Detection of *Salmonella* sp., *Vibrio* sp. and total plate count bacteria on blood cockle (*Anadara granosa*)

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**Abstract.** Blood cockle (*Anadara granosa*) has high level of zinc and protein, which is beneficial for therapeutic function for malnourished particularly stunting case in children. Zinc in animal foods is more absorbable than that from vegetable food. Blood cockle (*Anadara granosa*) is rich in nutrient and an excellent environment for the growth of microorganisms. This research aimed to identify the contamination of *Salmonella* sp., *Vibrio* sp. and total plate count bacteria on blood cockle (*Anadara granosa*). This was observation research with laboratory analysis. *Salmonella* sp. and *Vibrio* sp. were detected from blood cockle. Total plate count was determined of the total amount of the bacteria. Results detected from 20 samples of blood cockle showed that all samples were negative of *Salmonella* sp. and 1 sample positive *Vibrio* sp. The result of total plate count bacteria was $< 5 \times 10^5$ colony/g sample.

**Keywords**: *Salmonella* sp., *Vibrio* sp., *Anadara granosa*

1. **Introduction**

Food that is free from health risks cause by damage, counterfeiting and contamination by microbial and chemical compounds as well sufficient spiritual needs[1,2]. Blood cockle (*Anadara granosa*) is a seafood commodity that has commercial value and favored by consumers in Indonesia and in Asia generally. Blood cockle (*Anadara granosa*) has high level of zinc and protein, which has therapeutic function for malnourished children, particularly those with stunting case. Zinc in animal foods is more absorbable than that from vegetable food.[3,4]. Blood cockle (*Anadara granosa*) is found on muddy substrates at river estuary with sloping beach topography to a depth of 20 m [5]. Blood cockle’s (*Anadara granosa*) way of life as filter feeder causes this commodity to have high potential in accumulating pollutants substances, both microbes and heavy metals. Water in the environment is contaminated by microbial or heavy metals, which will be absorbed into the tissues of blood cockle bodies [2].

There are three general group of pathogenic bacteria in shellfish. The first is the indigenous bacteria as natural microflora, e.g *Clostridium botulinum, Vibrio* spp., *Aeromonas hydrophila*. The second is the enteric (non indigenous) bacteria that are present due to fecal contamination as *Salmonella* spp., *Shigella* spp., *Escherichia coli, Staphylococcus aureus*. The third is bacterial group formed in contamination during processing, storage or preparation of cooking, such as *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Salmonella* spp.[10].

Majority of epidemic of food born-illness is due to pathogenic microorganisms that are generally undetectable by human senses (usually not showing colour or flavor changes or leaving taints in the food). This bacteria are capable of growing rapidly under favourable storage conditions [6,7]. Since
1980s, the incidence of Salmonella spp. infection have increased dramatically, causing high medical costs, and decreasing the productivity of the workers [8]. *Salmonella* sp. is a major reservoir in aquatic environment. Blood cockle and fishery product have been recognized as agent of food-borne pathogens [6,9].

*Vibrio* sp. is the causative agent of vibriosis that infect marine animals such as fish, shrimp and shellfish [11]. *Vibrio* sp. is mostly found in marine environment and detected in some types of species aquatic [12,13]. It is also known as a common food-borne pathogen in Asia. It is a food borne-pathogen and a major cause of gastroenteritis particularly from traditional consumption of raw or undercooked seafood [13,14]. The case of food-borne disease caused by pathogenic Vibrio sp. has been widely reported in various outbreaks [14]. *Vibrio* sp. was found to be 73% of clinical samples from hospitalized diarrhea patients within 1995-2001. Meanwhile, in Japan, within the period of 1996-1998, 20-30% cases of outbreaks occurred and in 1998 this case was reported as exceeding the number of cases caused by *Salmonella* [15]. The bacterial disease from seafood is mainly due to contaminated water [16].

This study was aimed at detection of *Salmonella* sp., *Vibrio* sp. and total plate count bacteria on blood cockle (*Anadara granosa*).

2. **Materials and Methods**

2.1. **Sample Collection**

Blood cockle samples were collected from traditional market in Sidoarjo region, Jawa Timur, Indonesia. The sample were alive in plastic containers and were transported immediately to Bacteriology Laboratory, Faculty of Heath Science, University of Maarif Hasyim Latif, Sidoarjo, Jawa Timur, Indonesia. The samples were analyzed immediately on reaching the laboratory.

2.2. **Reagents and Medium**

Reagents used in this study were kovac, NaCl 0.85%, Methyl Red, α-naphtol 5%, KOH 40%, and buffer phosphate pH 6.8. Bacterial medium for detection of *Salmonella* sp. used were Selenite F-Broth, MCA, SSA, KIA, and Biochemical Reactions. Bacterial medium for detection of *Vibrio* sp. used were APW, TCBS, and Biochemical reactions. Medium for total plate count bacteria were PCA.

2.3. **Preparation of Blood Cockle (*Anadara granosa*)**

The blood cockle samples was washed with sterile distilled water then mashed using a blender.

2.4. **Detection of Salmonella sp.**

An amount of 25 g mashed blood cockle samples were grown on selenite F-broth medium and incubated 37°C for 24 hours. Cultures on Selenite F-Broth grown on MCA and SSA were incubated for 37°C for 24 hours. Colonies taken that were not fermented lactose from MCA or small colonies and has a black node from SSA using ose then grown on KIA and incubated 37°C for 24 hours. Further grown on biochemical reactions medium and incubated 37°C for 24 hours [17].

2.5. **Detection of Vibrio sp.**

As much as 25 g mashed blood cockle samples were grown on 1% Alkaline Peptone Water (APW) and incubated at 37°C for 18-24 hours. Cultures on APW grown on TCBS were incubated at 37°C for 18-24 hours. Suspected colonies of yellow color sized 2-3 mm were taken with ose and streak back to TCBS medium for being purified and then incubated at 37°C for 18-24 hours. Furthermore, separate bacterial colonies were taken using ose and cultured in a TSBC medium and incubated at 37°C for 18-24 hours. Furthermore, they were grown on biochemical reactions medium and incubated at 37°C for 24 hours. The positive result of *Vibrio* sp. on KIA medium was indicated by yellow base and red slope section. In SIM medium, *Vibrio* sp. venom showed a positive result, namely the growth of bacteria that spread like roots. In Simmon Citrate medium showed negative result, namely the medium did not change its color. Further
testing of *string test was* as follows: object glass with 0.5% sodium deoxycholate plus bacteria was mixed using ose, then the ose was pulled up, and positive phases took place when fibrin yarn occurred [18].

2.6. **Total Plate Count Bacteria**

Total Plate Count (TPC) bacteria were enumerated using pour plate technique. Smoother sample of 25 grams was mixed with 225 mL buffer phosphate to obtain dilution of $10^{-1}$. The suspension samples were diluted to dilution $10^{-3}$. Suspension sample of 1 mL was taken using pipette. Each dilution was put into each petri dish. PCA was poured into each petri dish and incubated for 24-48 hours at $35^\circ \pm 2 \, \text{C}$. The number of bacterial colonies growth on PCA medium was counted by colony counter [17,18].

3. **Result and Discussion**

Detection of *Salmonella* sp., *Vibrio* sp. and total plate count on 20 samples blood cockle is presented in Table 1.

| Sample Code | TPC (colony/gram) | Detection *Salmonella* sp. | Detection *Vibrio* sp. |
|-------------|-------------------|-----------------------------|-----------------------|
| 1           | $1.0 \times 10^4$ | Negative                    | Negative              |
| 2           | $3.8 \times 10^3$ | Negative                    | Negative              |
| 3           | $5 \times 10^3$   | Negative                    | Negative              |
| 4           | $6.2 \times 10^3$ | Negative                    | Negative              |
| 5           | $4.7 \times 10^3$ | Negative                    | Negative              |
| 6           | $1.0 \times 10^4$ | Negative                    | Negative              |
| 7           | $3.4 \times 10^4$ | Negative                    | Positive              |
| 8           | $5.4 \times 10^3$ | Negative                    | Negative              |
| 9           | $4.5 \times 10^4$ | Negative                    | Negative              |
| 10          | $1.5 \times 10^4$ | Negative                    | Negative              |
| 11          | $3.7 \times 10^3$ | Negative                    | Negative              |
| 12          | $2.0 \times 10^4$ | Negative                    | Negative              |
| 13          | $4.0 \times 10^3$ | Negative                    | Negative              |
| 14          | $1.0 \times 10^4$ | Negative                    | Negative              |
| 15          | $9.3 \times 10^5$ | Negative                    | Negative              |
| 16          | $1.6 \times 10^4$ | Negative                    | Negative              |
| 17          | $2.7 \times 10^4$ | Negative                    | Negative              |
| 18          | $1.2 \times 10^4$ | Negative                    | Negative              |
| 19          | $6.1 \times 10^3$ | Negative                    | Negative              |
| 20          | $7.0 \times 10^3$ | Negative                    | Negative              |

The detection result from 20 samples of blood cockle (*Anadara granosa*) showed that all samples were negative *Salmonella* sp. and 1 sample positive *Vibrio* sp. The result of Total Plate Count bacteria was $< 5 \times 10^5$ colony/g sample.

Shellfish was studied in this research because shells are widely consumed and favored by the public. Food safety is a condition and effort required to prevent food from possible biological, chemical and other contaminants that may interfere, harm and endanger human health. Shellfish are said to be good and feasible for consumption by humans if they do not contain harmful bacteria, for example, *Salmonella* sp. and *Vibrio* sp.[19].

Water can be a carrier of pathogenic bacteria that is harmful to health. Health hazards or risk related to water pollution in general can be classified into two, the direct and indirect hazards. Indirect hazards may
occur as the consequences of consuming fish, which is contaminated or contain harmful pollutant substances [19].

In this study, one sample was found to be positively contaminated with *Vibrio* sp. The incidence of microorganisms in shell fishes depends on the quality of water from which these animals are obtained. Periwinkle, oyster, mussels and clams are often tread in sewage polluted tidal estuaries water from which the shellfishes accumulate high levels of pathogens. Contamination of periwinkles could be as the result of processing. Food handlers could contaminate the meat during sucking. Periwinkles are filter-feeders, passing large amount of water through their bodies taking in suspended solids along with food. During this process, they pick up soil and water microorganisms, including pathogens if present. The inner tissues of healthy plants and animals are free of microorganism. However their surfaces are usually contaminated with a variety of microorganisms [20].

The result of Total Plate Count bacteria from 20 samples blood cockle was <5×10⁵ colony/g sample. Shellfish carry a resident population of bacteria that has been found to fluctuate between 10⁷ and 10⁶ bacterial counts. The consumption of mollusca occasionally gives rise to some serious diseases, such as poisoning (paralytic shellfish poisoning), gastroenteritis, enteric fever, cholera, and typhoid fever. The high level of moisture, rich nutrients, including amino acids and other extracts such as nitrogenous compounds, digestible protein and psychrophilic organisms, render seafood early perishable. Most often, spoilage occurs within a short period of time even under refrigeration [20]. Handling seafood safely is important to reduce the risk of foodborne illness. Seafood must be kept in ice or in the refrigerator after being bought or collected from the river. If seafood is not to be consumed within two days after being taken from the river, it must be stored in the refrigerator or wrapped tightly in plastic, aluminum foil or moisture-proof paper and stored in the freezer [21,22].

Proper handling of fish between capture and delivery to the consumer is a crucial element in assuring final product quality. Factor of standart sanitation, handling, time and temperature of holding fish influence quality. In some cases, fish is considered free of pathogenic bacteria that have public health significance when it was firstly caught. The presence of harmful bacteria to human generally indicates poor sanitation in handling and processing and the contamination is from of human or animal. *Salmonella* have been found in fish washed with polluted water and from fish-holds washed with polluted water [23].

### 4. Conclusion

The detection result from 20 samples of blood cockle (*Anadara granosa*) showed that all samples was negatively contaminated with *Salmonella* sp. and one sample was positively contaminated with *Vibrio* sp. The result of Total Plate Count bacteria was <5×10⁵ colony/g sample. Total plate count on 20 samples of blood cockle did not exceed the maximum limit required by SNI 7388:2009 about the maximum limit of contamination on food.

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