Original Article

Antibiogram and Heavy Metal Resistance Pattern of Vibrio Alginolyticus Isolated From Asian Seabass (Lates Calcarifer) Hatchery

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Abstract

This paper was described antibiogram and heavy metal resistance pattern of Vibrio alginolyticus isolated from Asian Seabass (Lates calcarifer) hatchery. V. alginolyticus was recognized as a causative agent of vibriosis in Asian seabass and may lead to huge loss to the fish farmer. Therefore, this study was carried out to investigate the suitable of antibiotic to against V. alginolyticus. In the present study, a total of 14 antibiotics; oxolinic acid (2µg), ampicillin (10µg), erythromycin (15µg), furazolidone (15µg), lincomycin (15µg), colistin sulphate (25µg), oleandomycin (15µg), doxycycline (30µg), fosfomycin (50µg), florfenicol (30µg), flumequine (30µg), tetracycline (30µg), fosfomycin (50µg) and spiramycin (100µg) as well as four heavy metals; mercury (Hg^{2+}), cadmium (Cd^{2+}), chromium (Cr^{6+}) and copper (Cu^{2+}) were applied in the present study. Based on the antibiotic sensitivity test result showed that oxolinic acid the most effective antibiotic in controlling V. alginolyticus in which 93.3% of the present bacterial isolates were sensitive to it. This was followed by furazolidone (86.7%), nitrofuratoin (80.0%), tetracycline (73.3%), doxycycline (73.3%) and florfenicol (73.3%). On the other hand, all the present bacterial isolates were resistant to lincomycin. In the heavy metal tolerance test, all the present bacterial isolates were resistant to Hg^{2+}, Cd^{2+}, Cr^{6+} whereas only 26.7% of them were resistant to Cu^{2+}.

Key Words: Antibiogram, Heavy metal, Vibrio alginolyticus, Asian Seabass, Lates calcarifer.

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Introduction

Asian seabass, Lates calcarifer (Bloch) is a native fish species in Indo-Pacific region (Greenwood, 1976) and an important species for aquaculture in Asia-Pacific region (Kumar, et al. 2007). It becomes a popular fish species among Malaysian aquaculturist due to high value and
huge demand from local and abroad seafood market. Production of *L. calcarifer* was recorded 20,000 tons per year with the value more than 65 million dollars. However, bacterial diseases were recognized as a significant constraint of the development of Asian seabass culture in worldwide especially vibriosis. Vibriosis was reported as a fatal disease to many marine fish and invertebrate such as shrimp (De la Peña, et al. 1993), crab and lobster (Bowser, et al. 1981), mollusks (Nottage, et al. 1989) and fish (Hustvedt, et al. 1992). Till present, the available information of vibriosis due to *Vibrio alginolyticus* is still scarce and does not allow prevention and control of the disease in hatchery. Therefore, this study was carried out to provide valuable database for fish farmer in selecting the suitable antibiotic for treatment or prophylactic purpose.

**Materials and Methods**

Asian Seabass, *Lates calcarifer* fingerling with the size 10 to 15cm and water samples of *L. calcarifer* nursery tank were collected from commercial Asian Seabass hatchery located at Setiu, Terengganu, Malaysia. The water parameters of the sampling sites were measured using pH meter (YSI, USA). The temperature, dissolved oxygen, pH and salinity of the sampling sites were 30.41°C, 6.66mg/l, 8.73 and 30.31ppt, respectively.

Water samples were collected from *L. calcarifer* fingerling water reservoir tank in four replicates. One millimeter of water sample was serially diluted in sterile physiological saline and plated on two medium; Tryptic Soy Agar (TSA) and Thiosulphate Citrate Bile Salt (TCBS) (Merck, Germany).

Ten diseased *L. calcarifer* fingerling were randomly sampled from nursery tank. Swab was aseptically taken from organs such as eyes, kidney, liver and abdominal fluid of the fish using sterile cotton bud and spread onto TCBS medium.

All the inoculated media were incubated at room temperature for 24 to 48h. The bacterial colonies that grew on the selective media were further selected for the identification test. The bacterial isolates were identified using conventional biochemical tests (Holt, et al. 1994) and confirmed with commercial identification kit (BBL, USA).

The present isolates (n= 150) were cultured in tryptic soy broth (TSB) (Oxoid, England) for 24h at room temperature. The bacterial cells were then centrifuged at 14,500 rpm for 5min by using minispin (Eppendorf, Germany). The concentration of the bacterial cells were adjusted into 10⁶ colony forming unit (CFU) by using saline and monitored with Biophotometer (Eppendorf, Germany) before being swabbed on the prepared Mueller Hinton agar (Oxoid, England). Antibiotic susceptibility test was conducted according to Kirby–Bauer disk diffusion method using Mueller-Hinton agar (Oxoid, England) (Bauer, et al. 1966). Antibiotics tested including oxolinic acid (2µg); OA 2, ampicillin (10µg); AMP 10, erythromycin (15µg); E 15, furazolidone (15µg); FR 15, lincomycin (15µg); MY 15, colistin sulphate (25µg); CT 25, oleandomycin (15µg); OL 15, doxycycline (30µg); DO 30, florfenicol (30µg); FFC 30, flumequine (30µg); UB 30, tetracycline (30µg); TE 30, fosfomycin (50µg); FOS 50 and spiramycin (100µg); SP 100 (Oxoid, England). Interpretation of the results namely sensitive (S), intermediary sensitive (I) and resistance (R) was made in accordance to the standard measurement of inhibitory zones in millimeter (mm).

MAR index (multiple antibiotic resistance) of the present isolates against the tested antibiotics was calculated based on the formula as follows (Sarter, et al. 2007):

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\text{MAR index (multiple antibiotic resistance)} = \frac{X}{(Y \times Z)}
\]

Where:

- \(X\) = total of antibiotic resistance case;
- \(Y\) = total of antibiotic used in the study;
- \(Z\) = total of isolates.
A MAR index value of equal or less than 0.2 was defined as those antibiotics were seldom or never used for the animal in term of treatment whereas the MAR index value higher than 0.2 is considered that animal have received high risk exposure to those antibiotics.

Heavy metal resistance test was carried out as described by Miranda and Castillo (1998). Bacterial tolerance to four elements of heavy metal, i.e. mercury (Hg\(^{2+}\)), cadmium (Cd\(^{2+}\)), chromium (Cr\(^{6+}\)) and copper (Cu\(^{2+}\)) was determined by agar dilution method. Overnight bacterial suspension was spread onto plates of TSA medium incorporated with different concentrations of HgCl\(_2\), CdCl\(_2\), K\(_2\)CrO\(_4\) and CuSO\(_4\) (Fluka, Spain). By two-fold dilutions, concentration of both Cd\(^{2+}\) and Cr\(^{6+}\) were ranging from 25 to 400µg/mL while concentration of Hg\(^{2+}\) and Cu\(^{2+}\) were ranging from 2.5 to 40µg/mL and 150 to 2400µg/mL, respectively. For the purpose of defining metal resistance, the isolates were considered as resistant if growth was obtained at concentration of 10µg/mL (Hg\(^{2+}\)), 100µg/mL (Cd\(^{2+}\) and Cr\(^{6+}\)) and 600µg/mL (Cu\(^{2+}\)).

The MAR value of the present study was 0.40. All the present bacterial isolates were found resistant to Hg\(^{2+}\), Cd\(^{2+}\) and Cr\(^{6+}\). However, Cd\(^{2+}\) was found able to inhibit the growth of 80% of present bacterial isolates at concentration of 400µg/mL. On the other hand, 73.3% of the present bacterial isolates were found sensitive to Cu\(^{2+}\).

**Results**

The total plate count of *Vibrio alginolyticus* from the water sample in the Asian seabass hatchery was 1.3X10\(^4\) colony forming unit (CFU)/ml. In the present study, majority (more than 70%) of the present bacterial isolates was found to be sensitive to nitrofuratoin, furazolidone, tetracycline, doxycycline, florfenicol and oxolinic acid (Figure 1). Whereas the percentage of present bacterial isolates sensitive to colistin sulphate, oleandomycin, fosfomycin, erythromycin, lincomycin, ampicillin, flumequine, and spiramycin was ranged from 0% to 60%. All the present bacterial isolates were found to be resistant to lincomycin. Overall, the total of sensitive case was 50.5% whereas 40% and 9.5% were reported as resistance and intermediary sensitive case. The MAR value of the present study was 0.40. All the present bacterial isolates were found resistant to Hg\(^{2+}\), Cd\(^{2+}\) and Cr\(^{6+}\). However, Cd\(^{2+}\) was found able to inhibit the growth of 80% of present bacterial isolates at concentration of 400µg/mL. On the other hand, 73.3% of the present bacterial isolates were found sensitive to Cu\(^{2+}\).

**Discussion**

Vibriosis due to *Vibrio alginolyticus* was recognized as major problem in aquatic animal farming. It was reported attacking various aquatic animals such as tiger shrimp, *Penaeus monodon* (Lee, et al. 1996), larvae of the catarina scallop, *Argopecten ventricosus* (Sainz, et al. 1998), gilt-head sea bream (*Sparus aurata* L.) (Balebona, et al. 1998), shrimp (George, et al. 2005) and giant freshwater prawn (Khuntia, et al. 2008). Till present, there have few reports on *V. alginolyticus* in Malaysia aquaculture. For instance, Idris et al. (2009) reported the virulence of *V. alginolyticus* in Asian seabass. *V. alginolyticus* was also reported in other aquatic animals in Malaysia. For example, mantis shrimp (Musa and Lee, 2008a), diseased seaweed, *Gracilaria changii* (Musa and Lee, 2008b) and oyster, *Crassostrea iridea* (Musa, et al. 2008). As our knowledge, this is first report on *V. alginolyticus* isolated from Asian seabass in Malaysia.

In the present study, nitrofuratoin, furazolidone, tetracycline, doxycycline, florfenicol and oxolinic acid were to be effective in controlling
V. alginolyticus in Asian seabass. Thus, fish farmer may use these antibiotics for prophylactic and treatment purpose in Asian seabass culture. However, the mentioned antibiotics can not kill all present strains of V. alginolyticus. The result of present study showed that oxolinic acid was only found can control up to 90% of the total present bacterial strains. Therefore, further study should be carried out to find out those antibiotics which can inhibit the growth of all strains of V. alginolyticus. For example, in the study of Thakur et al. (2003) reported that all the bacteria strains of Vibrio spp. including V. alginolyticus isolated from moribund shrimp were sensitive to erythromycin, streptomycin and chloramphenicol. Another study of Musa and Lee (2008a) revealed that all strains of V. alginolyticus isolated from diseased mantis shrimp were sensitive to chloramphenicol, oxytetracycline and furazolidone.

In additional to antibiotic test, high percentage of heavy metal resistance case was observed among isolated V. alginolyticus to the tested heavy metals. This may due the location of our study carried out was surrounded by agricultural activities. Subsequently, the agricultural wastes such as fertilizer consists of heavy metal residues may seep into water source of the Asian seabass hatchery in which our study was conducted. Therefore, bacteria may develop heavy metal resistance gene after exposed to heavy metal residues for a certain period. Till present, there have very few databases on heavy metal resistance pattern of bacteria isolated from aquaculture site in the literature. Therefore, comparison of heavy metal resistance pattern of the present bacterial isolates to the other study can not make.

References


