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RESEARCH ARTICLE

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Comparative study on antibiogram of *Vibrio* spp. isolated from diseased postlarval and marketable-sized white leg shrimp (*Litopenaeus vannamei*)

Abstract This paper compared antibiotic sensitivity between *Vibrio* spp. isolated from diseased postlarval and marketable-sized white leg shrimp (*Litopenaeus vannamei*). Recently, white leg shrimp become target species of shrimp culture among shrimp farmers in Malaysia to replace tiger shrimp (*Penaeus monodon*) culture. However, the baseline information on antibiogram of pathogenic bacteria especially *Vibrio* spp., the causative agent of vibriosis in white leg shrimp culture is not well established. Therefore, this study was conducted to reveal the antibiogram of *Vibrio* spp. isolated from diseased postlarval and marketable-sized white leg shrimp. The information gained from this study is useful for shrimp farmers in selecting appropriate antibiotic during disease outbreak. Antibiogram of present bacterial isolates was determined through disk diffusion method against 21 antibiotics (oxolinic acid 2 μg, ampicillin 10 μg, erythromycin 15 μg, furazolidone 15 μg, lincomycin 15 μg, oleandomycin 15 μg, amoxicillin 25 μg, colistin sulphate 25 μg, sulphamethoxazole 25 μg, chloramphenicol 30 μg, doxycycline 30 μg, florfenicol 30 μg, flumequine 30 μg, kanamycin 30 μg, nalidixic acid 30 μg, novobiocin 30 μg, oxytetracycline 30 μg, tetracycline 30 μg, nitrofurantoin 50 μg, fosfomycin 50 μg, and spiramycin 100 μg). A total of 47 *Vibrio* spp. isolates (*Vibrio parahaemolyticus,* 24, and *V. alginolyticus*, 23) from postlarval white leg shrimp and 49 *Vibrio* spp. isolates (*Vibrio parahaemolyticus*, 13, *Vibrio alginolyticus*, 28, and luminous *Vibrio parahaemolyticus*, 8) from marketable-sized white leg shrimp were successfully identified. Results of antibiotic sensitivity test from the present study showed that more than 80% *Vibrio* spp. from diseased postlarval and marketable-sized white leg shrimp, respectively, were sensitive to 14 out of the 21 tested antibiotics. *Vibrio* spp. isolated from marketable-sized white leg shrimp were found more susceptible to the tested antibiotics than *Vibrio* spp. isolated from postlarval white leg shrimp. This was due to 100% sensitive case against eight antibiotics found among *Vibrio* spp. isolated from marketable-sized white leg shrimp, whereas only three antibiotics were found sensitive to all *Vibrio* spp. isolated from postlarval white leg shrimp. Furthermore, the multiple antibiotic resistance (MAR) index indicated that marketable-sized white leg shrimp were not under high risk exposure to the tested antibiotics. On the other hand, the postlarvae were highly exposed to the tested antibiotics.

Keywords antibiogram, *Vibrio* spp., white leg shrimp (*Litopenaeus vannamei*), postlarval, marketable size

1 Introduction

White leg shrimp (*Litopenaeus vannamei*) is a popular cultured shrimp among shrimp farmers in western countries where 90% of the total shrimp production is from white leg shrimp (Wurmann et al., 2004). By the year 2000, white leg shrimp culture had been introduced by Malaysian shrimp farmers as an alternative to problematic tiger shrimp (*P. monodon*) culture. With high growth rate and disease resistance, white leg shrimp become favorite targets among Malaysian shrimp farmers. However, bacterial disease especially vibriosis has always been a major constraint of shrimp culture development in Malaysia. Until present, there is no scientific report on vibriosis in the white leg shrimp culture in Malaysia, although great damages of this disease are frequently occurred to the white leg shrimp cultures. Recently, Aguirre-Guzma et al. (2001) claimed that the vibriosis caused 85% mortality in white leg shrimp larvae in America and infected various types of aquatic animals at

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100% mortality of the cultured aquatic animals, including mantis shrimp (*Squilla* sp.) (Najiah and Lee, 2008), brine shrimp (*Artemia franciscana*) (Soto-Rodriguez et al., 2003), kuruma prawn (*Marsupenaeus japonicus*) (Liu et al., 1996), ridgeback prawn (*Sicyonia ingentis*), rock lobster (*Jasus verreauxi*) (Diggles et al., 2000), tiger prawn (*P. monodon*) (Lavilla-Pitogo et al., 1990), cobia fish (*Rachycentron canadum*) (Liu et al., 2004), grouper (*Epinephelus coioides*) (Lee et al., 2002), red drum (*Scaienops ocellatus*) (Liu et al., 2003), salmonids (Zhang and Austin, 2000), seahorse (*Hippocampus* sp.) (Alcaide et al., 2001), summer flounder (*Paralichthys dentatus*) (Soffientino et al., 1999), abalone (*Haliotis tuberculata*) (Nicolas et al., 2002), Japanese abalone (*Sulculus diversicolor*) (Nishimori et al., 1998), and pearl oyster (*Pinctada maxima*) (Pass et al., 1987). Therefore, our study was to investigate the species of *Vibrio* spp. found in the diseased postlarval and marketable-sized white leg shrimp in Malaysia as well as their antibiogram, which is beneficial to the shrimp farmers in terms of white leg shrimp farm health management.

2 Materials and methods

2.1 Bacterial isolation and identification

Both diseased postlarval and marketable-sized *L. vannamei* were collected from an *L. vannamei* nursery tank at a commercial hatchery and a growing pond, respectively, in Pahang, Malaysia. One thousand *L. vannamei* postlarvae were randomly sampled from the nursery tank. They were divided into four groups. Each group contained 250 postlarvae in 100 mL of sterile physiological saline homogenized using a sterile glass rod. One ml of the sample was serially diluted in sterile physiological saline and placed on seven media of Tryptic Soy Agar (TSA), Mac Conkey (MC), Thiosulphate Citrate Bile Salt (TCBS), Eosin Methylene Blue (EMB), Glutamate Starch Pseudomonas (GSP), Xylose Lysine Deoxycholate (XLD), and Baird Parker (BP), which was provided by Merck, Germany. A hundred of diseased marketable-sized white leg shrimp were sampled randomly from the growing pond. Hepatopancreas of the marketable-sized *L. vannamei* was homogenized in 1 mL of sterile physiological saline using sterile glass rod. All the inoculated media were incubated at room temperature for 24 to 48 h. The bacterial colonies growing on the media were further screened and identified using conventional biochemical tests (Holt et al., 1994) and confirmed with a commercial identification kit (BBL, USA).

2.2 Antibiotic susceptibility test and multiple antibiotic resistance (MAR) index determination

The present isolates were cultured in tryptic soy broth (TSB) (Oxoid, England) for 24 h at room temperature. The bacterial cells were then centrifuged at 14500 $r \cdot min^{-1}$ for 5 min by using MiniSpin (Eppendorf, Germany). The concentration of the bacterial cells were adjusted to $\times 10^6$ colony forming unit (CFU) by using saline and monitored by Biophotometer (Eppendorf, Germany). The bacterial inoculums were swabbed on Mueller Hinton Agar (Oxoid, England). Antibiotic susceptibility test was conducted according to Kirby–Bauer Disk Diffusion Method (Bauer et al., 1966). The antibiotics tested included oxolinic acid (2 µg), ampicillin (10 µg), erythromycin (15 µg), furazolidone (15 µg), lincomycin (15 µg), oleandomycin (15 µg), amoxicillin (25 µg), colistin sulphate (25 µg), sulphamethoxazole (25 µg), chloramphenicol (30 µg), doxycycline (30 µg), florfenicol (30 µg), flumequine (30 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), oxytetracycline (30 µg), tetracycline (30 µg), nitrofurantoin (50 µg), fosfomycin (50 µg), and spiramycin (100 µg) (Oxoid, England). Interpretation of the results, namely, in sensitivity (S), intermediary sensitivity (I), and resistance (R), was made in accordance with the standard provided by Clinical and Laboratory Standards Institute (CLSI, 2006). The MAR index of the present isolates against the tested antibiotics was calculated by using the following formula (Sarter et al., 2007):

$$\text{MAR index (multiple antibiotic resistance)} = X/(Y \times Z),$$

where X = total of antibiotic resistance case, Y = total of antibiotic used in the study, and Z = total of isolates.

A MAR index value of $\leqslant 0.2$ was defined as an indication that the antibiotics were seldom or never been used for animals with regard to treatments, whereas the MAR index value of > 0.2 was considered as an indication that the animals received high risk exposure to the antibiotics.

3 Results

In the present study, both luminous and non-luminous *Vibrio parahaemolyticus* isolates were curved, rod shaped, and Gram-negative. They appeared dark green, humid shiny, and 2 to 4 mm in diameter on TCBS agar. The bacterial isolates were positive in lysine decarboxylase, indole production, and motility test but failed to utilize sucrose. On the other hand, the *V. alginolyticus* isolates were positive in oxidase and gelatinase test but negative in arginine dihydrolase test. Biochemical test results showed that the bacterial isolates were able to utilize mannitol, mannose, sucrose, and leucine but unable to utilize cellobiose and lactose. Furthermore, all *Vibrio* spp. isolates were sensitive to 0/129 vibriostat at 10 µg and 150 µg per disk.

No other bacteria species were isolated from the diseased postlarval white leg shrimp except for *V.*

parahaemolyticus and *V. alginolyticus* found in the diseased postlarval white leg shrimp. Meanwhile, *V. parahaemolyticus*, luminous *V. parahaemolyticus*, and *V. alginolyticus* were successfully isolated from the diseased marketable-sized white leg shrimp. A total of 47 *Vibrio* spp. isolates (*V. parahaemolyticus*, 24, and *V. alginolyticus*, 23) were found in the postlarval white leg shrimp, whereas 49 *Vibrio* spp. isolates (*V. parahaemolyticus*, 13, *V. alginolyticus*, 28, and luminous *V. parahaemolyticus*, 8) were obtained from the marketable-sized white leg shrimp. Antibiotic sensitivity test results showed that more than 80% of *Vibrio* spp. from the post larval white leg shrimp were sensitive to oxolinic acid, erythromycin, furazolidone, lincomycin, colistin sulphate, chloramphenicol, florfenicol, flumequine, kanamycin, nalidixic acid, novobiocin, nitrofurantoin, fosfomycin, and spiramycin (Fig. 1). Whereas, most of the bacterial isolates obtained from the marketable-sized white leg shrimp were sensitive to oxytetracycline, tetracycline, oxolinic acid, erythromycin, furazolidone, chloramphenicol, florfenicol, flumequine, kanamycin, nalidixic acid, novobiocin, nitrofurantoin, doxycycline, and spiramycin (Fig. 2). *Vibrio* spp. isolated from the marketable-sized white leg shrimp was found more susceptible to the tested antibiotics that from the post larval white leg shrimp. This was due to 100% sensitivity of *Vibrio* spp. to eight antibiotics (oxolinic acid, chloramphenicol, florfenicol, kanamycin, nalidixic acid, novobiocin, nitrofurantoin, and doxycycline) found in *Vibrio* spp. isolated from the marketable-sized white leg shrimp. However, the *Vibrio* spp. isolated from the post larval white leg shrimp was found sensitive to only three antibiotics (kanamycin, nalidixic acid and florfenicol). Overall, MAR index indicated that marketable-sized white leg shrimp were not under high risk exposure to the tested antibiotics (Table 1). On the contrary, the postlarval white leg shrimp were deemed to be highly exposed to the tested antibiotics.

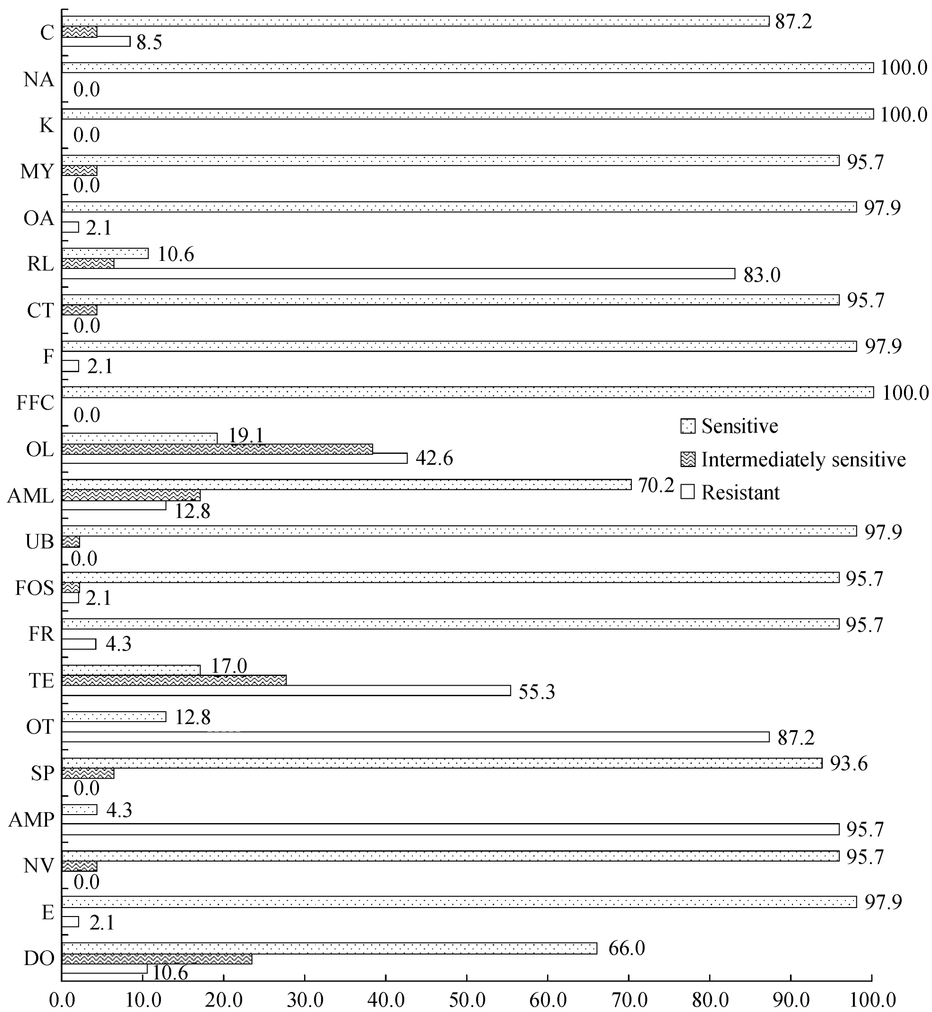

Fig. 1 Total percentage sensitivity of *Vibrio* spp. isolated from postlarval white leg shrimp to 21 antibiotics

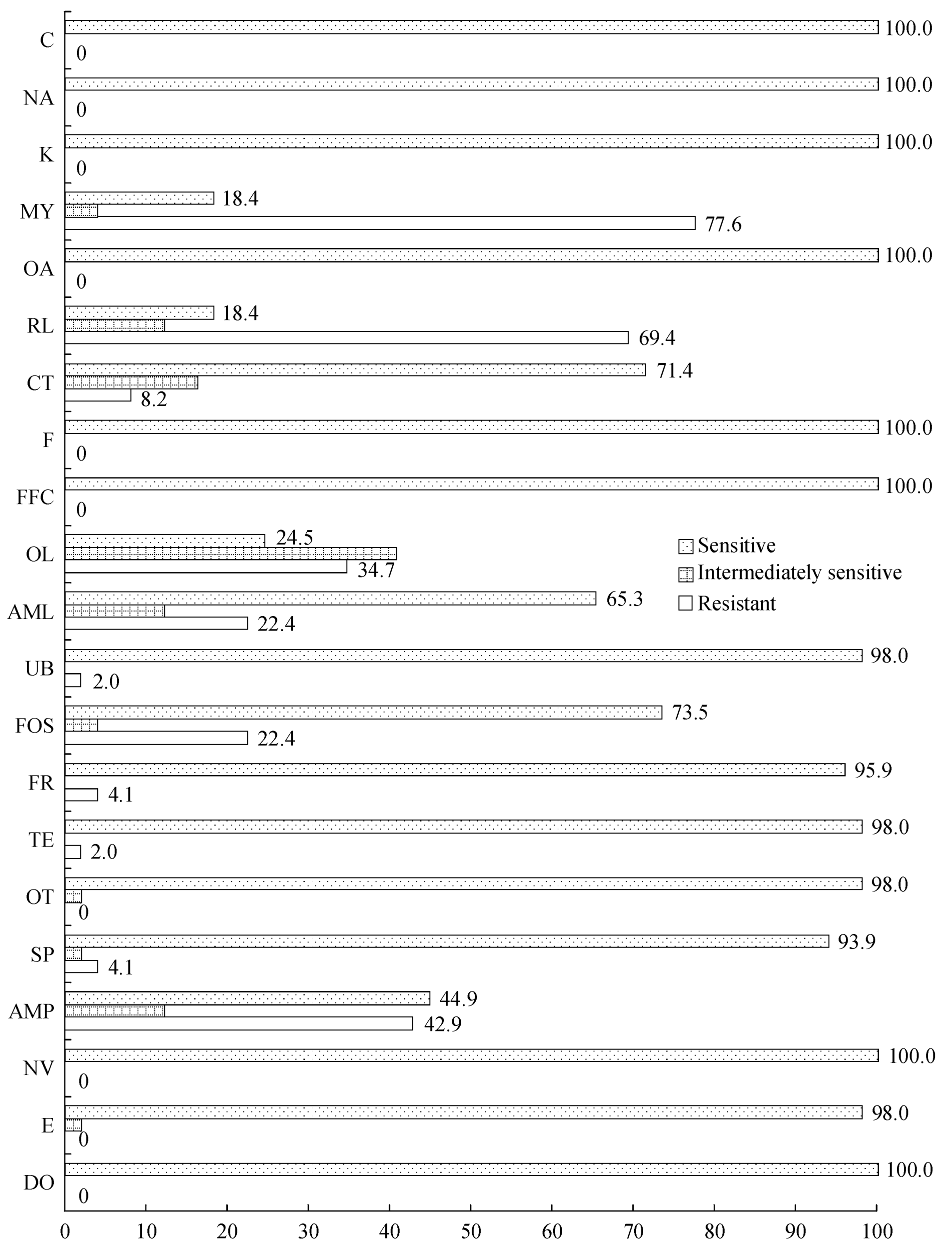

Fig. 2 Total percentage sensitivity of *Vibrio* spp. isolated from marketable-sized white leg shrimp to 21 antibiotics

Table 1 Multiple antibiotic resistance (MAR) value of *Vibrio* spp. isolated from post-larval and marketable-sized white leg shrimp

bacterial isolates	MAR value	
	postlarval white leg shrimp	marketable-sized white leg shrimp
V. alginolyticus	0.30	0.10
V. parahaemolyticus	0.09	0.11
luminous *V. parahaemolyticus*	–	0.19
total bacterial isolates	0.26	0.17

4 Discussion

Based on the results of the present study, we concluded that the postlarval white leg shrimp were infected with vibrios in which *V. parahaemolyticus* and *V. alginolyticus* were causative of the infection, while *V. parahaemolyticus*, luminous *V. parahaemolyticus* and *V. alginolyticus* were causative agents in the marketable-sized white leg shrimp. Recently, Najiah et al. (2008) observed that vibrios in tiger shrimp (*Penaeus monodon*) from three farms in three states (Kedah, Terengganu, and Johore) in Malaysia were due to

V. harveyi. However, no luminous *V. harveyi* was isolated from the diseased postlarval and marketable-sized white leg shrimp. This is the first report on the vibrios in white leg shrimp in Malaysia that *V. parahaemolyticus*, luminous *V. parahaemolyticus*, and *V. alginolyticus* are the causative agents of the disease.

Vibrio spp. isolated from the marketable-sized white leg shrimp was found more susceptible to the tested antibiotics than that from the postlarval white leg shrimp, which resulted from the more frequent application of antibiotics in the white leg shrimp hatchery compared to the culture-pond. *Vibrio* spp. isolated from the marketable-sized white leg shrimp was found 100% sensitive to eight antibiotics (oxolinic acid, chloramphenicol, florfenicol, kanamycin, nalidixic acid, novobiocin, nitrofurantoin, and doxycycline), whereas *Vibrio* spp. isolated from the postlarval white leg shrimp was found sensitive to only three antibiotics (kanamycin, nalidixic acid, and florfenicol). Therefore, shrimp farmers may use these antibiotics for prophylactic and treatment purposes in the shrimp hatchery. However, Malaysian government has banned oxolinic acid, chloramphenicol, and nitrofurantoin as antimicrobial agents in aquaculture. Based on this study, the farmers can make decisions on appropriate antibiotics to their farms. Furthermore, Malaysian shrimp farmers may also refer to praactices against vibriosis in shrimp cultures of other Asian countries. For instance, in India, chloramphenicol, erythromycin, and streptomycin were used to combat the antibiotic resistant-vibrios vibrios in shrimp culture (Karunasagar et al., 1994), whereas the farmers in Thailand tend to use kanamycin and carbenicillin for that purpose (Nakayama et al., 2006). Types of antibiotics used for the treatment of vibrios in shrimp culture vary among countries. For example, nitrofurantoin, novobiocin, and sulfonamide were applied in shrimp culture to the vibrio treatment in Taiwan, China (Liu et al., 1997), while the shrimp farmers in the Philippines applied many types of antibiotics to the vibrio treatment, including oxytetracycline, furazolidone, oxolinic acid, chloramphenicol (Tendencia and de la Pena, 2001), kanamycin, gentamycin, carbenicillin, and ampicillin (Nakayama et al., 2006). However, in Java Island, Indonesia, only tetracyclin, ampicillin and other beta-lactams were applied to treat their infected shrimps with vibriosis (Teo et al., 2000, 2002). In Mexico, ampicillin, amikacin, carbenicillin, cephalotin, and oxytetracycline were allowed in the vibriosis treatment in shrimp culture (Molina-Aja et al., 2002).

In general, the multiple antibiotic resistance (MAR) index indicated that the postlarval but not marketable-sized white leg shrimp were highly exposed to the tested antibiotics, which also implicit that the marketable-sized white leg shrimp are safe for human consumption.

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