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ANALYSIS OF *Escherichia coli* BACTERIA CONTENT IN FISH MEAT WITH VITEK 2 COMPACT

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ABSTRACT

Transmission of *Escherichia coli* in food can occur through contact with infected workers, as well as tools and materials used during food processing. One way to determine the presence of *E.coli* in processed fish balls is to perform a bacteriological test. The purpose of this study was to detect the presence or absence of bacteria in fish balls so that it can be seen whether these meatballs are in the hygienic category and are suitable for consumption or not. This type of research is a laboratory experiment. This research was conducted at the Maluku Provincial Health Laboratory on 26-28 January 2022. The sample used in this study was fish balls taken by purposive sampling (taken according to research needs) for laboratory testing. Based on the results above, it shows that the *E.coli* bacteria found in the meatball samples during the phenotyping test with Vitek 2 compact both at a dilution of 10-1 to 10-3 were not identified because they had not passed the maximum limit. Based on the results obtained, it was found that the fish balls made by service participants in Mamala country were still in accordance with the criteria of the Indonesian National Standard (SNI) No.7388:2009 regarding the maximum limit of *E.coli* bacterial contamination in processed meat products made by the government for the protection of consumers regarding the quality of the product in circulation and declared to meet the requirements for the maximum threshold of microbial contamination in food if the value is 3/g for the status fit for consumption. The condition of the value of the test results of the fish balls sample under study is due to the fact that food makers or processors when processing fish balls still use the correct sanitation and hygiene principles and according to procedures.

Keywords: *Escherichia coli*, Meatball, Vitek 2 compact

I. INTRODUCTION

Safe food is food that is not contaminated by chemical contaminants, biological contaminants, and physical contaminants. Food sanitation must always be maintained. If the food consumed is contaminated or unsafe, it will be one of the causes of health problems in our bodies. Events that often occur in cases of food poisoning are contamination caused by biological contaminants such as bacteria. Bacterial contamination of food can cause congenital diseases in the form of infections. Infection is caused by ingesting food contaminated with bacteria into the body. Contamination in food can be through several factors, namely place factors, equipment factors, people/ processors and food ingredients factors [1].

Processing of meatballs from fish can be done yourself, starting from processing raw materials to the appropriate boiling

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process in order to avoid bacteria that cause infection, considering that meatballs are one of the most popular processed products. So far, in the manufacture of meatballs, the main ingredient is meat. The meat used can be beef, chicken or other meat [2]. In the study, the type of meat used was tuna meat. Fish meat was chosen because fish is a very important source of protein for human nutritional needs [3]. Fish and processed fish products are highly perishable foodstuffs. In general, people like fish balls a lot. However, the obstacle faced is that the meatballs are easily degraded by spoilage bacteria and do not last long. Transmission of Escherichia coli in food can occur through contact from infected workers, as well as tools and materials used during food processing. According to Sanjaya & Apriliana (2013) in [4] *E.coli* can be one of the causes of disease transmission through food (Foodborne disease), namely diseases caused by consuming contaminated food and drinks.

One way to determine the presence of *E.coli* in processed fish balls is to perform a bacteriological test using a phenotypic test through Vitek 2 Compact. Vitek 2 Compact accommodates the same colorimetric reagent card. It is this colorimetric reagent card that will be incubated and interpreted automatically. The function of this test is to detect the presence or absence of bacteria in fish balls so that it can be seen whether these meatballs are suitable for consumption or not. *E.coli* bacterial contamination Indonesian National Standard SNI No.7388:2009 concerning the maximum limit of *E.coli* bacterial contamination in processed meat products made by the government for the protection of consumers regarding the quality of products in circulation is declared to meet the requirements for the maximum threshold of microbial contamination in food if the value is <3 /g [5] while based on Perkemenkes No.1096/MENKES/PER/VI/2011 which is 0/g [4]. Based on the description above, the purpose of this study was to identify the presence or absence of *Escherichia coli* bacteria in the processed homemade fish balls because of community service products with Vitek 2 Compact.

2. RESEARCH METHOD

Time and Place

This research was conducted at the Maluku Provincial Health Laboratory on 26-28 January 2022.

Method

This type of research is a laboratory experiment. The sample used in this study was fish balls. The determination of the number of samples used in this study was based on the purposive sampling method (taken according to research needs) in the form of fish meatball products processed by the Mamala country community for laboratory testing. The variables in this study were fish balls, *E. coli* bacteria, and the maximum number of germs in food. After the sample was taken, the sample was stored in an ice box and immediately taken to the laboratory to be checked for *E.coli* content. Treatments were analyzed in duplicate (repeated measurements on the same sample).

Research Procedure

The research instrument used was a sample or specimen of fish balls obtained from the results of community products in PkM activities and then observations were made by isolation and identification. Escherichia coli bacteria from fish ball samples in 1% peptone water buffer were grown on Brilliant Green Bile Broth (BGBB) media (E. Merck, Darmstadt, Germany) then incubated at 37^0 C for 18-24 hours [6]. A positive result is indicated by the presence of gas bubbles in the Durham tube and a green color change to cloudy green. After being positive, it was then

planted on Eosin Methylene Blue Agar (EMBA) media (E. Merck, Darmstadt, Germany) by streaking and incubation at 37 C for 24 hours. Typical colonies of *E.coli* on EMBA media are metallic green. In EMBA media, colonies of *E.coli* bacteria are metallic green, this is due to the ability of the bacteria to ferment lactose and methylene blue, while bacteria belonging to the *Enterobacter aerogenes* species will be pink to colorless.

Escherichia coli positive bacteria then performed microscopic test with gram staining. *E.coli* bacteria have gram-negative characteristics, pink color, and small rod-shaped appearance, arranged singly or in short pairs [7]. After the gram staining test, then continued with the phenotypic test with vitek-2. Gram negative bacterial colonies were taken from MacConkey agar and Gram positive bacteria from nutrient agar. The colonies were then dissolved in 3 mL of 0.45% NaCl solution pH 4.5, homogenized to form a suspension according to the McFarland standard 0.5–0.63 as measured by VITEK® 2 Densi CHEK™ Plus. The GN (Gram Negative) card is inserted into the suspension tube and placed in the cassette, then inserted into the Vitek 2 Compact. The results of the identification of gram negative bacteria were obtained after being incubated for 3–10 hours while Gram positive for 2–8 hours. According to CLSI 2014 explained that the Vitek-2 Compact system (bioMerieux, Marcy I'Etoile, France) is a system for identifying and testing semi-automatic resistance for bacteria so that it is possible to determine MIC quickly by analyzing bacterial growth kinetics using a card for test. The Vitek-2 method has proven to be very good in detecting the presence of bacterial resistance to a product because the results are very accurate and nothing is subjective. In a study conducted by Spanu et al., in [8] the vitek-2 method has a sensitivity of 98.1% and a specificity of 99.5%. Then, the data obtained analyzed descriptively. The results of the isolation and identification of *E.coli* bacteria isolated from fish ball products are presented in the form of tables and figures.

3. RESULT AND DISCUSSION

This study used one type of fish ball sample and then the suspension was added to Buffered Pepton Water (BPW) with a ratio of 1:10. Bacteria were grown on *Eosin Methylene Blue Agar* (EMBA) media as primary isolation. Typical colonies of *Escherichia coli* that grew were replanted into other EMBA media as secondary isolation to separate *Escherichia coli* colonies from other types of coliform bacteria in primary isolation. The results of the isolation and identification of the samples showed that the EMBA media changed color to metallic green as shown in Figure 1.

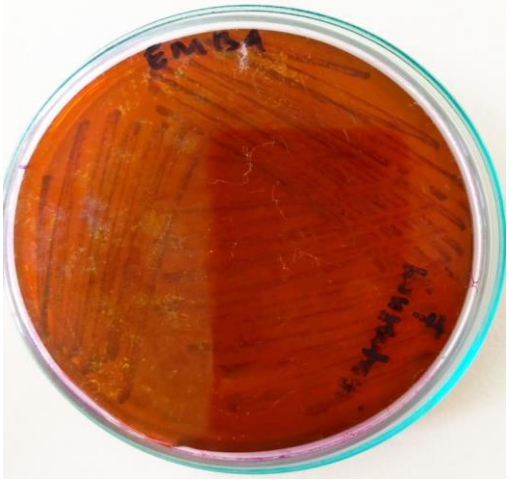

Figure 1. Presumptive *E.coli* on EMBA Media

The growth results of *Escherichia coli* on EMBA showed metallic green colonies, 2 - 3 mm in diameter with a black dot in the center of the colony [9]. EMBAs are selective media and differential media. This medium contains eosin and methylene blue, which inhibit the growth of gram-positive bacteria, so this medium was chosen for gram-negative bacteria. These bacteria form metallic colonies due to the reaction between bacteria and Methylene

blue. EMBA also contains lactose carbohydrates, in the presence of lactose carbohydrates gram-negative bacteria are differentiated based on their ability to ferment lactose. The color of the media before fertilizing the bacteria was red-purple. Metallic green color changes in EMBA media because *E. coli* can ferment lactose which results in an increase in acid levels in the media. High acid levels can precipitate methylene blue in EMBA media. From the results of this study, it can be seen that some samples showed high coliforms, but not all high coliform counts indicated the presence of E. coli bacteria. According to [10] if it is only known that the number of coliforms is high, and then the environment, which is an indication of pollution, which can indirectly be an agent for other pathogens, most likely causes it.

Colonies suspected of *Escherichia coli* were confirmed using the gram stain test as shown in Figure 2.

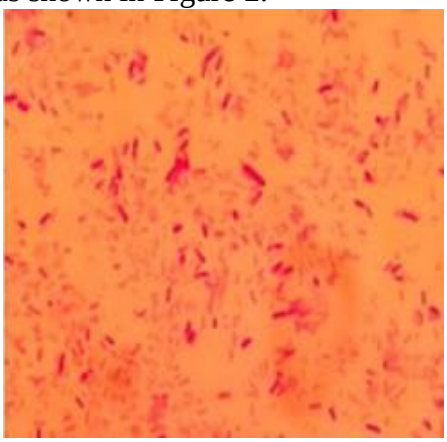

Figure 2. Colonies of *E. coli* bacteria with gram staining (arrows)

Based on the results of the identification that has been carried out, positive results of *E. coli* bacterial colonies are obtained by showing metallic green characteristics which are characteristic of bacterial colonies that ferment strong lactose, especially *E.coli* producing metallic green colonies. In gram staining, the results showed the characteristics of a short rod-shaped, pink, and solitary when observed under a microscope. The results of the Gram staining carried out obtained several bacterial morphologies that were visible under a microscope [11].

There are bacteria with the shape of bacilli, including long and short bacilli. The bacteria that were taken and continued for biochemical tests were short bacilli-shaped bacteria and were red in color, because the morphology of these bacteria was in accordance with the characteristics of the gram-negative bacteria group. Gram-negative bacteria show a red color because it is caused by the concentration of lipids and the thickness of the peptidoglycan layer on the bacterial cell wall. In gram-negative bacteria cell walls alcohol also increases cell wall porosity by dissolving outer layer lipids so that the crystal violet complex can be easily removed from the peptidoglycan layer which is not strongly crosslinked, therefore the alcohol washing process facilitates the release of the unbound crystal violet complex and makes cells colorless or loses color so that they can absorb the red rival dye, namely safranin [12].

In addition to isolation and identification on selective media and gram staining to detect Escherichia coli bacteria, the next test carried out was a phenotype test to determine the amount of Escherichia coli bacterial contamination in processed fish balls. The observations were made with the Vitek-2 Test which is shown in the following Table 1.

Based on Table 1, it shows that the *E.coli* bacteria found in the meatball samples during the phenotyping test with vitek-2 both at a dilution of 10-1 to 10-3 were not identified because they had not passed the maximum limit. Through the results obtained, the fish balls made by service participants in the Mamala country are still in accordance with the Indonesian National Standard (SNI) No.7388:2009 concerning the maximum limit of *Escherichia coli* bacterial contamination in processed meat products made by the government for the protection of consumers

regarding quality. Products in circulation are declared to meet the requirements for the maximum threshold for microbial contamination in food if the value is 3/g. The results showed that the fish ball samples had a suitable status for consumption. The condition of the value from the test results of the fish ball sample is due to the fact that when processing fish balls it still uses the correct sanitation and hygienic principles and according to the procedure. The presence of these low bacterial colonies was due to low activity during product manufacturing, a better hygienic processing system and a better sanitation system in the production room. The process of selecting fresh fish, good storage and handling will determine the quality and quality of meatballs [13].

Table 1. Phenotypic Test of *E. coli* with Vitek-2

Sample	Dilution	Result	Maximum Limit
Bakso Ikan	10^{-1}	Negatif *E. coli*	Neg / 25 g
	10^{-2}	Negatif *E. coli*	Neg / 25 g
	10^{-3}	Negatif *E. coli*	Neg / 25 g

Efforts that need to be made so that fish balls are protected from contamination with *Escherichia coli* bacteria are to maintain the quality of raw materials to avoid contamination, to control raw material storage, to process raw materials that maintain hygienic elements and when the presentation process must be clinically tested for cleanliness. In order to keep the food safety system running properly, it is very necessary to carry out food supervision. This food safety system is one of the efforts to protect the public in a sense of security and safety to consume a product. In addition, food products from fish are also good for consumption because they have high nutritional value so that they can increase their selling value in the market [14].

In addition, by paying attention to environmental cleanliness in a state of good sanitation, fish ball processors make the resulting product free from bacteria. Likewise, the use of contaminant-free tools and materials can make processed products said to be hygienic. Cooking utensils and utensils used in food preparation can also be a source of contamination. For example, knives or cutting boards used to cut raw materials, such as raw meat, can be contaminated with pathogens. If the equipment is used again without being cleaned properly, especially if it is used for cooked or ready-to-eat food, these pathogens can move and become a serious threat to food/food [15].

Furthermore, in the processing of fish balls that do not contain bacteria, it may be because the existing bacteria have died during the heating process or the making of meatballs with the water used is clean water (not contaminated with bacteria) so it is safe for consumption. Food that is safe for consumption is food that does not contain ingredients that can endanger health or cause disease or poisoning, namely biological hazards, chemical hazards, and physical hazards. Biological hazards are hazards due to the presence of living things such as microbes, pests and the like. Danger caused by microbes / bacteria, viruses, and molds [5].

4. CONCLUSION

After conducting research on the identification of *Escherichia coli* bacteria in processed fish balls made during service by the Mamala community, no Escherichia coli bacteria were found in the meatballs after being examined in the laboratory using an isolation test and identification then when a phenotype test was carried out

as a confirmation test with Vitek-2 it is stated that the fishball product meets the requirements because it complies with the maximum limit of microbial contamination in food. The author's suggestion is that further research is expected on testing other types of bacteria that are contaminated in fish ball products so that it can be ensured that the products made are truly safe for consumption and fit to be marketed.

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