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Analysis of plasmid profiles of *Escherichia coli* bacteria and their resistance to several antibiotics

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ABSTRACT

Escherichia coli is a bacteria belonging to the family Enterobacteriaceae. *Escherichia coli* is a harmful gram bacterium living in the digestive tract. These bacteria also live in water and soil, commonly referred to as coliform. This research aims to analyze the plasmid profile of *Escherichia coli* bacteria and its resistance to some antibiotics. To explore the plasmid profiles of *Escherichia coli* in a recent research electrophoresis gel, the antibiotic used in its resistance tests is the Amoxicillin, Tetracycline antibiotic, Ciprofloxacin, Sulfamethoxazole, and Streptomycin antibiotic. The electrophoresis results in a difference in the number of plasmid profiles with 1 to 2 ribbons of plasmid DNA different sizes from ˜1500 bp to ˜2300 bp. Antibiotic resistance tests have occurred in Amoxicillin, Ciprofloxacin, and some resistance samples to the Streptomycin antibiotic.

Keywords: antibiotics, *Escherichia coli*, plasmid, resistance

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INTRODUCTION

The presence of processed traditional food products, especially the people of Palembang, such as pempek, makes this food preferred because it is famous for its deliciousness. Pempek is a product of processed fish meat which is white in color, has a chewy texture and elastic. The best fish used in its manufacture are snakehead fish *(Chana striata)* or belida fish *(Notopterus chitala)*. These processed products have advantages but can pose a risk to health if the processing is not hygienic so that it is possible to be contaminated by bacteria.

Contamination caused by bacteria often found in food was *Escheichia coli* which can cause diseases of the digestive tract such as diarrhea. *Escherichia coli* is generally known as normal bacteria that exist in the digestive including the oral cavity, esophagus, stomach, rectum, and anus in humans. This bacterium is often associated as a secondary infection that worsens the condition of the host after primary infection by other disease agents (Elfidasari, 2011)**.** *Escherichia coli* bacteria used in this study were isolated from belida fish-based pempek. The purpose of the isolation of these food products was to analyze the resistance of several antibiotics to the *Escherichia coli* plasmid DNA obtained.

The *Escherichia coli* in processed food show a bacteria residue in the processed fish-based products circulated in several shops in Palembang. Antibiotic resistance is usually triggered by factors like the frequent use of antibiotics, overuse of new antibiotics, and long periods of antibiotics. The rise in antibiotic resistance has led to a test of antibiotic resistance of *Escherichia coli*. This test is made because it is feared that the bacteria *Escherichia coli* contained in processed fish-based products spread around the city of Palembang has become resistant to several antibiotics, so in this study, five antibiotics were used to test its resistance. Antibiotics used Amoxicillin, Tetracycline, Ciprofloxacin, Sulfamethoxazole, and Streptomycin. The antibiotic used is selected according to its mechanism and is widely used in research on antibiotic resistance to gram-negative bacteria such as *Escherichia coli*. The study uses Amoxicillin antibiotics to test sensitivity or bacterial resistance based on or no resistance zones by the discus paper method (Maida & Lestari, 2019). The results showed that all four bacteria tested to a large diameter of the support zones. It was either 0.03-0.44 mm or its resistance level the intermediate was their medication based on CLSI (*Clinical and Laboratory Standards Institute*) (CLSI, 2016). In research (Syafriana et al., 2020), Tetracycline tests antibiotic resistance. The use of this antibiotic includes the antibiotic that people often use and belongs to a broad spectrum of antibiotics by attacking bacterial protein synthesis. His research suggests that the Tetracycline antibiotic is still sensitive to test insulation by 75%, so they are still effective against *Escherichia coli* bacteria (Ogawara, 2019). The study used Ciprofloxacin antibiotics to test resistance and antibiotic sensitivity in the ISK treatment (Muslim et al., 2020). This choice of antibiotics is due to reports of antibiotics that have resistance to the handling of the ISK case. His research suggests that antibiotics Ciprofloxacin are mainly resistant (72.2%) and a small percentage sensitive (22,2%). Research by (Yamamoto et al., 2010) suggests that low doses are discouraged because they can cause increases in bacterial resistance. Research (Yogita et al., 2018) uses a Sulfamethoxazole antibiotic combined with Trimethoprim to see the frequency and percentage of its resistance levels made in RSUP Sanglah Denpasar, showed results that the bacteria tested were sensitive to these antibiotics, with a 100% sensitivity rate.

In research (Sukertiasih et al., 2021), a combination of antibiotics Sulfamethoxazole and Trimethoprim are used to treat pneumonia. The results suggest that the combination of antibiotics is still recommended for treatment therapy caused by gram-negative bacteria. Research results show a pretty high resistance rate of Sulfamethoxazole and Trimethoprim antibiotics. When combined, the resistance rate changes into a lower sensitivity, so the use of Sulfamethoxazole is usually combined with Trimethoprim to reduce the risk of antibiotic resistance. (Syahputra et al., 2018). In research (Monica et al., 2013). Streptomycin's use is based on the frequent use of antibiotics for treating the fish for a bacterial infection that reality presents in the field. (Suardana et al., 2014) also reports that the level of bacterial resistance *Escherichia coli* came from chicken feces on Streptomycin antibiotics by 42.9%. The study further studies on Streptomycin antibiotics (Januari et al., 2019), aiming to identify antibiotic resistance levels of *Escherichia coli* bacteria isolated from the chicken sold in Bogor's traditional market. The results showed considerable resistance to Streptomycin antibiotics up to 98%. The

opposition is suspected because the antibiotic is widely used and has a broad spectrum of workforce. But unlike the research done by (Reuben & Owuna, 2013) notes that Streptomycin antibiotics have a low resistance to *Escherichia coli* O157:H7. The differences in resistance from any research above could be due to a different *Escherichia coli* strain and antibiotics in a few different samples.

MATERIALS AND METHODS

Materials

The tools used in this study were an electrophoresis device set (Nanopac), micro pet (DragonLab), *vortex* (Mx-s), *centrifugate* (hanil model m15r), Mini Plasmid Kit, autoclave (SS XFS-280A 18 Liter), ruler (Butterfly), petri dish (Pyrex), incubator (memmert), needles ose, test tube (Pyrex), tube rack, Erlenmeyer (Pyrex), aluminum foil (Total wrap), measuring glass (Pyrex), beaker glass (Pyrex), hotplate, laminar airflow, Bunsen, match, rubbing alcohol, tag, heat-resistant plastic, disk paper, tissue (Tessa), cotton, thread, Kassa sterile, scissors. The materials used were food product containing Belida fish, NA (*Nutrient Agar*) medium, *Mueller Hinton Agar* (MHA) medium, NB (*Nutrient Broth*) medium, physiological NaCl, antibiotic solution (Amoxicillin, Tetracycline, Ciprofloxacin, Sulfamethoxazole, Streptomycin), agarose 1% (invitrogen), TBE buffer (invitrogen), PD1 Buffer (Geneaid), TrueBlue Lysis buffer (Geneaid), PD2 Buffer (geneaid), PD3 Buffer (Geneaid), W1 Buffer (Geneaid), Wash Buffer (Geneaid), Elution Buffer (Geneaid), Aquades (Sterile), RNAse (Geneaid), *Orange Loading buffer* (Invitrogen), marker DNA leader (Invitrogen).

Methods

Sampling

The samples were processed food product in the form of pempek made from Belida fish as many as five samples taken from several places at random in Palembang. The standard bacteria plasmid was *Escherichia coli ATCC* 25922 obtained from the INA-Lab, Padang.

Isolation and identification of Escherichia coli

The mashed food samples were cultured in Lactose Broth (LB) media and incubated at 37ºC for 24 hours. The presence of coliform in the sample when it produces gas in the Durham tube and the media becomes cloudy. Then the confirmation was tested by being inoculated back into Eosin Methylene Blue Agar (EMBA) media using the streak method to determine the presence of *Escherichia coli* at 37ºC for 24 hours with the appearance of shiny metal green colonies.

Bacterial rejuvenation

Previously identified bacteria are aseptically inoculated into a reaction tube containing NA (*Nutrient Agar*) to tilt one of three pure propagation groups. After that, it inoculated at 37ºC temperatures for 24 hours, then re-isolated into the NB (*Nutrient Broth*) and incubated for 24 hours. After that, these cultures were ready for the next test of antibiotic resistance and plasmid isolation.

DNA plasmid isolation

The process of isolation of plasmid DNA using Presto™ Mini Plasmid Kit (Geneaid). 1.5 mL of bacterial culture in each sample was transferred to a microtube centrifugation at a speed of 14-16,000 rpm for 1 minute. Then the supernatant was discarded and replicated four times. Then 200µl PD1 Buffer and 2µl True Blue Lysis Buffer were added into the microtube. The mixture was transferred to a microtube containing pellets and vortexed until dissolved. Then 200µl of PD2 Buffer was added while stirring for 2 minutes. Then 300µl of PD3 Buffer was added and centrifuged at 14-16,000 rpm for three minutes. Then all the supernatants were transferred to the PDH column which had been placed in the

collection tube and centrifuged at 14-16.000 rpm for 30 seconds. 600µl wash buffer was centrifuged at 14-16,000 rpm for 30 seconds and 50µl Elution Buffer was added. Then centrifuged at 14-16,000 rpm for two minutes. The resulting plasmid DNA was then tested by electrophoresis (Rotinsulu et al., 2019).

Plasmid separation with agarose gel 1%

As much as 0.4 mg of agarose was dissolved in 40 mL of TBE Buffer 1x to obtain Agarose gel 1%. The mixture was heated in the oven for 2 minutes to dissolve the Agarose. The Agarose solution was allowed to stand until it reached room temperature. Then 3 µL of ethidium bromide (EtBr) solution was added and poured into the mold in the electrophoresis box which already contained TAE buffer 1x. DNA samples and DNA markers were inserted into electrophoresis wells. Electrophoresis was carried out at a voltage of 80 V for 60 minutes. After that, visualization was carried out under UV light on a UV-Trans illuminator (Rotinsulu et al., 2019).

Antibiotic resistance test

Colonies of bacteria found in NA (*Nutrient Agar*) for sterile tilt are drawn using ose wire, moved to the physiological NaCl, and stirred until the bacteria colony was mixed up until it got the same frequency as Mc. Farland 0,5. The solution is then taken as much as 100ml into a petri dish, adds an MHA (*Mueller Hinton Agar*) solution, and is flattened by turning a petri dish into an 8; wait till the compactor. Place the discus paper dipped in antibiotic solution on the MHA (*Mueller Hinton Agar*) surface and is incubated at 37ºC temperatures 24 hours. There was a suppression zone diameter measurement (Yaddi et al., 2020).

Data Analysis

The data obtained were analyzed descriptively by presenting the test results for the presence of *Escherichia coli* bacteria in processed products of Belida Fish (*Chitala lopis*) and *Eschercihia coli* that are resistant to several antibiotics in the form of tables and figures.

RESULTS AND DISCUSSION

The results of isolation of *Escherichia coli* on the all samples in Lactose Broth (LB) media gave the media a cloudly color and formed gas in the dirham tube. These showed the presence of coliform bacteria. The bacteria were inoculated again with a selective medium, namely Eosin Methylene Blue Agar (EMBA). This medium is used for the isolation and differentiation of enteric (coliform) bacteria. The results of the inoculation that produced blackish purple colonies with a metallic green luster were *Escherichia coli*. This media contains lactose so that it can distinguish groups of bacteria based on the their ability to ferment lactose. Escherichia coli is one of the bacteria that can ferment lactose quickly and can produce a lot of acid which can produce metallic shiny green colonies.

The results of plasmid isolation and electrophoretic profiles of plasmid DNA against 5 isolates and standard plasmids of *E.coli* in Table 1 showed the plasmid DNA of *E.coli* isolates AL 01 (a) and isolate AL 04 (d) each had two types of plasmids. One plasmid measuring 1,700 bp and another plasmid measuring 2,300 bp. Isolate AL 02 (b) and isolate AL 03 (c) each had only one type of plasmid located at 2,300 bp and isolate AL 05 (e) located at 1,500 bp. Meanwhile, the standard bacterial plasmid (f) had three types of plasmids located at 2,000 bp, 1,500 bp, and 1,000 bp. In general, *E. coli* plasmids that had been isolated had sizes ranging from 1,500-2,300 bp. This corresponds to the size of the plasmid between 1 kbp to 200 kbp (Hardianto et al., 2015), Based on the type of plasmid that had been isolated, it showed a low copy number of plasmid where the plasmid has low replication ability so that in one cell it only contains one or several of the same plasmid.

A plasmid is a stable DNA molecule of a lowered bacteria chromosome and replicates independently (Wibowo, 2011). A plasmid is a circular DNA molecule free of bacteria or extra chromosomes and small amounts. A plasmid is a component that can transfer many bacterial genes from one bacterial cell to another (horizontal transfer or conjugation of genes). It is also known that bacteria with plasmid may most likely cause resistance. Furthermore, resistance can result from gram-negative

bacteria such as *Escherichia coli* can produce a beta-lactamase enzyme. It can impede the workings of antibacterial mechanisms, and the nature of bacterial resistance is transferred through plasmid. Any plasmid bacteria resistant to antibiotics will bring one or more resistance genes. A plasmid that can lower the spirit of resistance is commonly called plasmid R.

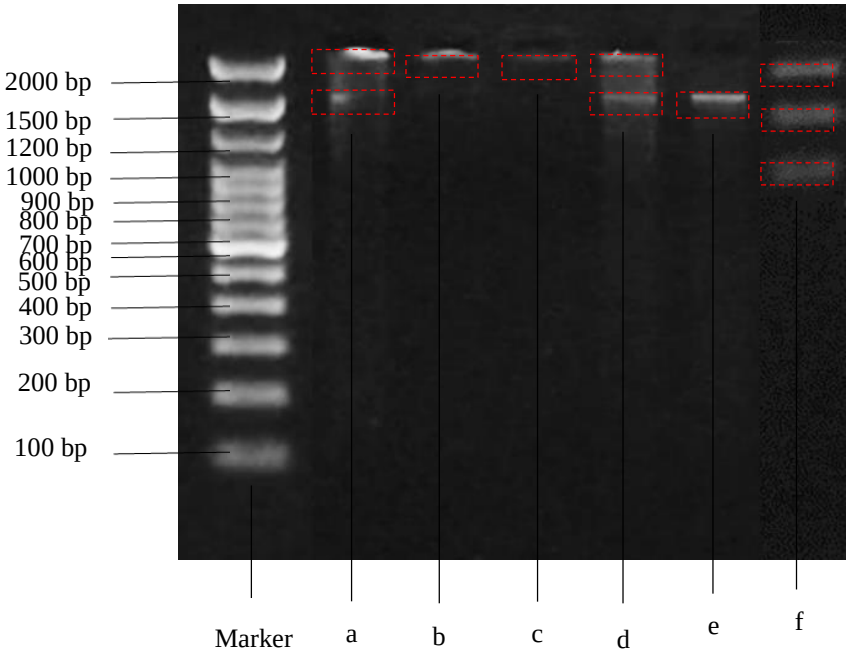

Figure 1. The Results of The Visualization of The Bacterial Plasmid DNA Test Using Electrophoresis Methods (a): isolate AL 01 (b): isolate AL 02 (c); isolate AL 03 (d): isolate AL 04 (e); isolate AL 05 and (f): standard *E. coli* plasmid

According to (Hardianto et al., 2015), many methods have been developed to isolate plasmids since the discovery of plasmids. Several ways such as alkaline lysis, lysis by heating, the use of cesium chloride, microwave and chromatography. The most commonly used plasmid isolation method is alkaline lysis. This method has a simple process, relatively inexpensive and high reproducibility. In this method, there is a resuspension process on cell pellets with Tris-EDTA and glucose solutions; cell lysis through a solution of SDS (Sodium Dodecyl Sulfate) and sodium hydroxide which causes the cell walls and cell membranes of bacteria to break; and the neutralization process through a solution of potassium acetate (pH 4.8) and glacial acetic acid cause chromosomal and plasmid DNA to renaturate (Yadav et al., 2011).

The plasmid profile of *Escherichia coli* bacteria (Figure 1) is known through electrophoresis methods. Electrophoresis is a technique for separating cellular molecules above their size through an electric field siphoned through a medium containing a sample to be separated. The electrophoresis would provide a track record of DNA ribbons. Some of the primary components in the electrophoresis usage, the TBE Buffer solution, serve to maintain its pH in the medium separator. As an electrolyte medium of electrifying, gel agarose serves as an independent media to maintain the conditions of sample similarities during the separation process. A UV light visualization was run electrophoresis with 50 V electric tension for 1 hour and 20 minutes (Harahap, 2018). Electrophoresis readings were made on a UV-Trans illuminator or gel doc from the sample by comparing the tape from the piece with a known scale called the marker. The marker being used is masker 1,500 bp. The results of electrophoresis visualization in all the samples contained DNA ribbons of varying thickness and the range in tape size ˜ 1,500 bp and

˜2,300 bp. In general, bacterial plasmids are double-strand DNA molecules separated from chromosomes with very small sizes from 1 kbp to more than 200 kbp which contain the genes that cells need to survive under certain conditions (Hardianto et al., 2015). Plasmids can be seen when the genes they contain give new traits to the host such as resistance plasmids, virulence plasmids, degradative plasmids, sex plasmids, and col-plasmids (Wibowo, 2011).

Table 1. Results of antibiotic resistance

Sample Codes	Amox	Kp	Tetra	Kp	Cipro	Kp	Sulfa	Kp	Strepto	Kp
AL 01	0	R	20 mm	S	0	R	26 mm	S	15 mm	S
AL 02	0	R	22 mm	S	0	R	25 mm	S	0	R
AL 03	10 mm	R	20 mm	S	15 mm	R	20 mm	S	10 mm	R
AL 04	12 mm	R	17 mm	S	18 mm	R	27 mm	S	14 mm	I
AL 05	10 mm	R	15 mm	S	16 mm	R	22 mm	S	16 mm	S

Information:
AL: Sample Codes; Kp: Sensitivity; R: Resistance
S: Sensitive; I: Intermediate; Amox: Amoxicillin; Tetra: Tetracycline; Cipro: Ciprofloxacin; Sulfa: Sulfamethoxazole; Strepto: Streptomycin

Table 2. Range of antibiotics inhibition zones (CLSI, 2016)

Antibiotics	Sensitive	Intermediate	Resistance
Amoxicillin	≥ 18 mm	14 – 17 mm	≤ 13 mm
Tetracycline	≥ 15 mm	12 – 14 mm	≤ 11 mm
Ciprofloxacin	≥ 26 mm	22 – 25 mm	≤ 21 mm
Sulfamethoxazole	≥ 16 mm	11 – 15 mm	≤ 10 mm
Streptomycin	≥ 15 mm	12 – 14 mm	≤ 11 mm

Antibiotics are drugs used to treat bacterial infections (Savitri et al., 2019). This antibiotic is a material secreted by microorganisms and has an antagonistic attitude toward the growth and life of other organisms. The increasingly widespread use of antibiotics has led to a host of bacteria resistant to antibiotics on the market.

According to Barnard and Jonathan in research (Utami, 2017), resistance or refusal to protest the changes that occur and are not appropriate. Infection by antibiotic-resistant bacteria will harm patients because they become difficult to treat and affect health care costs. Antibiotic resistance is a consequence of misusing antibiotics and developing such microorganisms. It is also the case with a genetic mutation that leads to resistance to antibiotics (Syah et al., 2020). Among the bacteria that have been highly resistant to antibiotics is the *Escherichia coli* bacteria. These bacteria are good bacteria that, if given too much, would become pathogenic bacteria that cause infection. Treating diseases caused by *Escherichia coli* bacteria, β-Lactam class antibiotics are commonly used, Tetracycline and other antibiotic classes as in research (Nurjanah et al., 2020).

The level of bacterial resistance to antibiotics according to the standard for assessing the diameter of the antibiotic inhibition zone based on the CLSI (Clinical Laboratory Standards Institute). These levels are grouped into three categories, namely sensitive, intermediate, and resistance (Table 2). A bacterium is said to be sensitive to antibiotics if the bacteria can be inhibited properly and a clear zone

is formed when tested. Intermediate category if the bacteria can be inhibited but with a weaker inhibitory power, and the resistant category is if the bacteria can be inhibited but show very weak inhibition or no inhibition is formed at all (CLSI, 2016).

In this study of antibiotic resistance tests 5 antibiotics represent each class of antibiotics after used for disease therapy caused by the *Escherichia coli* bacteria Amoxicillin, Tetracycline, Ciprofloxacin, Sulfamethoxazole, and Streptomycin antibiotics. The resistance tests show resistant, sensitive, and intermediate samples to some antibiotics of the five test samples. The five samples tested using Amoxicillin and Ciprofloxacin antibiotics show that the entire piece has been resistant to both antibiotics. For testing, the Tetracycline and the Sulfamethoxazole antibiotics show that the whole sample was sensitive to both antibiotics. And the last test of Streptomycin antibiotics suggests that the two samples tested have already been resistant to the antibiotic samples of AL 02 and AL 03, and two samples are sensitive to the antibiotic, those AL 01 and AL 05, and the AL 04 samples indicate the intermediate result of the testing of the antibiotics.

The results of this antibiotic resistance test showed that *Escherichia coli* bacteria were resistant to the antibiotics Amoxicillin, Ciprofloxacin, and in some samples were resistant to Streptomycin. Judging from the nature of the plasmid, that plasmid is defined as a double-stranded circular DNA molecule located outside the bacterial chromosome (extra-chromosomal) and capable of autonomous replication, and has an important role in the spread of resistance genes. If a bacterium has a larger plasmids, the more likely it is that a gene mutation will occur. This causes bacteria to easily become resistant to antibiotics.

Therefore, if it is associated with susceptibility tests that have been carried out against various antibiotics, it can be seen that within one species there are variations between species in carrying gene types that vary greatly in function, such as heavy metal resistance, sensitivity to mutagens, resistance to bacteriophages and antibiotics. This was obtained from the results of the electrophoresis that had been carried out, where from the five *E. coli* plasmids that were successfully isolated, the plasmid sizes varied as well as the pattern of sensitivity of the bacterial plasmids to the tested antibiotics

CONCLUSION

Research suggests that plasmid appears in the previously identified *Escherichia coli* bacteria. Based on the results of visualized DNA plasmid isolation with electrophoresis, DNA ribbons are produced from the size of this ˜ 1,500 bp and ˜ 2,300 bp. Antibiotics resistance tests show resistance to bacteria *Escherichia coli* against Amoxicillin, Ciprofloxacin antibiotics, and some samples resistance to Streptomycin antibiotics.

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