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Occurrence of *Plesiomonas shigelloides* in Cultured Red Hybrid Tilapia (*Oreochromis niloticus*) from Tropical Rivers, East Coast Malaysia

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Abstract: A total of 182 isolates of *Plesiomonas shigelloides* were identified from 40 healthy red hybrid tilapia, *Oreochromis niloticus* cultured at two important rivers in Terengganu, Malaysia namely Como River and Terengganu River from east coast Malaysia. *P. shigelloides* count in Digestive Tract Content (DTC) and Muscle (MUS) of red hybrid tilapia cultured at Terengganu River was 1000-fold higher than Como River. Antibiotic susceptibility test was also performed on *Plesiomonas shigelloides* isolates. The incidence of antibiotic resistance was higher in *Plesiomonas shigelloides* isolated from red hybrid tilapia cultured at Terengganu River compared to Como river. Thus, the findings of the study indicate that *P. shigelloides* from tilapia muscle and an intestine could be an alarming for serious public health risk to consumers.

Key words: Red hybrid tilapia, *Plesiomonas shigelloides*, digestive tract content, muscle

INTRODUCTION

Various microorganisms such as viruses, bacteria and protozoa can penetrate into fish body that reared in polluted water. Thus, the presence of pathogenic microorganisms in food fish represents a safety concern, particularly if fish is eaten raw or semi-cooked. *Plesiomonas shigelloides* is regarded as a potential human enteropathogen. For instance, there were cases of *P. shigelloides* isolated from human diarrhoeic stools as reported in Bangladesh (Albert *et al*., 1999), Malaysia (Rahizan *et al*., 1999), Taiwan (Yeh and Tsal, 1991). So far, most human infections with *P. shigelloides* are transmitted through waterborne. Hence, a study was carried out to investigate the presence of *P. shigelloides* in fish digestive tract and muscle of cultured red hybrid tilapia, *Oreochromis niloticus* in Terengganu, Malaysia. Antibiogram *P. shigelloides* isolates was also determined.

MATERIALS AND METHODS

Two commercial red hybrid tilapia cultured sites were chosen; Como River and Terengganu River. Terengganu River is a very complex system with increasing environmental impacts of sand mining and sewage treatment plant activities. In contrast, Como River is located in an isolated place, far from human habitats and activities.

At each sampling location, 9 sites (L_n) were chosen for water quality sampling and another 4 sites for fish sampling (S_n). The coordinate of the selected locations were shown in Table 1.

A total of 10 healthy red hybrid tilapia, *Oreochromis niloticus* were sampled randomly from each fish sampling sites. Fish were collected using a fish scoop and were put into separate sterile plastic bags with aeration and immediately transported to Fish Disease Laboratory, Universiti Malaysia Terengganu (UMT).

Table 1: Water quality sampling sites of Terengganu river and Como river

Location	Coordinate
Terengganu river	
L_1	N05°13.586'E103°02.374'
L_2	N05°13.587'E103°02.373'
L_3	N05°13.586'E103°02.372'
L_4	N05°13.689'E103°02.367'
L_5	N05°13.689'E103°02.364'
L_6	N05°13.687'E103°02.373'
L_7	N05°13.782'E103°02.361'
L_8	N05°13.781'E103°02.354'
L_9	N05°13.781'E103°02.348'
Como river	
L_1	N05° 01.790'E102°50.568'
L_2	N05° 01.790'E102°50.570'
L_3	N05° 01.785'E102°50.537'
L_4	N05° 01.753'E102°50.537'
L_5	N05° 01.757'E102°50.544'
L_6	N05° 01.761'E102°50.551'
L_7	N05° 01.680'E102°50.508'
L_8	N05° 01.680'E102°50.510'
L_9	N05° 01.675'E102°50.513'

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At the laboratory, the fish were put under sedation using 250 mg L^{-1} MS222 (Tricaine methanesulfonate) before humanely killed. Digestive Tract Content (DTC) and Muscle (MUS) samples were aseptically taken out and homogenized using sterile physiological saline. Ten-fold serial dilutions were made from each sample (from 10^{-1} to 10^{-20}). The dilutions were plated onto a selective agar, Inositol Brilliant Green Bile Salt agar (IBB, Hi Media) and incubated at 30°C for 24-48 h. Typical *P. shigelloides* colonies were selected and sub-cultured on the Tryptic Soy Agar (TSA) for further oxidase test. Bacterial identification was confirmed using BBL Crystal Identification Kit Enteric/Non fermenter ID Kit (BBL, USA). *P. shigelloides* strains isolated in the present study were also subjected to antibiotic susceptibility test against 7 antibiotics (OXOID, England) namely ampicillin (AMP), 10 μg; chloramphenicol (C), 30 μg; furazolidone (FR), 15 μg; kanamycin (K), 30 μg; Nalidixic Acid (NA), 30 μg; oxytetracycline (OTC), 30 μg and sulfamethoxazole (RL), 25 μg following a modified Kirby-Bauer disk-diffusion standard method (Bauer *et al*., 1966). The results were recorded by measuring the diameter of the inhibition zones and compared with standards for antimicrobial disk susceptibility tests as described by National Committee of Clinical Laboratory Standards (1993).

Water samples (500 mL) were taken approximately 20 cm below the water surface by using Van Dorn water sampler (KC Denmark) from each identified locations. Water samples collected were then transported in ice box to the laboratory for bacteriological analysis. All quantifications were made in triplicate. Temperature, salinity, Dissolved Oxygen (DO), Total Dissolved Solid (TDS) and pH were measured *in situ* by using the Hydrolab® (YSI 6600 multiparameter). Instrument was calibrated prior to use according to manufacturer's instructions.

RESULTS

Water quality parameters at all sampling sites were shown in Table 2. Temperature, TDS values, pH was higher in Como River than Terengganu river except for higher Dissolved Oxygen (DO). Salinity readings however, were similar at all locations.

Mean bacterial count from MUS and DTC sampled from fish at both locations were shown in Table 3. Bacterial counts of *P. shigelloides* in both MUS and DTC were 1000-fold higher in fish cultured at Terengganu River than fish in Como River.

Isolates of *P. shigelloides* from Como River were found to be highly resistant to oxytetracycline when compared to other antibiotics. The percentage antibiotic resistance of the isolates observed between MUS and DTC of red hybrid tilapia from Como River were: nil (0) for both chloramphenicol and kanamycin; 53.0 and 33.0% for oxytetracycline; 13.0 and 0.0% for nalidixic acid; 40.0 and 31.0% for furazolidone; 4.0 and 26.0% for ampicillin and 2.0 and 0.0% for sulfamethoxazole. However, isolates of *Plesiomonas* spp. from Terengganu River were found to be resistant to ampicillin, furazolidone and oxytetracycline. The percentage of antibiotic resistance were: chloramphenicol 30.0 and 22.0%; oxytetracycline 25.0 and 7.5%; nalidixic acid 15.0 and 10.0%; kanamycin 7.5 and 0.0%; furazolidone 57.0 and 47.0%; ampicillin 57.0 and 45.0% and sulfamethoxazole both 2.5%.

Table 2: Water quality parameters recorded at Terengganu and Como river

	Parameters				
Location	Temp. (°C)	TDS (g L^{-1})	Salinity (ppt)	DO (mg L^{-1})	pH
Terengganu river					
L_1	25.70	0.02	0.01	6.16	7.31
L_2	25.64	0.02	0.01	4.98	7.09
L_3	25.71	0.02	0.01	6.07	6.57
L_4	25.73	0.02	0.01	5.75	6.50
L_5	25.71	0.02	0.01	5.23	6.81
L_6	25.69	0.02	0.01	5.17	6.82
L_7	25.79	0.02	0.01	4.82	7.06
L_8	25.73	0.02	0.01	4.78	7.10
L_9	25.71	0.02	0.01	4.08	7.03
Como river					
L_1	30.45	0.16	0.01	4.50	7.51
L_2	30.45	0.16	0.01	4.37	7.34
L_3	30.48	0.16	0.01	4.34	7.39
L_4	30.48	0.16	0.01	4.29	7.33
L_5	30.47	0.16	0.01	4.27	7.31
L_6	30.48	0.16	0.01	4.23	7.21
L_7	30.49	0.16	0.01	4.21	7.27
L_8	30.50	0.16	0.01	4.20	7.23
L_9	30.49	0.16	0.01	4.19	7.29

Table 3: Mean bacterial count of *P. shigelloides* isolated from muscle (MUS) and digestive tract content (DTC) of red hybrid tilapia from Como and Terengganu river

	Count (CFU g^{-1})	
Station	MUS	DTC
Como river		
S_1	8.30×10^{13}	3.24×10^{13}
S_2	11.6×10^{13}	2.81×10^{13}
S_3	7.69×10^{13}	2.60×10^{13}
S_4	8.75×10^{13}	2.49×10^{13}
Terengganu river		
S_1	3.33×10^{16}	3.19×10^{16}
S_2	2.81×10^{16}	2.97×10^{16}
S_3	2.57×10^{16}	2.76×10^{16}
S_4	1.98×10^{16}	2.75×10^{16}

DISCUSSION

Fish and shellfish are natural habitat of *P. shigelloides* (Krovacek *et al*., 2000). Van Damme and Vandepitte (1980) found that isolation rates of these

bacteria from fish are as high as compared to mammals. *P. shigelloides* has been isolated from the intestines of 59% of freshwater fish in Zaire while 10.2% of freshwater fish in Japan (Arai *et al*., 1980). Fish infected with *P. shigelloides* were reported to suffer from catarrhal and hemorrhagic enteritis, hepatopancreatic degeneration, ventricular hemorrhage, renal edema, gall bladder dilation and skin pathology (Bardon, 1999).

In the present study, bacterial count in both intestine (DTC) and muscle (MUS) of cultured red hybrid tilapia at Terengganu River was higher than Como River. These could be due to sand pumping activities as well as sewage activities that drained into the river which indirectly expose the fish to the bacteria. In addition, overfeeding could also results in higher bacteria loads in water (Karakassis *et al*., 2000). For example, red hybrid tilapia cultured at Terengganu River were fed 5 times per day as compared to red hybrid tilapia at Como River which were fed 2 times per day. Accumulation of feed could deteriorate the surrounding water quality and this would contribute to higher bacterial population particularly at the base of ponds as reported by Schubert and Pelz (1993). It is probable that poor water quality may have induced weakness in the fish, resulting in a greater susceptibility to bacterial infection. Poor water quality could also induce stress to fish which is manifested in elevated cortisone level, a hormone known to be a very potent immunosuppressant (El-Shafai *et al*., 2004).

According to the Guzman *et al*. (2004), retention of foreign bacteria in the digestive tract of freshwater fish species is not only depend on water quality and the ingestion of contaminated food. Other factors, such as other bacteria or toxins in digestive tract may inhibit the presence or growth of bacteria (Jalal *et al*., 2009, 2010).

In the present study, *P. shigelloides* from cultured red hybrid tilapia in Como River were resistant to oxytetracycline, whereas, *P. shigelloides* from cultured red hybrid tilapia in Terengganu River were resistant to ampicillin, furazolidone and oxytetracycline. The results of antibiotic resistance and multiple drug resistance found are fairly consistent with many reported studies of fish pathogens and aquaculture environments (Schmidt *et al*., 2000; Hatha *et al*., 2005; Jacobs and Chenia, 2007). Thus, our study warrants frequent monitoring on antibiotic use in cultured tilapia as frequent and excessive uses of antibiotics could pose hazard to consumers. According to El-Shafai *et al*. (2004), the major public health concern could be the risk of *P. shigelloides* entering the wound of people who handled and processed the infected fish. Daskalov (2006) recommended that the food should be processed by smoking, oxidizing high hydrostatic pressure, cooling, heating, alcohol and chloride treatment to prevent bacterial contamination.

In the future, *in vivo* experimental study on the effect of *P. shigelloides* in red hybrid tilapia should be conducted as the fish could still tolerate up to 10^{13} up to 10^{16} CFU g^{-1} bacteria in its body and yet remained healthy. Thus, the isolation of *P. shigelloides* from tilapia muscle and intestines in this study connotes a serious public health risk to consumers.

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