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The Presence of Pathogenic Bacteria in School Children's Snack Food Samples in Medan City

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Abstract

Purpose: Good and healthy food must be free from harmful and toxic substances, such as microbial contamination, chemicals, and other materials. The term School Children's Snack Food (PJAS) is a food that is found and routinely consumed by children in the school environment, food must meet the requirements of microbial contamination limits based on SNI (Indonesian National Standard) safety testing. The cause of food poisoning that is still a serious problem in various countries including Indonesia is pathogenic bacteria. The purpose of this study is to determine the presence of pathogenic bacteria in school children's snack food samples in Medan City in the Pematangsiantar area. **Methodology:** The methods used were the Nearest Approximate Count (JPT) of *Escherichia coli, Bacillus sp. Number Test, Staphylococcus sp*. Number Test, Salmonella Identification, and Total Plate Count (TPC). **Findings:** The test results on JPT Escherichia coli fried meatballs include SD M (9.9 ± 3.8) MPN/g, SD 5 (8.68 ± 2.73) MPN/g, SD IT (5.08 ± 0.66) MPN/g, and SD 4 (4.26 ± 2.44) MPN/g. The results of JPT Escherichia coli in fried meatballs in SD 1 and SD 2, as well as JPT E. coli ojek in all elementary schools, namely < 3 MPN/g. **Significance:** Conclusion that the snack food for elementary school children (fried and fried meatballs) in Pematangsiantar did not contain contamination with pathogenic bacteria *Escherichia coli, Bacillus sp., Staphylococcus sp., Salmonella*, and Total Plate Count (TPC).

Keywords: *Indonesian national standard, pathogenic bacteria, snack food.*

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Introduction

Food is a basic human need that has a great influence on the existence and survival of life, both in terms of quantity and quality (Béné et al., 2019; Hossain et al., 2018). The importance of nutritional value in food depends on food safety, namely the Indonesian National Standard that is enforced. Information and data on the status of processed food snacks for school children in Pematangsiantar are needed to improve food safety and quality. Pematangsiantar City is one of the cities in North Sumatra Province. Because of the strategic location of Pematangsiantar, it is crossed by the Sumatra Highway which is only 128 km from Medan and 50 km from Parapat is often a crossing city for tourists who want to go to Lake Toba. As a tourism support city in the surrounding area, this city has 8 star hotels, 10 jasmine hotels and 268 restaurants. The industrial sector that is the backbone of the city's economy located in the middle of Simalungun Regency is a large and medium industry. Of the total economic activities in 2000 which reached Rp1.69 trillion, the industrial market share reached 38.18% or Rp646 billion. The trade, hotel and restaurant sectors followed in second place, with a contribution of 22.77% or Rp 385 billion. In the city of Pematangsiantar there is the HKBP Theological College, whose campus is located on Jl. Sangnawaluh No. 6. There is also Simalungun University or abbreviated as USI and HKBP Nommensen University which is often called Nommensen. There are also large private schools such as Methodist, Sultan Agung, Kalam Kudus, SMA Kampus Nommensen, Taman Orphan, Taman Siswa, Taman Ashan, Taman Siswa, SMK Parbina Nusantara, SMA Budi Mulia, SMA Bintang Timur and SMA Seminary, Surya or often called with Surya Computer, SMA-SMK PELITA. These private schools have produced outstanding students who compete in national sports events. In total, Pematang Siantar has 160 Elementary Schools, 43 First Level Advanced Schools, 28 Public High Schools, and 7 Universities/Academies.

The extraordinary incidence of food poisoning in Indonesia for the period 2000-2015 is the result of a study using a systematic and quantitative study approach, stating that the KLB is increasing every year (Arisanti et al., 2018). Food poisoning of 60% is suspected to be caused by bacteria, without being proven by the results of laboratory tests that the cause of KLB occurs due to bacteria. The highest causative pathogenic bacteria in cases of food poisoning include Escherichia coli, Bacillus cereus, Staphylococcus sp., Salmonella. The most cases of food poisoning occur in residential houses, schools, and factories. The most common causes of food poisoning are found in household foods, food services, and processed snack foods. The most extraordinary events occurred in household cooking, and the highest deaths were caused by eating processed foods in the household industry.

Based on the above background, a study was conducted to study the existence of pathogenic bacteria in snacks that are often consumed by children in elementary schools in Pematangsiantar. This is based on the Microbiology Criteria in Processed Food in the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 16 of 2016, which is in accordance with the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 24 of 2015 concerning Guidelines for the Development of Safe Food Villages.

Method

Data collection methods

Time and place of research

The research was conducted from December 2023 to February 2024. Testing for the presence of pathogenic bacteria Escherichia coli Nearest Approximate Number (JPT), Bacillus sp. Count, Staphylococcus sp. Count, Salmonella Identification, and Total Plate Count (TPC) in snack food samples of Pematangsiantar elementary school children were carried out at the Biology Laboratory of the Science Education Study Program, Faculty of Teacher Training and Education, HKBP Nommensen University.

Research Implementation

Sample preparation

Sample handling was carried out by selecting 2 types of food, namely motorcycle taxis and fried meatballs in each elementary school (SDN 1 Pematangsiantar, SDN 2 Pematangsiantar, SDN 4 Pematangsiantar, SDN 5 Pematangsiantar, SD IT Pematangsiantar, and SD M Pematangsiantar). Samples were obtained by buying snacks directly from sellers. Each type of sample is wrapped in sterile plastic, labeled, and put into an aseptic Marina brand cooler box. Food samples were weighed ± 40 g each and put into sterile plastic bottles, labeled, and transferred into liquid nitrogen boxes.

Research Tools and Materials

The tools used in this study include Erlemeyer, vortex, Beaker glasses, Petri dishes, Durham tubes, test tubes, and other glassware. The materials used in this study are Pepton Dilution Fluid (PDF), Lactose Broth double concentration, Lactose Broth single concentration, Brilliant Green Bile Broth (BGBB), Eosin Methylene Blue Agar (EMBA), Blood Agar, Mannitol Salt Agar (MSA), Buffered Peptone Water (BPW), Salmonella Shigella Agar (SSA), Nutrient Agar (NA), Plate Count Agar (PCA), crystal violet dye, iodine, 95% alcohol, and safranin dye.

Testing the Nearest Approximate Quantity (JPT)

Escherichia coli

The sample was weighed 10 g aseptically and placed in a glass vial containing 90 mL PDF, then homogenized with vortex for 1 minute so that it was obtained by dilution 10-1 to 10-2. The results of the 10-2 dilution were inoculated into 3 series of tubes equipped with Durham tubes on Lactose Broth double concentration and Lactose Broth single concentration media. The samples were incubated at a temperature of 37oC for 24 – 48 hours, and the formation of gas and turbidity in each tube was observed. Next, the positive result test was inoculated into a test tube equipped with a Durham tube on BGBB medium, and incubated at 37oC for 24 – 48 hours. Observation was made of affirmation tests with positive results of gas formation and turbidity in each tube. The positive results of the BGBB culture were then streaked in EMBA media, then incubated at a temperature of 37oC for 48 ± 2 hours. A positive result in EMBA media will form a metallic green colony. Finally, Gram staining was carried out, E. coli colonies were counted, and matched on the MPN Table of the 333 tube series according to the Thomas Formula.

1. Bacillus sp.

Aseptically weighed 10 g of the sample and placed in a glass vial containing 90 mL of Peptone Dilution Fluid (PDF). The sample is homogenized using a vortex for 1 min. 1 mL of dilution results of 10-1 samples were pipetted and inoculated with the streak plate method on Blood Agar medium. The sample was then incubated with the position of the Petri dish upside down which was carried out at a temperature of 36oC ± 1oC for 48 hours. Observed colonies growing on the Blood Agar medium. Reisolation was carried out on growing colonies suspected to be Bacillus sp. The results were observed by looking at the characteristics of specific colonies that grew on the Blood Agar medium, which were gray in color with uneven and slightly wavy colony edges. Gram staining was carried out which is suspected to be a colony of Bacillus sp., and counted Specific colonies of Bacillus sp. Finally, it is identified with BBL Crystal.

2. Staphylococcus sp.

10 g of samples were prepared by aseptic method and put into a glass bottle containing 90 mL of Peptone Dilution Fluid (PDF), in order to obtain a homogeneous 10-1 dilution result using vortex for 1 minute. Pipetted 1 mL of 10-1 dilution and inoculated using the pour plate method on MSA media. Then incubated at 37oC for 48 hours with the Petri dish upside down. The colony that grew was then observed, the bacteria suspected to be Stapylococcus sp. reculture. Furthermore, the position of the Petri dish up-side down was incubated at a temperature of 37oC for 24 hours. Specific bacteria in Mannitol Salt Agar medium are characterized by colony characteristics and the medium is yellow, with small to medium colony sizes, smooth surfaces. Staphylococcus sp. then it was chosen for Gram coloring. Finally, Staphylococcus sp. Confirmed staining. Salmonella Identification Testing

Aseptic prepared 10 g of samples containing 90 mL of Buffered Peptone Water (BPW) in glass bottles. The sample is homogenized using a vortex for 1 minute, and put into an incubator at a temperature of 37oC ± 1oC for 18 ± 2 hours. A 1 mL sample resulting from a 10-1 dilution was inoculated using the pour plate method on SSA media. The sample in a Petri dish with the inverted position is then put into an incubator at a temperature of 37oC ± 1oC for 45 ± 48 hours. Observed colonies.

Research Variables

The variables in this study consist of independent variables and bound variables. The independent variable was the presence of pathogenic bacteria in food samples of snack food for elementary school children in Pematangsiantar, Simalungun, North Sumatra. Meanwhile, the bound variables were the number of pathogenic bacteria Escherichia coli, Bacillus sp., Staphylococcus sp., Salmonella Identification, and Total Plate Count (TPC). Methods are the second section of an IMRAD paper. Its purpose is to describe the experiment in such retail that a competent colleague could repeat the experiment and obtain the some or equivalent results. Provide sufficient detail to allow the work to be reproduced. Research Design

The research was carried out by exploration and laboratory tests. The presence of pathogenic bacteria was tested on snack food samples of elementary school children in Pematangsiantar, Simalungun, North Sumatra using the method of Nearest Estimated Number (JPT) of Escherichia coli, Bacillus

sp. Count, Staphylococcus sp. Count, Salmonella Identification, and Total Plate Count (TPC). Testing for the presence of pathogenic bacteria using RAL (Complete Random Design) with 5 replicates based on the formula: (n – 1) (t – 1) ≥ 15.

Information:

n : number of repetitions t : number of treatments (Federer, 1999).

Data Collection Methods

The data collection method is carried out by means of exploration and laboratory tests, namely data obtained directly from snack samples taken in the field and the results of testing snack food samples of elementary school children in Pematangsiantar.

Results and Discussion

The results of the test for the presence of pathogenic bacteria Escherichia coli Nearest Estimated Number (JPT), Bacillus sp. Numbers, Staphylococcus sp. Numbers, Salmonella Identification, and Total Plate Count (TPC) in 2 types of snack food samples (motorcycle taxis and empek-empek) taken in six elementary schools in. Based on the research that has been carried out, it was found that the snack food products of elementary school children in Pematangsiantar were positive for E. coli. Positive EMBA media is characterized by the characteristics of growing E. coli colonies, which are medium in size, smooth surface, flaky, metallic green, and have been confirmed by Gram staining (Figure 1).

(a)  (b) 

Figure 1. E. coli bacteria (a) Colonies of Escherichia coli bacteria are metallic green growing on EMBA media; and (b) Staining of Escherichia coli bacterial cells in the form of short rods Magnification 10x100

The results of the Nearest Estimated Number (JPT) of E. coli in elementary school snack food in Pematangsiantar showed that 4 out of 12 samples contained E. coli exceeding the SNI limit, which is < 3 MPN/g that has been set (Figure 2). The mean values ± standard deviation of JPT E. coli results ranged from (0.08 ± 0.18) MPN/g to (9.9 ± 3.8) MPN/g. Bacillus sp. on school children's snack food (PJAS)

The results of the isolation did not find Bacillus sp. bacteria. in all the samples tested, but in the Blood Agar medium (Figure 3a) grew small gray colonies. Researchers are interested in continuing the identification of bacteria growing on the medium. Staining results Grams were found to be coccus-shaped bacterial cells (Figure 3c). Identification was carried out using BBL Crystal (Becton Dickinson Microbiology Systems,

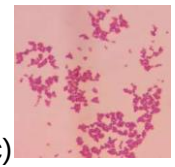
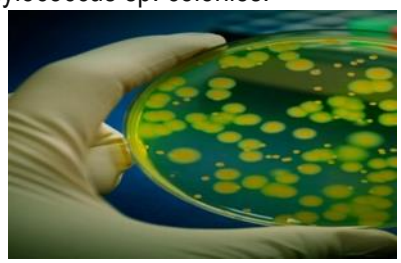
(a)  (b)  (c) 

Figure 3. Results of bacterial identification in school children's snack food (a) Colonies suspected to be Bacillus sp. gray grows on Blood Agar medium; (b) Results of colony reisolation suspected to have been grown on NA media;

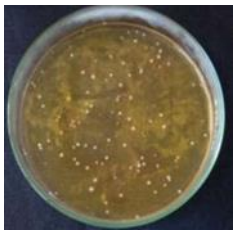
(c) Staining of bacterial cells found in the form of a magnification coccus 10x100;

Staphylococcus sp. on school children's snack food (PJAS)

Based on the Staphylococcus sp. positive results were obtained for Staphylococcus sp. Marked in MSA media, namely growing small to medium-sized colonies, smooth surfaces, yellow colonies with a medium that turns yellow, and has been confirmed by Gram staining on samples suspected of growing Staphylococcus sp. colonies.



A



B

Figure 4. Results of bacterial identification in school children's snack food (a) Staphylococcus sp. yellow and MSA media that turns yellow; and (b) Stature of Staphylococcus sp. group-shaped coccus Magnification 10x100 and Pseudomonas sp

The samples that have been tested were found to be 7 out of 12 samples in accordance with the characteristics of Staphylococcus sp. colonies. Staphylococcus sp. contained in school children's snack food (Figure 5) obtained the average value of ± standard deviation, including in the PJAS sample of SD IT motorcycle taxis (7.4 ± 1.14) x 101 colonies/g, SD 2 (6 ± 1.58)x 101 colonies/g, and SD 1 (2.6 ± 0.55) x 101 colonies/g, and for the results of the PJAS sample of fried meatballs, namely from SD 5 (2 ± 0.71) x 101 colonies/g, SD 4 (1.6± 0.55) x 101 colonies/g, SD M (1.2 ±0.45) x 101 colonies/g, and SD 2 (0.2 ± 0.45) x 101 colonies/g. Based on the test results, it is stated that it is still eligible for consumption if it does not exceed. Identification of Salmonella in school children's snack food (PJAS).

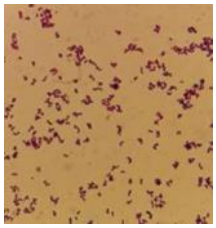


Figure 5. Salmonella positive Salmonella sp

Testing for the identification of Salmonella positive Salmonella sp. It is characterized by the growth of colonies on SSA media, namely colonies of small size, smooth surface, round shape with flat edges, transparent in color with black in the middle. By tests conducted on the sample did not find colonies growing on SSA media such as the characteristics of Salmonella colonies Sp. so that no confirmation test was carried out Nearest Estimated Number (JPT) of Escherichia coli in school children's snack food (PJAS)The final section of an IMRAD paper. Its purpose is to fit the results from the current study into the preexisting fabric of knowledge. The important points will be expressed as conclusions. This should explore the significance of the results of the work, not repeat them. A combined *Results and Discussion* section is often appropriate. Avoid extensive citations and discussion of published literature.

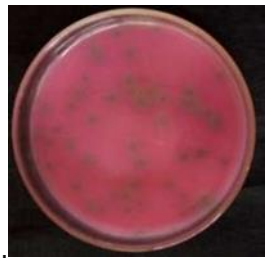


Figure 6. Salmonella Identification test

The results of the Salmonella Identification test on snack food for elementary school children in Pematangsiantar (Table 1) were declared eligible for consumption because the results showed negative Salmonella sp. Table 1. The results of the Salmonella bacteria Identification Test tested from 6 samples of fried meatballs and 6 samples of fried foods with 5 repetitions found that there was no Salmonella growing on the SSA medium so that all samples were declared Negative for Salmonella according to the SNI requirements, namely qualified (MS) for consumption Salmonella Identification Results.

Table 1. Salmonella Identification test

No.	School	Motorcycle Taxis	Fried Meatballs	Qualified (MS)
1.	SD 1	Negative	Negative	MS
2.	SD 2	Negative	Negative	MS
3.	SD 4	Negative	Negative	MS
4.	SD 5	Negative	Negative	MS
5.	SD IT	Negative	Negative	MS
6.	SD M	Negative	Negative	MS

The Total Plate Count in PJAS based on the results of bacteria grown in PCA media can be calculated as 1 bacterial colony cell (Figure 7). Total Plate Count on Testing Snack Food Samples for Elementary School Children in Pematangsiantar (Table 1) obtained a < result of 1 x 105 CFU/g. When compared to the SNI value, the test results on the sample are declared to be safe for consumption.

Figure 7. 102 dilution bacterial colonies growing on PCA media

Table 2. The results of the Total Plate Count (TPC) on PCA media were obtained by all samples tested with 5 repetitions under the SNI requirement, namely < 1 x 105 CFU / g so that it meets the requirements (MS) for consumption Average Results ± PJAS Deviation Standards.

Table 2. Total Plate Count (TPC) on PCA media

1.	SD 1	$(4.2 \pm 0.3) \times 10^2$	$(4.04 \pm 0.37) \times 10^4$	$< 1 \times 10^5$	MS
2.	SD 2	$(1.05 \pm 0.05) \times 10^3$	$(3.65 \pm 0.29) \times 10^3$	$< 1 \times 10^5$	MS
3.	SD 4	$(3.22 \pm 0.31) \times 10^2$	$(9.95 \pm 0.21) \times 10^4$	$< 1 \times 10^5$	MS
4.	SD 5	$(6.21 \pm 0.4) \times 10^2$	$(4.32 \pm 0.35) \times 10^3$	$< 1 \times 10^5$	MS
5.	SD IT	$(5.62 \pm 0.21) \times 10^2$	$(4.05 \pm 0.15) \times 10^3$	$< 1 \times 10^5$	MS
6.	SD M	$(4.5 \pm 0.7) \times 10^2$	$(4.01 \pm 0.65) \times 10^3$	$< 1 \times 10^5$	MS

The Nearest Estimated Number of Escherichia coli that exceeded the SNI limit in the research on children's snack food (fried meatballs) in elementary schools in Pematangsiantar occurred in SD M (9.9 ± 3.8) MPN/g, SD 5 (8.68 ± 2.73) MPN /g, SD IT (5.08 ± 0.66) MPN/g, and SD 4 (4.26 ±2.44) MPN/g. This shows that the results do not meet the food safety requirements for consumption because they can be the causative agent of food poisoning cases. Escherichia coli is one of the microorganisms of sanitation indicators. According to Adams and Motoarjemi (2003) E. coli pathogenic bacteria that contaminate food can occur due to a lack of attention to sanitation, both from snack food, snack handlers, places of sale, and appliances used, as well as utensils that are reused without being washed, especially utensils used for cooked or ready-to-eat food. Disease-causing pathogens that contaminate food can be a serious threat. According to research that has been conducted by Riyanto and Asep (2012) regarding factors that can affect the content of E. coli in food, namely there is a meaningful relationship between E. coli and clean food processing, equipment, ingredients, and means of selling snack food in elementary schools. According to Mudey et al. (2010) knowledge and behavior have a great influence on food quality and the level of risk of contamination due to bacteria. The application of good personal hygiene by respondents still contains E. coli in the food can occur because the food storage process is not right. Food stored in open spaces is susceptible to being contaminated with E. coli by 2 times. Bacillus sp. not found in all samples tested. The identification results showed that Staphylococcus lentus bacteria were found. According to Shaker et al. (2018) S. lentus is a pathogenic bacterium and has the ability to cause disease

and is resistant to antibiotics. Staphylococcus lentus was found in several operating rooms in three hospitals in Iraq. Staphylococcus sp. contained in snack food must be in accordance with SNI in the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 16 of 2016, namely < 1 x 102 colonies / g. Testing of Staphylococcus sp. which has been carried out on samples of school children's snack food in Pematangsiantar gave the result that 7 out of 12 samples contained Staphylococcus sp. Although the results are known to contain Staphylococcus sp. bacteria, the results are still below the SNI that Identification of Salmonella in snack food samples of elementary school children in Pematangsiantar based on Table 4.1. It is known that no positive results were obtained. This is in accordance with the maximum limit of SNI Salmonella, which is Negative / 25 g in the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 16 of 2016. Packaged and unpackaged processed meat and meat in Tehran contained Salmonella sp., and found that Salmonella sp. on DAG The presence of Salmonella sp. occurs because there is an attachment of food to the causative factors, such as water sanitation and equipment which includes the provision of clean water, replacement of dirty rinse water, the availability of garbage dumps with closed conditions, and clean traders' equipment. Research by Kumalasari et al. (2017) states that contamination is determined from the distance of the source of pollutants, the presence of vectors, and garbage cans. Salmonella sp. closely related to distance. The Total Plate Count (TPC) test of bacteria in food samples of school snacks of motorcycle taxis and fried meatballs (Table 4.2) showed results that were below SNI < 1 x 105 CFU/g according to with Regulation Number 16 of 2016 which has been stipulated by the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia. The mean values ± the highest and lowest standard deviations in the PJAS samples were (9.95 ± 0.21) x 104 CFU/g and (4.5 ± 0.7) x 101 CFU/g.

Conclusion

Based on the results and discussion in the previous chapter, it can be concluded as follows: Snack food for elementary school children that was sampled randomly at each location in elementary school, namely samples (fried and fried meatballs) in Pematangsiantar did not contain contamination with pathogenic bacteria Escherichia coli, Bacillus sp., Staphylococcus sp., Salmonella, and Total Plate Count (TPC) that exceeded the SNI threshold, except for the testing of JPT E. coli on fried meatballs in SD M, SD 5, SD IT, and SD 4 that exceeds the SNI limit.

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Conflict of interests

I convey in the results of this study that there is no interest during the research until the results are obtained

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