



Prevalence of *Listeria* spp. in Fresh Shrimp from Local Markets and Its Susceptibility to Antibiotics

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ABSTRACT

This study aimed to determine the prevalence of *Listeria* spp. in fresh shrimp in Bogor, Indonesia, and to determine the antibiotic susceptibility patterns of isolated *Listeria* spp. A quantitative descriptive approach was employed. A total of 25 samples of fresh shrimp were obtained from randomly selected traditional markets (n = 18) and modern markets (n = 7). The results revealed that 14 samples (56.0%) were positive for *Listeria* spp. Among them, 11 (78.6%) and 3 (21.4%) were selected from traditional markets and modern markets, respectively. Thirty-six isolates, which upon biochemical characterization included 1 (2.8%) *Listeria innocua*, 26 (72.2%) *Listeria grayi*, 1 (2.8%) *Listeria welshimeri*, and 8 (22.2%) *Listeria*-like strains, were identified. The most common isolates were *L. grayi*, while the least common were *L. innocua* and *L. welshimeri*. The disk diffusion assay determined the susceptibility of the 36 strains to 10 commercial antibiotics. In general, 100% of the 36 isolated *Listeria* spp. were resistant to amoxicillin and nebacetin, and almost 100% were susceptible to chloramphenicol. However, antibiotic multiple resistance (AMR) was observed, with all *Listeria* strains resistant to more than two antibiotics. No isolate was resistant to only one antibiotic. The present study provides the first baseline data on the prevalence of *Listeria* spp. in fresh shrimp in Indonesia and the susceptibility of these isolates to antibiotics.

Keywords: Antibiotic, Fresh Shrimps, *Listeria* Spp., Prevalence, Susceptibility.

INTRODUCTION

Listeria sp. is a bacteria having commonly contaminates a variety of food (Hagens & Loessner, 2007; Hagens & Offerhaus, 2008; Kazi & Annapure, 2016; Sillankorva et al., 2012). According to Hitchins and Jinneman (2003), the *Listeria* genus has six species, and there are *Listeria monocytogenes*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria ivanovii* and *Listeria grayi*. Based on an epidemiology study, only just *L. monocytogenes* became pathogenic to humans. However, there are reports that other *Listeria* species can seriously infect humans, there are *L. seeligeri* (Rezai et al., 2018), *L. innocua* (Perrin et al., 2003), and *L. Ivanovii* (Guillet et

al., 2010; Snapir et al., 2006). Listeriosis is a disease caused by *L. monocytogenes*. This *Listeria* is an opportunistic pathogen, affecting mainly the very young and old, immune-compromised patients as well as pregnant women (Hagens & Loessner, 2010). However, it is characterized by a high mortality rate, up to 30% (Aguilera et al., 2022), in Canada, expected to be 20 – 30% (Sweezey et al., 2019).

While in the United States of America, the mortality rate caused by listeriosis is expected to be 16% (Costley Jessie, 2019). Food-borne listeriosis that is caused by *L. monocytogenes* is often associated with fresh food with minimal process, such as milk products or salad, or processed food that is stored at low temperature (coolant temperature). Given the nature of *L. monocytogenes* widely distributed in water sources as well as soil, being commonly found in the environment (Ivanek et al., 2006; Todar, 2009), resistant to treatment and can growth at low temperatures (Madden et al., 2018) made it became important food-borne pathogen. Seafood can be a vehicle for the transmission of various diseases, one of which is listeriosis caused by *L. monocytogenes*. Contamination can occur along the food chain including cultivation, harvesting, processing, handling/preparation, distribution, and marketing.

Some countries have a “zero-tolerance” policy for *Listeria monocytogenes* in ready-to-eat seafood from national and foreign producers. The fish industry in Indonesia supplies both local consumption and export markets. The majority of Indonesian fishery products are exported to several countries such as the United States, Europe, Japan, and some other countries. Indonesian exports of fishery products had refused by the USFDA because contaminated with *Listeria* in 2012 and 2013 about 0.6% and 11.8%, respectively (Wen et al., 2018). Based on those data, Indonesian exports of fishery products that are contaminated with *Listeria* are frozen shrimp and frozen shrimp cooked, which are 8.7% and 91.3%, respectively.

Fishery products especially fresh shrimp local consumption marketed in Indonesia are typically sold at two types of markets i.e. modern markets and traditional markets. The modern markets, which are in-door markets, often display fresh products under refrigeration. In contrast, traditional markets usually display in the open air at ambient temperatures. Since there is not much information regarding *Listeria* species in fresh shrimp in Indonesia, this study was carried out to determine the prevalence of *Listeria* spp. in fresh shrimp and susceptibility to antibiotics of *Listeria* strains isolated from fresh shrimp collected from modern markets and traditional markets in Bogor, Indonesia.

The study aims to determine the prevalence of *Listeria* species in fresh shrimp marketed in Indonesia, specifically focusing on Bogor. It seeks to compare the prevalence of *Listeria* spp. in fresh shrimp sold in modern markets, which typically display products under refrigeration, versus traditional markets, where products are displayed at ambient temperatures. Additionally, the study aims to assess the antibiotic susceptibility of *Listeria* strains isolated from fresh shrimp collected from both types of markets in Bogor, Indonesia.

RESEARCH METHODS

This research employed a quantitative descriptive approach to evaluate the presence of *Listeria* bacteria in shrimp from traditional markets (n = 18) and modern markets (n = 7) in Bogor, Indonesia. Shrimp samples were randomly collected, placed in sterile bags, and transported to the laboratory in a cool box with ice to maintain a temperature of 4°C until analysis. The USDA method described by Hitchins and Jinneman (2003) was used to detect *Listeria*. Briefly, 25 g of each sample was added to 225 ml of Buffer *Listeria* Enrichment Broth (BLEB) in a plastic bag, homogenized at room temperature, and then incubated for 24 hours at 30°C. After incubation, 100 µL of the BLEB was plated onto the surface of Palcam Agar (Oxoid) and incubated at 37°C for 24-48 hours. Five suspected *Listeria* colonies on Palcam agar were isolated and grown in Tryptone Soya Broth containing 0.5% yeast extract (TSBYE) at 37°C overnight. The cultures were then streaked on Tryptone Soya Agar containing 0.5% yeast extract (TSAYE) for purification and confirmation of *Listeria*.

The techniques recommended by Hitchins and Jinneman (2003) were followed to confirm and identify *Listeria* species. Colonies suspected to be *Listeria* on Palcam agar exhibited bluish-gray colonies and produced a black zone of esculin hydrolysis. These putative *Listeria* colonies were characterized using Gram staining, motility, and catalase tests, hemolysis characteristics on blood agar, and carbohydrate utilization. *Listeria* spp. were identified as Gram-positive bacilli or *coccobacilli*, catalase-positive, and showing motility at 28°C as umbrella-like growth on SIM agar (Oxoid). Hemolytic-positive colonies were identified as *Listeria monocytogenes*, *L. ivanovii*, and *L. seeligeri*, while *L. innocua*, *L. grayi*, and *L. welshimeri* were hemolytic negative. For the carbohydrate utilization test, isolated colonies from TSAYE were transferred into test tubes containing 5 mL of Purple Carbohydrate Base (Oxoid) with 0.5% D-glucose, maltose, mannitol, xylose, and rhamnose, and incubated at 37°C for 7 days. Positive reactions were indicated by a yellow color (acid fermentation), mostly occurring within 24 to 48 hours.

Antibiotic susceptibility patterns of all confirmed *Listeria* isolates were determined using the standard disc method. The Clinical Laboratory Standards Institute method (Afhami et al., 2020) was employed for antibiotic susceptibility testing with Mueller Hinton Agar (MHA). Colonies of *Listeria* spp. from TSAYE (Oxoid) were transferred to 10 mL of saline water to achieve turbidity equivalent to 0.5 McFarland standard (approximately 108 CFU mL⁻¹) and inoculated onto the entire surface of dried MHA (Oxoid) plates using a sterile cotton swab. Antibiotic susceptibility discs purchased from Oxoid were placed on the surface of MHA plates and incubated at 37°C for 24 hours. The diameter (in mm) of the zone around each disc was measured and interpreted according to CLSI guidelines (2020) and Hardy Diagnostics (2019) to classify the antibiotic sensitivity of each isolate.

RESULTS AND DISCUSSION

Out of the 25 samples of fresh shrimp, 56.0 % (14/25) were positive and 44.0 % (11/25) were negative for *Listeria* species (Table 1). When comparing samples taken from traditional markets and modern markets, 78.6 % of positive samples for *Listeria* (11/14) originated from traditional markets, and 21.4 % (3/14) samples from modern markets. Positive samples based on the kind of market shown are 61.1 and 42.9 % for the traditional market and modern market, respectively. Thirty-six bacterial strains isolated from 14 of 25 (56.0 %) fresh shrimp samples were identified, which, upon biochemical characterization, as members of the genus *Listeria*, as shown in Table 2. Fresh shrimp samples examined harbored 4 *Listeria* species: a single isolate of *L. innocua* (2.8%), 26 isolates of *L. grayi* (72.2 %), a single isolate of *L. welshimeri* (2.8 %), and 8 isolates of *Listeria*-like strain (22.2%). The most common isolates were *L. grayi* while the least isolates were *L. innocua* and *L. welshimeri*. However, *L. monocytogenes* was not found in all of the fresh shrimp samples was observed.

Table 3 shows the number of isolates for each category (with percentage) of antibiotics tested. In General, one hundred percent of 36 isolated *Listeria* spp. were resistant to amoxicillin and nebacetin. More than 80% were resistant to ampicillin, erythromycin, and kanamicin precisely is 86.1, 91.7, and 83.3%, respectively. All strains of *Listeria* spp. none resistant to chloramphenicol but otherwise, 94.4% are susceptible and 5.6% are intermediate. However, antibiotic multiple resistance (AMR) was observed, all of the *Listeria* strains were resistant to more than two antibiotics. In addition, no isolate was seen as resistant to only one antibiotic.

Table 1. Detection of *Listeria* spp. in the samples of fresh shrimps

Detection of <i>Listeria</i>	No. of samples (% of samples)								
	Based on total samples			Based on positive samples			Based on kind of market		
	TM	MM	Total	TM	MM	Total	TM	MM	Total
Positive	11 (44/0)	3 (12.0)	14 (56.0)	11 (78.6)	3 (21.4)	14 (100.0)	11 (61.1)	3 (42.9)	–
Negative	7 (28.0)	4 (16.0)	11 (44.0)	–	–	–	7 (38.9)	4 (57.1)	–
No. of samples	18 (72.0)	7 (28.0)	25 (100.0)	11 (78.6)	3 (21.4)	14 (100.0)	18 (100.0)	7 (100.0)	–

TM = Traditional market; MM = Modern market

Table 2. Prevalence of *Listeria* spp. in the samples of fresh shrimps

Sample nomor	Origin	<i>Listeria</i>	No. of isolates (% of samples contaminated)			
			<i>L. innocua</i>	<i>L. grayi</i>	<i>L. welshimeri</i>	<i>Listeria</i> -like strain
1	TM	+	1 (2.8)	1 (2.8)	-	-
2	TM	+	-	1 (2.8)	-	1 (2.8)
3	TM	+	-	3 (8.3)	-	-

4	TM	-	-	-	-	-
5	TM	+	-	2 (5.6)	-	-
6	TM	+	-	-	-	1 (2.8)
7	MM	-	-	-	-	-
8	MM	+	-	5 (13.9)	-	-
9	TM	-	-	-	-	-
10	TM	-	-	-	-	-
11	TM	+	-	1 (2.8)	-	-
12	TM	+	-	4 (11.11)	-	-
13	TM	+	-	2 (5.6)	1 (2.8)	1 (2.8)
14	TM	+	-	4 (11.11)	-	1 (2.8)
15	TM	-	-	-	-	-
16	TM	-	-	-	-	-
17	TM	+	-	-	-	1 (2.8)
18	TM	-	-	-	-	-
19	MM	+	-	2 (5.6)	-	2 (5.6)
20	MM	+	-	-	-	1 (2.8)
21	MM	-	-	-	-	-
22	MM	-	-	-	-	-
23	MM	-	-	-	-	-
24	TM	-	-	-	-	-
25	TM	+	-	1 (2.8)	-	-
Total			1 (2.8)	26 (72.2)	1 (2.8)	8 (22.2)

TM = Traditional market; MM = Modern market; + = Positive; - = Negative

Table 3. Antibiotic Susceptibility Profiles of *Listeria* spp. Isolated from Samples of Fresh Shrimp

Anti-biotics	<i>L. innocua</i> (n = 1)			<i>L. welshimeri</i> (n = 1)			<i>L. grayi</i> (n = 26)			<i>Listeria</i> -like strain (n = 8)			Total (n=36) No. of isolates (% of samples)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amp	0	0	1	0	0	1	4	0	22	1	0	7	5 (13.9)	0 (00.0)	31 (86.1)
Amx	0	0	1	0	0	1	0	0	26	0	0	8	0 (00.0)	0 (00.0)	36 (100.0)
Chlp	1	0	0	1	0	0	25	1	0	7	1	0	34 (94.4)	2 (05.6)	0 (00.0)
Ern	0	0	1	0	0	1	0	3	23	0	0	8	0 (00.0)	3 (08.3)	33 (91.7)

Tetra	1	0	0	1	0	0	20	6	0	5	1	2	26 (72.2)	8 (22.2)	2 (05.6)
Tri-Sm	1	0	0	0	1	0	6	17	3	1	6	1	8 (22.2)	24 (66.7)	4 (11.1)
Knm	0	0	1	0	0	1	0	5	21	0	1	7	0 (00.0)	6 (16.7)	30 (83.3)
Gtm	1	0	0	0	1	0	9	15	2	3	5	0	13 (36.11)	21 (58.3)	2 (05.6)
Nbc	0	0	1	0	0	1	0	0	26	0	0	8	0 (00.0)	0 (00.0)	36 (100.0)
Cont	1	0	0	0	1	0	3	16	7	0	6	2	4 (11.1)	23 (63.9)	9 (25.0)

Amp = Ampicillin; Amx= Amoxicillin; Chlp = Chloramphenicol; Ern = Erythromycin; Tetra = Tetracycline; Tri-Sm = Trimethoprim-Sulfamethoxazole; Knm = Kanamycin; Gtm = Gentamicin; Nbc = Nebacatin/neomicin, Cont = Contrimozazole; S: Susceptible; I: Intermmediate; R: Resistan. Almost all *L. grayi* isolates have the same pattern of susceptibility to antibiotics, except for ampicillin, trimethoprim-sulfamethoxazole, gentamicin, and cotrimoxazole some isolates showed a susceptible response but otherwise are resistant. While *Listeria*-like strain isolates showed, just only one isolate had a contrast response for ampicillin and trimethoprim-sulfamethoxazole. Likewise, all of the isolates from the same sample have the same pattern of susceptibility to antibiotics, but there is one exception. Two isolates from the same sample showed different responses for ampicillin, one showed a susceptible effect and the other one showed a resistant effect.

Discussion

The general occurrence of *Listeria* spp. in fresh shrimp is limited reported in many countries. This study has shown that contamination of *Listeria* spp. Is as much as 56.0% higher than the finding of the previous research in Indonesia by Murtiningsih and Sunarya (2020), who reported a prevalence of 13.0% (n=31) on samples shrimps. While Dhanasheree *et al.* (2003) reported that only 9.1% (n=11) of fresh shrimp samples in India. Wong *et al.* (2011) reported the prevalence of the major foodborne pathogen in imported seafood from many countries in Baton Rouge, LA, 4.1 % of 38 shrimp samples were positive for *L. monocytogenes*, and 8.2% (14/38) were positive *Listeria* spp. Similar to ANZFSC (2014) which detected 3.0% of samples of cooked shrimps (n=380) positive for *L. monocytogenes*. While *L. monocytogenes* was not found in all of the fresh shrimp samples we were observed, in close agreement with the prevalence reported in Indonesia (Goh Ee et al., 2020) and India (Dhanasheree *et al.* 2003). Otherwise, none of *Listeria* spp. on shrimp and prawn frozen samples reported by Md.Rokibul *et al.* (2013).

Percentage the prevalence of *Listeria* spp. Detected in traditional markets is higher than in modern markets. Our observation was that the markets where samples were purchased is very

crowded, and fresh shrimp were sold in the open air without ice, products in direct contact with the seller's hands, and the floor around it was very dirty. Otherwise, conditions in the modern markets are cleaner than the traditional markets, and fresh shrimp are sold with ice in a cold room. Thus, we are tempted to speculate about the high rates of *Listeria* spp. Because of bad handling cross-contamination occurs.

The present results of this study suggest that fresh shrimp is potentially a source of *Listeria* contamination and infection. The microorganism, because of its ubiquity in nature, may be introduced into food-processing environments and ultimately may result in the contamination of food products. Fish products and other seafood, including shrimp, have a risk to a contaminated with *Listeria*, which may be from water sources that they are cultivated. These indigene bacteria can also contaminate products during the processing step, either by cross-contamination in industry or because these bacteria are naturally present in many food industrial environments or in human skin (Pilet & Leroi, 2011). The prevent or reduce this contamination, the consistent application of good agricultural practices is needed at every stage of food supply, from the farm to the table, and should be implemented by all individuals involved with handling and processing.

Positive samples were examined harbored 4 *Listeria* species that are *L. innocua*, *L. grayi*, *L. welsimeri*, and a *Listeria*-like strain dominated by *L. grayi*. Different to the invention of Murtiningsih and Sunarya (2020) their finding was dominated by *L. innocua* besides *L. welsimeri*, dominated by *L. innocua*. A report on the species of *Listeria* in fresh shrimp is very limited. As a reference, Chen *et al.* (2010) reported species found on catfish fillet samples: *L. monocytogenes*, *L. innocua*, *L. seligeri*, *L. welshimeri* and *L. innocua*. Jamali *et al.* (2015) reported 4 species *Listeria* isolated from fish samples in Iran there are *L. monocytogenes*, *L. innocua*, *L. seeligeri*, and *L. ivanovii*. We identified as *Listeria*-like strain because that fitted the description of the genus *Listeria* although it could not be assigned to any of the known species. It caused we identified by biochemical characterization based on Hitchin and Jinneman (2013) that the *Listeria* genus has six species. Another study found a new species of *Listeria* there are *Listeria marthii* (Graves *et al.*, 2010) and *Listeria rocourtiae* (Leclercq *et al.*, 2010).

However, The results of pattern antibiotic susceptibility are similar to the findings by Issa *et al.* (2011). Their findings indicate that isolated *L. monocytogenes* from foods were highly resistant (more than 80%) to ampicillin, rifampicin, and tetracycline. However, in contrast to tetracycline, our findings regarding test antibiotics to 36 isolated *Listeria* spp. against tetracycline were obtained to be 72.2% susceptible. Our finding is similar to Troxler *et al.* (2000) in that they were reported to effect susceptible or intermediate to 93 strains of *Listeria* isolated from various sources against tetracycline. Chloramphenicol and streptomycin have the same effect by Issa (2011), and our findings demonstrate no resistance, but in contrast to erythromycin, which Issa (2011) found none resistant while our findings are resistant. Troxler *et al.* (2000) obtained the same result for chloramphenicol, who reported sensitivity or immediate to 93 strains of *Listeria*

isolated from various sources. For trimethoprim and co-trimoxazole, our findings are intermediate or resistant to all *Listeria* spp. Isolates, including *L. grayi*, are in agreement with Toxler *et al.* (2000).

We observed all of the *Listeria* strains are antibiotic multiple resistance (AMR). Antibiotic multiple resistance incidents on *Listeria* spp. were also reported by Walsh *et al.* (2001), Shen *et al.* (2006), and Jamali *et al.* (2015). According to Allen *et al.* (2016), in particular, food chain factors may influence AMR through i) horizontal exchange of AMR genes, ii) induction of AMR-related stress responses through sub-lethal exposure to factors controlling microbial growth in food, and iii) recurring exposure to disinfectants in the food processing environment.

CONCLUSION

In conclusion, we have demonstrated in this study that the fresh shrimps we collected were positive for *Listeria* spp. contamination both to traditional markets and modern markets. Species found in samples of fresh shrimp are *L. innocua*, *L. grayi*, *L. welshimeri*, and *Listeria*-like strains, which are dominated by *L. grayi*. Of all the samples, *L. monocytogenes*. The result of the susceptibility of antibiotics demonstrated that isolated *Listeria* spp. from fresh shrimp samples are resistant to many antibiotics, there are ampicillin, amoxicillin, erythromycin, kanamycin, and nebacetin. Isolated *Listeria* spp. were susceptible to chloramphenicol, streptomycin, and tetracycline. All of *Listeria* spp. are AMR, which have more than 3 resistant to antibiotics. The present study provides the first baseline data on the prevalence of *Listeria* spp. in fresh shrimp in Indonesia and the susceptibility of these isolates to antibiotics. The data may be useful for all people concerned with the antibiotic susceptibility of *Listeria* spp.

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