Antibiotic Resistance Bacteria in Coastal Shrimp Pond Water and Effluent

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Abstract The rapid growth of shrimp farm activities began since last 40 years. It is due to high demands and can generate economy to the country. In Malaysia, thousands tonnes of shrimp were produced every year for local demand and export as well. However high demand in this industry, causes problem of water pollution in shrimp ponds which subsequently contaminated discharge from the pond due to presence of bacteria. In this study, it was found that shrimp farm water and effluent containing pathogenic bacteria which is resistant to certain antibiotic. These antibiotic resistance bacteria could be harmful to human. *Vibrio alginolyticus, Vibrio parahaemolyticus, Shigella flexneri* and *E. coli* were detected in the water and effluent from the shrimp farm. Total of *Vibrio* in 3 and 6 months pond were higher than allowable limit, which is 1625 and 2650 cfu/ml, respectively. *E. coli* in this study was recorded at low concentration, however dramatically increased in 4 months pond (438 cfu/ml) before plunging to 13 cfu/ml in 6 months pond. It can be concluded that the higher number of

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pathogenic bacteria (>1000 cfu/ml of *Vibrio*) were detected in shrimp pond water and effluent can cause illness to human health.

Keywords Pathogenic bacteria · Antibiotic resistance bacteria · Water and wastewater · Shrimp farm · Aquaculture

1 Introduction

Coastal shrimp farming activities became a fastest growing food producing in many countries [1–3]. It is due to high demands and helps to generate income in the country. However, there is negative impact on the environment if this activity release untreated effluent. The untreated effluent will be discharged directly into the receiving water bodies. Therefore, river water will be polluted and can increase human health risk due to chemical, physical and biological contaminants in wastewater.

Antibiotic resistance bacteria is one of the major health risks associated with shrimp pond water and the wastewater. In this study, the antibiotic resistance bacteria is categorized as pathogenic to human. *Pseudomonas aeruginosa* is one of the leading nosocomial pathogens worldwide. *P. aeruginosa* represents a phenomenon of bacterial resistance and practically is known as mechanisms of antimicrobial resistance [4]. *P. aeruginosa* is an extremely adaptive organism and it can grow on a wide range of substrates and quickly responds to environment alterations.

There are many types of bacterias could be found in shrimp ponds water and their effluent. Previous studies showed that *Vibrios* are the most commonly species found in shrimp farms. Table 1 shows the types of bacterias commonly found in shrimp water and their effluent.

The main objective of this study is to investigate the concentration of antibiotic resistance bacteria presence in the coastal shrimp pond water and their effluent.

Table 1 Bacteria commonly found in shrimp pond water and effluent

No.	Bacteria	References
1	*Vibrio* and *Photobacterium*	[14]
2	*Vibrio*	[15, 16]
3	*Vibrio parahaemolyticus*	[21, 22]
4	*Vibrio harveyi*	[23]
5	*Vibrio nigripulchritudo*	[24]
6	*V. alginolyticus*, *V. Parahaemolyticus*, *V. Alginolyticus*	[17]
7	*Salmonella and Vibrio*	[18]
8	*E. coli*, *Salmonella*, *Shigella*, *Vibrio*	[13]

Table 2 Characteristics of the shrimp farm

Pond criteria	Farm details
Shrimp type	Tiger shrimp
Total of ponds	14
Pond in operation	6
Total land area	12 ha
Total pond area	8 ha
Age of farm	7 year
Shrimp production (each pond)	6 tonnes
Source of water	Brakish river water
Use of probiotics	Yes
Nearby farm	No

2 Materials and Methods

2.1 Sampling Area

Water samples were collected from a selected coastal shrimp farm in Selangor, Malaysia from November 2013 to January 2014. Table 2 shows the characteristics of the identified farm.

2.2 Sampling

Water samples were collected in 500 ml steriled bottles and stored at 4 °C until analysed process is been done in the laboratory. The bottles were labelled and the measurement processes were conducted within 24 h. Samples were collected in each pond, including detention pond, inlet and outlet. Sampling was conducted during a fine day.

2.3 Water Quality Analysis

On-site measurement of water quality parameters such as pH, turbidity, Dissolved Oxygen (DO), temperature and salinity were conducted using a portable water quality probe (HORIBA). Biochemical oxygen demand (BOD_5), ammonium nitrogen (NH_4^+–N) and Total Suspended Solid (TSS) were analysed in laboratory in accordance to Standard Methods [5].

2.4 Microbial Sample and Analysis

Identification of *Shigella flexneri* and *E. coli* showed that 1–2 mm pink and yellow of colonies on XLD agar, respectively. Identification of *Vibrio* was using TCBS agar (OXOID). *Vibrio alginolyticus* was appeared on the media in yellow colonies while *Vibrio parahaemolyticus* was appeared in green colonies. One loopful of sample was streaked on the agar, sealed, labeled and incubated invertedly at 37 °C for 24 h [6]. The concentration of antibiotic resistance bacteria was calculated as colony forming unit (cfu) per milliliter (ml).

3 Result and Discussion

3.1 Water Quality Analysis

The most important activity for the control of noxious crisis in the shrimp farming system is water quality assessment [6]. The Dissolved Oxygen value in the water indicates the growth situation for pollution conditions and aquatic organisms. As shown in Table 3, DO in 3 months pond showed the lowest concentration compared to other ponds. Detrimental to aquatic life happened when the DO is low [7]. Low DO conditions can be attributed to the presence of numerous microorganisms in 3 months pond.

Detention pond was recorded the lowest pH compared to other ponds. The pH range between 6.5 and 9.0 is suitable for aquaculture production [6]. It is showed that water condition in detention pond still not quite suitable yet to being route to the other ponds. The extreme low or high of pH will affect the survival rate of shrimps in the pond.

Temperature recorded in all ponds were found suitable as it is still in the range between 28 and 32 °C where is within the optimum water temperature for the growth of shrimp [6]. The average water temperature recorded during day time in the black tiger shrimp pond is 28 °C [8]. Salinity recorded in Bangladesh shrimp farm wastewater was under 9 psu [3] while in this study, the salinity is ranging between 1.82 and 3.11 psu.

Table 3 Water quality parameter

Parameter	Inlet	Det.	1 month	3 months	4 months	6 months	Outlet
pH	6.76	6.20	8.61	7.88	8.05	7.92	7.91
Turbidity (ntu)	13.00	14.00	18.50	23.34	53.84	43.33	94.67
DO (mg/l)	5.14	8.08	7.57	4.86	7.29	7.56	6.31
Temperature (°C)	29.64	31.52	30.87	30.54	30.95	31.15	30.94
Salinity (psu)	1.82	2.10	2.10	3.06	3.11	3.04	2.88

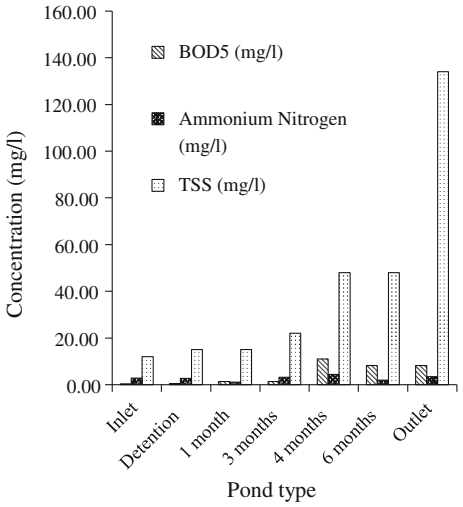

Fig. 1 BOD_5, ammonium nitrogen and TSS

Aquatic life also need nitrogen. They get the required nitrogen by eating plants or by consuming other animals that eat the plants. If the level of ammonium nitrogen in surface waters are too high, they could be toxic to some aquatic organisms. Figure 1 shows that 1.06–4.4 mg/l of ammonium nitrogen were generated. Shrimp farming produced 0.5–1.8 g ammonium/kg shrimp [9]. It shows that the concentration of ammonium nitrogen in the wastewater was considerably low. When more concentration of ammonium nitrogen, it will accelerate the corrosion of pipelines and it causes the chlorination process becomes complicated due to existence of chloramines [10].

The growth of plant and algae usually rise as much nitrogen can be found as nutrient. This occurred when the ammonium nitrate was moderate. This will have a ripple effect on other properties of water quality, such as improving biochemical oxygen demand levels and significantly decreased dissolved oxygen. Increased amount of nitrification will occurred if the level of dissolved oxygen decreased when ammonium nitrogen is high. The BOD_5 of the inlet, 1, 3 months and detention pond were low with the range between 0.36 and 1.38 mg/l. However it was increased for 4 and 6 months pond and outlet with 11.04, 8.22 and 8.16 mg/l, respectively. It was suggested the BOD_5 in shrimp farm is 9.4 mg/l [11].

Total suspended solid discharges during the harvest draining process was 134 mg/l. The average of TSS produced in shrimp pond outlet is 138.5 mg/l [9]. The TSS significantly higher in outlet but still under the limit. The range of TSS were found between 121 and 147 mg/l from shrimp farm wastewater in Ha-Long Bay [12].

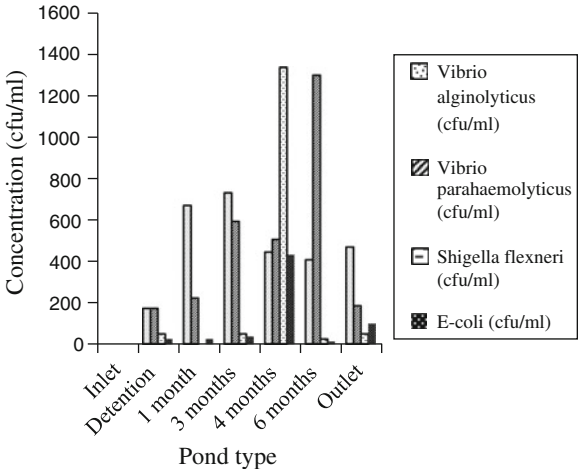

Fig. 2 Pathogenic bacteria detected in shrimp farm

3.2 Bacteria Identification

Shigella flexneri, *E. coli* and *Vibrio parahaemolyticus* are life-threatening bacteria and the leading cause of foodborne illness associated with the consumption of seafood. *Shigella flexneri*, *E. coli* and *Vibrio parahaemolyticus* are pathogenic bacteria and a major cause food-borne illness related to seafood intake [13].

Vibrio was known as pathogenic to human and can cause disease through food consumption. Many studies have been done on occurrence and prevalence of *Vibrio* in shrimp farm [2, 14–18]. Figure 2 shows the *Vibrios* found in this study were ranged between 350 and 1726 cfu/ml. Optimal level for water quality parameters for *Vibrio* is <1000 cfu/ml [6]. It's indicated that the total of *Vibrio* in 3 and 6 months pond are higher than allowable limit.

A total of 11.4 % strains isolated from wastewater were *E. coli* [19]. The presence of *E. coli* in this study were recorded at low concentration, however dramatically increased in 4 months pond (438 cfu/ml) before plunging to 13 cfu/ml in 6 months pond. *E. coli* normally produced from feces of warm-blooded animal and used as an indicator of water pollution. The concentration should be below than 1000 MPN/ml in wastewater analysis [20]. This result indicated that antibiotic resistance bacteria which also pathogenic could be released to the environment and produced potential harm to human health.

4 Conclusion

Based on the findings of this study, it can be concluded that the higher number of pathogenic bacteria (>1000 cfu/ml of *Vibrio*) were detected in shrimp pond water and effluent. These were supported by the increasing of the turbidity level and

E. coli concentration towards harvesting time. Although if quality met the standard of shrimp farm activities, but it still can be harmful to human if the effluent is excessively discharge to the water bodies.

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