The Resistance Of Bacteria Of Vibrio Sp. Isolated From The Dumai Sea Waters Against Antibiotics

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Abstract: Vibrio sp is one type of bacteria that infects a lot of biota, this bacterium is very easy to spread, no exception affecting fish both marine and fresh aquaculture. This research was conducted in April - May 2018. The purpose of this study was to analyze the resistance of Vibrio sp. in the sea waters of Dumai against antibiotics. The research method used is the survey method, by taking 5 sampling points that are in different conditions. The test antibiotics used were: Chloramphenicol, Penicillin and Isoniazid. The results showed that the density of Vibrio sp. ranging from 1.3 x 103 - 1.6 x 103 CFU / ml. Number of colonies of Vibrio sp. highest at station 4, lowest at station 5. Results of the resistance test for Vibrio sp. for antibiotics as many as 32 isolates of Vibrio sp. showed that in Chloramphenicol antibiotics there were 31 bacterial isolates classified as intermediate resistances with inhibition zones ranging from 12-19.8 mm, and 1 isolate including sensitive with a 21 mm inhibition zone. In Penicillin antibiotics all bacterial isolates were included as resistant with inhibitory zones ranging from 1.6 to 9 mm. And on Isoniazid antibiotics all bacterial isolates included resistance with inhibitory zones ranging from 0.9 to 6.2 mm.

Index Terms: Vibrio sp, Dumai waters, resistance, antibiotics.

1. INTRODUCTION

THE development of Dumai City into an industrial city and a port will cause disruption of coastal ecosystems in Dumai City, including the mangrove ecosystem and the biota that live in it. There are microorganisms that are beneficial to human life, but many are detrimental to humans. For example can cause various diseases, parasites or even can cause damage due to contamination by microorganism. Vibrio is one type of bacteria that infects many biota, this bacterium is very easily spread. The distribution of these bacteria causes several diseases in biota, including marine biota. Several cases of Vibrio bacteria have caused illness and death in fish and marine invertebrates, both in the larval and adult phases (El Manama et al., 2006). The direct impact of pathogenic bacteria can cause disease, parasites, spoilage and toxins that can cause the death of biota that inhabit these waters. According to Feliatra et al (2019) the presence of types of pathogenic bacteria such as Vibrio sp. will cause disease in fish farming so it needs to be anticipated for its prevention. The purpose of this study was to determine the distribution of vibrio bacteria in Dumai waters and the level of resistance to antibiotics. The quality of a waters can be reviewed based on chemical, physical and biological aspects. From the biological aspect, one of them is bioindicator bacteria. Bacteria as a bioindicator can indicate a waters that have been contaminated or not.

2 METHOD

This research was conducted in April - May 2018. Water sampling was conducted in the Dumai Sea Waters of Dumai City, Riau Province. Isolation and counting activity of Vibrio sp. and bacterial resistance test Vibrio sp. Antibiotics were carried out at the Marine Microbiology Laboratory, Department of Marine Sciences, Faculty of Fisheries and Maritime Affairs, University of Riau.

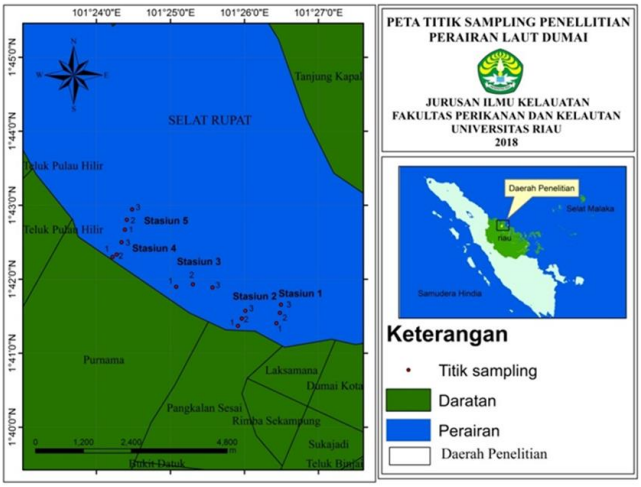

Figure 1. *Research Location Map.*

Sampling was carried out at five stations, namely: Station 1 around the Mangrove Area, Station 2 around the Settlement. Station 3 is around the Harbor, Station 4 is around Industry, and Station 5 is around the Sea which is far from community activities. To reduce the level of error and to get complete data, sampling was done 3 times. Calculation of the number of bacterial colonies of Vibrio sp. by using the total plate count method. Bacterial resistance test is carried out on 3 types of antibiotics (Choloramphenicol, Penicilin, Vancomycin) which have different spectra.

3 RESULT & DISCUSSION

3.1 General Condition of Research Area

Dumai City is one of the City in Riau Province. The city of Dumai is on the coast of the eastern island of Sumatra. The Dumai region is in a position between 1010.23 ".37" -1010.8 ".13´ east longitude and 10.23" .23´-10.24 ".23´ LU. Based on this position, the Dumai time zone is UTC + 7. Dumai has an area of 1,727,385 km2. The boundaries of Dumai City include the north bordering the Rupat Strait, the east bordering Bukit

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Batu District, Bengkalis Regency, the south bordering Mandau and Bukit Batu District, Bengkalis District, and the west bordering Tanah Putih and Bangko District , Rokan Hilir Regency. The climate in Dumai is a tropical climate with two seasons, namely the rainy season from September - February and the dry season in March - August. The average air temperature is between 21 - 350C and the average rainfall is between 100 - 300 mm (Dumai.go.id, 2018).

3.2. Water Quality Parameters

The condition of the quality of a waters is very important for the life of organisms as well as for bacteria. In general, bacterial growth is influenced by water quality parameters which include temperature, pH, salinity, brightness, current speed, dissolved oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), nitrate (NO3), and ammonia (NH3). The results of water quality measurements can be seen in Table 1.

TABLE 1.
AVERAGE WATER QUALITY MEASUREMENT RESULTS

No	Parameter	Station					Quality standards KEPMEN LH No. 51 of 2004 [5]
		1	2	3	4	5	
1	pH	7,3	7	7	6,7	6,7	7-8,5
2	Salinity (PPT)	26	21	24,7	20	25	natural
3	Temperature (°C)	30,1	30,5	30,6	30,6	30,7	natural
4	Brightness (cm)	58,3	54,3	67,5	74,2	88,3	natural
5	DO (mg/L)	8,4	8,6	8,2	8,0	8,2	>5
6	Flow Speed (m/s)	0,2	0,07	0,19	0,12	0,06	-
7	COD (mg/L)	28000	36666,7	24000	28000	24000	-
8	BOD (mg/L)	4,529	3,019	2,752	3,833l	1,027	20
9	Nitrate (mg/L)	0,172	0,179	0,145	0,144	0,131	0,008
10	Ammonia (mg/L)	1,407	1,348	1,625	1,734	1,585	0,3

Based on Table 1 it can be seen that the results of water quality measurements show pH, salinity, temperature, brightness, DO and current velocity and COD in Dumai sea waters have met the quality standard while nitrate and ammonia exceed water quality standards according to Decree of the Minister of Environment No. 51 of 2004.

3.3. Bacteria Density Vibrio sp.

Bacteria Vibrio sp. isolated from seawater samples from the Dumai sea waters of Riau Province, the results can be seen in Table 2 below.

TABLE 2.
DENSITY OF VIBRIO SP BACTERIA IN THE DUMAI SEA WATERS

Station	Sampling Point	Number of vibrio sp bacteria (CFU / ml)
1	1	1.8×10^3
	2	1.3×10^3
	3	1.3×10^3
average		1.4×10^3
2	1	1.4×10^3
	2	1.51×10^3
	3	1.6×10^3
average		1.5×103^3
3	1	2.1×10^3
	2	1.0×10^3
	3	1.5×10^3
average		1.5×10^3
4	1	2.0×10^3
	2	1.4×10^3
	3	1.6×10^3
average		1.6×10^3
5	1	1.2×10^3
	2	1.6×10^3
	3	1.3×10^3
average		1.3×10^3

CFU: Colony Forming Unit

Based on Table 2, it can be seen that the density of Vibrio sp. in Dumai sea waters ranging from 1.3×10^3 to 1.6×10^3 CFU / ml. Density of Vibrio sp. highest at station 4 lowest at station 5. Taslihan et al (2004), the minimum threshold for the presence of the bacterium Vibrio sp. in water is 10^4CFU / ml, if this threshold is exceeded then the mass death of shrimp farming in a pond can occur. Bintari (2016) states that Vibrio is very potential to develop as an opportunistic pathogen. Hatta et al. (2011) added that this condition can occur if there is an increase in organic material sourced from feed and feces that encourages microflora to develop into opportunistic pathogens. Vibrio Cholera bacteria are commonly found in waters contaminated by human feces or warm-blooded animals in the river or in densely populated waters. Murray et al., (2002), states that waters can be considered as one of the media for cholera transmission caused by Vibrio Cholera bacteria. Feliatra et al (2014) that the pathogenicity of some bacteria depends on the condition of the environment, water quality is a very influential factor in hatchery and aquaculture. Changes in water quality such as temperature, pH, ammonia, alkalinity and dissolved oxygen cause fish to experience stress thereby reducing the fish's ability to defend itself from disease. In Humans it is known that, Vibrio bacterial infections can cause gastrointestinal problems, loss of limbs, can even cause death. These bacteria are interesting organisms and their effects range from a massive global epidemic such as cholera to a rare but deadly infection, Vibrio vulnifucus. According to the US Centers for Disease Control and Prevention (CDC), there are 12 species of Vibrio bacteria that can cause disease in humans. About 80 percent of infections occur between May-October when the waters become warmer, and usually occur after someone eats a type of seafood with a shellfish. Symptoms that can arise due to Vibrio bacterial infection include diarrhea which is often accompanied by stomach cramps, nausea, vomiting, fever, and chills. Usually these symptoms appear within 24 hours after the contaminated food

is ingested and can last up to three days. Vibrio bacteria can also cause skin infections when open wounds are exposed to brackish water or sea water.

3.4. Test Results of Vibrio sp Bacterial Resistance to Antibiotics

The results of the bacterial resistance test Vibrio sp. against antibiotics in bacterial isolates with an average value can be seen in Table 3.

TABLE 3

AVERAGE RESULTS OF RESISTANCE TESTS OF VIBRIO SP BACTERIA AGAINST ANTIBIOTICS

Isolate name	Inhibited Zone Diameter(mm)											
	Chloramphenicol				Penicillin				Isoniazid			
	U1	U2	U3	R	U1	U2	U3	R	U1	U2	U3	R
V1	23	13,5	15,7	17,4	0,5	2,9	1,4	1,6	1	1,9	0,5	1,1
V2	28,6	15,6	10,2	18,1	3,9	2,1	0,7	2,2	0,5	3,6	1.1	1,7
V3	23,8	21,8	13	19.5	2,6	0,5	3	2	3,3	0,8	2,4	2,1
V4	25,6	14,7	17,5	19,2	3,8	1,6	1,8	2,4	2,8	1,2	0,7	1,5
V5	27,9	16,9	9,8	18,2	1,7	2,9	2,4	2,3	0,9	2,9	1,4	1,7
V6	15,6	18,1	14,6	16,1	6,9	1,6	0,5	3	2,4	3,1	1	2,1
V7	22	12,5	10	14,8	4,6	1,9	2,9	3,1	1	0,4	2,6	1,3
V8	26,1	7,2	12,9	15,4	6,5	4,6	1	4	1,8	1	0,9	1,2
V9	23,7	10	15,7	16,4	4,2	2	2,1	2,7	0,1	1	3,5	1,5
V10	30,8	14,7	11,3	18,9	14,4	2,8	3,8	7	4,5	0,3	1,8	2,2
V11	24,3	17,4	14	18,5	9,9	3,6	2,9	5,4	4,8	2,4	1,2	2,8
V12	16,4	10,1	18,3	14,9	2	4,6	1	2,5	1,5	1,3	2,2	1,6
V13	19,4	7,4	12,5	13,1	12,9	3,5	2,6	6,3	15,1	3,3	0,4	6,2
V14	21,8	9,9	10,2	13,9	0,6	3,5	4,9	3	0,2	1,8	0,7	0,9
V15	28,7	15,9	18,5	21	3,7	2,9	1,5	2,7	2	2,6	2	2,2
V16	24,7	10,2	16,9	17,2	5,1	1,2	3,7	3,3	0,7	2,7	1,9	1,7
V17	13,2	13,5	19,4	15,3	18,1	4,8	4,2	9	1,3	0,4	3,1	1,6
V18	21,2	10,9	15,6	15,9	2,2	3,5	0,9	2,2	3,8	0,1	2,7	2,2
V19	17	12,1	9	12,7	1,6	4,1	2,7	2,8	1,8	0,7	1,5	1,3
V20	23,7	11,7	14,1	16.5	4,8	2,4	1,4	2,8	2,1	0,4	0,8	1,1
V21	24,2	14,7	15,1	18	3,2	1,4	4,9	3,1	2	0,5	3,1	1,8
V22	22,2	11,9	12	15,3	6,6	3,1	2	3,9	3,8	1,4	0,4	1,8
V23	15,7	9,9	15,2	13,6	3,3	1,4	3,8	2,8	1,9	0,8	0,1	0,9
V24	16,3	11,2	8,7	12	2	3,8	2,5	2,7	2	1,5	3,5	2,3
V25	15,9	12,7	11	13,2	1,8	4,2	0,7	2,2	3,3	0,1	2,7	2
V26	26,2	13,7	15,1	18,3	2,8	0,7	2,1	1,8	3,4	1,1	1	1,8
V27	15,6	13,5	17	15,3	8,8	0,3	2	3,7	1,8	1,4	3,5	2,2
V28	22,1	16,5	9.2	15,9	2,3	2,2	1,8	2,1	0,9	2,9	0,6	1,4
V29	24,8	17,1	10	17,3	2,6	3,1	0,5	2	1,7	3,6	1,5	2,2
V30	21,9	14,9	11,5	16,1	5,1	0,6	4,4	3,3	2,1	1	1,8	1,6
V31	21,9	10,9	13,7	15,5	9,8	4,5	0,9	5	0,9	2,5	0,8	1,4
V32	21,2	25,3	12,9	19,8	4	1,8	5,8	3,8	3,9	2,4	0,6	2,3

U1: repetition 1st U2 : Repetition 2nd U3: repetition 3rd R : average

Based on the results of the bacterial resistance test Vibrio sp. to antibiotics in Table 5 can be seen the results of testing of Chloramphenicol antibiotics ranging from 12-21 mm, V15 isolates are sensitive to inhibition zones 21 mm while 31 other isolates are classified as intermidate with inhibition zones ranging from 12 to 19.8 mm. Chloramphenicol is a broad-spectrum antibiotic that is used for various kinds of infections by microorganisms. Chloramphenicol antibiotics work by inhibiting bacterial protein synthesis. Chlorampenicol cannot be used for other types of eye infections other than serious eye infections caused by bacteria. Chloramphenicol (chloramphenicol) is an antibiotic that has bacteriostatic activity that inhibits protein synthesis and is usually used in diseases caused by Salmonella bacteria and at high doses is bactericidal. Its anti-bacterial activity by inhibiting protein synthesis by binding to the 50S ribosome subunit, which is an important step in the formation of peptide bonds. Testing using Pinicilin antibiotics all isolates including resistant with zone diameters ranging from 1.6-9 mm. Penicillins are a group of antibacterial drugs that attack a wide range of bacteria. They were the first drugs of this type that doctors used. The discovery and manufacture of penicillins have changed the face of medicine, as these drugs have saved millions of lives. Penicilin is used to treat a wide variety of bacterial infections. It may also be used to prevent certain bacterial infections (such as rheumatic fever). This medication is a long-acting penicillin antibiotic. It works by stopping the growth of bacteria. Testing using Isoniazid antibiotics. all isolates including resistant with zone diameters ranging from 0.9-2.8 mm. Isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. At therapeutic levels isoniazid is bacteriocidal against actively growing intracellular and extracellular.Isoniazid works by attacking into bacteria and inhibiting cell metabolism and replication so that cells can be killed. Antibiotic sensitivity test is classified into three criteria according to National Committee for Clinical Laboratory Standards (NCCLS), namely resistance (R) if the zone of inhibition is 0-10 mm, intermediate (I) if the zone of inhibition is 11-1 9 mm, and sensitive (S) if the zone of inhibition is above 20 mm. The inhibitory response of bacterial isolates using the Cloramfinocol antibiotic showed that only 1 bacterial isolate was classified as Sensitive, while 31 other isolates included

Intermidate. Penicillin and Isoniazid Antibiotics show that all isolates of bacteria are resistant to these antibiotics. The ability of antibiotics to inhibit bacterial growth is the presence of antimicrobial compounds that attack bacteria will damage the cell wall or prevent the system, so that it will cause the formation of cells that are sensitive to osmotic pressure or known as trauma. Damaged cell walls will cause plasmolysis. If these protoplasts are placed in an environment with a certain osmotic pressure, they will take fluid quickly, expand and break. Cytoplasmic membranes in bacteria act as selective permeability barriers, carry active transport functions and then control the internal composition of cells. If this integrity function is damaged, then macromolecules and ions out of the cell then cell lysis occurs and even death occurs. Cytoplasmic membranes can be easily damaged by antimicrobial agents which result in inhibition of the growth of pathogenic bacteria (Jawetz et al., 2005). The different results are caused by the ability of each bacterium to fight antibacterial activity depending on the thickness and composition of the cell wall. According to Sari (2017), in his research there are differences in the composition and structure of cell walls in each bakeri. Gram-negative bacteria contain lipids in higher presentation than those contained in Gram-positive bacteria. This statement is in line with the opinion of Adithya (2017) that the structure of Gram-negative bacteria has an outer membrane membrane that covers the thin layer of peptidoglycan, the structure of this peptidoglycan is a double layer containing phospholid, protein and lipoposaccharides. Lipoposaccharides are located in the outer layer and are characteristic of Gram negative bacteria. The difference in the inhibitory ability of each antibiotic is caused by differences in the composition of the antibiotic composition, so that the growth of pathogenic bacteria becomes inhibited. Lestari et al (2016), that antibiotics are able to inhibit the growth of pathogenic bacteria because antibacterial agents are able to reduce pH to be low so that pathogenic bacteria are difficult to survive.

4 CONCLUSION

The results showed the density of Vibrio sp. at station 4 higher than stations 1, 2 and 3 and the lowest at station 5. The density of vibrio bacteria is still below the threshold in waters (103 / ml). The results of bacterial resistance tests to antibiotics showed that 1 isolate was sensitive, 31 isolates were Intermidate to the antibiotic Cloramfinicol, while the antibiotics Pinicilin and Isoniazid were all resistant bacterial isolates.

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