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Analysis of Contamination of Total Microbes, *Coliform*, *Escherichia coli*, and *Staphylococcus aureus* in Elementary School Children's Snacks in Jimbaran area, Bali, Indonesia

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ABSTRACT

School-age children like buying snacks after school. The snacks are sold on the roadside, therefore making them vulnerable to bacterial contamination, such as *Coliform, Escherichia coli*, and *Staphylococcus aureus* that cause foodborne diseases. Jimbaran is in South Kuta, Badung, Bali. There are 20 elementary schools in Jimbaran with 7,321 students. The purpose of this research was to determine pathogenic bacterial contamination in snacks. Sampling was done using Simple Random Sampling method, by selecting three types of snacks from five different elementary schools in Jimbaran. Testing pathogenic bacterial contamination using total plate count (TPC), Most Probable Number (MPN), and *Staphylococcus aureus* test. The results showed the highest average total microbes in cubit cake at Elementary School E is 34.06×10^4 CFU/g. The highest average of *Coliforms* in fried tofu at Elementary School B was 92.27×10^2 MPN/g. The highest average *E. coli* in *omelets roll at Elementary School C* was 63.93×10^2 MPN/g. The highest average *S. aureus* in cakkue cake at Elementary School D was 92.9×10^2 CFU/g. The conclusion were 45 samples had total microbial contamination, 22 samples were positive for *Coliform*, 16 samples were positive for *E. coli*, and 40 samples were positive for *S. aureus*. According to Indonesian Food Safety Standards (BPOM RI), 4.4% of the snacks tested were suitable for consumption, while 95.6% were not suitable for consumption.

Key words: *Snacks, Pathogenic bacteria, Safety standards*

Introduction

Snacks (Street Food) are food or drinks that are sold on the streets or public crowded places (Morano *et al.*, 2018). School-age children have a habit of buying snacks on the side of the road after school (Marissa *et al.*, 2019). Snacks sold in open locations are prone to contamination by microbes. Food contaminated with microbes makes these snacks unfit for consumption (Sharma *et al.*, 2020). The presence

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of microbes such as bacteria in snacks can be used as an indicator in determining the quality of a food microbiologically (Compaore *et al*., 2022). Several pathogenic bacteria found in food are *Coliforms*, *Escherichia coli*, and *Staphylococcus aureus* which can cause foodborne disease (Bintis, 2017). Illness due to foodborne disease shows several symptoms, such as diarrhea, vomiting, nausea, abdominal pain, fever, and headache (Switaj *et al*., 2015). Foodborne disease can be transmitted orally if swallowed, enters the digestive tract, and causes clinical symptoms. The body can lose fluids if the symptoms of foodborne disease in the form of diarrhea and vomiting last a long time (Carrique-Mas and Bryant, 2013).

Cases of foodborne disease in Indonesia in 2011 recorded as many as 18,144 people exposed to foodborne (Muna and Khariri, 2020). According to Pitriyanti *et al.* (2020), the most outbreaks due to food poisoning around educational institutions occurred in elementary schools, with 9 incidents. According to BPOM's annual report (2019), cases of poisoning in Bali occupy the fourth position in Indonesia with 373 cases (BPOM RI, 2020). The Bali Provincial Health Office (2020) also stated that there were 365 cases of outbreaks during 2019 caused by food poisoning.

Jimbaran is a village located in South Kuta District, Badung Regency, Bali Province. The Jimbaran area is one of the areas in the Province of Bali which has a very dense population of 50,530 people. There are 20 elementary schools (SD) in the Jimbaran area, including private elementary schools and public elementary schools with a total of 7,321 students (Badung Regency Central Bureau of Statistics, 2017). The number of elementary school students in the Jimbaran area demands a lot of supervision of food safety.

Dayanara *et al*. (2019) reported that empek-empek snacks from elementary schools on Sapeken Island, Sumenep, East Java were contaminated with *E. coli* bacteria. Nuryani *et al.* (2016) reported that as much as 71% of the snacks in the Public Elementary School canteen in the South Denpasar District were contaminated with *E. coli*. Some of the results of previous studies indicate that many snacks sold around elementary schools are still contaminated with pathogenic microbes. Therefore, it is necessary to test the snacks of elementary school children in the Jimbaran area. The purpose of this study was to obtain the total microbial contamination, *Coliform, Escherichia coli*, and *Staphylococcus aureus* found in the sample snacks of elementary school children in the Jimbaran.

Materials and Method

Research Location

This research was conducted in the Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Udayana University, Bukit Jimbaran for two months, from November 2022 to December 2022.

Sample Collection

The sampling of snacks was carried out using the simple random sampling method. Samples were taken by selecting three different types of snacks from five different elementary schools three repetitions for three weeks without any time interval so that a total 45 samples was obtained. The elementary school was the location for sampling and the types of snacks taken were

a. Elementary School A: Chicken balls, Meat balls, and Sempol
b. Elementary School B: Chicken balls, Meat balls, and Fried Tofu
c. Elementary School C: Fried Banana, Rolled Omelette, and Donuts
d. Elementary School D: Buns, Cakkue cake, and Rissoles
e. Elementary School E: Chicken balls, Fried Tempeh, and Cubit Cake

Samples were obtained by buying the snacks directly from the sellers. Based on National Standardization Agency (SNI) 19-0428-1998, sampling was carried out using a sharp-tipped tool (National Standardization Agency, 1998).

Total Plate Count Test

Total Plate Number Test (ALT) was done using Nutrient Agar media (National Standardization Agency, 2015). The suspension from the third dilution was then pipetted 1 ml and was put into a sterile Petri dish and added with Nutrient Agar (NA) media using the pour plate method, incubated at 37 °C for 24 hours. Colonies growing on the media were observed and counted with a range of the number of colonies showing 30-300 colonies (National Food and Drug Testing Center, 2014).

The total microbes are calculated using the following formula (National Center for Drug and Food

Testing, 2014) $N = \frac{\sum C}{(V\ (n_1) \times d)}$

Information :

N = Number of microbes in the sample
ΣC = Number of colonies on the Petri dish from dilutions that meet the calculation range
V = volume of inoculum put into each Petri dish
n_1 = Number of Petri dishes used in the first calculated dilution
d = Dilution corresponding to the first calculated dilution

The results of the total microbial count were compared with the Indonesian Food Safety Standards (BPOM RI) (2012).

Most Probable Number (MPN) Test

This test consisted of 3 stages, namely the prediction test using Lactose Broth (LB) media, the *Coliform* confirmation test using Brilliant Green Bile Broth (BGBB) media, and the *E. coli* confirmation test using Eosine Methylene Blue Agar (EMBA) media (National Standardization Agency, 2015). The positive results of *Coliform* were then matched to the MPN table series 3 tubes according to the Thomas Formula (National Standardization Agency, 2015) and compared with the National Standardization Agency (SNI) 7388: 2009 guidelines concerning the maximum limit of microbial contamination in food (National Standardization Agency, 2009). The positive results of *E. coli* were then matched to the MPN table series 3 tubes according to the Thomas Formula (National Standardization Agency, 2015) and compared with Indonesian Food Ssafety Standards (BPOM RI) (2012).

***Staphylococcus aureus* Number Test**

Number of *Staphylococcus aureus* using pour plate method with selective media Mannitol Salt Agar (MSA) (National Standardization Agency, 2015), incubated at 37°C for 24 hours. *Staphylococcus aureus* bacteria that grow on the media are marked with yellow colonies and observed and counted with a range of 30-300 colonies (National Standardization Agency, 2015), using the following formula (National Food and Drug Testing Center, 2014). The results of calculating the number of *S. aureus* were then compared with Indonesian Food Safety Standard (BPOM RI) (2012).

To confirm that bacteria is *E. coli* and *S. aureus* obtained, the test will continue with catalase test and Gram Staining.

Data analysis

Data is processed quantitatively with the Microsoft Excel application. Data were analyzed descriptively with tables and figures also compared with guidelines for contamination criteria in ready-to-eat food and home industry food according to Indonesian Food Safety Standard (BPOM RI) (2012) or the maximum limit of microbial contamination in food according to National Standardization Agency (SNI) 7388:2009.

Results

Total Microbes Results

The results of the total microbes on the types of snacks found in Elementary School, Jimbaran can be seen in Table 1.

The highest average total number of microbes was found in cubit cakes at Elementary School E was 34.06×10^4 CFU/g, while the lowest average total microbial count was found in Chicken Balls at Elementary School B was 85.1×10^3 CFU/g. The positive results of the total microbial test can be seen in Figure 1. Based on BPOM RI (2012), the maximum limit for microbial contamination in all tested samples is 1×10^5 CFU/g. The results showed that as many as 15 samples were suitable for consumption, while 30 samples were not suitable for consumption.

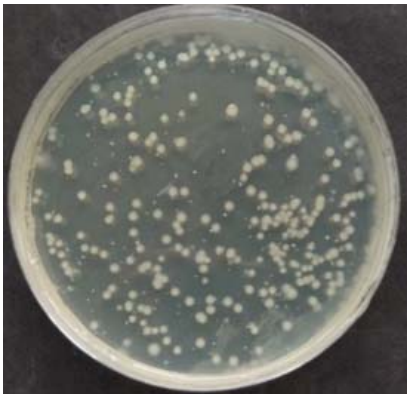

Fig. 1. Results of Total Microbes in Snacks

MPN of *Coliform*

The MPN of *Coliform* results in the different types of snacks found in Elementary School, Jimbaran can be seen in Table 2. The highest average number of MPN of *Coliform* was found in Fried Tofu from Elementary School B was 92.27×10^2 MPN/g, while

the lowest was found in Rissoles from Elementary School D and Cubit Cake from Elementary School E was 0 MPN/g. Based on SNI 7388:2009, the maximum limit for *Coliform* contamination in Chicken Balls, Meat Balls, Sempol, Fried Tempeh, Fried Tofu, and Fried Banana is 10 MPN/g, while the maximum in Donuts, Buns, Cubit Cake, Rolled Omlette, and CakKue Cake was 20 MPN/g. The results showed that 23 samples were suitable for consumption, while 22 samples were not suitable for consumption.

MPN *Escherichia coli* Results

The MPN results of *Escherichia coli* on the types of snacks found in Elementary School, Jimbaran can be seen in Table 3. The highest MPN average for *E. coli* was found in Rolled Omelette from Elementary School C was 63.93 × 10^2 MPN/g, while the lowest was found in Chicken Balls from Elementary School A and Elementary School B, also Rissoles from Elementary School D was 0 MPN/g. The maximum limit for *E. coli* contamination in elementary school snacks according to Indonesian Food Safety Standard is <3 MPN/g (BPOM RI, 2012). The results showed that 29 samples were suitable for consumption, while 16 samples were not suitable for consumption.

Table 1. Results of Total Microbes in Types of Snacks

No	School's Name	Type of Snack	Total Microbes (CFU/g)			Average (CFU/g)	BPOM Standard (CFU/g)
			Week I	Week II	Week III		
1	Elementary School A	Chicken Balls	44 × 10^3	146 × 10^3	52,4 × 10^4	238 × 10^3	1 × 10^5
		Meat Balls	106 × 10^1	41,6 × 10^4	44,8 × 10^4	28,83 × 10^4	
		Sempol	50 × 10^1	32,0 × 10^4	124 × 10^3	148,16 × 10^3	
2	Elementary School B	Chicken Balls	35 × 10^1	192 × 10^3	63 × 10^3	85,1 × 10^3	
		Meat Balls	83 × 10^1	162 × 10^3	76,8 × 10^4	31,02 × 10^4	
		Fried Tofu	292 × 10^1	176 × 10^3	224 × 10^3	134,3 × 10^3	
3	Elementary School C	Fried Banana	32,0 × 10^2	71 × 10^3	44,0 × 10^4	171,4 × 10^3	
		Rolled Omlette	220 × 10^3	46 × 10^3	57,6 × 10^4	280,6 × 10^3	
		Donuts	34,0 × 10^2	42 × 10^3	33,2 × 10^4	125,8 × 10^3	
4	Elementary School D	CakKue cake	87 × 10^3	286 × 10^3	32,0 × 10^4	23,1 × 10^4	
		Rissoles	230 × 10^3	164 × 10^3	194 × 10^3	19,6 × 10^4	
		Buns	48 × 10^3	186 × 10^3	42,0 × 10^4	21,8 × 10^4	
5	Elementary School E	Chicken Balls	231 × 10^3	101 × 10^3	216 × 10^3	182,6 × 10^3	
		Fried Tempeh	85 × 10^3	190 × 10^3	216 × 10^3	163,6 × 10^3	
		Cubit Cake	228 × 10^3	146 × 10^3	64,8 × 10^4	34,06 × 10^4	

Table 2. MPN of *Coliform* in different Types of Snacks

No	School's Name	Types of Snacks	MPN *Coliform* (MPN/g)			Average (MPN/g)	SNI Standard (MPN/g)
			Week I	Week II	Week III		
1	Elementary School A	Chicken Balls	0	0	10 × 10^1	3,3 × 10^1	10
		Meat Balls	0	10 × 10^1	0	3,3 × 10^1	10
		Sempol	0	29 × 10^1	0	9,7 × 10^1	10
2	Elementary School B	Chicken Balls	0	10 × 10^1	0	3,3× 10^1	10
		Meat Balls	0	0	29 × 10^1	9,7× 10^1	10
		Fried Tofu	4,32 × 10^3	≥18,98 × 10^3	438 × 10^1	92,27 × 10^2	10
3	Elementary School C	Fried Banana	29 × 10^1	190 × 10^1	76 × 10^1	98,3 × 10^1	10
		Rolled Omelette	29 × 10^1	76 × 10^1	≥18,98 × 10^3	66,77 × 10^2	20
		Donuts	0	190 × 10^1	10 × 10^1	66,7 × 10^1	20
4	Elementary School D	CakKue Cake	0	76 × 10^1	4 × 10^1	26,7 × 10^1	20
		Rissoles	0	0	0	0	20
		Buns	0	0	76 × 10^1	25,3 × 10^1	20
5	Elementary School E	Chicken Balls	0	29 × 10^1	0	9,7 × 10^1	10
		Fried Tempeh	4 × 10^1	0	29 × 10^1	11,0 × 10^1	10
		Cubit Cake	0	0	0	0	20

The positive results of *E. coli* in the snacks of Elementary School in Jimbaran can be seen in Figure 2. The positive results of *E. coli* are indicated by the metallic green color formed on the EMBA media. Microscopically, this bacterium is red in color because it is a Gram Negative and rod-shaped bacterium. The positive result was then confirmed using the catalase test. The catalase test on *E. coli* bacteria showed a positive result which was indicated by the formation of bubbles which can be seen in Figure 3.

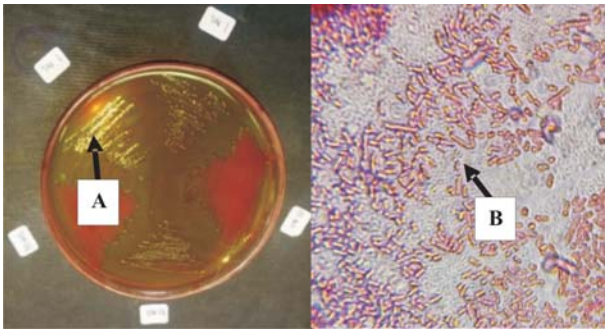

Fig. 2. Positive *Escherichia coli* Results on Cak Kue Snacks (A) Colony *E. coli* (Macroscopic), (B) Microscopic *E. coli*

Staphylococcus aureus count

The results of the *Staphylococcus aureus* found in snacks from Elementary School, Jimbaran can be seen in Table 4. The highest average test for *S. aureus* contamination found in Cak Kue Cake from Elementary School D was 92.9×10^2 CFU/g, while the lowest average was 20.4×10^2 CFU/g in Cubit Cake

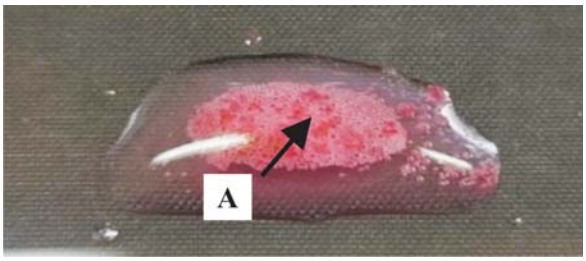

Fig. 3. *Escherichia coli* catalase test results (A) Positive result (bubble)

from Elementary School E. The maximum limit for *S. aureus* contamination in school children's snacks (PJAS) according to Indonesian Food Safety Standard was 1×10^2 CFU/g (BPOM RI, 2012). The results showed that there were 5 samples of snacks that were suitable for consumption, while 40 samples were not suitable for consumption.

The positive results of *S. aureus* in the snacks can be seen in Figure 4. Macroscopically, *S. aureus* bacteria form yellow colonies surrounded by yellow zones on Mannitol Salt Agar (MSA) media. These bacteria appear microscopically in shape-like grapes and are purple in color because they are Gram Positive bacteria. Confirmation of *S. aureus* was done by catalase test. The results of the catalase test on *S. aureus* bacteria can be seen in Figure 5. The catalase test on *S. aureus* showed positive results which were indicated by the presence of bubbles.

Discussion

The test results for total microbes (Table 1), *Coliform*

Table 3. Results of MPN of *Escherichia coli* in different Types of Snacks

No	School's Name	Types of Snacks	MPN *E. coli* (MPN/g)			Average (MPN/g)	BPOM Standard (MPN/g)
			Week I	Week II	Week III		
1	SD No.4 Jimbaran	Chicken Balls	0	0	0	0	< 3
		Meat Balls	0	4×10^1	0	$1{,}3 \times 10^1$	
		Sempol	0	29×10^1	0	$9{,}7 \times 10^1$	
2	SD No.7 Jimbaran	Chicken Balls	0	10×10^1	0	$3{,}3 \times 10^1$	
		Meat Balls	0	0	0	0	
		Fried Tofu	0	11.6×10^2	438×10^1	18.47×10^2	
3	SD No.8 Jimbaran	Fried Banana	29×10^1	27×10^1	76×10^1	44.0×10^1	
		Rolled Omlette	10×10^1	10×10^1	$\geq 18{,}98 \times 10^3$	63.93×10^2	
		Donuts	0	21×10^1	0	7.0×10^1	
4	SD No.11 Jimbaran	CakKue Cake	0	76×10^1	0	25.3×10^1	
		Rissoles	0	0	0	0	
		Buns	0	0	21×10^1	7.0×10^1	
5	SD No.12 Jimbaran	Chicken Balls	0	29×10^1	0	9.7×10^1	
		Fried Tempeh	4×10^1	0	0	1.3×10^1	
		Cubit Cake	0	0	0	0	

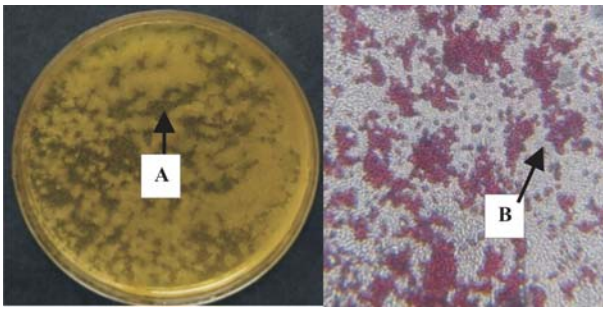

Fig. 4. Positive Results of *Staphylococcus aureus* in School Children's Snacks, Mannitol Salt Agar (MSA) media
(A) Colony of *S. aureus* (Macroscopic), (B) Microscopic of *S. aureus*

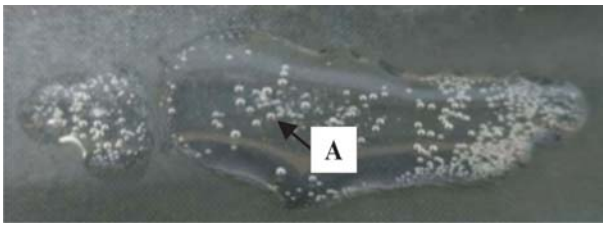

Fig. 5. *Staphylococcus aureus* catalase test results
(A) Positive result (bubble)

(Table 2), *E. coli* (Table 3.), and *S. aureus* (Table 4) for each snack sample from the first to the third week experienced fluctuations caused by the seller factor who did not pay attention to the hygiene of processing, serving, and sanitation of the environment where they sell snacks. This is supported by Vitria *et al.* (2013), the high total microbes in food are influenced by processing methods, equipment washing methods, and seller hygiene. According to Rahim *et al.* (2020), *Coliform* contaminates food due to the seller's poor hygiene and poor environmental sanitation, such as dusty roads or close to landfills. Nuryani *et al.* (2016) said that food storage, processing, and sanitation facilities are related to the presence of *E. coli* in snacks. Kadariya *et al.* (2014) reported that the causes of *S. aureus* contamination in snacks can come from unclean containers or equipment, environmental sanitation is not clean, and sellers do not maintain the hygiene of their hands when processing.

Based on the survey conducted, sellers of rissoles, cakkue cake, fried tofu, fried bananas, fried tempeh, and cubit cakes sell cakes in an unhygienic way. Sellers do not wash their hands before processing these snacks so that these snacks can be contaminated with pathogenic bacteria such as *Coliform*, *E. coli* and *S. aureus*. The Donuts are stored in an open place so that these snacks are contaminated with insects such as ants. Chicken Balls, Meat Balls, and Sempol sellers showed that the environmental sanitation in which the snacks were sold was poor, the presentation of the snacks was not hygienic, and the equipment used was not clean enough. This survey supported by Riyanto and Abdillah (2012) that contamination of pathogenic bacteria in snacks is caused by sellers who do not apply personal hygiene such as not washing their hands with soap before processing, serving food that is not covered

Table 4. Results of Total Number *Staphylococcus aureus* in different Types of Snacks

No	School's Name	Types of Snacks	Total Number *S. aureus* (CFU/g)			Average (CFU/g)	BPOM Standard (CFU/g)
			Week I	Week II	Week III		
1	Elementary School A	Chicken Balls	$58{,}8 \times 10^2$	74×10^1	33×10^1	$231{,}7 \times 10^1$	
		Meat Balls	192×10^1	89×10^1	$150{,}4 \times 10^2$	$59{,}5 \times 10^2$	
		Sempol	98×10^1	0	$122{,}4 \times 10^2$	$44{,}0 \times 10^2$	
2	Elementary School B	Chicken Balls	0	$17{,}52 \times 10^3$	42×10^1	$59{,}8 \times 10^2$	
		Meat Balls	0	$90{,}4 \times 10^2$	41×10^1	$31{,}5 \times 10^2$	
		Fried Tofu	47×10^1	208×10^1	$119{,}2 \times 10^2$	$48{,}2 \times 10^2$	
3	Elementary School C	Fried Banana	41×10^1	$69{,}6 \times 10^2$	$139{,}2 \times 10^2$	$70{,}9 \times 10^2$	1×10^2
		Rolled Omelette	$64{,}8 \times 10^2$	132×10^1	$15{,}12 \times 10^3$	$76{,}4 \times 10^2$	
		Donuts	79×10^1	$117{,}2 \times 10^2$	224×10^1	$49{,}1 \times 10^2$	
4	Elementary School D	CakKue Cake	45×10^1	143×10^2	$131{,}2 \times 10^2$	$92{,}9 \times 10^2$	
		Rissoles	$90{,}8 \times 10^2$	166×10^1	$88{,}0 \times 10^2$	$65{,}1 \times 10^2$	
		Buns	$72{,}8 \times 10^2$	$74{,}4 \times 10^2$	$112{,}8 \times 10^2$	$86{,}6 \times 10^2$	
5	Elementary School E	Chicken Balls	$94{,}4 \times 10^2$	186×10^1	$45{,}6 \times 10^2$	$52{,}8 \times 10^2$	
		Fried Tempeh	0	$118{,}4 \times 10^2$	$126{,}4 \times 10^2$	$81{,}6 \times 10^2$	
		Cubit Cake	0	84×10^1	$52{,}8 \times 10^2$	$20{,}4 \times 10^2$	

causing contamination from dust and insects. Rolled Omlette are only wrapped in plastic with the top and bottom open so that they are vulnerable to contamination by pathogenic bacteria. Kurniasih *et al.* (2015) reported that the sellers do not use clean and dry containers when serving food and places where food is served that are not clean cause snacks to be contaminated with pathogenic bacteria. The sellers also only use newspaper or plastic covers so that the snacks are not covered properly.

Snacks contaminated with *E. coli* and *S. aureus* bacteria can cause digestive tract infections (Foodborne Diseases), such as diarrhea (Tanih *et al.*, 2015). Diarrhoea can cause symptoms such as abdominal pain, vomiting, fever, lack of appetite, fatigue, and weight loss. Diarrhea can also cause a sudden decrease in body fluids and electrolytes, causing various complications, such as fluid loss, organ damage, and even death (Machava *et al*., 2022). There are six types of *E. coli* can infect the digestive infection, such as *Enteropathogenic E. coli* (EPEC), *Enterohaemorrhagic E. coli* (EHEC), *Enterotoxigenic E. coli* (ETEC), *Enteroaggregative E. coli* (EAEC), *Enteroinvasive E. coli* (EIEC), and *Diffusely Adherent E. coli* (DAEC) (Pakbin *et al.*, 2021). Staphylococcus aureus can cause diseases because of Staphylococcal Toxin contaminate food (Al-Bahry*et al*., 2014). Snacks contaminated with *E. coli* can also cause urinary tract infections, cholera, dysentery, and gastrointestinal (Nisa *et al*., 2019), while *S. aureus* can also cause inflammation of the oral cavity, necrosis, and abscess formation (Tong *et al*., 2015).

Conclusion

Based on this research, it can be concluded that all 45 samples of snacks are contaminated with total microbes, 23 samples were positive for *Coliform*, 16 samples were positive for *Escherichia coli*, and 40 samples were positive for *Staphylococcus aureus*. All the entire sample tested, snacks for elementary school children in Jimbaran were safe to eat as much as 4.4%, while snacks that were not suitable for consumption were as much as 95.5%.

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