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Pathogenic food-borne bacteria in Shellfish and shrimp from the largest traditional seafood market in Surabaya, Indonesia

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ABSTRACT

The study assessed the levels of *Escherichia coli, Salmonella*, and *Vibrio cholerae* in seafood (*Anadaragranosa, Pernaviridis, Anadara antiquata, Penaeus monodon, Litopenaeus vannamei*) collected from the main traditional seafood market (Pasar Pabean), Surabaya, Indonesia. The aim of the study was to determine and evaluate the level of bacterial contamination if any, found in shellfish and shrimp sold at Pabean market. The total number of *Escherichia coli* in green mussel (*Pernaviridis*) samples was about 21,500 CFU/mL and the lowest was recorded in tiger prawn samples *(Penaeus monodon)* which was 2,800 CFU/mL. The highest *Salmonella* concentration was obtained from blood clam (*Anadara granosa*) samples which contained 470 CFU/mL however it was not present in shrimp samples. The highest concentration of *Vibrio cholerae* was obtained from the antique ark (*Anadara antiquata*) samples and was about 120 CFU/mL but was absent in green mussels samples, tiger prawn, and vannamei shrimp. In all, it can be concluded that the concentration of *Escherichia coli* obtained from all the seafood samples in this research exceeds the threshold for human consumption. All the shellfish samples contained *Salmonella* but *Vibrio cholera* was only present in two species including blood clam and antique ark.

***Key words**: Seafood contamination, Bio-monitoring, Safety standards, Pathogenic bacteria*

Introduction

The Fisheries sector has an important role in meeting human nutritional needs. As an important source of animal protein, aquaculture is rapidly increasing globally to meet increasing demand and cover for animal protein deficiency (Alkhunni *et al*., 2017). For example, according to Iwamoto *et al*., (2010), the consumption of seafood in the United States has increased in the past decades as an average American recently consumed approximately 16.5 pounds of seafood yearly, compared to the 10-12 pounds consumed in the 1980s (NOAA, 2005). Similar to other nations, the level of national fish consumption has been on a yearly rise in Indonesia (Ministry of Maritime Affairs and Fisheries of the

Republic of Indonesia, 2018) hence the targeted fish consumption per capita has increased; about 38.14 kg per capita in 2014, 40.9 in 2015, 43.88 in 2016, about 47.12 in 2017 and in 2018 50 kg per capita was recorded. In this year 2019, the Ministry is expecting the level of national fish consumption to reach 54.49 per capita. In order to meet these demands without compromising safety, the hygiene level of fishery products must be considered since seafood is sometimes associated with diseases in humans, especially if seafood products are consumed raw or undercooked. The presence of various human pathogenic bacteria in seafood products can be related to the level of environmental sanitation and water pollution. Thus, it can be seen that the bacteria detected in seafood products reflects the level of safety or otherwise conditions of water bodies or fish production sites (Alkhunni *et al*., 2017).

It is important to study the level of pathogen contamination in seafood to ensure a better understanding of the environment and the distribution of pathogens in the food chain. However, only a few studies have reported on the safety of commercially available seafood in traditional markets in large urban areas like Surabaya. This study aims to evaluate the level of contamination of *Escherichia coli*, *Salmonella* and *Vibrio cholera* in seafood from the main traditional seafood market "Pasar Pabean" in Surabaya, Indonesia. Since the "Pasar Pabean" is the main traditional seafood market in the second largest city of Indonesia, this study is expected to be a reference in monitoring safety standards regarding the quality, and hygiene of fishery products.

Materials and Methods

Sample collection and preparation

Seafood samples were collected from Pabean traditional market in Surabaya city, East Java from May-July 2019. After sampling, the seafood samples were cleaned and dried. The samples were then placed in a sterile plastic bag, properly packaged and put in a cool box and transported to the laboratory. During transportation, the temperature was maintained at 4 °C. The microbiological analysis started within 24 hours after sample collection. Each seafood sample used for analysis in this study weighed up to 90 grams.

Detection and analysis of *Escherichia coli*

Detection and analysis of *Escherichia coli* contamination in each seafood sample was carried out by serial dilution. After sample preparation, about 90 g of sample was weighed and put in 10 mL of sterile water. The sterile water was centrifuged until the sample became homogeneous. The sample in the bottle was then pipetted up to 1 mL and transferred into a new sterile tube in order to obtain a 10^{-2} dilution factor since coloniesat 10^{-1} dilution were too dense and difficult to analyse. About 1mL of sample was pipetted and poured into a sterile Petri dish as the selective media *eosin methylene blue agar* (EMB) and the Petri dish was homogeneously shaken slowly. The sample was incubated at 37°C for 24 hours. The *E. coli* colonies that grew were "metallic green" and were analysed by the *Total Plate Counts* (TPC) method and the unit for growing colonies was the colony formingunit (CFU / g) as in Figure 1.

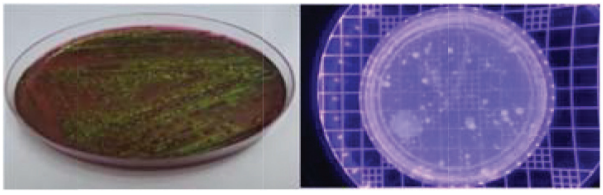

Fig. 1. **A**: *E. coli* colony (metallic green) on EMB media; **B**: Observation of the *E.coli* colonies by *totalplate count* on the colony counter.

Detection and analysis of *Salmonella*

A 1 ml volume of the sample was homogenized, spread into *Salmonella shigella* agar (SSA) media and incubated at 37 °C for 24 hours. Calculations were performed using the total plate counts (TPC) method and for bacterial confirmation, Salmonella colonies growing in SSA media were taken with ose and streaked for single colony on sterile SSA media. With this method, growing colonies are known to be solid black (Figure 2).

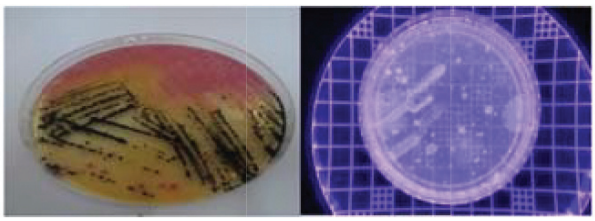

Fig. 2. **A**: Salmonella colonies on *Salmonella Shigella Agar* (SSA) media; **B**: Observation of the *Total Plate Count* of the Salmonella colony on the counting microscope.

Detection and analysis of *Vibrio cholerae*

Detection of the amount of *V. cholerae* contamination was carried out with the same procedure for Salmonella analysis but for the observation of *V. cholerae*, 1 mL sample was spread on thiosulfate citrate bile salts (TCBS) selectively and incubated at 37 °C for 24 hours. In this method, colonies of bacteria that grow turn greenish in colour (Figure 3).

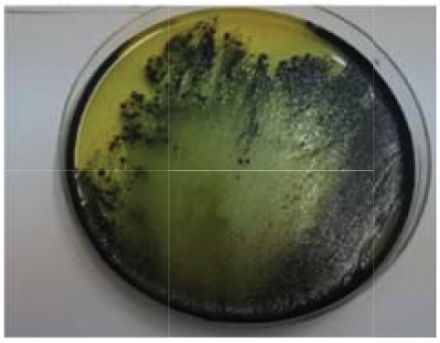

Fig. 3. *Vibrio cholerae* colonies on thiosulfate media citrate of sucrose bile salt (TCBS) agar.

Data Analysis

The data obtained was numerically described since it needed no statistical package.

Results

Total number of *Escherichia coli*

The highest number of *E. coli* colonies was found in green shellfish samples (*P. viridis*) and it was 21,500 CFU/mL and the lowest in tiger prawn samples (*P. monodon*), which had 2,800 CFU/mL *E. coli* (Table 1). The total number of *E. coli* colonies found in blood clams (*A. granosa*), antique ark *(A. antiquata*), and vannamei shrimp *(L. vannamei*) also had total number of colonies that exceeds the quality standards of different countries.

The total number of *Salmonella* in seafood (shrimp and shellfish)

The total amounts of *Salmonella* obtained from the samples were equally higher than the threshold recommended by different nations. The green mussels *(P. viridis*), blood shells (*A. granosa*), and antique ark (*A. antiquata*) had 380 CFU/mL, 470 CFU/mL, and 190 CFU/mL *Salmonella* respectively (Table 2). However, *Salmonella* was not detected in the two shrimp species.

Table 1. Total number of *Escherichia coli* colonies in seafood samples (shrimp and shellfish)

Sample name	Total *Escherichia coli* (CFU/mL)
Green mussels (*P. viridis*)	21,500
Antique ark (*A. antiquata*)	19,800
Blood clam (*A. granosa*)	18,200
Tiger prawn (*P. monodon*)	3,400
Vannamei shrimp (*L. vannamei*)	2,800
Acceptable limits	< 3 CFU/g (Indonesian) SNI 2009
Food Standards in Other Countries	< 2-5 CFU/g (Australia) Australian Food Standards (2015
	< 10 CFU/g (Europe) Official Journal of the European Union (2005)

Table 2. Total number of *Salmonella* colonies in seafood samples (shrimp and shellfish)

Sample name	Total *Salmonella* (CFU/mL)
Blood clam *(A.granosa)*	470
Green mussels *(P.viridis)*	380
Antique ark *(A.antiquata)*	190
Tiger prawn *(P.monodon)*	ND
Vannamei shrimp *(L.vannamei)*	ND
Acceptable limits	Negative/25g (Indonesia) SNI (2009)
Food Standards in Other Countries	Negative/g (Australia) Food Standards Australia (2015) Negative/25g (Hongkong) Microbiological Guidelines for Food (2014) Negative/25g (Europe)' Official Journal of the European Union (2005)

*ND: Not Detected

Total number of *Vibrio cholerae* in seafood (shrimp and shellfish)

The total number of *Vibrio cholerae* colonies detected in blood clam samples (*A.granosa*), and antique ark (*A. antiquata*), were 90 CFU/mL and 120 CFU/mL, respectively as tabulated in Table 3.

Discussion

With the global rapid growth of the Fisheries sector, it can be regarded as a sector with high potential to meet human nutritional needs. The reports from different countries including Indonesia reveal that

the level of fish consumption is increasing on yearly basis. Based on this fact, the appraisal of Fishery products for the Indonesian populace has been of high importance since these products form a significant part of their diets.

Table 3. Total number of *Vibrio cholerae* colonies detected in seafood samples (shrimp and shellfish)

Name sample	Total *Vibrio cholerae* (CFU/mL)
Blood clam *(A.granosa)*	90
Green mussels *(P.viridis)*	ND
Antique ark *(A.antiquata)*	120
Tiger prawn *(P.monodon)*	ND
Vannamei shrimp *(L.vannamei)*	ND
Acceptable limits	Negative/25g (Indonesia)
Food Standards in	SNI (2009)
Other Countries	Negative/g (Australia) Food Standards Australia (2015) Negative/25g (Hong Kong) Microbiological Guidelines; Food (2014) Negative/25g (Europe) Official Journal of the European Union (2005)

*ND : Not Detected

However, up to date, data on the bacterial levels in seafood products including shellfish and shrimp from traditional markets in Surabaya city are yet to be available. The results of this study indicate that the total number of *Escherichia coli* colonies in seafood products (shellfish and shrimp) had a high variation in the samples. The total number of *E. coli* colonies in all samples exceeds the quality standard values of the Indonesian National Standard (2009) on the quality of fish and fishery products which should be limited to less than (<) 3 CFU /g. The high amount of *E. coli*from this study (2,800-21,500 CFU /mL) may have been caused by fecal contamination in the aquatic environment and poor post-harvest handling resulting in the presence of *E.coli* in seafood products sold in traditional markets. Contamination by *E. coli* is generally associated with faeces of warm-blooded animals; contaminant indicators derived from human and animal faeces can be used to detect the presence of *E. coli* in food products such as seafood (Savichcheva and Okabe, 2006).

In terms of total number of *Salmonella* colonies, the total number of *Salmonella* colonies detected in shellfish samples ranged from 190-470 CFU/mL which is an indication that such seafood products are dangerous for human consumption, especially if consumed raw or undercooked. According to Indonesian National Standard (2009), Fishery products must be free from Salmonella contamination and the same thing applies to the quality control standards of several countries (Table 2). The reports of EFSA (2015) indicate that *Salmonella* is the most common cause of gastroenteritis in humans. The presence of these microorganisms in the environment and Fishery products can be an indicator of inadequate sanitation in seafood production and post-harvest handling (Li *et al*., 2009; Budiati *et al*., 2013).

The findings of this study have proven that samples of shellfish can be used as a bio-indicator of aquatic pollution. According to Kumar *et al*., (2009) the reason for the high prevalence of S*almonella* in shellfishes is because they are filter-feeders, usually filtering large amounts of water and sediments, hence they are able to accumulate various pollutants such as heavy metals, pesticides, synthetic dyes, and pathogenic bacteria in their tissues. *Salmonella* can be divided into more than 2,500 serovars (Agbaje *et al*., 2011). Out of the 2,500 serovars, only certain serovars are dominant in the aquaculture environment. The Worthingtonserovarfor example was previously detected in fish, shrimp, shellfish, crab, lobster, squid and octopus in India (Shabarinath *et al*., 2007; Kumar *et al*., 2009).

The total number of *Vibrio cholerae* colonies was only detected in samples of blood clam and antique ark which were 90 and 120 CFU/mL respectively. Similar to *Salmonella*, it is also necessary for food products including seafoodto be free from *V. cholerae*. *Vibrio cholerae* is a Gram-negative bacteria originating from the marine or estuary environment. According to the World Health Organization (WHO) report *V.cholerae* is the major cause of diarrheal disease due to the enterotoxin it produces and forms colonies in the small intestine.Consumption of raw or undercooked meat and poor quality of water can equally enhance the spread of cholera. *V.cholerae* is often found on water surfaces that have been contaminated with faeces containing these bacteria, therefore the presence of *E.coli* and *V.cholerae* can serve as indicators of water pollution (Hadi and Alamudi, 2019). Moreover, besides *V.cholerae*, several species of *Vibrio* are also pathogenic in human such as *V. parahaemolyticus* and *V. vulnificus*. A Comparison of the amount of *E.coli, Salmonella*, and *V.cholerae* in seafood from various regions is pre-

Table 4. Record of *Eschericia coli, Salmonella,* and *Vibrio cholerae* in seafood from different regions

Location	Sample Species	*Escheria coli* (CFU/mL)	*Salmonella* (CFU/mL)	*V. cholerae* (CFU/mL)	Reference
Beijing, China	*Mytilus galloprovincialis* and *Solen marginatus*	16	ND	ND	Fusco *et al*., (2013)
Malaysia	*Tilapia massambica*	ND	Positive (43% sample)	ND	Budiati *et al.,* (2013)
Bangladesh	Frozen fish (Jew fish, Tongue fish, Queen fish, Ribbon fish and Cuttle fish)	5	ND	ND	Sanjee and Karim (2016)
India (2003-2006)	Shrimp	ND	Positive (26.7% sample)	ND	Kumar *et al.,* (2009)
India (1997)	*Penaeus indicus*	NS	21.2%	NS	Hatha and Lakshmanaperumalsamy (1997)
	Penaeus monodon	NS	11.1%	NS	
Buleleng, Bali, Indonesia	Abalone *(Haliotissquamata)*	4-7	ND	ND	Wiradana *et al*., (2019)
Maputo Bay, Mozambique	*Meretrixmeretrix*	110-170000	Positive (33% samples)	ND	Kronkvist (2006)
	Eumarcia Paupercula	400-310	Positive (67% samples)	ND	
East Java Coast, Indonesia	*Anadaragranosa*	Negative-4800	ND	ND	Soegianto and Supriyanto (2008)
Marmara Sea, Turkey	*Mystilusgalloprovincialis*	<10-590	ND	ND	Yilmaz *et al*., (2005)
	Venus gallina	48-960	ND	ND	
Sea of Galilee and Mediterranean Sea	*Sarotherodon galilaeus*	ND	ND	Positive (>50% samples)	Senderovich *et al*., (2010)
	Oncorhynchus mykiss	ND	ND	1-7	Diler *et al*., (2000)
USA(1973-2006)	All seafood	33 illnesses	374 illnesses	1,393 illnesses	Iwamoto *et al.,* (2010)
Surabaya, East Java	*P. viridis*	21,500	380	ND	Current study
	A.	*antiquata*	9,800	190	120
	A.	*granosa*	18,200	470	90
	P. monodon	3,400	ND	ND	
	L. vannamei	2,800	ND	ND	

*ND = Not Detected; CFU = Colony Forming Unit. NS = Not studied

sented in Table 4. The differences in those results could be linked to study locations, seasons and other factors.

Conclusion

Based on the results of this study, the studied seafood were contaminated by food-borne pathogens including *E.coli, Salmonella*, and *V.cholerae* which shows the pollution level the studied environment and poor post-harvest sanitation by producers and sellers. The levels of contaminations by the studied bacteria were all above the quality standards of various countries, therefore this need to be corrected to safeguard public health in Surabaya.

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