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Abstract. The consumption of seafood and seafood products contaminated with *Salmonella* sp. can lead to illness, thus this has become a worldwide public health concern. The aim of this research was to detect *Salmonella* sp. in various seafood and seafood products from Surabaya traditional market. The study was conducted through a sample collection, followed by the pre-enrichment, enrichment, isolation, and the identification of *Salmonella*. A total of twenty-nine samples (fish, crustacean, bivalve, and seafood products) from five local markets in Surabaya were collected and analyzed. Twenty-seven (93.10%) samples were positive for *Salmonella* sp. All of the isolates were tested for an antibiotic-resistant profile by employing disc diffusion targeting seven antibiotics. A high prevalence of *Salmonella* sp. in the seafood in the traditional markets was apparent in this study. Among all of the isolates, there were eight strains resistant to at least one antibiotic that was tested. This study can be a basis for further research into managing antibiotic-resistant *Salmonella* sp. infection as an important food-borne illness of developing countries.

1. Introduction

Salmonella sp. is normally found in humans and animal digestive tracts. However, it is also widespread in the environment, can be consumed by humans and can cause illness [1]. The bacterium can reach the human gastrointestinal tract through the consumption of contaminated foods such as eggs, milk, poultry, meat, aquaculture products and seafood [2]. *Salmonella* naturally cannot be found in seafood. However, the contamination of it during handling and processing can be a serious problem for public health [3].

In recent years, many articles have reported the occurrence of *Salmonella* in fish products. Raufu et al. [2] said that 23 (11.5%) out of 200 fish samples were *Salmonella* positive in sub-Saharan Africa. In another study, Zhang et al. [3] reported that 217 (29.7%) out of 730 fishery products were *Salmonella* positive. On the other hand, many reports were focused on the occurrence of *Salmonella* in terrestrial animal products, such as raw eggs, raw meat, mayonnaise, turkey meat, broiler chicken and sausage [1]. Therefore, the occurrence of *Salmonella* in seafood and seafood products is a recent advance in detection as part of an overall mitigation strategy.

Human health surveillance programs are lacking in coastal communities due to their limited access to transport-wise and poor personal hygiene. On the other hand, many coastal areas of Indonesia are becoming centers of human activity, such as Jakarta and Surabaya. Surabaya, the second biggest city in Indonesia, is located in the East Java Province. A common part of the daily consumption of food by Surabaya's people is the seafood that can be obtained from fishermen, traditional markets or supermarkets. Thus, the data on food-borne pathogens related to seafood and seafood products have become important as part of a wider mitigation strategy. However, up until now, the data on the occurrence of *Salmonella* in seafood and seafood products is still limited.

Focusing on food-borne pathogens, particularly antibiotic-resistant bacteria, it is a major worldwide concern. Infected patients will need special treatment to be cured. Food-borne illnesses consist of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Listeria monocytogenes* [4]. Therefore, this study focused on the antibiotic-resistant profile of *Salmonella* is important.

The occurrence of *Salmonella* in fresh shrimp in five traditional markets in Surabaya reported a rate of occurrence of 36%. On the other hand, the data for *Salmonella* in other seafood (fish, cephalopods, shellfish, and seafood products) has not yet been reported. Therefore, the objective of this study was to determine the occurrence of antibiotic-resistant *Salmonella* in seafood and seafood products in Surabaya, East Java, Indonesia.

2. Materials and methods

2.1. The study area

The study area, Surabaya, located in East Java, is the second biggest city in Indonesia and is populated by 2,000,000 people. The sampling locations chosen consisted of traditional west, north, east, south and central Surabaya local markets selling seafood and seafood products.

2.2. Sample collection

A total of 29 samples (17 fish, 4 crustacea, 5 mollusks, and 3 fermented fish products) were collected from five traditional fish markets in Surabaya and analyzed for the presence of *Salmonella*. All of the samples were collected using a standard aseptic protocol and transported to the Education Laboratory of Faculty of Fisheries and Marine, Universitas Airlangga. The samples were analyzed directly upon arrival at the laboratory.

2.3. Salmonella isolation

For the isolation, 25 g of seafood sample was homogenized with 225 ml of peptone water and then pre-enriched at 37^{o}C for 18 h. The culture was taken from the pre-enrichment broth (1 ml) and then sub-cultivated in two selective enrichment broths; a Selenite Cystine Broth (Merck) and a Rappaport-Vassiliadis Broth (Oxoid). The broths were incubated for 24 h at 41^{o}C. A loopful sample from the enrichment broths was streaked on the selective plating media *Salmonella* Shigella Agar (Merck) and Xylose Lysine Deoxycholate (Merck) and incubated at 37 C for 24 h. The *Salmonella* presumptive colonies that showed a clear black-centered colony were taken for further purification steps. The next purification step was done by streaking the *Salmonella* presumptive colonies onto tryptic soy agar (Merck) incubated at 37^{o}C for 24 h. A single colony with demonstrated growth was then taken for Gram staining and biochemical testing for confirmation.

2.4. Profiling of the Antibiotic-Resistant Salmonella

An antibiotic-resistant test was conducted by Kirby-Bauer disc diffusion assay against seven antibiotics: chloramphenicol (30 µg), lincomycin (10 µg), azithromycin (15 µg), cefixime (5 µg), amoxicillin, trimethoprim/sulfamethoxazole (1.25/23.75 µg), erythromycin (15 µg), ciprofloxacin (5 µg), tetracycline (30 µg) and ampicillin (10 µg). Briefly, one loopful of purified Salmonella was cultured in tryptic soy broth (TSB) overnight, and the cell was separated from the broth through a centrifuge (Hettich Centrifuge, Germany) at 5,000 rpm for 5 minutes and then washed with normal saline twice. The cell was adjusted to McFarland standard number 1 and then streaked on Mueller Hinton Agar (Merck) with a sterile cotton butt. Antibiotic discs were placed on the lawn and incubated at 4^{o}C for 1 h prior to incubation at 37^{o}C for 24 h. The clear zone was measured and the resistance strain was decided by CLSI [5].

3. Results and discussion

3.1. Samples profile

A total of 29 samples were obtained from 5 traditional markets in Surabaya, consisting of fish, crab, shrimp, shrimp products and cuttlefish. Details of the traditional markets as a source of the samples have been shown in Table 1.

Table 1. Details of the seafood and seafood product samples.

Traditional Market	Fish and fish products	Crab	Shrimp and shrimp products	Cuttlefish	Shellfish
Babatan/Wiyung	10	-	2	1	1
Sutorejo	5	-	2	-	1
Kenjeran	1	-	-	-	1
Mulyorejo	-	-	1	-	-
Waru	1	1	1	-	1

3.2. Occurrence of Salmonella

Traditional markets or retail markets have become one site of *Salmonella* contamination that can affect public health concerns [6]. A total of 27 isolates out of 29 seafood and seafood product samples were found to be *Salmonella* positive based on a culture analysis. This represents a prevalence of 93%. The details of the data have been shown in Table 2.

Table 2. *Salmonella* prevalence in various seafood and seafood products in Surabaya.

Products	Number of positive samples/total number of samples	Percentage (%)
Fish	16/17	94,11
Crab	1/1	100
Shrimp and shrimp product	6/6	100
Cuttlefish	1/1	100
Shellfish	¾	75

In our study, the high prevalence of *Salmonella* in the fish samples (94%) was in agreement with [7], which reported that the prevalence of *Salmonella* in the fish samples was 90%. However, relatively lower results were reported by [3], [8] and [6] at rates of 29.7%, 43.8%, and 5% respectively. The high prevalence of shrimp in this study (100%) and shrimp products (100%) was also with an agreement with [9], at a rate of 58%. However, Yang et al. [10] reported a relatively low rate of *Salmonella* in the shrimp products (13.0%). The high prevalence of *Salmonella* in the shrimp products may occur due to *Salmonella* being a part of the cultural environment and natural flora [11]. On the other hand, the high rate of *Salmonella* in cuttlefish is on the contrary to previous reports. Laksmanan et al. [12] reported a rate of *Salmonella* in cephalopod products from India as 3.2%. The difference in the results may be caused by several factors, including sanitation, the isolation procedure and the sampling season [13].

Our finding in this study was that there is a high prevalence of Salmonella in the seafood and seafood products in Surabaya’s traditional markets. Gastroenteritis caused by Salmonella sp. is considered to be a worldwide public health problem and the first step to overcoming the problem is understanding the Salmonella prevalence, cross-contamination process and personal hygiene. Moreover, antibiotic resistance to Salmonella has also become a worldwide concern [2,3,6].

Therefore, further research related to antibiotic resistance profiling on Salmonella isolated from Surabaya's traditional markets is needed.

3.3. Antibiotic-resistant profile

Among the 33 isolates tested, there were eight isolates resistant to antibiotics (Table 3).

Table 3. Resistance profile of Salmonella strains isolated from the seafood of Surabaya Local Market.

No	Code	Products	Antibiotics									
			1	2	3	4	5	6	7	8	9	10
1	SBI04	*Pindang*	S	R	S	S	S	S	R	S	R	S
2	SBI07	*IkanCukul*	S	R	R	S	S	S	R	S	S	S
3	SBK01	*Anadara*	R	R	S	S	S	S	R	S	S	S
4	SBI01	*Belanak*	S	R	S	S	S	S	R	S	R	S
5	SSI01	*Banyar*	S	R	S	S	S	S	R	S	R	S
6	SSP01	*Kepiting*	S	R	S	S	S	S	R	S	S	S
7	STI02	*Jambal*	S	R	R	S	S	S	R	S	R	S
8	STI04	*Ikanlaut*	R	R	R	S	S	S	R	S	S	S

Note: R (Resistant), S (Susceptible), 1. Chloramphenicol (30 µg); 2. lincomycin (10 µg); 3. azithromycin (15 µg); 4. Cefixime (5 µg); 5. Amoxicillin; 6. Trimethoprim/sulfamethoxazole (1.25/23.75 µg); 7. Erythromycin (15 µg); 8. Ciproflaxin (5 µg); 9. Tetracyclin (30 µg) and 10. Ampicillin (10 µg)

The resistance test results showed that 12.12% of the isolates were resistant to tetracycline and that 100% were resistant to erythromycin. These results are in contrast to the studies conducted by Nguyen et al. [14] that obtained the result that 53.3% were resistant to tetracycline, 43.8% resistant to ampicillin, chloramphenicol 37.5%, and 31.3% for trimethoprim/sulfamethoxazole. The testing of the antibiotic resistance of the Salmonella isolates also showed that there were traditional markets with samples that were resistant to antibiotics. Resistance to antibiotics is one of the world's focuses on attention because if the antibiotic-resistant pathogen infects someone, then the treatment is much more difficult and/or longer. The resistance test results showed that the majority of the isolates were sensitive to the antibiotics used; however, there were 8 isolates that were resistant to at least one antibiotic, and even resistant to more than two antibiotics (multidrug resistant).

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