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Occurrence and level of *Listeria monocytogenes* and *Salmonella* in sushi at retails in Greater Jakarta area, Indonesia

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Abstract

Sushi is ready-to-eat food consisting of cooked vinegared rice and raw fish. Since sushi does not undergo heat treatment, inadequate control in the production may lead to microbial contamination. This study aimed to determine the prevalence and level of *Listeria monocytogenes* and *Salmonella* contamination in sushi sold in Greater Jakarta (Jakarta, Bogor, Depok, Tangerang, and Bekasi) area, Indonesia using the MPN-PCR method. Samples of sushi (n = 120), consisting of nigiri (rice with raw fish on top) (n = 57) and maki (rice with raw fish inside) (n = 63), were obtained from retail outlets in Jabodetabek. The results showed that both sushi products were more frequently contaminated with *L. monocytogene*s (14.2%) than *Salmonella* (2.5%). The contamination levels for *L. monocytogene*s and *Salmonella* were 3-1100 and 3.6-11 MPN/g, respectively. The highest prevalence of *L. monocytogenes* was found in maki sold in supermarkets (66.7%) and nigiri in kiosks (22.2%). In addition, maki and nigiri from restaurants have the highest prevalence of *Salmonella* at 2.2 and 3.8%, respectively. These findings indicate that contamination of *L. monocytogenes* and *Salmonella* in sushi sold at retail in Jabodetabek may pose a health risk to consumers and more study is needed to determine the source of contamination along the processing of sushi.

1. Introduction

Sushi has become one of the most popular cuisines due to changes in lifestyle in big cities that demand fast food. Increased income and education also changed the preference to consume food, including Indonesian, particularly the people in Jakarta and its surrounding area (Jakarta, Bogor, Depok, Tangerang, and Bekasi). Moreover, the perception of consumers of sushi as a nutrient-dense and healthful food adds value to sushi products; therefore, sushi businesses are growing in Jakarta. The Central Bureau of Statistics reported that in 2015 there were 5.64% Japanese restaurants of which 7.5 and 8.5% were located in the DKI Jakarta and West Java regions, respectively (BPS, 2016).

Sushi is a traditional food from Japan consisting of fish and rice. It is generally served as raw fish placed on rice, known as nigiri or a combination of rolled rice with fish inside or other ingredients such as eggs and vegetables, then wrapped with sheets of seaweed (nori), called maki (Feng, 2012). Sushi products are increasingly diverse due to the broader consumers, including Indonesia. Hence, nowadays, sushi is also prepared using thoroughly processed fish, eggs, shrimp, vegetables, and others. Sushi made from raw fish directly by the hands of the preparators could be contaminated with pathogenic bacteria due to the lack of good manufacturing practices implementation. Anggraeni (2017) reported that the prevalence of *Salmonella* in tuna loin products in Indonesia was 26% (20/77), indicating the possibility of pathogen contamination in fish as the raw material for sushi. In addition, salmon, as an imported commodity for the raw materials of sushi in Indonesia, is also at high risk of being contaminated by pathogenic bacteria. This is supported by the finding of Wang *et al.* (2011) that 20.6% (13/63) of salmon

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exported to the United States for 2009-2010 contained *L. monocytogenes*, *Salmonella* and *Shigella.*

There was no report available regarding the presence of *L. monocytogenes* and *Salmonella* in sushi at retail in Indonesia. Therefore, this study aimed to determine the prevalence and level of bacterial contamination of *L. monocytogenes* and *Salmonella* in sushi sold in restaurants, kiosks, and supermarkets in Greater Jakarta area in Indonesia. Furthermore, this information is essential to estimate the potential risk to public health.

2. Materials and methods

2.1 Sample collection

The samples were ready-to-eat sushi from restaurants, supermarkets, and kiosks located in DKI Jakarta, West Java (Bogor, Depok and Bekasi), and Banten (Tangerang, Tangerang Selatan) obtained between March to May 2019. Samples from restaurants and kiosks were obtained upon ordering. On the other hand, samples from supermarkets have been displayed in the refrigerated showcase for a maximum of 4 hrs. There were 120 sushi samples consisting of 57 nigiri and 63 maki containing either tuna or salmon. The sushi outlets (n = 111) consist of 98 restaurants, seven kiosks, and six supermarkets, which means some sushi outlets provide more than one sushi product. Samples were placed in sterile plastic bags and then stored in a cool box containing ice maintained at ±4°C for the sample transportation to the laboratory. Bacterial analysis of *L. monocytogenes* and *Salmonella* was performed as soon as the samples reached the laboratory.

2.2 Detection and enumeration of Listeria monocytogenes

Analysis of *L. monocytogenes* referred to ISO 11290 -1 (ISO, 2017a) with modifications at the confirmation stage using the polymerase chain reaction (PCR) method (Paziak-Domańska *et al.,* 1999). A 25 g sushi sample was added to 225 mL half Fraser (HF) broth (Oxoid, UK) and homogenized using a stomacher at 200 rpm for 1 min. The homogenate samples were incubated at 30±1° C for 24 hrs. A 0.1 mL homogenate was inoculated into 10 mL Fraser broth (FB) (Oxoid, UK) and incubated at 37°C for 48±3 hrs. The samples were then inoculated on PALCAM agar (Oxoid, UK) and CHROMAgar *Listeria* (CHROMAgar, France), then were incubated at 37±1°C for 24±3 hrs. Three to five typical colonies were inoculated on tryptone-soya-yeast-extract agar (TSYEA) (Oxoid, UK) and after incubation at 37±1°C for 24 hrs they were confirmed using the polymerase chain reaction (PCR) method.

Enumeration of *L. monocytogenes* was performed using the Most Probable Number (MPN) method adapted from Chen *et al.* (2015). For each 10^{-1}, 10^{-2} and 10^{-3} dilutions of each sample, 1 mL was transfered into three test tube sets containing 10 mL FB and incubated at 30±2°C for 48±2 hrs. The turbid cultures were streaked on PALCAM agar and incubated at 37±1°C for 24±3 hrs. Typical colonies were inoculated on TSYEA and then incubated at 37±1°C for 24 hrs. The isolates were subjected to PCR detection for confirmation. The MPN values were determined based on the MPN table (Blodgett, 2006).

2.3 Detection and enumeration of Salmonella

Salmonella was detected using ISO 6579-1 (ISO, 2017b) with modifications (Gwida and Al-Ashmawy, 2014). Approximately 25 g of sushi sample was added to 225 mL buffered peptone water (BPW) (Oxoid, UK) and homogenized using a stomacher at 200 rpm for 1 min. The homogenate samples were incubated at 37±1°C for 24 hrs. A 0.1 mL homogenate from BPW was inoculated into 10 mL Rappaport-Vassiliadis soya peptone (RVS) broth (Oxoid, UK) and incubated in a water bath at 41.5±1°C for 24±3 hrs. Homogenate was inoculated into xylose-lysine-desoxycholate (XLD) agar (Oxoid, UK) and incubated at 37±1°C for 24±3 hrs. Typical colonies of *Salmonella* were incubated on tryptone soya agar (TSA) (Oxoid, UK) at 37±1°C for 24 hrs and were confirmed using the PCR method (Rahn *et al.,* 1992).

Enumeration of *Salmonella* was performed using the MPN method referring to ISO/TS 6579-2 (ISO/TS, 2012). Approximately 25 g of sushi sample was added to 225 mL BPW and homogenized using a stomacher at 200 rpm for 1 min. A 2.5 mL of homogenate was placed into a first raw 12-well microtiter plate. Then, 0.5 mL of homogenate from the first row was transferred into the different well containing 2 mL BPW, and serially diluted in triplicate wells of BPW. The homogenate samples were incubated at 37±1°C for 18±2 hrs. After incubation, 20 µL of BPW from each raw of 12-well microtiter plates was plated on modified semisolid Rappaport–Vassiliadis agar/MRSV (Oxoid, UK) plates and incubated at 41.5°C for 24±3 h. A loopful of media from the leading edge of white zones from the MRSV plate was streaked onto XLD agar plates (Oxoid, UK) for confirmation of *Salmonella*.

2.4 Confirmation of Listeria monocytogenes and Salmonella

Confirmation of *L. monocytogenes* and *Salmonella* began with the extraction of genomic DNA from typical bacterial colonies using the DNA extraction kit protocol from Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). The amplification was performed by targeting

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the *hly*A gene for *L. monocytogenes* and the *inv*A gene for *Salmonella*. The oligonucleotide pairs and PCR protocol for the amplification are shown in Table 1. The PCR reactions were conducted using a reactant mixture consisting of DreamTaq Hot Start Green PCR Master Mix (Thermo Fisher Scientific, USA), *hly*A and *Inv*A gene primers (forward and reverse), DNA samples, also DNA amplification using a thermal cycler (Biorad, USA). The gel electrophoresis of PCR products (voltage 70 V, current 35 mA) was performed using 1.5% agarose gel and TBE1X buffer for 35 mins. The samples were stained with SYBR gold nucleic acid gel stain (Invitrogen, USA) and visualized with UV-transilluminator (Biorad, USA). *Listeria monocytogenes* (ATCC 19114) and *Salmonella enterica* serovar Enteritidis (ATCC 13076) were used as positive controls.

3. Results and discussion

3.1 Occurrence of Listeria monocytogenes and Salmonella in sushi

Listeria monocytogenes and *Salmonella* were confirmed in 14.2% (17/120) and 2.5% (3/120) of the sushi samples, respectively (Table 2). The highest prevalence of *L. monocytogenes* was found in sushi sold in supermarkets (40%), followed by those sold at kiosks (17.7%) and restaurants (12.3%). Meanwhile, *Salmonella* was found only in sushi sold in restaurants and absent in sushi from supermarkets and kiosks.

The isolation results showed that the number of suspect *L. monocytogenes* on the PALCAM was higher than those on CHROMAgar *Listeria*. However, all 17 isolates confirmed as *L. monocytogenes* were obtained from CHROMAgar *Listeria*. This result aligns with El Marrakchi *et al.* (2005), who showed that CHROMAgar *Listeria* was more selective than the PALCAM. This was evidenced by the higher percentage of confirmed *L. monocytogenes*, i.e., 87.5% than in PALCAM of 3.8%. In addition, Jamali *et al.* (2013) stated that CHROMAgar *Listeria* had higher sensitivity and specificity than PALCAM. They found that 12.4% of ready-to-eat food samples were confirmed to have *L. monocytogenes* as compared to 8.8% on PALCAM.

CHROMAgar *Listeria* is a chromogenic selective agar medium capable of distinguishing *L.*

Table 1. The nucleotide sequences of primers and protocol used in PCR analysis.

Gene	Primer	Sequence (5'-3')	PCR Product	Protocol	References
InvA	*InvA*-F	GTGAAATTATCGCCACGTTCGGGCAA	284 bp	2 mins in 95°C with 35 amplification cycles (15 s in 95°C, 30 s in 60°C, 1.5 mins in 72°C), and 10 mins in 72°C	Rahn *et al.* (1992)
	InvA-R	TCATCGCACCGTCAAAGGAACC			
hlyA	*hlyA*-F	GCAGTTGCAAGCGCTTGGAGTGAA	456 bp	1 min in 95°C, with 35 amplification cycles (30 s in 95°C, 30 s in 64°C, 30 s in 72°C), and 4 mins in 72°C	Paziak-Domanèska *et al.* (1999)
	hlyA-R	GCAACGTATCCTCCAGAGTGATCG			

Table 2. Occurrence of *L. monocytogenes* and *Salmonella* in sushi samples from restaurants, kiosks, and supermarkets in Greater Jakarta area.

Sushi Sample	Sushi Outlets	Number of samples	Number of samples with suspect pathogen on		The number of samples (%) with confirmed pathogen based on the *hlyA* gene from:	
L. monocytogenes			PALCAM	CHROMagar™ *Listeria*	PALCAM	CHROMagar™ *Listeria*
Nigiri	Restaurant	46	11	10	5/46 (10.9)	6/46 (13)
	Kiosk	8	1	2	1/8 (12.5)	1/8 (12.5)
	Supermarket	3	2	3	2/3 (66.7)	2/3 (66.7)
Maki	Restaurant	52	13	12	5/52 (9.6)	6/52 (11.5)
	Kiosk	9	5	4	1/9 (11.1)	2/9 (22.2)
	Supermarket	2	0	0	0/2 (0)	0/2 (0)
	Total	120	31	30	14/120 (11.7)	17/120 (14.2)
Salmonella			XLD Agar		Number of samples (%) with confirmed pathogen based on *invA* gene	
Nigiri	Restaurant	46	5		1/46 (2.2)	
	Kiosk	8	2		0/8 (0)	
	Supermarket	3	1		0/3 (0)	
Maki	Restaurant	52	4		2/52 (3.8)	
	Kiosk	9	2		0/9 (0)	
	Supermarket	2	0		0/2 (0)	
	Total	120	14		3/120 (2.5)	

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monocytogenes from other *Listeria* spp. The phosphatidylinositol-specific phospholipase C (PIPL-C) enzyme activity is produced only by *L. monocytogenes* and *L. ivanovii*. Therefore, this medium is more specific for the isolation of pathogenic *Listeria*. Other studies reported that CHROMAgar *Listeria* was more selective and sensitive to isolating *L. monocytogenes* specifically in food (Hedge *et al.,* 2007; Ritter *et al.,* 2009; Al-Wasify *et al.,* 2011). Conversely, PALCAM is only known to distinguish *Listeria* spp. from other bacteria through the hydrolysis of esculin using esculinase; hence, it cannot distinguish *L. monocytogenes* colonies from other *Listeria*. Overall, 17 of the 120 samples were confirmed to contain *L. monocytogenes* based on the *hly*A gene. This gene encodes the listeriolysin O (LLO) protein through its hemolytic activity, a virulence factor of *L. monocytogenes*. This gene was reported to be possessed by all clinical isolates of *L. monocytogenes*, and relevant for identifying *L. monocytogenes* (Groves and Welshimer, 1977; Golsteyn Thomas *et al.,* 1991).

In this study, 14 of the 120 samples were suspected of *Salmonella* on XLDA. Isolation of *Salmonella* in the fishery product using XLDA showed the highest efficiency (13.8%) as compared to Hektoen Enteric (10.2%), Bismuth Sulfite (6.8%) and Brilliant Green (3.2%) (Kumar *et al.,* 2010). Gwida and AL-Ashmawy (2014) reported that 21 and 12% of suspected XLDA isolates were confirmed on *Salmonella* based on PCR and biochemical methods, respectively. Only three of the 14 suspect *Salmonella* on XLDA were confirmed as *Salmonella*, based on the possession of the *inv*A gene. The *inv*A gene is a conserved gene belonging to the genus *Salmonella*. This gene has been known and widely applied to detect *Salmonella*. The gene regulates bacterial invasion to penetrate the intestinal epithelial tissue (Galan and Curtiss 1991).

The prevalence of *L. monocytogenes* in sushi (14.2%) in this study is higher than those reported by other studies. The prevalence of *L. monocytogenes* in sushi reported in several studies ranges from 2-13%. There were 2.4% in Germany (Atanassova *et al.,* 2008) and 2% in the United Kingdom (Meldrum *et al.,* 2010). Furthermore, *L. monocytogenes* was found in 12.1% of minced tuna and 7.7% of fish roe (Miya *et al.,* 2010). In Australia, the isolation rate of *L. monocytogenes* was 2.9% and 3.2% in sushi samples taken during winter and summer, respectively (NSW Food Authority, 2008). Cross-contamination of *L. monocytogenes* might originate from the ingredients and food handlers during sushi preparations (Yap *et al.,* 2019). The study revealed that food handlers used the same napkin to wipe their hands during sushi preparation and after washing their hands. This resulted in *L. monocytogenes* cross-contamination from fish to the hands.

The prevalence of *Salmonella* in sushi products in this study was 2.5%. The isolation rate is also higher than Atanassova *et al*. (2008), who reported an isolation rate of 0.8% (1/125). On the contrary, it has a lower isolation rate than sushi samples in Malaysia. i.e. 12.8% (19/149) (Puah *et al.,* 2017). Many studies, however, reported the absence of *Salmonella* in sushi (NSW Food Authority, 2008; Muscolino *et al.,* 2014; FEHD, 2015; Liang *et al.,* 2016; Batista *et al.,* 2017).

The occurrence of *Salmonella* in ready-to-eat food sushi varies depending on the type of food (Liang *et al.,* 2016). The difference in the sanitary conditions at the facility for handling, storage, and refrigerator for displaying sushi may also contribute to *Salmonella* contamination (Seow *et al.,* 2012). Contamination of *Salmonella* might be due to the lack of hygiene practices and cross-contamination between sushi ingredients during its preparation. Barralet *et al.* (2004) reported the contamination of *Salmonella* Singapore in sushi products sold at retail in Queensland, Australia. The cross-contamination during sushi preparation and the temperature of sushi products when placed in a display refrigerator, might have been the cause of the above outbreak.

This study also showed that two sushi samples from restaurants were contaminated with *L. monocytogenes* and *Salmonella*. Contamination of *L. monocytogenes* and *Salmonella* can occur throughout the processing process, both from the environment, processing equipment, food serving, and cross-contamination of the sushi ingredients. The possibility of contamination during the processing must be a significant concern. Hereafter, understanding the flow of the sushi processing is crucial to prevent pathogen contamination of the final product. This includes the ingredients of sushi products, processing equipment, and environment, up to the food handlers involved in the processing (Hansen *et al.,* 2006; Hu *et al.,* 2006; Chen *et al.,* 2010).

3.2 Prevalence of Listeria monocytogenes and Salmonella by type of sushi outlets

This study reported the first-ever of *L. monocytogenes* and *Salmonella* prevalence in sushi sold in Indonesia. Sushi from East Jakarta had the highest prevalence for *L. monocytogenes,* where four samples were from restaurants, and one sample was from supermarkets. The highest prevalence of *Salmonella* was found in sushi from the Bekasi area, and all positive samples came from restaurants (Table 3). Contamination of *L. monocytogenes* and *Salmonella* was not found in the sushi samples sold in Central Jakarta and Depok.

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The sushi outlets in this study are categorized into three types, i.e., restaurants, kiosks, and supermarkets. Sushi restaurants generally have a kitchen separated from the serving area. Meanwhile, sushi kiosks usually have a sushi processing area equipped with a table, a refrigerator and a freezer to store sushi ingredients. The serving area at the kiosk is usually not separated from the processing area; accordingly, customers could watch the food handlers preparing sushi. Sushi is generally made in the kitchen and displayed in a refrigerated showcase in supermarkets.

The highest isolation frequency of *L. monocytogenes* was found in sushi samples sold in supermarkets, while the highest isolation frequency of *Salmonella* was found in sushi sold in restaurants. In the supermarkets, sushi was placed in the refrigerated showcase with temperatures ranging between 3-8°C and replaced every 4 hrs. *L. monocytogenes* can grow at refrigeration temperatures. Eicher *et al.* (2020) reported the number of *L. monocytogenes* in sushi made with salmon increased by 1 log cycle and 1.7 log cycle after three days at 5 and 8°C, respectively. Since supermarkets generally sell sushi and other foods, high cross-contamination of *L. monocytogenes* might arise. Cross-contamination between other food ingredients, equipment, or food contact with the surface is suspected to be a source of *L. monocytogenes* contamination. Miguéis *et al.* (2016) stated *L. monocytogenes* contaminated 20.2% (23/114) of sashimi samples sold in non-Japanese restaurants in Portugal.

There were no detailed observations of the environmental conditions in the sushi outlet or equipment reported in this study. However, multiple studies have found *L. monocytogenes* and *Salmonella* contaminated sushi products. The contaminations are related to sanitation, equipment during processing, hygiene during preparation, and cross-contamination along production (Wu *et al.,* 2015; Hoel *et al.,* 2017; Madden *et al.,* 2018).

Sushi production areas in restaurants, supermarkets, and kiosks are the most likely places for *L. monocytogenes* to be found (Tompkin, 2002; Kovačević *et al.,* 2012). Contamination generally comes from equipment and surfaces that contact with sushi ingredients, such as cutting boards and sushi rollers (Holah *et al.,* 2004; Barros *et al.,* 2007). In addition, sushi processing environments such as floors and drains can also be a source of contamination (Leong *et al.,* 2014; Rückerl *et al.,* 2014). Leong *et al.* (2017) and Madden *et al.* (2018) reported that 3.8% (n = 4667) and 6.3% (n = 1203) samples from processing environments were positive for *L. monocytogenes* contamination. The presence of *L. monocytogenes* contamination in equipment and the environment is possibly caused by inadequate sanitation processes. Moreover, it is supported by their ability to adhere to the equipment and environment surfaces by forming biofilms (Borucki *et al.,* 2003; Barros *et al.,* 2007; Yan *et al.,* 2010).

The highest occurrence of *Salmonella* was found in sushi sold in restaurants (3.1%). *Salmonella* contamination in sushi is likely related to the handling and processing procedures. The pathogen may also exist in raw materials such as fish. Raw fish is an appropriate substrate for bacterial growth and can be contaminated during processing such as filleting and slicing. In this study, contamination of *Salmonella* was detected in sushi made with salmon and tuna, at the rate of 2.9 and 8.7%, respectively (Table 4). *Salmonella* contamination could also come from the environment, processing equipment, serving practices, and the water used for processing.

Several research results on the prevalence of *Salmonella* in fishery products have been reported. USFDA reported that during the period 1990-1998, *Salmonella* contamination was found in fishery products both imported and domestic markets by 6.9% (833/12,080) (Heinitz *et al.,* 2000). Kumar *et al.* (2010) also reported that the prevalence of *Salmonella* of 24.8% (108/436) was found in fishery products originating from fish landing sites and domestic markets in India.

Table 3. Prevalence of *L. monocytogenes* and *Salmonella* in sushi samples from various areas.

Location	Number of samples	The number of samples (%) with confirmed:	
		L. monocytogenes	*Salmonella*
East Jakarta	10	5 (50)	0 (0)
West Jakarta	13	2 (15.4)	0 (0)
North Jakarta	13	1(7.7)	0 (0)
South Jakarta	30	2 (6.7)	0 (0)
Central Jakarta	14	0 (0)	0 (0)
Tangerang	15	5 (33.3)	1 (6.7)
Bekasi	8	1 (12.5)	2 (25)
Bogor	9	1 (11.1)	0 (0)
Depok	8	0 (0)	0 (0)
Total	120	17 (14.2)	3 (2.5)

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Furthermore, 14.6% (210/1,440) of ready-to-eat shrimp products originating from the domestic market in Nigeria have confirmed *Salmonella* (Beshiru *et al.,* 2019).

The poor knowledge and lack of good hygiene practices of food handlers are also sources of contamination for *L. monocytogenes* and *Salmonella*. This was proved by Yap *et al.* (2019), finding that contamination of *L. monocytogenes* in the fish to the final product occurs through the hand of waiters. The waiters always use the same napkin to wipe their hands during sushi processing, even after washing their hands.

Sushi is a ready-to-eat food that does not undergo processing and has a short shelf life. Subsequently, the application of low temperatures for both raw materials and final products must be conducted to prevent bacterial growth. However, this temperature provides opportunities for the growth of *L. monocytogenes* in sushi products. Liu *et al.* (2016) reported that the number of *L. monocytogenes* increased by 3-4 log cycles in raw tuna stored at 5-7°C for 14 days. As a psychrotrophic bacteria, *L. monocytogenes* can grow and multiply at refrigeration temperatures. Lorentzen *et al.* (2012) reported an increase in the number of bacteria in nigiri sushi stored at 4°C after one day of storage. Low temperatures are known to inhibit the growth of *Salmonella* in ready-to-eat food products such as sushi. The level of *Salmonella enterica* ser. Weltevreden decreased by 1.63 log cycle and 2.22 log cycle, respectively after being stored at 5-7°C for 14 days (Liu *et al.,* 2016).

3.3 Prevalence of Listeria monocytogenes and Salmonella by types of fish

The sushi samples consisted of 57 nigiri and 63 maki samples of salmon or tuna. The prevalence of *L. monocytogenes* in the nigiri was higher than that in maki, but *Salmonella* was more frequently encountered in maki than nigiri (Table 4). As for the types of fish, sushi made from tuna was more likely to contain *L. monocytogenes* and *Salmonella* than salmon. The tuna used for sushi products in Indonesia generally is the catch of domestic fishermen which is pooled by suppliers. The suppliers then trade the tuna as frozen tuna cuts to restaurants, supermarkets, or kiosks. Pathogenic bacteria from the natural environment, equipment, workers, and cross-contamination can contaminate tuna during capture, landing, pooling, distribution, processing, and sushi preparation.

Contamination of *Salmonella enterica* ser. Paratyphi B (dT+) and *S. enterica* ser. Weltevreden in frozen tuna imported from Indonesia used as raw material for sushi has caused salmonellosis outbreaks in the USA (Hassan *et al.,* 2018). The source of this *Salmonella* contamination probably comes from unsanitary and unhygienic practices during the phases of fishing, handling of fish on land, and processing. Contaminated water, carrier or unhealthy food handlers, cross-contamination from processing equipment, or animals present in the environment during handling and processing have potential as sources of contamination. Another report mentioned that tuna imported from India for sushi has caused an outbreak in the USA (CDC, 2012). The results of an FDA investigation at the frozen tuna processing facility owned by the producer stated that the lack of HACCP control in the processing unit, such as control over storage temperatures and poor sanitation practices, was the cause of contamination in the frozen tuna products.

Sushi made with salmon was also found to contain *L. monocytogenes* and *Salmonella*. Salmon sold in Indonesia is generally an imported product from Norway. Medrala *et al.* (2003) reported that 4.3-15.4% of exported salmon from Norway contains *L. monocytogenes*. This bacterial contamination originates from the salmon farming environment via transmission from land to the cultivation area. Vogel *et al.* (2001) conducted a study on *L. monocytogenes* contamination in smoked salmon processing units and reported the possibility of contamination from salmon used as raw material. The results also indicated that the *L. monocytogenes* strain detected in the final product was also found in the raw material, which may be a source of contamination. Therefore, fish raw materials used for sushi processing may contain *L. monocytogenes* and *Salmonella* contamination. The initial quality of raw materials for sushi processing is critical to producing safe sushi. Moreover, hygiene factors, storage temperatures, and good processing practices must be optimally applied (Atanassova *et al.,* 2008; Hoel *et al.,* 2015).

Table 4. Prevalence of *L. monocytogenes* and *Salmonella* in sushi samples based on type of fish.

Fish Type	Sushi Type	Number of samples	The number of samples (%) with confirmed:	
			L. monocytogenes	*Salmonella*
Salmon	Nigiri	34	4 (11.8)	1 (2.9)
	Maki	41	4 (9.8)	0 (0)
Tuna	Nigiri	22	5 (22,7)	0 (0)
	Maki	23	4 (17.4)	2 (8.7)
Total		120	17 (14.2)	3 (2.5)

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3.4 Level of Listeria monocytogenes and Salmonella contamination in sushi

The level of *L. monocytogenes* contamination in the sushi samples in this study ranged from 3-1100 MPN/g (Table 5), with an average of 160.8 MPN/g. Three of the 17 sushi samples contained more than 100 MPN/g *L. monocytogenes*, of which two samples were nigiri and one sample was maki sushi. Sushi samples with *L. monocytogenes* contamination levels of more than 100 MPN/g were sold in South Jakarta, West Jakarta, and North Jakarta restaurants. Miya *et al.* (2010) reported *L. monocytogenes* in sushi ingredients such as minced tuna and salmon roe sold at retail in Japan, with contamination levels ranging from 0.3 to 15 MPN/g.

The contamination levels of *Salmonella* in sushi samples were in the range of 3.6-11 MPN/g, with an average value of 8.5 MPN/g. Yang *et al.* (2015) reported the contamination level of *Salmonella* in raw fish products in retail markets in China, where 19.4% (6/31) were contaminated at a level of 10 MPN/g, and one sample was contaminated at a level of >110 MPN/g. The sample with higher *Salmonella* content in this study was sushi made from tuna. Substandard handling of tuna from the time the fish is caught to the final product might cause contamination of tuna from the domestic market.

The Codex Alimentarius Commission CAC/GL 61-2007 recommends the contamination level of *L. monocytogenes* in ready-to-eat food. There are two microbiological criteria in this regulation: absence/25 g for ready-to-eat food that can support the growth of *L. monocytogenes* and no control of bacterial growth before consumption. The second one is 100 CFU/g for ready-to-eat food that can support the growth of *L. monocytogenes* during the shelf life or in foods that do not support the growth of *L. monocytogenes* (CAC, 2007). Countries in the European Union, Australia, New Zealand, and the United States of America enforce these requirements (FDA, 2008; FSANZ, 2014). Meanwhile, the requirement for *Salmonella* contamination in sushi products is negative within 25 g (FEHD, 2001). In Indonesia, the microbiological criteria for *L. monocytogenes* and *Salmonella* in sushi have not been established.

Listeria monocytogenes contamination has caused foodborne illnesses. Listeriosis case has a serious severity, much higher in susceptible populations including pregnant women, infants, the elderly and immunocompromised people. In 2020, the European Food Safety Authority (EFSA) and European Centre For Disease Prevention and Control (ECDC) reported that listeriosis occurring in 27 Member States (MS) in the EU with 0.42 notification cases per 100,000 population, a decrease of 7.1% as compared to the rate in 2019 and it is a serious foodborne disease under EU supervision (EFSA/ECDC, 2021). *L. monocytogenes* was the causative agent of sixteen foodborne outbreaks at the EU level, involving 120 cases of illness, 83 hospitalizations and 17 deaths in 2020. The cases most commonly occurred in the age group over 64 particularly in the age group over 84 years. The most common implicated food vehicles for the strong evidence associated with listeriosis foodborne outbreaks in 2020 were fish and fishery products.

Table 5. Level of contamination of *L. monocytogenes* and *Salmonella* in sushi samples.

No	Code of sample	Type and outlet	Pathogen confirmed	Level of contamination (MPN/g)
1	JTAS	Nigiri, Supermarket	*L. monocytogenes*	28
2	JTWS	Nigiri, Restaurant	*L. monocytogenes*	15
3	JTIS	Nigiri, Restaurant	*L. monocytogenes*	93
4	JTTS	Maki, Restaurant	*L. monocytogenes*	23
5	JTSK	Nigiri, Restaurant	*L. monocytogenes*	3.6
6	JBSK	Maki, Restaurant	*L. monocytogenes*	3
7	JBZS	Nigiri, Restaurant	*L. monocytogenes*	1100
8	JSSY	Nigiri, Restaurant	*L. monocytogenes*	1100
9	JSSM	Nigiri, Restaurant	*L. monocytogenes*	35
10	JUWS	Maki, Restaurant	*L. monocytogenes*	240
11	BOHS	Maki, Kiosk	*L. monocytogenes*	3.6
12	TOS1	Maki, Restaurant	*L. monocytogenes*	7.4
13	TOS2	Maki, Restaurant	*L. monocytogenes*	3.6
14	TRSB	Nigiri, Kiosk	*L. monocytogenes*	3.6
15	TTS	Maki, Restaurant	*L. monocytogenes*	9.2
16	TSLH	Nigiri, Supermarket	*L. monocytogenes*	43
17	BKST	Maki, Restaurant	*L. monocytogenes*	23
18	BKST	Maki, Restaurant	*Salmonella*	11
19	BKSI	Nigiri, Restaurant	*Salmonella*	11
20	TTS	Maki, Restaurant	*Salmonella*	3.6

https://doi.org/10.26656/fr.2017.8(2).421

Meanwhile, 21 cases of listeriosis that caused outbreaks occurred in the Netherlands and Belgium throughout 2017 (1 case), 2018 (8 cases), and 2019 (12 cases) (EFSA/ECDC, 2019). This listeriosis case caused 2 pregnant women and 17 patients ranging from 64 to 94 years to be hospitalized. This case caused 3 patients to die and 1 pregnant woman to miscarry in the Netherlands. In Belgium, 1 patient was reported, aged 97 -year-old woman and a perinatal case. This case occurred after consuming RTE meat products produced by an industry in the Netherlands and all these RTE meat products were recalled.

4. Conclusion

Some of the sushi products sold in restaurants, kiosks, and supermarkets in Greater Jakarta area in Indonesia are contaminated by *L. monocytogenes* and *Salmonella*. The prevalence and level of *L. monocytogenes* in sushi were higher than those of *Salmonella*. Presence of the pathogens in sushi suggests potential risks to consumers. A more study to determine the source of contamination along the processing of sushi sold at retail will be conducted to reduce the probability of pathogen contamination in sushi products.

Conflict of interest

The authors declare no conflict of interest.

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