THE IDENTIFICATION OF *Escherichia coli* BACTERIA IN SKEWERED MEATBALLS IN CENTRAL GORONTALO CITY

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ABSTRACT

Skewered meatballs are a type of snack food made from flour and meat, formed into balls and then boiled. Meatballs are a gel product made from protein derived from meat, including beef, chicken, fish, or shrimp. The quality of meatballs can vary significantly due to differences in raw materials and additional ingredients used, the proportion of meat to flour, and the manufacturing process.The aim is to identify *Escherichia coli* bacteria in skewered meatball snacks in the Central City of Gorontalo. This research method uses a qualitative descriptive approach. It is a type of descriptive research.The study's results identified *Escherichia coli* bacteria in skewered meatballs in the Central City of Gorontalo, where 2 out of 5 samples tested positive for *Escherichia coli*. The study concludes that the skewered meatballs sold in the Central City of Gorontalo contain *Escherichia Coli*. The skewered meatballs sold in the Central City of Gorontalo still lack cleanliness and hygiene such as the selling area, and the containers or tools used are not given suffuient attention.

Keywords : Identification, Escherichia Coli Bacteria, Skewered Meatballs

INTRODUCTION

Food can be a place for microbes to grow, especially if it contains high amounts of water or protein. Snacks are foods or drinks made by food vendors when they are going to be sold, or served in ready-to-eat form. In serving them, snacks must prioritize cleanliness and hygiene so that their quality is maintained. However, snacks still carry the risk of causing health problems [1].

Bacterial contamination of food due to negligence in maintaining cleanliness can occur through human contact during the production process or when food is consumed, because food comes into contact with many people who handle it. In addition, cooking utensils and storage places that are used continuously for a long period of time can also be a source of bacterial growth and cause contamination. Contaminated meat or poultry is an important thing to consider in the food processing process. Therefore, food safety and cleanliness must always be maintained. If the food consumed is contaminated or dangerous, this can trigger health problems. Most cases of food poisoning are caused by contamination from biological factors [2].

Microbesare very small living organisms that cannot be seen with the naked eye and can only be observed using a microscope. The main types of microorganisms include

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bacteria, fungi, protozoa, algae, and viruses [2].

One type of snack that is very popular is skewered meatballs, which are made from a mixture of flour and meat, shaped into rounds, boiled, seasoned with special spices, then grilled directly over charcoal and served with peanut sauce. This snack is popular among school children because it is affordable, tastes good, and looks attractive. However, the food safety aspect of meatball skewers needs to be considered because they are generally sold openly on the side of the school road and left uncovered for long periods of time [3].

One of the main causes of foodborne illness is the transmission of infection through contaminated raw meat. The meat used to make meatballs can come from beef, chicken, fish or shrimp. The main ingredients in making meatballs include ground meat, tapioca flour, spices, and table salt (NaCl). Each meatball usually weighs 25–30 grams and is round like a marble. Chewy texture is one of the characteristics of cooked meatballs. The quality of meatballs is greatly influenced by the ratio of meat and flour, processing techniques, and the raw materials and additives used [3].

Standardization of meatballs is important because of the high number of consumers, so food safety must be truly maintained. Food safety includes the efforts and conditionsnecessary to prevent biological, chemical and other types of contamination that could endanger human health [4].Food poisoning is generally caused by bacteria due to poor sanitation or hygiene. One of the bacteria that is often used as a sanitation indicator is *Escherichia Coli*, because this bacteria naturally lives in the human intestine and is generally not pathogenic, so it is safe to use for testing [5].

Escherichia coli is a bacterial flora commonly found in the human intestine and can cause diseases, such as diarrhea. These bacteria are members of *the Enterobacteriaceae family*, are gram-negative, and have a short rod shape also known as coccobacillus. *Escherichla Coli*moves using flagella [6].

Escherichia Coli(10–20%) is one of the bacteria that causes foodborne illness in developing countries. This bacteria generally lives in the human digestive system [7]. *E. coli* infection can occur through direct contact, consumption of undercooked meat, or processed meat products, because the structure of meat supports bacterial growth. Initial infection in the large intestine by *E. coli* can cause diarrhea. Diarrhea is characterized by defecating more than three times in a short period of time, with soft to liquid textured stools [8].

These bacteria are gram-negative, have a short rod shape, and are often referred to as coccobacilli. *Escherichia Coli* has flagella measuring 0.4 -0.7 µm x 1.4 µm and is equipped with a ring. This bacterium has a length of about 2 µm, a diameter of 0.7 µm , and a width of between 0.4 and 0.7 µm, and is facultative anaerobic. *Escherichia Coli* colonies are round, smooth, and have clear boundaries. This bacterium has 150 types of O antigens, 50 types of H antigens, and 90 types of antigens. Some O antigens can also be found in other microorganisms, such as *Shigella*. Some infections related to this O

antigen can occur in urinary tract infections and diarrhea [6].

Gram staining findings show that *Escherichia Coli bacteria* have a round, convex shape and smooth edges, with a microscopic shape in the form of round cells (cocci) and a single cell arrangement [6].

The time required for *Escherichia Coli* to grow varies, from 30 to 87 minutes, depending on the temperature. The time required for *E. coli cells* to divide is called the generation time. The shortest generation period is 30 minutes, while the ideal temperature for the development *of E. coli* is 37 °C. Because its presence in water indicates that the water is contaminated with feces that may contain other dangerous intestinal pathogens, *Escherichia Coli* is also used as an indicator of drinking water quality. Although most *E. coli bacteria* in water are harmless, some pathogenic strains, such as enterotoxigenic and those producing higa-toxin (*enteromorhagic*), are sometimes found.

Escherichia Coli is usually benign and can be found in the human digestive tract. However, if *E. coli* that was initially non-pathogenic acquires additional virulence genes from other microbes through gene transfer (transformation), plasmid transfer (conjugation), or gene transfer via bacteriophage (transduction), then this bacterium will become pathogenic. Diseases caused by pathogenic *E. coli* vary depending on their virulence and pathogenesis mechanisms. Pathogenicity refers to the ability of an organism to cause disease. *E. coli* can cause disease symptoms when it encounters entering the host body, survives, and attacks the immune system which eventually causes disease. This pathogenic mechanism occurs through several stages, like other harmful bacteria, which include colonization in intestinal surface cells (mucosal cells), cell division, destruction of intestinal cells, penetration of intestinal cells to enter the bloodstream, attachment to target organs, and finally damage to organs. Most strains of pathogenic *E. coli* damage host cells externally, while *EIEC* is an intracellular pathogen that is able to invade and multiply in intestinal mucosal cells and macrophages [9].

According to the World Health Organization (WHO), foodborne illness can occur in two ways: intoxication or infection. Food intoxication occurs due to toxins found in food by bacteria, while food infection occurs when bacteria enter the body through contaminated food and the body reacts to the bacteria. Both can cause problems with the digestive system. The bacteria that most often cause food infections are Salmonella and *E. coli*. These bacteria generally appear due to the sale of food that does not pay attention to hygiene and safety standards.

According to WHO data in 2022, diarrhea is the second highest cause of death in children under the age of five. Each year, an estimated 1.7 billion cases of diarrhea occur in children, resulting in around 525,000 deaths. UNICEF noted that 5% of under-five deaths due to diarrhea occur in the Southeast Asia region. In 2015, Indonesia reported 8,600 under-five deaths due to diarrhea, ranking it 12th out of 15 countries with the highest under-five mortality rates in the world, as well as the highest in Southeast Asia.

In Indonesia, diarrhea is a common disease and can be categorized as an

extraordinary event because it is often associated with death. In 2019, the number of diarrhea patients from all age groups who received treatment at health facilities reached 3,176,079 people, while in 2017 the figure was higher at 4,274,790 people. In that year, 21 extraordinary events (KLB) of diarrhea were recorded, spread across 12 provinces and 17 districts/cities.

In 2020, the coverage of diarrhea services for toddlers in Indonesia was 40.07%, and the number of recorded cases also increased to 4,504,524 people. There were 10 outbreaks in 8 provinces and 8 districts/cities. In 2021, the coverage of services for toddlers increased to 40.90 %. While in 2022, the number of diarrhea cases decreased slightly to 4,485,513 people. In 2023, service coverage for toddlers with diarrhea was recorded at 40%. Nationally, the incidence of diarrhea reaches 270 per 1,000 population.

According to Filemon (2024), in Indonesia, especially Manado City, food sales in public places are still wide open, so many street vendors sell on the side of the road. One of the snacks that is popular with residents, especially students, is meatball satay. However, this food is at risk of causing health problems if contaminated and consumed [11].

In Gorontalo City, meat consumption is still relatively low, mainly because the price of meat products, especially high-quality ones, is quite expensive. Therefore, efforts are being made to maximize the use of low-quality meat such as offal, or to educate residents to be interested in consuming less popular types of meat, in order to increase its economic value.

In Gorontalo City, there are currently many meatball entrepreneurs. Usually, meatballs are sold by street vendors. This shows that the level of meatball consumption in the city is starting to increase. The growth of this meatball business is certainly related to the food consumption patterns of residents and consumer characteristics, especially residents of Gorontalo City.

One of the bacteria that can contaminate food and cause diarrhea or food-borne illness is the *Escherichia Coli bacteria*.This bacteria is a microorganism that naturally lives in the digestive tract of humans and livestock. However, there is a type of *E. coli* that is pathogenic, namely *E. coli* O157: H7, which is able to find shiga toxins and cause serious infections.

E. coli bacteria that exceeds safe limits can cause health problems such as diarrhea, meningitis, and even hemolytic uremic syndrome (HUS) [8].

How to identify *Escherichia Coli bacteria* is as follows:

1) Gram Staining

An ose is used to take the isolate and place it on a glass object. Then, ethanol drops are applied (until the blue color disappears, then rinsed with distilled water for 5 seconds), iodine (for 1 minute, rinsed with distilled water for 5 seconds), and crystal violet (for 1 minute, rinsed with distilled water for 5 seconds). The preparation is then examined under a microscope after drying. Because *Escherichia Coli* is a gram-negative bacteria, this bacteria will appear red [15]

2) TSIA Test

The TSIA test aims to differentiate bacterial types according to their ability to ferment lactose, sucrose, glucose, and produce sulfide. If the bacteria find gas that causes air

bubbles at the bottom, changes the base color to yellow, and finds H2S, the test findings are considered positive. For *Escherichia Coli* bacteria , the TSIA test findings will be positive if the color changes to yellow, finds gas at the bottom of the tube, but does not find H2S [16].

3) Indole Test

Tryptone broth medium with tryptophan substrate was used for indole test. Conversion of tryptophan to indole found a positive response. After adding 15 drops of Kovac's reagent (hydrochloric acid, p-dimelanminobenzaldehyde, butanol), a cherry red ring formed on the surface of the culture, indicating a positive indole test finding in *E. coli* [16].

RESEARCH METHODS

This study uses a qualitative approach that aims to identify *Escherichia Coli bacteria* in meatball skewer snacks. And this type of research uses descriptive research that aims to identify *Escherichia Coli bacteria* in meatball skewer snacks.

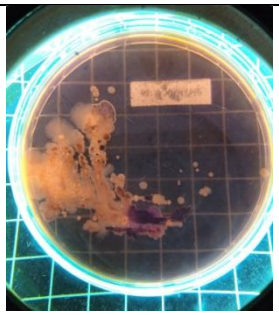
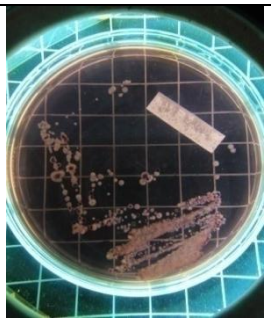
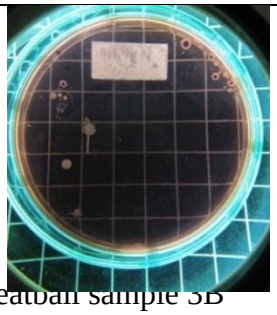
The research location was carried out in Kota Tengah District, Gorontalo City and the identification examination of *E. coli bacteria* was carried out at the Integrated Laboratory of Bina Mandiri University, Gorontalo, with the research time being carried out in October 2024. The samples used in this study were 5 samples of meatball skewers in Kota Tengah District, Gorontalo City.

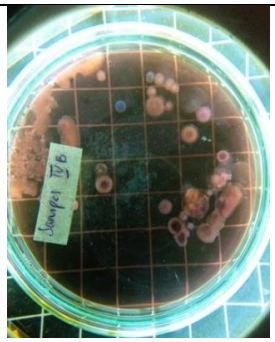
RESEARCH RESULT

Five meatball skewer samples from Kota Tengah District, Gorontalo City, were analyzed according to research findings. The following is an explanation of the findings of *Escherichia Coli bacteria* on the meatball skewers.

1. Macroscopic Observation Findings are displayed in the following table:

Table 4.1 Macroscopic Observations

No.	Picture	Information
1	 Meatball sample 1A	Round shape Regularly edged Medium sized colony Metallic green colonies
2	 Meatball sample 2A	Round shape Regularly edged Colonies are pink or light purple in color.
3	 Meatball sample 3B	Smooth round shape Regularly edged Medium sized colony Shiny metallic green colonies

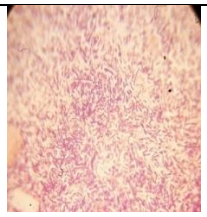
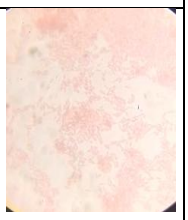
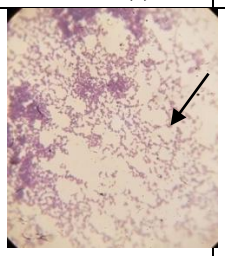
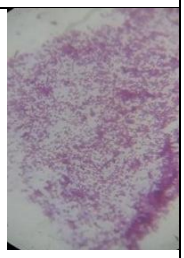
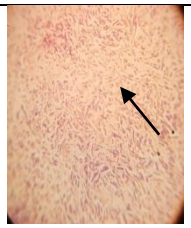
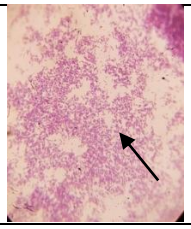
4	 Meatball sample 4B	Smooth round shape Regularly edged Colonies are pink or light purple in color.
5	 Meatball sample 5B	Smooth round shape Regularly edged Colonies are pink or light purple in color.

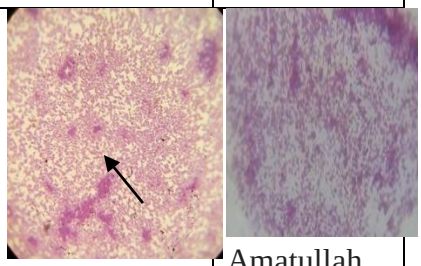
(Data Source: Secondary Data, 2025)

According to Table 4.1, it shows that of the 5 samples of meatball skewers studied, 2 samples of meatball skewers were suspected of containing *Escherichia Coli bacteria*, colony growth on EMBA media was round, smooth and had regular edges, colonies tended to be medium sized, had shiny metallic green colonies.and 3 samples of skewered meatballs were not infected with *Escherichia Coli bacteria* ,the colony growth was round, smooth and had regular edges, and had pink or light purple colonies.

2. Microscopic Observation Findings
   a. Gram Staining
      Microscopic observation findings with gram staining of the bacterial isolates obtained, observed microscopically,the following findings were obtained:

Table 4.2 Microscopic Observations

No	Sample Code	Picture	Comparator
1	Meatball 1A	 Gram Negative Bacteria (-)	 Dita, DP, & Kholik, K. (2023)
2	Meatball sample 2A	 Gram Positive Bacteria (+)	 Amatullah, LH, Afifah, DN, & Jannah, SN (2023).
3	Meatball sample 3B	 Gram Negative Bacteria (-)	 Dita, DP, & Kholik, K. (2023)
4	Meatball sample 4B		

		Gram Positive Bacteria (+)	Amatullah, LH, Afifah, DN, & Jannah, SN (2023).
5	Meatball sample 5B	 Gram Positive Bacteria (+)	Amatullah, LH, Afifah, DN, & Jannah, SN (2023).

(Data Source: Secondary Data, 2025)

According to Table 4.2, Gram staining is carried out to see the morphology of *Escherichia Coli bacteria.*which grew on EMBA media microscopically from 5 samples of meatball skewers studied, 2 samples of meatball skewers were suspected of containing *Escherichia Coli bacteria.*with a rod-shaped and red color, and 3 samples of skewered meatballs did not contain *Escherichia coli bacteria.*rod-shaped and purple in color.

DISCUSSION

According to research findings, the identification of *Escherichia coli bacteria* In meatball skewers, several meatball skewers sold in Central City, Gorontalo City, were found to be infected with *Escherichia Coli bacteria.*because seen from the hygiene and sanitation is not comfortable and clean. Because meat is an excellent substrate for the development of germs, *Escherichia Coli bacterial infections* can also be transmitted through direct contact, undercooked meat, and processed meat products. Diarrhea can be caused by primary infection in the large intestine caused by *Escherichia Coli bacteria*.

From the laboratory examination findings, it is suspected that there is *Escherichia Coli bacteria.*namely 2 samples of meatball skewers sold in Kota Tengah District, Gorontalo City, it can be seen from the findings of observations that have been carried out by researchers that there are several causes of bacterial contamination, one of which is seen from the cleanliness of the seller's environment, the processing method and the place or tool used by the seller is one of the causes of bacterial contamination. And seen from the findings of the researcher's interview with the seller of meatball skewers in Kota Tengah District, Gorontalo City, the seller said that he often pays attention to environmental cleanliness and always pays attention to the place or tool used, and the seller also pays more attention to the place when selling, so that customers feel comfortable and are not disturbed by the dirt around. Environmental cleanliness is very important, because not maintaining cleanliness will cause disease for humans

Observation of *Escherichia Coli bacteria* Macroscopically, it is done using a *colony counter tool* to see the morphology of the colony and the color of the colony, after macroscopic observation, it is continued with microscopic examination by doing gram staining, the first thing to do is take the preparation and drip 1 drop of distilled water, then heat the ose above the bunsen then take a little colony in a petri dish then apply it on the

object glass that has been dripped with distilled water then flatten it and wait until dry, after that drip with a solution of carbolic violet crystals, let it stand for 30 seconds, then rinse with running water, then drip lugol, let it stand for 30 seconds and rinse with running water, after that drip 96% alcohol until there is no more purple solution that fades, then drip safranin, let it stand for 30 seconds to 1 minute and rinse with running water, then dry it using tissue and do not wipe the top of the object glass, after dry look under a microscope with 100x magnification and give oilmersi oil, after that observe the findings.

Observation findings of *Escherichia Coli bacteria* on meatball skewers macroscopically and microscopically. In macroscopic observations, it was found that meatball samples 1A and 3B contained *Escherichia Coli bacteria.*with a smooth round colony shape and regular edges, tending to be medium in size and have a shiny metallic green color, the type of bacteria is gram-negative and rod-shaped, meatball samples 2A, meatball 4B, and meatball 5B did not contain *Escherichia Coli bacteria.*with a round colony shape, regular edges and a pink or light purple color, a gram-positive and rod-shaped type of bacteria.

According to the findings of 5 samples of meatball skewers sold in Kota Tengah, Gorontalo City, two samples of meatball skewers are suspected of containing *Escherichia Coli bacteria* , meaning that personal hygiene and sanitation in the environment around the seller are not clean, and these bacteria can also be contaminated through direct contact with the seller such as hands when processing or serving these meatball skewers not washing hands first and can also be contaminated through storage or equipment used by the seller so that it can have bad consequences or cause diseases in the human intestines or cause diarrhea. Good hygiene also includes washing hands before and after activities. In addition, sellers must also pay more attention to cleanliness around them when selling. Meatballs on skewers are generally made from meat that can be contaminated by bacteria.because when these ingredients are stored or used in an open or dirty place, bacteria can contaminate the meatballs.

The findings of the examination of five meatball skewer samples showed the presence of two types of bacteria, namely gram-positive and gram-negative bacteria, according to microscopic observations. *Escherichia Coli* has an important role in the body, because it lives in the large intestine and finds colicin which helps protect the digestive system from harmful bacteria. However, *E. coli* can become pathogenic if its number increases in the digestive tract or moves from its original environment to other parts of the body. In addition, there are various types of pathogenic *E. coli* that find enterotoxins and can cause diarrhea, which are classified according to their level of virulence [12]

E. coli contamination in food and drinks can be prevented by cooking food thoroughly, because this bacteria is sensitive to high temperatures. In addition, it is important to maintain personal hygiene and sanitation, such as washing hands before and after handling food, maintaining body hygiene, and ensuring the cleanliness of food utensils [12]

Escherichia Coli is a bacteria that is naturally found in the digestive tract of humans and livestock. However, some types of pathogenic *E. coli can cause infections and foodborne illnesses because they contain shiga toxin.E. coli* contamination that exceeds the threshold is often associated with health problems such as diarrhea, meningitis, and Hemolytic Uremic Syndrome (HUS). These bacteria can grow at temperatures up to 44°C and require a minimum temperature above 7–8°C to thrive. Storing food at low temperatures for long periods can inhibit the growth of *E. coli* [13].

Escherichia Coli bacterial infection can be fatal because it can cause septicemia and worsen the condition of the disease. In humans, *E. coli infection* can occur through direct contact, consumption of undercooked meat, or processed meat products, considering that meat is a medium that supports the growth of microorganisms [13].

Escherichia Coli belongs to the coliform bacteria group, usually found in the large intestine and in human and animal feces. Therefore, *E. coli* is often used as an indicator of sanitation. These bacteria are rod-shaped, gram-negative, and do not form spores. *E. coli* is also opportunistic, meaning it can cause infections in individuals with weakened immune systems, although it is not harmful to healthy people [14].

In food microbiology, the term *sanitation indicator bacteria* refers to microorganisms that indicate the presence of human or animal waste contamination in food. The presence of these bacteria indicates that the food has come into contact with human or animal intestinal waste during processing [14].

Escherichia Coli, a common cause of diarrhea, is widely distributed globally. Its ability to adhere to epithelial cells of the small or large intestine is influenced by genetic factors in plasmids, as well as the toxins it produces, which are mediated by plasmids or phages. This bacterium can grow well in almost all common culture media [14].

RESEARCH LIMITATIONS

The limitations of this study include the fact that the researcher only examined macroscopic and microscopic data, and did not examine the data too deeply in this study , such as conducting biochemical tests.

CONCLUSION

From the results of the research that has been done, it can be concluded that According to the research findings, *Escherichia Coli* bacteria were found in two samples of meatball satay sold in Kota Tengah District, Gorontalo City. *E. coli* bacteria are classified as gram-negative, red rod-shaped with sizes ranging from 0.4 to 0.7 µm × 1.4 µm. These bacteria are part of the normal flora in the digestive system, known as fast lactose fermenters, and find positive indole.

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