ANALYSIS OF *COLIFORM* BACTERIAL CONTAMINATION IN TOMATO SAUCE, SNACK FOOD AT JALAN TOTO CENTRAL GORNTALO CITY

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ABSTRACT

The purpose of this study was to determine the results of the analysis of *Coliform* bacterial contamination in tomato sauce snacks on Jalan Toto Tengah, Gorontalo City.

This research method is descriptive observational with a qualitative approach. The method in this study uses the MPN method which consists of the estimator test, reinforcement test and Gram staining and uses the MPN 333 table according to Thomas's formula.

The results showed that all samples of tomato sauce tested positive for *Coliform* bacteria and did not meet the standards based on BPOM Standard number HK.00.06.1.52.4011 in 2009 concerning the maximum limit of microbial and chemical contamination in food with a maximum APM *Coliform* of 100 APM/g. estimator test all samples change color to yellow. Meanwhile, in the reinforcement test results, 1 sample had metallic green bacterial colonies and 5 other samples had pink colonies and for Gram staining, rod-shaped Gram negative bacteria were obtained.

Keywords: Contamination, Coliform Bacteria, Tomato Sauce, Snacks

INTRODUCTION

Food is one of the most important basic needs for humansconsisting of carbohydrates, proteins, fats, vitamins, minerals and water. Food has a function for human life, namely to maintain body processes in growth and development and replace damaged body tissues and play a role in mechanisms in the body's defense against various diseases [18].

Food is not only beneficial for humans, but also very good for humansgrowth of pathogenic microbes. Microbial growth in food can cause health problems that can be caused by food poisoning. This poisoning can be caused by dirt or bacterial contamination [1]. Bacterial contamination of foodstuffs can mainly be caused by poor hygiene and sanitation conditions at food processing sites.Opportunities for food contamination can occur at any stage of food processing. Unhygienic food management can result in the presence of ingredients in food that can cause health problems for consumers [16].

Contamination of pathogenic bacteria in food and beverages can cause various diseases including typhoid, diarrhea, food poisoning and so on. Foodborne diseases are a common public health problem.

According to the World Health Organization (WHO), millions of people around the world experience food-borne

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diseases. Every year, about 70% of cases of diarrheal disease occur due to contaminated food. Diarrhea is also the second leading cause of death in children under the age of five, accounting for 525,000 (0.030%) children each year [30].

The cause of KLB (Extraordinary Events) of food poisoning is the highest, namely, in processed food, catering and household preparations services caused bv contamination bv microorganisms. In Indonesia, there were 10 outbreaks of diarrhea in 2018 spread across 8 provinces, 2 provinces including Central Sulawesi Province with 13 cases and the Case Fatility Rate (CFR) of 7.69% and Papua Province with 122 cases and Case Fatility Rate (CFR) is 22.95% [13].

Diarrhea is still a public health problem in developing countries such as Indonesia. In 2017 the number of diarrhea sufferers served in health facilities was 4,274,790 and there was an increase in 2018 to 4,504,524 sufferers or 62.93% of the estimated diarrhea in health facilities. The national incidence of diarrhea for all ages is 270/1,000 population [13].

According to the Gorontalo City Health Office (2020), the prevalence of diarrhea was recorded at 2,377 cases. Diarrhea that occurs is caused by contamination of agents in the form of microbes. Hygiene and sanitation of food processors are one of the risk factors that cause outbreaks of food poisoning [6]

One of the causes of outbreaks is street food. Based on the results of observations that have been made to sellers of special snacks around Jalan Toto Tengah, Wongkaditi sub-district, Gorontalo City, which is one of the places that is visited by many people, both just enjoying the afternoon atmosphere and those looking for food. During the sales process they are in an open space, where the tomato sauce storage is open and they

usually don't pay attention to the location

of the place of sale. This can be the cause of the occurrence of microbial contamination of food caused by air.

Snack food is food served by traders which is usually consumed directly without further processing or preparation which is sold by street vendors on the streets and crowded places. The nutritional quality is not high at a relatively low price, which is very popular with the public[14].

Street food is food and drink made or served at the point of sale or served as ready-to-eat food which is usually sold by street vendors. According to the Food and Agriculture Organization (FAO) it is defined as food and beverages prepared or sold by street vendors or in crowded places. Generally, snacks that are sold are directly eaten or consumed [9].

According to the Directorate of Nutrition (2011), safe snacks are food and drinks that are free of germs (pathogenic microbes), chemicals and hazardous materials which when consumed do not cause human health problems. The selection of snacks that are safe for consumption can be done in several ways, namely:

Choose snacks that are closed or in packaging, choose snacks that are in good condition or before the expiration date, observe whether the food is brightly colored or much different from the original color. Snacks, crackers, noodles and ice cream that are too brightly colored may have added unsafe coloring agents. Likewise, the color of processed beef, which remains red, is the same as the fresh meat. Also pay attention to the quality of the food, is it still fresh, or is it moldy which can cause poisoning. Moldy food indicates that the preservation process is not perfect, or the food has expired, observe the composition.

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There are bad effects that result from consuming unsafe food, namely: infection is caused because in food there are germs or pathogenic microorganisms that can cause health problems such as cholera, typhus, abdominalis and so on. The spread of infectious diseases can be caused because the seller who processes food has previously been exposed to a disease or is suffering from a certain disease and can also be caused by poor cooking methods.

Food poisoning is the onset of clinical symptoms caused by eating certain foods. Food poisoning can be classified as follows, namely poisoning due to food containing toxic chemicals and infection due to microorganisms that develop either in the mucosa or other tissues [27].

Snack food has become an inseparable part of people's lives, both in urban and rural areas. Street food is a type of food that is sold by street vendors on roadsides, stations, markets, in residential areas and in similar locations [19]. Various types of snacks such as meatball noodles, chicken noodles, fried rice, dumplings and so on are usually equipped with tomato sauce.

Tomato sauce is a complementary food in the form of pasta that is often served with other foods. Tomato sauce is basically a product that can last a long time because of the low pH of the product, the addition of benzoic acid and the heating treatment during the production process. However, the tomato sauce production process that does not meet the standards can result in microbial contamination of the tomato sauce and packaging bottles[21].

To get the benefits of lycopene, it is recommended that you eat tomatoes that have been cooked. Processed tomatoes have a higher lycopene content than unprocessed tomatoes. Tomato processing will increase the lycopene content of fresh tomatoes. By nature, lycopene is tightly bound to cell walls and fibers, making it difficult for our digestion to digest. Boiling tomatoes will reduce the concentration of lycopene bonds in the walls and cells [29].

Lycopene is a naturally occurring substance found in large amounts in tomatoes. Lycopene acts as an antioxidant and has an influence in reducing the risk of various chronic diseases including cancer. The lycopene content in tomatoes increases in the body if the tomatoes are processed first such as into juices, sauces, and so on [29].

Tomato sauce is a food ingredient that can be a good growth medium for the proliferation of bacteria. Small amounts of contamination if left in the food with sufficient temperature and time, it will grow and develop so that it becomes a lot that can cause serious outbreaks. Transmission of disease through food, one which is food infection where of microorganisms enter and multiply in food that can cause disease. The most common types of microorganisms are Salmonella, Shigella, and Escherichia Coli which are pathogenic bacteria [1].

One of the microbes that often contaminate foodstuffs is bacteria from the *Coliform* group. Therefore, *Coliform* bacteria are an early sign to take steps to prevent food and drink diseases (foodvorne diseases).

Coliform bacteria is one type of bacteria that is widely used as an indicator of the quality of food and water sanitation. These bacteria can produce various kinds of toxins that can cause disease if the amount is excessive in the body [10].

Coliform bacteria are a group of intestinal bacteria, namely bacteria that live in the human digestive tract. *Coliform* bacteria are indicator bacteria and are included in the group of microorganism

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bacteria that are widely used as indicators [20]. *Coliform* bacteria, which consist of Serratia, Hafnia, *Citrobacter*, *Enterobacter*, *Klebssiella*, and *Escherichia Coli* are a group of bacteria used as an indicator of the quality of contaminated water, food and dairy products [20].

Coliform bacteria are bacteria belonging to the Enterobacteriaceae family that are capable of fermenting lactose. Coliform bacteria are generally found in human and animal feces which can be found in large numbers. The bacterial genera included in Coliform are Escherichia coli, Klebsiella spp, Enterobacter Citrobacter spp, spp. Coliform bacteria such as Citrobacter spp species, Enterobacter spp and Klebsiella spp can also be found in the environment such as soil, vegetation or water surfaces, whose presence is not always associated with faecal contamination, these bacteria belong to the group of non-fecal Coliform bacteria [26].

Coliform bacteria can contaminate foodstuffs that are not stored properly. This can cause health problems. Health problems caused by the *Coliform* bacteria group have several symptoms in human digestive tract disorders such as diarrhea, vomiting, and fever [22].

Based on the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number HK.00.06.1.1.52.4011 of 2009 concerning the maximum limit of microbial and chemical contamination in food with *Coliform* APM a maximum of 100 APM/g.

According to previous research conducted by Karlah, et al (2014) which examined 12tomato sauce samples all showed positive results for *Coliform* bacteria and Escherichia Coli bacteria. Meanwhile, research conducted by Arini and Rahayu (2017) obtained from 10 samples of dumpling sauce from campus are traders in Surakarta, all positive samples were contaminated with *Coliform* and Echerichia Coli bacteria.

Most probable number (MPN) is a method of calculating the number of microorganisms from the growth of in a specific liquid microorganisms medium in a series of tubes to obtain qualitative data (MPN/ml/g).Most probable number is a multilevel dilution test used to measure contamination of target microorganisms with an estimate of the Indonesian national standard with the 012332.1 series describing MPN as a method for calculating the number of microbes using liquid medium in test tubes [20].

The unit used is generally per 100 ml, so if there is an MPN value of 10/100 ml in a water sample, it is estimated that the sample contains 10 *Coliforms* in 100 ml. The smaller the MPN value, the higher the quality of the drinking water and fit for drinking [25].

The MPN method uses a liquid medium in a test tube, in this case the calculation is based on the number of positive tubes. Positive tube observations can be seen by observing the presence of turbidity or gas formation in the Durham tube [25].

Dilute the sample to a certain level so that the appropriate concentration of microorganisms is obtained and when planted in a tube produces positive growth. The larger the number of samples included, the lower the dilution carried out [20].

The MPN method system that is often used is the sysmetric dilution system using many tubes in parallel for each dilution carried out. The more the number of tubes that are used frequently, the more precise the value obtained [20]. The MPN method

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uses three stages of testing, namely as follows:

The estimator test is a test used for the fermentative nature of *Coliform* in the sample. In the estimation test, each tube produces gas during the incubation period, it is suspected that it contains *Coliform*. If gas is not formed within 24 hours then incubation is continued for up to 48 hours. This test is to detect the fermentative nature of *Coliform* in the sample and must be confirmed by a confirmatory test to rule out the presence of other organisms that give a positive result in lactose fermentation. The aim is to find bacteria that ferment lactose and form gas at 370C using Lactose Broth media [20].

This test is a follow-up test from the previous test, this test is carried out to confirm that the gas formed is caused by *Coliform* bacteria and not caused by the cooperation of several species to produce gas. The aim was to determine whether the gas-forming fermentation in the initial test was really caused by *Coliform* bacteria at 370C. Confirmation test can use EMBA media [20].

This test is carried out to determine what examination results are really positive, then the microbes from the confirmation test results are positive. Completeness test was carried out to ensure confirmation test (confirmation) by making microscopic observations of *Coliform* characteristics such as rodshaped, Gram negative, not spore. The estimator test if it does not show the presence of *Coliform* no longer needs to be carried out at the complete test stage [20].

RESEARCH METHODS

This research approach is a descriptive qualitative approach which aims to determine the analysis of *Coliform* bacterial contamination in tomato sauce

for snacks on Jalan Toto Tengah, Gorontalo City.

The type of research used is descriptive observational, namely research by directly observing the object to be studied, then described descriptively to determine the presence or absence of *Coliform* bacteria in tomato sauce.

The type of data in this study. Researchers used primary data with the results of preliminary studies or initial observations. collecting data (questionnaires) to street food vendors around Toto Tengah street, Gorontalo City and the results of the tomato sauce examination carried out in the laboratory. Meanwhile, secondary data with observation results in the form of medical record data obtained from hospitals, health centers and at the Health Service accompanied by documents/literature such books, journals and others as in Wongkaditi Village, Gorontalo City.

In this study, the population is all street food vendors on Jalan Toto Tengah, Gorontalo City. The sample used is all tomst sauce used by street food vendors on Jalan Toto Tengah, Gorontalo City. The sampling method used is total sampling; where all members of the population are sampled in this study.

The data analysis technique used is descriptive, that is, it describes the presence or absence of *Coliform* bacterial contamination in tomato sauce which is presented in the form of pictures and narratives.

RESEARCH RESULT Table. 1 Estimator Test Results

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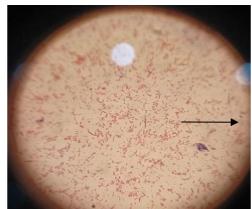
Sample	Incubation period	Tube combination	<i>coliform</i> APM/100g	BPOM Coliform APM/g., regulatory standards	Note.
А	2x24 hours	3-1-1	>1100	100	TMS
В	2x24 hours	1-1-1	1100	100	TMS
С	2x24 hours	3-0-2	>1100	100	TMS
D	2x24 hours	1-1-1	1100	100	TMS
Е	2x24 hours	2-2-1	>1100	100	TMS
F	2x24 hours	2-1-1	>1100	100	TMS

(Source: Primary Data, 2021)

Based on Table 4.1, the results of the MPN value on the estimator test for street food tomato sauce on Jalan Toto Tengah, Gorontalo City showed that 6 of the tomato sauce samples did not meet the standard because they had exceeded the threshold set by the standard based on the Regulation of the Head of the Food and Supervisory Republic Drug of the Republic of Indonesia. Indonesia Number HK.00.6.1.52.4011 of 2009 concerning the maximum limit of microbial and chemical contamination in food with a maximum APM Coliform of 100 APM/g.
 Table. 2 Amplifier Test Results

sample	Colony color	Observation result	Morphol ogical shape
А	Pink	Gram Negative	stam
В	Pink	Gram Negative	517111
С	Metallic green	Gram Negative	stem
D	Pink	Gram Negative	stam
Е	Pink	Gram Negative	stem
F	Pink	Gram Negative	stam

(Source: Primary Data, 2021)



Picture. 1 Gram negative *Coliform* Bacteria

(Source: Secondary Data, 2021)

Based on Table 4.2 the growth of *Coliform* bacteria on Eosin Methylene Blue Agar (EMBA) media, there is 1 sample having a metallic green bacterial colony, namely sample C which is a characteristic of faecal *Coliform* bacteria and 5 other samples having a pink and transparent bacterial colony namely samples A, B, D, E and F which are the characteristics of non-fecal *Coliform* bacteria.

Based on Figure 4.1, the results of the examination using Gram staining with observations on a microscope were found to be Gram negative bacteria with red and rod-shaped characteristics.

DISCUSSION

Tomato sauce is a product made from a mixture of tomato powder obtained from tomatoes that have been processed first. Making tomato sauce is processed using additional natural spices or without using additional chemical ingredients (Tampubulon, 2019)

Tomato sauce is a food ingredient that can be a good growth medium for the proliferation of bacteria. Small amounts of contamination if left in the food with sufficient temperature and time, it will grow and develop so that it becomes a lot

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that can cause serious outbreaks. Transmission of disease through food, one of which is food infection where microorganisms enter and multiply in food that can cause disease. The most common types of microorganisms are Salmonella, Shigella, and Escherichia Coli which are pathogenic bacteria [1].

Based on the results of the research that has been done, it was found that 6 samples that were tested were positively contaminated with *Coliform* bacteria. the results are declared not to meet the standards based on the Regulation of the Head of the Food and Drug Supervisory Republic of Indonesia Number HK.00.6.1.52.4011 of 2009 concerning the maximum limit of microbial and chemical contamination in food with a maximum APM *Coliform* of 100 APM/g.

The estimator test using Lactose Broth (LB) media showed that from the 6 samples there was turbidity in Lactose Broth (LB) media, the occurrence of turbidity indicated that the sample contained *Coliform* bacteria.

According to Kamaliah (2017) explained that the positive results that occurred on LB media were characterized by the presence of turbidity caused by bacteria fermenting lactose into lactic acid. Turbidity occurs due to increased lactic acid so that the lactose component coagulates, this clot is what causes the formation of turbidity.

Lactose Broth(LB) is one of the selective breeding media, in that media it contains lactose and bile salts (bilesalt) which are used to identify growing *Coliform* bacteria. *Coliform* bacteria will form gas or a cloudy yellow color change when grown on Lactose media.

Lactose Broth (LB) media was used to determine the presence or absence of *Coliform* bacteria based on the formation of acid and gas caused by the fermentation of lactose by *Coliform* bacteria. The formation of acid can be seen from the turbidity that occurs in Lactose Broth (LB) media and the gas produced can be seen from the Durham tube changing the presence of air bubbles [11].

The presence of *Coliform* bacteria in large numbers in tomato sauce indicates that there are enteropathogenic or toxigenic microbes that are harmful to health.

Coliform bacteria contamination in tomato sauce snacks on the Toto Tengah road, Gorontalo City can occur due to several factors, namely the equipment used is not hygienic, the place for tomato sauce is usually left open and also the condition of the place for traders who are on the roadside and in open places that can potentially be contaminated by microbes through air and dust pollution.

This is also in accordance with research conducted by Febria Agustina, et al (2009) which states that factors that can bacterial contamination cause or contamination include the materials used. the tools used, the use of hands and the surrounding environment. Meanwhile, according to Kurniadi Y, et al (2013) is a tool used by traders usually after use is stored in an open state so as to allow increased contamination of microorganisms.

In line with research conducted by Nadifah Fitri, et al (2013) also stated that *Coliform* bacteria can come from storage containers that are not clean, often left open and the way the sauce is served is mixed with water.

In another study conducted by Ratih and Leka (2016) stated that other factors that can cause bacterial contamination are the manufacturing materials, the tools used, the use of hands and the surrounding environment.

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Food handlers need to use gloves, the source of contamination with pathogenic bacteria can be through the use of unclean hands from preparing food to serving. Pollution of pathogenic bacteria can also be caused by a dirty environment, exposure to dust from vehicle fumes can be a potential source of pollution.

Coliform are a group of bacteria that are rod-shaped and are gram-negative. *Coliform* in food indicates that the food has been contaminated and it is possible that the food also contains Escherichia coli bacteria. However, this is not an absolute result because there can also be other non-pathogenic bacteria that can cause a positive *Coliform* test result.

Based on the results of the reinforcement test, sample C had blackish red colonies with metallic green killat which was a colony of Fecal *Coliform* bacteria which was suspected to be Escherichia Coli bacteria and other samples A, B, D, E and F had transparent pink colonies which were from non-fecal *Coliform* bacterial colonies.

According to Musdalifa (2018) explaining that the faecal *Coliform* group is Escherchia Coli bacteria because these bacteria produce more acid in lactose medium, form indole, but do not produce acetone. while the non-fecal *Coliform* group produces less acid, does not form indole but produces acetone.

The Amplifier Test uses Eosin Methylene Blue Agar (EMBA) Media which is an advanced test to see bacterial colonies. From the results of a positive estimator test then proceed with planting on EMBA media and incubating for 1x24 hours.

EMBA is a differential selective medium which contains eosin which can inhibit the growth of Gram positive bacteria and can only grow Gram negative bacteria. The EMBA has the advantage that it contains lactose, lactose and the coloring agent eosin and methylene blue is able to distinguish between lactose fermenting and non-fermenting bacteria and the presence of eosin and methylene blue helps to sharpen this difference. Microbes that ferment lactose produce dark colored colonies with a green flash.

As for other microbes that grow their colonies will be colorless. A good bacterial colony to grow on this medium and confirm the contamination is Escherchia Coli. Colonies of Escherchia Coli on EMBA media will appear blackish with a metallic green flash due to the high quality of the acid produced and the dye that is on the growth surface. Then proceed with Gram staining to see the morphology of the bacteria.

After carrying out the Amplifier test, the Gram staining process was carried out and observed under a microscope which was red, Gram negative and in the form of bacilli. Gram staining aims to determine the morphology of *Coliform* bacteria. The cell walls of Gram-negative bacteria contain lipids that are so high, this causes when washing with a bleach solution causes the enlargement of the pores and increased permeability of the dye. While the cell walls of Gram-positive bacteria contain low lipids, so that during the process of adding alcohol, dehydration and shrinkage of these pores causes the dye to remain bound and the cells turn purple [12].

Food contamination by pathogenic microbes, such as in tomato sauce can cause health problems for consumers. To reduce the risk of disease infection for consumers, it is necessary to pay attention to several things, including sanitation and cleanliness of food transportation equipment or carts, food hygiene, proper and correct food storage, washing hands

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before touching food and paying more attention to the physical changes that occur in the tomato sauce. [21].

There are several ways that can be done to prevent the occurrence of *Coliform* bacterial contamination, namely maintaining environmental cleanliness, not mixing leftover food with raw food ingredients, cooking the sambal until it is completely cooked, and in serving it the seller is expected to always wash his hands first. In addition there are several other ways to prevent contamination of E.coli bacteria in food, namely, washing hands before and after preparing food and before eating, washing fruits and vegetables thoroughly, avoiding crosscontamination by using clean utensils, pans, and serving plates, keep raw meat away from other foods and from other clean items, drink only pasteurized dairy products (avoid raw milk, don't prepare food if you have diarrhea,

CONCLUSION

Based on the results of the study, it was found that tomato sauce was positive for *Coliform* bacteria and was declared not to meet the standard because it had exceeded the maximum limit set based on the Regulation of the Head of the Food and Drug Supervisory Republic of Indonesia Number HK.00.6.1.52.4011 of 2009 concerning the maximum limit of microbial contamination. and chemicals in food with the minimum number (APM) *Coliform* maximum 100 APM/g.

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