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Total Analysis of Bacteria Contaminations, *Staphylococcus aureus*, and Salmonella in Urutan Babi Sold in Several Sub-district in Gianyar, Bali, Indonesia

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ABSTRACT

Urutan babi (pork sausage) is a traditional Balinese food sold in various areas of Bali Island, one of which is in Gianyar Regency. In general, urutan babi are sold with a little regard for environmental sanitation in the sales area. The feared condition could result in bacterial contamination, including *Salmonella* sp. and *Staphylococcus aureus* which can cause various disease problems, especially in the human digestive system. This study aims to determine the total contamination of bacteria, *Staphylococcus aureus,* and *Salmonella* in urutan babi which are sold in Gianyar Regency and to determine the feasibility of urutan babi samples in accordance with food safety standards according to BPOM. Samples of urutan babi were obtained from several sellers of urutan babi spread across four sub-districts in Gianyar Regency, namely Gianyar, Sukawati, Tegallalang, and Ubud Districts. Sampling method was using a purposive sampling method of 30% of the total traders in each sub-district, while the microbial testing method used pour plate and streak plating methods and used selective media. Parameters tested included Total Plate Count (TPC), *Staphylococcus aureus*, and the presence of *Salmonella* in the urutan babi. The results showed that the microbial and *S. aureus* contamination rates for 3 weeks fluctuated between 10^2 and 10^7 CFU/g, while the *Salmonella* test result was negative. Based on the test, as many as 55 out of 66 samples (83%) exceed the consumption eligibility standards for the microbial contamination test (TPC), all samples exceed the consumption eligibility standards for the *S. aureus* contamination test, while all samples did not exceed the consumption eligibility standards for the *Salmonella* contamination test according to BPOM standards.

Key words: *Food safety, BPOM standards, Pathogenic bacteria, Balinese traditional food*

Introduction

The Province of Bali is one of the regions in Indonesia that is identical with customs and culture, besides that the Province of Bali is also famous for its variety of traditional culinary delights which are characterized by being rich in spices. One of the areas in Bali Province which is visited by many tour-

1. Student

ists because of its customs, culture, and culinary variety is Gianyar Regency. Traditional Balinese food can be found in every corner of Gianyar Regency, including *sate lilit, ayam betutu, lawar,* and *urutan*. Urutan babi or traditional Balinese sausage is a Balinese food which is still made traditionally untill this day. Urutan babi is a food made from pork and lard which is cut into small pieces and mixed with various "*base genep*" spices, namely shallots, garlic, coriander, cumin, aromatic ginger, chilies, salt, pepper, shrimp paste, turmeric, ginger, and galangal. The mixture of pork, lard and spices is then put into the pig's intestines to form a sausage-like shape and it can be processed by steaming, boiling, smoking, drying or frying (Gardipa *et al*., 2019; Sumardani *et al*., 2020).

Urutan babi is a favorite food for local Balinese people and tourists. This food is usually used as a daily side dish or used in celebrations or religious ceremonies. Because the demand is quite high, urutan babi are very easy to find in various places, including restaurants and markets. However, in the selling process itself, urutan babi are often sold without using food covers at room temperature, and some traders also only pay little attention to environmental sanitation around the sales area. This can result in an increased risk of contamination of urutan babi by various types of microbes that can cause health problems, especially for the digestive system, including *Staphylococcus aureus* and *Salmonella* sp. (Kurniadi *et al*., 2013).

Staphylococcus aureus is a type of Gram-positive bacteria that are round in clusters resembling like a grapes and are non-motile, while *Salmonella* sp. is a type of Gram-negative bacteria in the form of rods and is motile (Soedarto, 2019; Apriani *et al*., 2019). *Staphylococcus aureus* can grow easily in dirty environments or on media that rich in protein, while *Salmonella* sp. is an enteric pathogenic bacteria that develops well in the digestive system of animals and humans. Both of these toxic bacteria are able to reproduce well at room temperature and very easily contaminate food. There are more than 200 types of diseases that can be caused by consuming food that has been contaminated with toxic bacteria. Contaminated food that is sold freely is feared to cause an increasingly widespread of disease resulting in food borne disease (Muna and Khariri, 2020). Badan Pengawas Obat dan Makan (BPOM) as the organisation who set food safety standards in Indonesia states that in Indonesia there have been 128 extraordinary cases of food borne disease with 18,144 victims in 2011. The United States on the other hand also has data on the number of cases of food poisoning, where each year it has been recorded 48 million cases of food poisoning (Quinto *et al*., 2019). Food borne disease caused by bacterial contamination to date has the highest number of extraordinary events and death rates compared to virus and parasite contamination (Nurmawati *et al*., 2019). Rahayu *et al*. (2014) confirmed that urutan babi obtained from the Denpasar City area and sold openly at room temperature had *S. aureus* contaminants that exceeded the contamination threshold set by BPOM regulations. So far, a test for contamination of urutan babi in Gianyar Regency has never been carried out, so the purpose of this study was to determine the total contamination of bacteria, *S. aureus* and Salmonella in urutan babi in Gianyar Regency and to determine the feasibility of samples of urutan babi according to food safety standards. according to BPOM.

Research Method

The research was carried out at the Microbiology Laboratory in Biology Department, Faculty of Mathematics and Natural Sciences, Udayana University from December 2022 to January 2023. Samples were obtained from 4 different sub-district in Gianyar Regency, namely 6 sample from Gianyar sub-district, 6 sample from Ubud sub-district, 5 sample from Sukawati sub-district, and 5 sample from Tegalalang sub-district with the coordinates of sampling as shown in Table 1.

Testing for total microbial contamination (TPC) and *Staphylococcus aureus* contamination was carried out using a pour plate dilution series and platting method using selective media based on modified SNI 2897:2008 (BSN, 2008). Nutrient Agar (NA) media was used in TPC test, while Mannitol Salt Agar (MSA) was used for *S. aureus* contamination test. The test sample on the Petri dish was incubated at 37 ^{o}C for 24 hours. *S. aureus* bacteria on MSA media is characterized by the growth of typical colonies with golden yellow colonies characteristics and a change in the color of the media to yellow. Colonies that grow from the incubation results are observed with the assumption that 1 colony comes from 1 microbial cell, then the colonies are counted using the total microbial calculation formula:

Colonies per gram = Total colonies per Petri dish x $\frac{1}{\text{dilution series}}$

Table 1. The coordinates of the urutan babi sampling location

No	Location	Sample Code	Coordinate
1	Gianyar	G1	8°32′34.2"S 115°19′20.2"E
		G2	8°32′29.5"S 115°19′36.4"E
		G3	8°32′46.3"S 115°19′31.0"E
		G4	8°33′57.5"S 115°17′51.0"E
		G5	8°31′52.1"S 115°19′03.1"E
		G6	8°32′02.2"S 115°19′00.7"E
2	Ubud	U1	8°32′26.0"S 115°16′21.4"E
		U2	8°31′43.0"S 115°15′07.6"E
		U3	8°31′39.0"S 115°15′23.9"E
		U4	8°32′30.0"S 115°15′43.5"E
		U5	8°30′12.3"S 115°15′49.0"E
		U6	8°30′17.6"S 115°16′19.6"E
3	Sukawati	S1	8°36′41.7"S 115°15′11.8"E
		S2	8°37′11.7"S 115°15′41.3"E
		S3	8°36′58.1"S 115°15′14.1"E
		S4	8°37′09.3"S 115°15′23.7"E
		S5	8°35′57.0"S 115°16′58.9"E
4	Tegalalang	T1	8°26′08.4"S 115°16′45.0"E
		T2	8°27′43.9"S 115°16′25.9"E
		T3	8°28′45.2"S 115°16′43.3"E
		T4	8°28′13.5"S 115°16′37.0"E
		T5	8°26′58.2"S 115°16′48.4"E

Explanation:
G = Gianyar S = Sukawati
U = Ubud T = Tegalalang

Testing for Salmonella contamination was carried out through 3 stages, namely pre-enrichment, enrichment, and platting based on modified SNI 2897:2008 (BSN, 2008). The pre-enrichment stage was carried out by adding 10 g of urutan babi sample to 90 mL of Buffered Peptone Water (BPW) medium and homogenize, then the suspension was incubated at 37 °C for 24 ± 2 hours. The results of the BPW incubation were then carried out at the enrichment stage by taking 100 µL using a micropipette and then mixing it into 10 mL of Rappaport Vassiliadis (RV) media and homogenize. The RV media containing the sample was then incubated at 42°C for 24 ± 2 hours. After the enrichment stage, the platting stage was carried out by taking 1 Ose of RV media and inoculating it in a Petri dish containing XLD media using the streak method. The Petri dish containing the sample was incubated upside down using a temperature of 37 °C for 24 hours. Salmonella bacteria on XLD media are characterized by pink or clear colonies with a black core.

The test results for *S. aureus* and Salmonella were followed by a confirmation test in the form of a Gram stain test and a catalase test which was guided by SNI 2332.9:2011 (BSN, 2011). The results of the Salmonella contamination test were followed by a biochemical test as the final confirmation test using Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSIA) media. The LIA and TSIA media which had been inoculated by bacterial colonies were then incubated at 37°C for 24 hours. The results of the biochemical test were observed based on the change in the color of the media. The results of a positive Salmonella analysis can only be declared positive for Salmonella contamination if all the results of the reaction of the biochemical test media comply with the criteria without any test showing non-compliance with the provisions stated in the biochemical test reading guide in SNI 2897-2008 (BSN, 2008).

Results

The results of the TPC test and contamination of *S. aurues* within 3 weeks showed contamination rates that fluctuated between 10^2 CFU/g to 10^7 CFU/g. The results of TPC testing on samples of urutan babi are guided by BPOM Regulation No. 13 of 2019 which states that the TPC limit for this sample is 1 x 10^4 CFU/g. Based on BPOM regulations, the sample that exceed the requirements for consumption in the first week is the G2 sample from Gianyar sub-district; samples U1, U3, U5, and U6 from Ubud sub-district; and sample T5 from Tegalalang sub-district. In the second week those who exceed the consumption requirements were samples G1, G2, and G4 from Gianyar sub-district; and samples T2 and T4

Fig. 1. TPC result of urutan babi in NA medium

from Tegalalang sub-district. Samples in the third week as a whole are not eligible. Overall, 55 out of 66 samples (83%) did not meet the eligibility requirements according to BPOM. The results of the bacterial contamination test were indicated by the growth of bacterial colonies on NA media as shown in Figure 1 and the total number of microbes which are presented in detail in Table 2.

The results of testing for *Staphylococcus aureus* contamination in samples of urutan babi are guided by BPOM Regulation No. 13 of 2019 which states that the limit for *S. aureus* contamination in the sample is 1 x 10^2 CFU/g. Based on the standards set by BPOM, none of the samples tested in the first, second, and third weeks exceed the food eligibility requirements because they had exceeded the *S.aureus* contamination limit. The results of the *S. aureus* contamination test are presented in detail in Table 3.

The growth of *S. aureus* bacteria on MSA selective media was shown by a change in the media which was originally red in color as shown in Figure 2a to yellow as shown in Figure 2b and the presence of typical colony growth to yellowish white as shown in Figure 2c.

Catalase test results on MSA media showed positive catalase results as indicated by the presence of bubbles which were the result of the decomposition of H_2O_2 into H_2O and O_2 as shown in Figure 3a. The results of the Gram stain test showed that the bacteria observed were Gram positive and staphylococcal in shape as shown in Figure 3b.

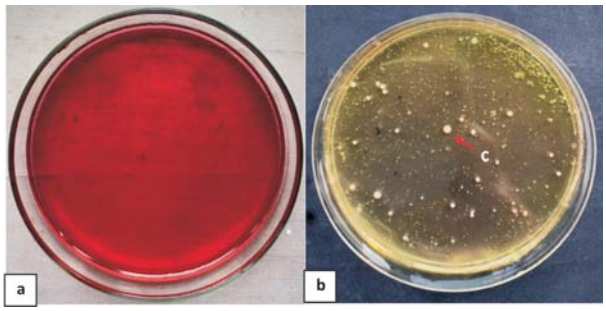

Fig. 2. *S. aureus* contamination test results of urutan babi samples on MSA media (a) MSA media control, (b) Test result of MSA media, (c) *S. aureus* colonies

The results of testing for *Salmonella* contamination in samples of urutan babi are guided by BPOM Regulation No. 13 of 2019 which states that the *Salmonella* contamination limit in the sample is required to be negative/25g. Based on the standards set by BPOM, all samples tested in the first, second, and

Table 2. Total Plate Count (TPC) results of urutan babi samples

No	Region	Sample Code	Total Plate Count (CFU/g)		
			1st week	2nd week	3rd week
1	Gianyar	G1	18 x 10^6	42 x 10^3	24 x 10^7
		G2	59 x 10^3	81 x 10^3	22 x 10^7
		G3	60 x 10^6	33 x 10^4	18 x 10^7
		G4	38 x 10^4	31 x 10^3	20 x 10^7
		G5	71 x 10^6	11 x 10^4	25 x 10^6
		G6	41 x 10^6	38 x 10^4	22 x 10^6
2	Ubud	U1	60 x 10^2	37 x 10^4	12 x 10^7
		U2	50 x 10^6	11 x10^5	29 x 10^7
		U3	50 x 10^3	26 x 10^4	11 x 10^7
		U4	31 x 10^4	29 x 10^4	21 x 10^7
		U5	19 x 10^3	12 x 10^5	33 x 10^6
		U6	84 x 10^3	59 x 10^4	32 x 10^7
3	Sukawati	S1	48 x 10^4	15 x 10^5	26 x 10^7
		S2	22 x 10^5	12 x 10^5	23 x10^7
		S3	72 x10^4	26 x 10^4	28 x 10^7
		S4	13 x 10^5	12 x 10^5	25 x 10^7
		S5	78 x 10^4	92 x 10^4	24 x 10^7
4	Tegalalang	T1	32 x 10^4	15 x 10^5	64 x 10^6
		T2	63 x 10^4	77 x 10^3	11 x 10^7
		T3	71 x 10^4	47 x 10^4	24 x 10^7
		T4	26 x 10^4	32 x 10^3	23 x 10^7
		T5	23 x 10^3	16 x 10^5	22 x 10^7

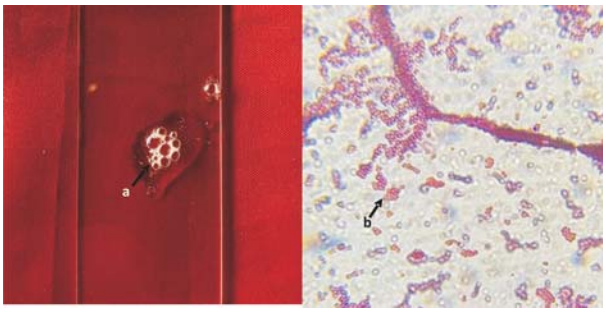

Fig. 3. Confirmation test results for *S.aureus* colonies on MSA media (a) Positive result of catalase test in the form of bubbles, (b) *S.aureus* microscopically at 1000x magnification

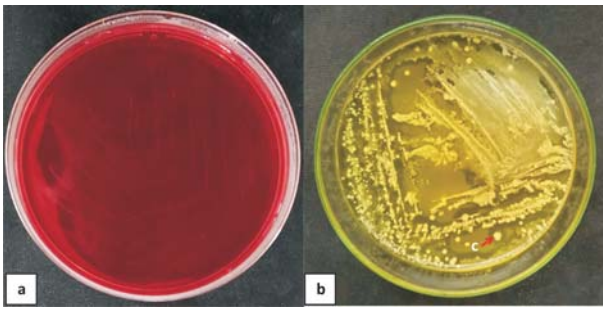

Fig. 4. Results of Salmonella contamination test of urutan babi samples on XLD media (a) Control of XLD media, (b) Test results of XLD media, (c) Bacterial colonies

third weeks not exceed the food eligibility requirements because they did not exceed the *Salmonella* contamination limit. The results of the *Salmonella* bacteria contamination test are presented in detail in Table 4.

There was a color change in the XLD selective media which was originally red as shown in Figure 4a to yellow as shown in Figure 4b and there was growth of yellowish white colonies as shown in Figure 4c, both of which did not characterize the growth of *Salmonella*.

Catalase test results on MSA media showed positive catalase results as indicated by the presence of bubbles which were the result of the decomposition of H_2O_2 into H_2O and O_2 as shown in Figure 5a. The results of the Gram stain test showed that the observed bacteria were Gram negative and bacillus shaped as shown in Figure 5b.

The results of the *Salmonella* biochemical test showed a change in the color of the LIA media which was originally purple (Figure 6a) to red in the slant and turned yellow in the butt with H_2S production (Figure 6c), the color change indicates that there

Table 3. Results of *S. aureus* contamination test on urutan babi

No	Region	Sample Code	Total of *S.aureus* (CFU/g)		
			1st week	2nd week	3rd week
1	Gianyar	G1	17×10^3	26×10^4	50×10^4
		G2	24×10^3	16×10^4	54×10^4
		G3	38×10^6	50×10^4	42×10^4
		G4	15×10^4	94×10^3	49×10^4
		G5	47×10^4	12×10^4	25×10^4
		G6	30×10^4	18×10^4	11×10^4
2	Ubud	U1	63×10^6	47×10^4	20×10^7
		U2	47×10^6	49×10^4	34×10^4
		U3	26×10^4	13×10^4	39×10^4
		U4	13×10^4	23×10^4	36×10^4
		U5	35×10^3	29×10^4	24×10^4
		U6	55×10^2	16×10^4	27×10^4
3	Sukawati	S1	71×10^4	15×10^5	33×10^4
		S2	96×10^5	17×10^4	31×10^4
		S3	33×10^4	35×10^4	70×10^3
		S4	12×10^3	40×10^2	56×10^6
		S5	73×10^6	34×10^4	29×10^7
4	Tegalalang	T1	26×10^4	95×10^4	63×10^4
		T2	31×10^6	11×10^4	67×10^4
		T3	12×10^4	53×10^4	13×10^5
		T4	11×10^4	32×10^3	42×10^5
		T5	35×10^3	75×10^4	61×10^3

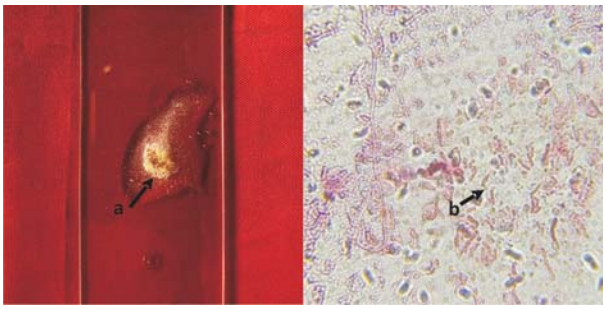

Fig. 5. Confirmation test results for bacterial colonies on XLD media (a) Positive result of catalase test in the form of bubbles, (b) Bacteria in the form of bacillus at 1000x magnification

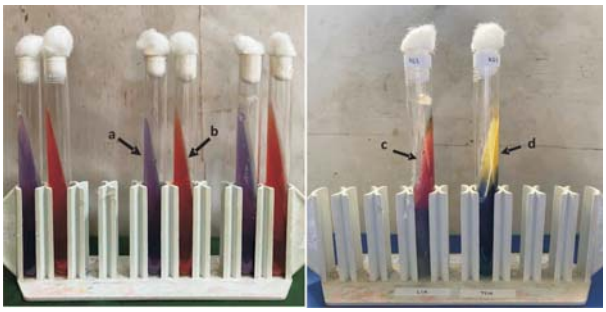

Fig. 6. Biochemical test results on urutan babi samples (a) LIA media control, (b) TSIA media control, (c) R/A results on LIA media, (d) A/A results on TSIA media

is acidification and lysine deamination so that it can be called R/A. In TSIA media, there was a change in the color of the media from which was originally red (Figure 6b) to yellow in the butt and slant and producing H2S (Figure 6d), the color change indicates that acidification has occurred in the butt and slant so that it can be called A/A.

Discussion

Tests for total microbial contamination (TPC) and *Staphylococcus aureus* which were carried out within 3 weeks showed contamination rates that fluctuated between 10^2 CFU/g to 10^7 CFU/g, where as many as 55 out of 66 samples (83%) in the TPC test were de-

Table 4. Results of *Salmonella* bacterial contamination test on urutan babi

No	Region	Sampel Code	Salmonella Contamination			BPOM Requirements Limit no.13 of 2019	Explanation
			1st week	2nd week	3rd week		
1	Gianyar	G1	negative	negative	negative	negative	Q
		G2	negative	negative	negative		Q
		G3	negative	negative	negative		Q
		G4	negative	negative	negative		Q
		G5	negative	negative	negative		Q
		G6	negative	negative	negative		Q
2	Ubud	U1	negative	negative	negative		Q
		U2	negative	negative	negative		Q
		U3	negative	negative	negative		Q
		U4	negative	negative	negative		Q
		U5	negative	negative	negative		Q
		U6	negative	negative	negative		Q
3	Sukawati	S1	negative	negative	negative		Q
		S2	negative	negative	negative		Q
		S3	negative	negative	negative		Q
		S4	negative	negative	negative		Q
		S5	negative	negative	negative		Q
4	Tegalalang	T1	negative	negative	negative		Q
		T2	negative	negative	negative		Q
		T3	negative	negative	negative		Q
		T4	negative	negative	negative		Q
		T5	negative	negative	negative		Q

Explanation:
Q = Quallify

clared exceed the eligibility standard consumption, while all samples in the *S. aureus* test were also declared exceed the eligibility standards for consumption. The conditions of microbial contamination and *S. aureus* which were classified as high in the samples tested could be caused by various factors that could trigger microbial growth, including intrinsic factors and extrinsic factors (Bakara *et al*., 2014). Intrinsic factors that can affect microbial growth in the urutan babi include the nutrients contained in the urutan babi, water content, and the presence of substances inhibiting bacterial growth in the urutan babi. Nutrient content is one of the microbial growth factors in food products such as urutan babi. Urutan babi is food made from meat and lard, where these ingredients are excellent media for the growth of various kinds of microbes because they contain high amounts of nutrients and water (Bonnet *et al*., 2020). Pork has a nutritional content similar to beef which includes protein, essential amino acids, minerals, vitamins and so on. Like meat in general, the highest nutritional content found in pork is protein. Proteins found in animal meat contain essential amino acids, where these compounds are one of the main requirements that can support the development of *S. aureus* bacteria (Schelin *et al*., 2011).

The urutan babi tested also contained substances that inhibited bacterial growth in the form of spices which contain secondary metabolites that act as antimicrobial agents, including shallots, garlic, coriander, cumin, aromatic ginger, chilies, pepper, shrimp paste, turmeric, ginger, and galangal. However, the content of secondary metabolites in the spices used in the urutan babi samples was still not able to inhibit bacterial growth. According to Li *et al*. (2017), there are several factors that can affect the ability of an antimicrobial substance to inhibit bacterial growth, namely the concentration of the antimicrobial substance used; time and temperature of storage of antimicrobial substances; and the nature of the microbes that will be inhibited growth. The spices used in the urutan babi samples had different concentrations, the temperature and storage time of the spices to be used were also different, apart from that the antimicrobial levels in each dose of the spices used were also unknown. These things allow for differences and decreases in antimicrobial performance in each sample of the urutan babi tested so that the results of the microbial contamination test with a high contamination rate are obtained. The nature of the microbes that are inhibited also greatly affects the performance of antimicrobials, one of which is the *S.aureus* bacterium which has a very good adaptability to antimicrobials that causes resistance to various types of antimicrobials used to inhibit its growth (Afifurrahman *et al*., 2014). These things can explain the cause of the acquisition of microbial contamination test results and *S.aureus* in the samples which are classified as high.

Extrinsic factors that can affect microbial growth in urutan babi samples include the environmental conditions around the place of sale; serving method; and storage methods, where these factors are closely related to hygiene and sanitation (Preetha and Narayanan, 2020). Some traders sell urutan babi near the main road where vehicles often pass by so that road dust which may contain microbes such as *S. aureus* can easily stick to food (Rahayu *et al*., 2014). Several samples of urutan babi were obtained from traders who sell in markets where there is stagnant water in the surrounding environment, where stagnant water can be a source of contaminants. Stagnant water is a very popular place for bacteria to breed, this happens because there is no movement or aeration in the water (Bédard *et al*., 2018). There were also sellers who sold urutan babi with stalls positioned side by side with stalls selling vegetables and fruits. Vegetables and fruits are food ingredients that often attract insects such as flies because these foods quickly decompose. Flies are insects that are used as indicators of environmental hygiene because a dirty environment with an unpleasant odor attracts flies. Most of the bacterial contamination that occurs in urutan babi is caused by the transmission of bacteria through the hands of food servers. Most traders ordering urutan babi immediately serve or wrap the urutan babi order using hands without gloves or food tongs after finished holding the money given by the customer. Money is a medium of exchange that is used for transactions in everyday life so that money often passes from hand to hand, this increases the potential of money as a place for the development of various kinds of microbes (Vriesekoop *et al*., 2016).

The results obtained from biochemical testing on samples suspected of being contaminated with Salmonella using Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSIA) slant media showed that the LIA media obtained R/A results which showed the characteristics of Salmonella, which are being able to deaminate lysine and being able to ferment glucose

(Percival *et al*., 2014). In the TSIA media test results obtained A/A results, this indicates that the tested bacteria are capable of fermenting glucose, lactose, and sucrose contained in the TSIA media, in this test it is proven that the tested bacteria are not *Salmonella* because *Salmonella* can only ferment glucose (Fàbrega and Vila, 2014). Based on the test results on the XLD media and the confirmation test which confirmed that the bacteria tested were not Salmonella, the samples tested were classified as eligible (Q) based on the standards set by BPOM, which were required to be negative/25g (BPOM RI, 2019). *Salmonella* is a type of bacteria that is capable of growing well in the digestive tract of warm-blooded animals such as the intestines of pigs, but the urutan babi sample tested did not contain *Salmonella* bacteria, this could be due to several reasons. *Salmonella* is a type of mesophilic bacteria that can live in environments with temperatures up to 54 ^{o}C and can develop well at optimum temperatures in the range of 35-37 ^{o}C. *Salmonella* will die if exposed to temperatures that exceed 54 ^{o}C, where at 54 ^{o}C *Salmonella* will die after being exposed to heat for 1 hour and will die after heating for 15 minutes using a temperature of 60 ^{o}C (Agustin and Chandraini, 2021). All of the urutan babi samples tested were heated by frying the urutan using cooking oil. Cooking oil has a boiling point at 176 ^{o}C and in general the optimum frying temperature using cooking oil reaches a temperature of 176-190 ^{o}C (Setiarto, 2021). Based on this, it can be ascertained that the frying temperature is one of the factors to avoid the presence of *Salmonella* bacteria in the urutan babi sample, but the presentation hygiene factor also remains a factor that needs to be considered so that the urutan babi is protected from *Salmonella* contamination.

Conclusion

Based on the research results, it can be seen that the urutan babi tested showed microbial and *Staphylococcus aureus* contamination are fluctuating between 10^{2} CFU/g to 10^{7} CFU/g within 3 weeks of testing. A total of 55 samples out of 66 samples (83%) did not meet the consumption eligibility standards for the microbial contamination test (TPC) and all samples exceed the consumption feasibility standards for the *Staphylococus aureus* contamination test. The results of the *Salmonella* contamination test showed that all samples were declared free from contamination by Salmonella bacteria and met the appropriate standards for consumption according to BPOM standards.

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