarum</i>
Novobiocin	Sensitive	Sensitive	Sensitive	Sensitive
Oxytetracycline	Semi-Sensitive	Semi-Sensitive	Semi-Sensitive	Semi-Sensitive
Ampicillin	Resistant	Resistant	Resistant	Resistant
Erythromycin	Resistant	Resistant	Resistant	Resistant
Oxacillin	Resistant	Resistant	Resistant	Resistant
Streptomycin	Resistant	Resistant	Resistant	Resistant

bacteria in this study shows that the predominant genus is *Vibrio* bacteria, but in this study, no mortality from *Vibrio* bacteria was observed in oyster rearing tanks.

For controlling and reducing the losses caused by *Vibrio* species in aquatic animals using antibiotics as an effective agent has been suggested, but due to the resistance of bacteria, continuous monitoring of antibiotic susceptibility is essential for treatment in vibriosis. Typically, antibiotics that are used during the aquatic life cycle include Streptomycin, Chloramphenicol, Ampicillin, Oxytetracycline, Novobiocin, Erythromycin, and Trimethoprim as anti-vibrio-effective drugs [12]. Notably, In Iran, Oxytetracycline is the most common antibiotic used in aquaculture [13].

These study results are in alignment with the results of other studies such as Azam Moghimi et al. and Tendencia et al. that isolated bacteria include *V. harveyi*, *V. alginolyticus*, and *V. anguillarum* have been sensitive to oxytetracycline. The susceptibility of these bacteria to erythromycin has also been reported to be resistant and semi-sensitive, while they have been resistant to streptomycin [14,15].

Resistance to streptomycin, ampicillin, erythromycin and oxacillin antibiotics, according to a study by Manjusha et al. can be considered the transfer of plasmids from terrestrial bacteria to marine bacteria because these antibiotics are widely used for human use and may it enter the seawater through sewage and pollutants of human origin [16].

In this study, although *vibrio* pathogen was observed in the habitat of oysters, this bacterium cannot cause disease alone and requires a stressor in the environment. The study also found no mortality in *Pinctada margaritifera*, which revealed that bacteria are not the only cause of stress in the breeding reservoir of oysters.

CONCLUSION

In this study, 4 species of the *Vibrionaceae* include *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio splendidus*, and *Vibrio anguillarum* were isolated. For the first line to treatment and use of aquaculture, it is recommended to use novobiocin, oxytetracycline also can be used subsequently. as well as the proper use of antibiotics has a critical role in preventing bacterial antibiotic resistance.

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