Microbiological quality (pathogen *E. coli* bacteria) in the coastal environment of Dumai City, Riau Province

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Abstract. *Escherichia coli* is a water indicator microorganism. *E. coli* is found in almost water bodies such as lakes, rivers and seas that come from the feces of human and warm-blooded animals and waters contaminated with organic wastes. Microbiological quality is based on the presence of certain groups of microbes such as microbial pathogens (stomach disease), pollutants (especially *E. coli*), toxin producers etc. This research was conducted in October 2018. The purpose of this study was to calculate the density of *E. coli* bacteria in the coastal city of Dumai. This study used the survey method, with 5 stations with 3 sampling points at each station. Calculation of the number of *Escherichia coli* colonies used the MPN (Most Probable Number) method. The density of *E. coli* bacteria ranged from $2.37 \times 10^4$ to $1.1 \times 10^5$ CFU / 100 ml. The highest number of *Escherichia coli* bacterial colonies was at stations 2 and 3, the lowest was at station 5. The results of the *E. coli* bacteria resistance test for seven isolates of *E. coli* and the results of *Escherichia coli* bacteria resistance to antibiotics showed that there were seven isolates of Chloramphenicol, which included resistance with inhibitory zones ranging from 2.17 to 10.70 mm, 9 bacterial isolates including intermediates with inhibitory zones ranging from 11.90 - 19.33 mm, and one isolate including sensitive with a zone of inhibition of 22.77 mm. In the Penicillin and Isoniazid antibiotics all resistant bacterial isolates with inhibition zones ranged from 0.80 to 4.70 mm and 1.50 - 4.67 mm respectively. The coastal waters of the city of Dumai have passed the threshold for cultivation.

1. Introduction

Dumai Coast is one of the national and international shipping lines in Riau, as well as several industrial activities which consist of processing, disposal of processed industrial and household products. This gives a big influence on changes in environmental conditions around Dumai Waters (Dumai City Government). Pollution that occurs due to chemical, physical, and biological factors including industrial, domestic, and other activities have a negative impact on water resources,
including reducing the quality of water. This condition can cause interference, damage, and danger to living things that depend on water resources.

Indicator microorganisms can be differentiated into indicators of bacteria, viral indicators, and indicators of protozoa. Microbiologically, the pollution indicator bacteria are Coliform bacteria, Fecal coli, Fecal streptococcus, Clostridium and Pseudomonas, Coliform bacteria are the most important microbiological parameters for drinking water quality. The group of coliform bacteria, including Escherichia coli, Enterobacter aerogenes, and Citrobacter freundii, among the main bacteria is E. coli.

Escherichia coli is a water indicator microorganism. E. coli is found in almost all water bodies such as lakes, rivers, and seas originating from the feces of human and warm-blooded animals and waters contaminated with organic wastes. E. coli is generally habitat to the digestive tract of humans and animals [1], can be easily disseminated outside its original habitat through water and food intermediaries [2]. Under certain conditions, E. coli can cause infection, especially in patients with immune system disorders or in conditions where the gastrointestinal barrier is disrupted, even though non-pathogenic E. coli can cause infection [3].

Escherichia coli is commonly found in the digestive tract and is spread to all individuals. From the number of E. coli bacteria obtained, the condition of a polluted waters can be known because these bacteria are indicators of pollution. The problem of E. coli bacteria is that it endangers human health, a direct danger to human health can occur as a result of consuming polluted water both directly drunk, through food and can also be a result of the use of contaminated water for daily needs. Indirect hazards occur when E. coli bacteria are in waters so it contaminates the biota and eventually consumed by humans. This can cause disease for humans such as diarrhea and digestive tract disorders.

Many human activities carried out in the waters of Dumai will have an impact on the presence of bacteria, especially pathogenic bacteria. The existence of these microorganisms is beneficial for human life, but many also harm humans, for example, can cause various diseases or even cause damage due to contamination. Researchers are interested in conducting research on the density of E. coli bacteria, and the extent of their resistance to existing commercial antibiotics.

2. Materials and Methods
This research was carried out in September to October 2018. Water sampling was carried out in Dumai Sea Water in Dumai City, Riau Province. In this study the determination of the sampling point using a purposive sampling method, determined three sampling points for each station. Station 1 was in industrial area, station 2 was in residential area, station 3 was in port area, station 4 was in mangrove area, and station 5 was in area far from human activity (Figure 1). The activity of isolation and calculation of E. coli bacteria and heterotrophic bacteria and E. coli bacterial resistance to antibiotics was carried out in the Marine Microbiology Laboratory, Department of Marine Sciences, Faculty of Fisheries and Marine, University of Riau.

Calculation of the number of Escherichia coli bacterial colonies using MPN (Most Probable Number) series 3 tube method, through 3 stages, namely the Predictive Test (Presumptive test) using Lactose Broth (LB) media, Affirmation Test (Confirmed test) using Brilliant Bile Green Lactose Broth media (BBGLB), and Completed Test using Eosin Methylene Blue Agar (EMBA) media. The obtained isolates of Escherichia coli bacteria were tested for Gram, Catalase, Motility, Indol, H₂S, TSIA test, MR test, Citrate, and Sugar test (Lactose, Sucrose and Glucose) and E. coli bacterial resistance test against Chloramphenicol, Penicillin, and Isoniazid.
3. Results and Discussions

3.1. General condition of research area

Dumai City is one of the cities in Riau Province. Dumai City is on the coast of the eastern island of Sumatra. The Dumai area is in the position between 101°.23´-101°.8´ BT and 1°.23´-1°.24´ LU. Based on this position, the Dumai time zone is WIB. Dumai has an area of 1,727,385 km². The boundaries of Dumai City include the north bordering the Rupat Strait, the east bordering the Bukit Batu Subdistrict, Bengkalis Regency, the south bordering Mandau District and Bukit Batu District, Bengkalis Regency, and on the west bordering Tanah Putih District and Bangko District, Rokan Hilir Regency. The climate in Dumai is a tropical climate with two seasons namely the rainy season from September to February and the dry season from March to August. The average air temperature is between 21 - 35 °C and the average rainfall is between 100 - 300 mm [4].

3.2. Water quality parameters

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

Table 1 shows the result of measurements of water quality including pH, salinity, temperature, brightness, DO and current velocity and COD in the waters of Dumai have met the quality standards while nitrate and ammonia exceed the water quality standards according to Minister of Environment Decree No.51 of 2004 [5].
Table 1. Average water quality measurement results

| No | Parameter               | Station 1 | Station 2 | Station 3 | Station 4 | Station 5 | KEPMEN LH No. 51 of 2004 [5] |
|----|-------------------------|-----------|-----------|-----------|-----------|-----------|-------------------------------|
| 1  | pH                      | 7.3       | 7.7       | 6.7       | 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  | Transparency (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  | Current Velocity (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     | 3.8331    | 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                           |

3.3. *Escherichia coli* bacterial density

In Table 2 it can be seen that the density of *Escherichia coli* bacteria is different, the highest density of *E. coli* bacteria is at station 2 and station 3 with an average value of $1.1 \times 10^5$ CFU / 100ml due to several factors, namely at station 2 is a residential area, where there are many human activities, such as human waste disposal and household waste. At station 3 which is a port area so that the waste affects the density of bacteria. The density of *Escherichia coli* bacteria is lowest at station 5 with an average value of $2.37 \times 10^4$ CFU / 100ml due to this region is far from human activity. According to the Indonesian Ministry of Environment [6], the total content of *coli* and *E. coli* bacteria in seawater used for aquaculture must be under 1000 cfu / 100 ml. The coastal waters of the city of Dumai have passed the threshold for cultivation. On the sea has the potential for anti-bacterial pathogens including probiotic bacteria [7].

The high content of *E. coli* bacteria is inseparable from the habits of the community in collecting garbage / daily waste. Therefore, the disposal of waste both domestically and non-domestic in residential areas should be rearranged by the location of waste disposal, so that the flow of waste from each residential area can be well coordinated and does not cause diseases that disturb the lives of surrounding residents [8].

The most widely used bacterium as an indicator of sanitation is *E. coli* because these bacteria are commensal bacteria in the human intestine and are generally not pathogens that cause disease. But if the faecal *E. coli* is detected in the water, it shows that the raw water has been contaminated with human waste and may contain intestinal pathogens [9].
Table 2. Density of *Escherichia coli* bacteria in the Dumai Sea Waters

| Station | Sampling Point | Number of *Escherichia coli* bacteria (CFU / 100ml) |
|---------|----------------|-----------------------------------------------|
| 1       | 1              | $1 \times 10^4$                             |
|         | 2              | $1.6 \times 10^4$                           |
|         | 3              | $9.2 \times 10^2$                           |
|         | average        | $4.23 \times 10^4$                          |
| 2       | 1              | $1 \times 10^4$                             |
|         | 2              | $1 \times 10^4$                             |
|         | 3              | $1 \times 10^4$                             |
|         | average        | $1 \times 10^4$                             |
| 3       | 1              | $1 \times 10^4$                             |
|         | 2              | $1 \times 10^4$                             |
|         | 3              | $1 \times 10^4$                             |
|         | average        | $1 \times 10^4$                             |
| 4       | 1              | $1 \times 10^4$                             |
|         | 2              | $1 \times 10^4$                             |
|         | 3              | $3.5 \times 10^4$                           |
|         | average        | $7.45 \times 10^4$                          |
| 5       | 1              | $2.1 \times 10^4$                           |
|         | 2              | $2.1 \times 10^4$                           |
|         | 3              | $2.9 \times 10^4$                           |
|         | average        | $2.37 \times 10^4$                          |

CFU: Colony Forming Unit

*Escherichia coli* is generally habitat to the digestive tract of humans and animals [1] can be easily disseminated outside its original habitat through water and food intermediaries [2]. Under certain conditions, *E. coli* can cause infection, especially in patients with immune system disorders or in conditions where the gastrointestinal barrier is disrupted, even though non-pathogenic *E. coli* can cause infection [3]. Pollution in coastal waters generally occurs due to population concentration, tourism, and industrialization [10]. Concentration of population in coastal areas is a producer of household waste (domestic waste). Domestic waste generally consists of feces / stool, urine, waste water from other wastes (bathroom, laundry, and kitchen) [11].

3.4. Resistance of Escherichia coli bacteria to antibiotics

In Table 3 shows the results of testing of Chloramphenicol antibiotics that isolate bacteria A.EC.5, A.EC.6, A.EC.7, A.EC.9, A.EC.10, A.EC.11 and A.EC. 13 including resistance with inhibition zones ranging from 2.17 to 10.70 mm. While bacterial isolates A.EC.1, A.EC.2, A.EC.4, A.EC.8, A.EC.12, A.EC.14, A.EC.15, A.EC.16 and A.EC.17 including intermediates with inhibition zones ranging from 11.90-19.33 mm. And the isolation of A.EC.3 bacteria included sensitive with inhibition zones measuring 22.77 mm. On the average test results for Penicillin antibiotics that all bacterial isolates were included as resistant to the inhibition zone ranging from 0.80 to 4.70 mm. Likewise, testing of Isoniazid antibiotics that all bacterial isolates included were resistant to inhibition zones ranging from 1.50 to 4.67 mm. The results of the sensitivity test were seen according to the provisions of the National Committee for Clinical Laboratory Standards (NCCLS) [12]. Antibiotic susceptibility tests are classified into three criteria according to NCCLS [11], namely resistance (R) if the resistance zone
is 0-10 mm, intermediate (I) if the obstacle zone is 11-19 mm, and sensitive (S) zone is above 20 mm. According to Black [13] resistance is a condition of reduced influence of anti-infective drugs on bacteria or naturally bacteria are no longer sensitive to the administration of antibiotics. Resistance occurs when microorganisms such as bacteria, viruses, fungi and parasites change in such a way that making the medicines consumed to cure the infection becomes ineffective. 

*Escherichia coli* resistance to antibiotics has been widely reported. The results of the antimicrobial resistance study in Indonesia (AMRIN-Study) show that out of 2,494 individuals spread throughout Indonesia, 43 percent of *Escherichia coli* are resistant to various types of antibiotics. 

The development of bacterial resistance to antibiotics is strongly influenced by the intensity of antibiotic exposure in an area, uncontrolled use of antibiotics tends to increase the resistance of germs that were originally sensitive [14]. Heterotrophic marine bacteria have the potential as an antimicrobial for pathogenic bacteria in fish [15].

### Table 3. Average Results of Resistance Tests of *Escherichia coli* Bacteria against Antibiotics

| Isolate name | Chloramphenicol | Penicillin | Isoniazid |
|--------------|-----------------|------------|-----------|
|              | U1 | U2 | U3 | R   | U1 | U2 | U3 | R   | U1 | U2 | U3 | R   |
| A. E.C.1     | 27.5| 14.8| 15.7| 19.33| 5.1| 2.1| 4.2| 3.80| 1.8| 3.8| 2.9| 2.83|
| A. E.C.2     | 13.9| 16.9| 14.5| 15.10| 1.9| 2.8| 2.5| 2.40| 5.1| 1.3| 4.5| 3.63|
| A. E.C.3     | 24.7| 19.9| 23.7| 2277 | 0.5| 2.7| 1.6| 1.60| 2.9| 2.3| 2.8| 2.67|
| A. E.C.4     | 10.9| 18.4| 16.9| 15.40| 1.8| 1.7| 1.7| 1.73| 2.6| 4.7| 4.8| 4.03|
| A. E.C.5     | 3.8 | 15.6| 12.7| 10.70| 1.7| 2.1| 1.9| 1.90| 2.5| 2.1| 2.2| 2.27|
| A. E.C.6     | 2.2 | 13.2| 12.2| 9.20  | 2.1| 2.2| 2.2| 2.17| 1.1| 3.1| 1.9| 2.03|
| A. E.C.7     | 8.9 | 12.8| 9.2  | 10.30 | 1.9| 2.4| 2.1| 2.17| 1.9| 2.8| 2.7| 2.47|
| A. E.C.8     | 10.7| 12.9| 12.1| 11.90 | 2.7| 3.8| 7.6| 4.70 | 2.4| 2.4| 2.5| 2.43|
| A. E.C.9     | 12.8| 1.1 | 8.2  | 7.37  | 2.5| 3.6| 5.8| 3.97 | 0.8| 2.3| 1.9| 1.67|
| A. E.C.10    | 3.4 | 2.4 | 2.2  | 2.67  | 1.1| 1.1| 1.3| 1.17 | 1.3| 1.6| 1.6| 1.50|
| A. E.C.11    | 1.8 | 3.4 | 3.3  | 2.83  | 3.2| 1.3| 1.9| 2.13 | 2.9| 3.2| 3.1| 3.07|
| A. E.C.12    | 6.7 | 20.4| 17.7| 14.93 | 0.9| 2.8| 1.9| 1.87 | 2.5| 2.2| 2.3| 2.33|
| A. E.C.13    | 2.4 | 1.9 | 2.2  | 2.17  | 1.2| 0.1| 1.1| 0.80 | 1.8| 2.9| 2.8| 2.50|
| A. E.C.14    | 3.8 | 17.8| 15.4| 12.33 | 2.6| 3.7| 4.4| 3.57 | 2.6| 2.3| 2.6| 2.50|
| A. E.C.15    | 2.1 | 19.9| 19.8| 13.93 | 1.1| 4.8| 3.4| 3.10 | 3.8| 5.8| 4.4| 4.67|
| A. E.C.16    | 16.3| 20.9| 18.7| 18.63 | 2.8| 1.2| 8.9| 4.30 | 2.3| 3.8| 4.1| 3.40|
| A. E.C.17    | 16.2| 18.1| 18.1| 17.47 | 2.8| 2.3| 2.2| 2.43 | 1.2| 2.6| 3.3| 2.37|

U1: repetition1  U2: repetition 2nd  U3: repetition 3rd  R : average

### 4. Conclusion

The density of *E. coli* bacteria in the waters of Dumai ranged from $2.37 \times 10^4$ - $11 \times 10^4$ CFU / 100ml where the highest *E. coli* density was found at stations 2 and 3 and the lowest density at station 5. The results of the *E. coli* bacterial resistance test against 7 antibiotics of *E. coli* isolates were resistant to Chloramphenicol antibiotics, 9 bacterial isolates including intermediates and one sensitive isolate. In the Penicillin and Isoniazid antibiotics all isolates of resistant bacteria. In this study the testing was limited to the morphological and biochemical tests alone. It is hoped that in further research a more in-depth test will be carried out such as testing DNA sequences to find out more about the types of bacteria found in the Dumai Sea Waters. It needs the attention of the Dumai city government for handling waste in the coastal environment before being dumped into the waters of the sea.
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