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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (6): 293-297, 2010
ISSN 1028-8880

Bacteria Associated with Wild Mud Crab (*Scylla serrata*) from Setiu Wetland, Malaysia with Emphasis on Antibiotic Resistances

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Abstract: A study was carried out to investigate the presence of bacteria flora in wild mud crab (*Scylla serrata*) from Setiu Wetland as well as their antibiotic resistances. A total of 91 bacterial isolates consisting of 12 bacterial species were successfully isolated from mud crab. Oxolinic acid was found to be effective against all the bacterial isolates whilst the highest percentage of antibiotic resistance was shown by lincomycin (94.5%) followed by ampicillin (90.1%), amoxicillin (86.8%) and oleandomycin (78.0%). The study is very useful to evaluate the safety of mud crab for human consumption based on wild mudcrab-associated bacteria as well as their antibiotic resistances.

Key words: Wild mud crab, bacteria flora, antibiotic resistance, Malaysia

INTRODUCTION

Wild mud crab, *Scylla serrata* is a favorite local dish among Malaysians. This mud crab species has higher price compared to shrimp due to its juicy flesh quality and bigger size (Quinitio *et al*., 2001). In Malaysia, the culture and fattening of mud crab is quite slow due to lack of wild juveniles for culture and adult crabs for fattening process (Shelley, 2008). Microbial infections has been the major concern of aquaculturists worldwide. Various bacteria in marine and estuarine environment such as *Vibrio parahaemalyticus*, *Vibrio cholerae* and *Vibrio* species are potential human pathogens (Broza *et al*., 2007; Senderovich *et al*., 2010). The use of antibiotics in aquaculture practices influenced the bacteria population in the environment (Chelossi *et al*., 2003). Antibiotics such as oxytetracyline, oxolinic acid and quinolones are usually reported in fish farming (Tyrpenau and Rigos, 2004; Halling-Sorensen *et al*., 1998). As a result, presences of antibacterial residues in fish farms were reported in marine sediments and invertebrates (Weston, 1996; Brillantes *et al*., 2001). As crabs are in close contact with the milieu that rich in pathogenic bacteria, infection by bacteria can be vast (Hudson and Lester, 1994). In relation to the health of mud crabs and concerning food safety for human consumption, it was crucial to understand the susceptibilities of bacteria flora in the mud crabs to antibiotics. Due to the lacking of scientific documentation regarding this aspect, a study was conducted to isolate bacteria flora from mud crabs and to evaluate the bacterial resistances to antibiotics.

MATERIALS AND METHODS

Sampling area: The study was carried out from January 2008 to May 2008. The Setiu Wetland (N 05° 40' 38.6" E 102° 43' 03.2") is consisted of Setiu River and lagoon with numerous islands. The lagoon drains into the South China Sea via an opening at Kuala Setiu. Mangroves dominate the area and the area is rich with invertebrates and mollusks. Water temperature, dissolved oxygen and salinity were 30.5°C, 5.6 mg L^{-1} and 29.5 ppt, respectively.

Collection of mud crabs: A total of 50 live wild mud crabs weighed from 300 to 500 g were caught at Setiu Wetland, Malaysia. They were transported to Fish Disease Laboratory, University Malaysia Terengganu within an hour and were subjected to immediate analysis. The crabs were sacrificed according to RSPCA Guidelines (2006). Briefly the crabs were chilled in a refrigerator and then killed by spiking to destroy the nerve centres. Aseptically, each body parts namely abdominal contents, gill and

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hepato-pancreas was swabbed by using sterile cotton bud and streaked onto universal agar namely Trypticase Soy Agar (TSA) and selective agar such as Cytophaga Agar (CA), Glutamate Starch Pseudomonas (GSP), Thiosulphate Citrate Bile Salt Sucrose (TCBS) and Xylose Lysine Deoxycholate (XLD) agar (Merck, Germany). The plates were then incubated at 30°C for 24 to 48 h. Then, five colonies which represent different morphologies per plate were selected from each sample and restreaked three times onto nutrient agar plates to ensure pure bacterial culture. Phenotypic characteristics, Gram staining and oxidase production were determined for all isolates accordingly (Holt *et al*., 1994; Whitman and MacNair, 2004). Further identification was carried out using a commercial identification system kit (BBL Crystal, USA) following manufacturers instruction.

Antibiotic susceptibility testing: Antibiotic susceptibility test was conducted according to Kirby-Bauer disk diffusion method (Bauer *et al*., 1966) by using Mueller Hinton agar (Oxoid, England). The antibiotics were erythromycin (E), 15 μg; spiramycin (SP), 100 μg; oxytetracycline (OT), 30 μg; furazolidone (FR), 15 μg; kanamycin (K), 30 μg; nalidixic acid (NA), 30 μg; chloramphenicol (C), 30 μg; ampicillin (AMP), 10 μg; Sulphamethoxazole (RL), 25 μg; amoxicillin (AML), 25 μg; colistin sulphate (CT), 25 μg; Doxycycline (DO), 30 μg; florfenicol (FFC), 30 μg; flumequine (UB), 30 μg; fosfomycin (FOS), 50 μg; lincomycin (MY), 15; μg nitrofurantoin (F), 50 μg; novobiocin (NV), 30 μg; oleandomycin (OL) 15 μg; oxolinic acid (OA), 2 μg and tetracycline (TE) 30 μg (Oxoid, England).

RESULTS

In total 91 bacterial isolates were obtained from 50 wild mud crabs representing 12 bacterial species. *Aeromonas hydrophila* (54.9%) was the most frequently isolated bacteria from mud crab followed by *Vibrio parahaemolyticus* (19.8%), *V. alginolyticus* (4.4%), *V. cholera* (6.6%), *Chromobacterium violaceum* (4.4%), *Acinetobacter baumanii* (2.2%), *Pseudomonas aeruginosa* (1.1%), *Hafnia alvei* (2.2%), *Morganella morganii* (1.1%), *Escherichia coli* (1.1%), *Plesiomonas shigelloides* (1.1%) and *Shewanella putrefaciens* (1.1%). Table 1 showed most bacteria species were isolated from abdominal contents than from hepatopancreas and gills. A total of 62.0 and 12.4%, respectively, of the bacteria were classified as sensitive and intermediary sensitive (Table 2). On the other hand, the incidence of antibiotic resistance was 25.6%. All bacterial isolates in the present study were sensitive to oxolinic acid whereas more than 80.0% of the bacterial isolates were sensitive to kanamycin, furazolidone, erythromycin, chloramphenicol, doxycycline, florfenicol, flumequine, nalidixic acid, oxytetracycline and tetracycline. Most of the present bacterial isolates were found to be resistant to lincomycin (94.5%), ampicillin (90.1%), amoxicillin (86.8%) and oleandomycin (78.0%). Less than 10.0% of the bacterial isolates were found to be resistant to the rest of the antibiotics.

Table 1: Bacteria detected in abdomen, hepatopancreas and gills of wild mud crab

Bacteria	Abdomen	Hepatopancreas	Gill
A. hydrophila	+	+	+
V. parahaemolyticus	+	+	+
V. alginolyticus	+	+	+
V. cholerae	+	+	-
C. violaceum	-	+	-
A. baumanii	+	-	-
E. coli	+	-	-
P. shigelloides	+	-	-
H. alvei	+	-	-
P. aeruginosa	+	-	-
S. putrafaciens	+	-	-
M. morganii	+	-	-

+: Presence of bacterial species; -: Absence of bacterial species

Table 2: Susceptibility of 91 bacteria isolates of wild mudcrab against 21 antibiotics

	Sensitive		Intermediary sensitive		Resistant	
Antibiotic[a]	Incidence	%	Incidence	%	Incidence	%
OA2	91	100.0	0	0.0	0	0.0
AMP10	5	5.5	4	4.4	82	90.1
E15	75	82.4	9	9.9	7	7.7
FR15	81	89.0	7	7.7	3	3.3
MY15	2	2.2	3	3.3	86	94.5
OL15	7	7.7	13	14.3	71	78.0
AML25	4	4.4	8	8.8	79	86.8
CT25	11	12.0	38	41.8	42	46.2
RL25	23	25.2	29	31.9	39	42.9
C30	86	94.5	2	2.2	3	3.3
DO30	86	94.5	1	1.1	4	4.4
FFC30	86	94.5	2	2.2	3	3.3
UB30	88	96.7	0	0.0	3	3.3
K30	81	89.0	9	9.9	1	1.1
NA30	89	97.8	1	1.1	1	1.1
NV30	26	28.6	42	46.1	23	25.3
OT30	87	95.6	0	0.0	4	4.4
TE30	86	94.5	1	1.1	4	4.4
F50	64	70.3	22	24.2	5	5.5
FOS50	51	56.0	21	23.1	19	28.9
SP100	55	60.4	25	27.5	11	12.1
Total	1184	62.0	237	12.4	490	25.6

[a]Oxolinic acid 2 μg (OA2); Ampicillin 10 μg (AMP10); Erythromycin 15 μg (E15); Furazolidone 15 μg (FR15); Lincomycin 15 μg (MY15); Oleandomycin 15 μg (OL15); Amoxicillin 25 μg (AML 25); Colistin sulphate 25 μg (CT25); Sulphamethoxazole 25 μg (RL25); Chloramphenicol 30 μg (C30); Doxycycline 30 μg (DO30); Florfenicol 30 μg (FFC 30); Flumequine 30 μg (UB30); Kanamycin 30 μg (K30); Nalidixic acid 30 μg (NA30); Novobiocin 30 μg (NV30); Oxytetracycline 30 μg (OT30); Tetracycline 30 μg (TE30); Nitrofurantoin 50 μg (F50); Fosfomycin 50 μg (FOS50); Spiramycin 100 μg (SP100)

DISCUSSION

Bacteria isolated from the present study could be pathogenic and involved in disease transmission to human. Previous research on pathogenic microorganisms isolated from crabs include Faghri *et al.* (1984) who found tissues of several types of crabs such as tanner crab *Chionoecetes opilio*, Dungeness crab *Cancer magaster*, King crab *Paralithodes camtschatica* and Rock crab *Cancer irroratus* could serve as accumulation sites for human pathogens particularly in crabs collected from contaminated area. In their study, *Klebsiella* species and other enteric bacteria excluding *E. coli* were isolated from crab tissues. Hauxhurst *et al.* (1980, 1981) indicated that crab tissues contained higher number of bacteria than their surrounding environments. In addition, Tison *et al.* (1982, 1984) reported that the presence of *Vibrio* species from marine ecosystems have led to a reevaluation of the taxonomy of this group and the definition of several new species that are potential human pathogens. Lavilla-pitogo *et al.* (2001) observed significant diseases such as shell disease in tank-held mud crab *Scylla* sp. broodstock. In all three crab species, *S. tranquebarica*, *S. olivacea* and *S. serrata*, the total bacterial count (cfu/0.1 g) was around 10^4 to 10^6 with presumptive Vibrio count (cfu/0.1 g) around 10^3 to 10^5. *Vibrio vulnificus*, *V. splendidus* and *V. orientalis* were found to contribute to shell disease in crab as their aggregate formed on the shell causing gradual damage and perforation. They also observed that almost 67.0% of newly recruited crabs harbored mixed populations of bacteria in the hemolymph, mainly dominated by sucrose fermenting vibrios.

Most of the bacteria species found in the present study were comparable to bacteria found in cultivated oyster in Setiu Wetland (Najiah *et al.*, 2008). In the present study, the bacterial species found in the mudcrab were not quantitatively analyzed but qualitatively identified. According to Najiah *et al.* (2008), although, vibrio species are ubiquitous in estuarine and marine environment, the ability of these bacterial species sampled from cultivated oyster in Setiu Wetland to hemolyse blood could indicate the presence of some virulence factors.

The presence of *E. coli* in the present study further suggests that fecal contamination occurred in Setiu Wetland area. Being deposit-feeding animals which feed on plants and animal debris buried in mud, mud crabs may accumulate microorganisms from their environment and therefore could serve as a vector for disease transmission. These warrants further studies in the bacterial distribution at Setiu Wetland area.

There has been less attention paid to the risk of antibiotic use in fish farming to human health. Other than marine system (Weston, 1996), there has been a report on the occurrence of drug resistance microorganisms in freshwater eel farm system (Alcaide *et al.*, 2005). In addition to transfer of resistant microorganisms through consumption of contaminated mudcrab, there is a substantial risk to environmental contamination due to the practice of using medicated feeds to treat bacterial disease. Furthermore, antibiotics do not only act against pathogenic bacteria but also to the normal microbial flora in both animals and humans. Therefore, it is also important to monitor the antibiotic resistance incidence not only of pathogenic bacteria but also against normal bacteria flora. Oxolinic acid was the most effective antibiotic in inhibiting the bacteria present in mud crab. In the present study, relatively higher numbers of bacterial isolates were resistant to lincosamides (lincomycin), B-lactam (ampicillin, amoxicillin) and macrolides (oleondomycin). The antibiotic resistance might suggest a signal of antibiotic ineffectiveness in mud crab as well as disease-associated due to consumption of mud crab harvested from the study area. The loss of antibiotic susceptibility among the aquatic bacteria could also enhance by the physicochemical qualities of water and seasonal variation (Pathak *et al.*, 1993). Hence, it is still vital for the local authority to introduce strict guideline of antibiotics use and to establish surveillance of antimicrobial resistance of normal bacteria flora of the coastal animal inhabitants of Setiu Wetland. At present, there is less information, that allows an updated estimation on the degree of antibiotic resistance associated with Malaysian aquaculture (Lee *et al.*, 2010).

In the present study, the bacteria species found in the gills, abdomen and hepatopancreas did not cause mortality to mudcrabs. This could be due to the ability of the serum of *S. serrata* to agglutinate bacteria which further indicate the involvement of humoral agglutinins in host-defense response (Jayaraj *et al.*, 2010).

The present study concluded that wild mud crabs of Setiu Wetland contain antibiotic resistances bacteria. As a consequence, these bacteria could transfer their antibiotic resistance genes to bacteria from other aquaculture sites and other organisms in the food chain, including human. Therefore, comprehensive monitoring and regular analysis on crabs should be implemented to provide an early warning to the public for the presence of antibiotic resistances bacteria in wild mud crabs.

ACKNOWLEDGMENTS

The project was funded by Malaysian Ministry of Science, Technology and Innovation (Grant No. 03-01-12-SF0082). We thanked Universiti Malaysia Terengganu for providing the facilities and Mr. Lee Seong Wei and Mr. Soh Chong Poh for their assistance during sample collections.

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