

ANTIMICROBIAL SUSCEPTIBILITY OF *VIBRIO* SPECIES ISOLATED FROM BRACKISH WATER SHRIMP CULTURE ENVIRONMENT

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ABSTRACT

A comparative study regarding prevalence and antimicrobial susceptibility of *Vibrio* species in the black tiger shrimp (*Penaeus monodon* Fabricius) culture environment as well as market samples were analyzed. Total *Vibrio* counts were found to range up to 2.5×10^3 cfu/gm and 60 cfu/gm in shrimp and Gher water samples, respectively. Results revealed that the total vibrio count were found to be higher in samples taking from the market shops in Dhaka city than Gher shrimp samples and the live shrimps were not contaminated with *Vibrio* species but dead shrimps were found contaminated, which were collected from shrimp Gher. In the antimicrobial susceptibility test, highest 28.57% resistance among total *Vibrio* isolates was observed to penicillin and cephalixin. Among the seven isolates of *Vibrio* species from Gher water sample one isolate displayed multi-drug resistance (MDR). The main reason of antibiotic resistance could be the indiscriminate application of antibiotics in shrimp farming and release of shrimp pond effluent to estuarine ecosystems or post harvest contamination of shrimps with the antibiotic resistant bacteria through the environment and human handling.

Key words: Food safety, *Vibrio* species, *Penaeus monodon*, Prevalence, Antimicrobial resistance, Multiple drug resistance

INTRODUCTION

Food security is a complex issue, where various factors pose a condition of risk to fish food safety and they range from contamination from the environment where it is caught up to contamination by the consumer before eating (Vieira 1989). Black tiger shrimp may contain many bacteria of public health importance like *Vibrio* species. Presence of such pathogenic bacteria in shrimp beyond certain limits renders it unfit and dangerous for human consumption. Consumption of raw or undercooked seafood is the factor most commonly associated with infection (Butt *et al.* 2004).

Shrimps are one of the most important commodities of the global fishery trade (Bhaskar *et al.* 1995). The shrimp sector is the second largest export earner for Bangladesh. It provides

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millions of employment and earns more than USD 428 million annually (FSYB 2007). However, a lot of fishery resources are simply wasted due to lack of appropriate post harvest technology (James 1984).

The development of antimicrobial resistance among pathogenic bacteria has emerged as a major public health concern, which has led to an intensification of discussion about the prudent use of antimicrobial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al.* 2000). Antimicrobial agents have been applied to the shrimp feed and water in large quantities primarily to treat and prevent diseases in farmed shrimps. Consequently, antimicrobial agents persist in sediment and aquatic environments, leading to deteriorated environmental conditions and conferring antimicrobial resistance to the sediment bacteria. Of particular concern is the indiscriminate use of antibiotics leading to the development of multiple-antibiotic-resistant pathogenic bacteria in shrimps and humans (Zanetti *et al.* 2001). In this case, shrimps could serve as delivery vehicles of antimicrobial resistance to pathogenic bacteria from aquatic environments to humans and from one country to another.

The purpose of this investigation was to determine the prevalence of drug resistant *Vibrio* species in cultured black tiger shrimp and shrimp pond environment. The present study was also attempted to help the surveillance of the antimicrobial resistance status.

MATERIALS AND METHODS

The black tiger shrimp and Gher water samples were collected from Kaliganj Upazilla in Satkhira district of Bangladesh where most of the shrimp farming is carried out. The study area lies between the latitudes 22°27'34N and longitudes 89°01'37E. The samples were collected in July, 2011. Besides these, five shrimp samples were collected from the different markets of Savar and Dhaka city to compare the prevalence of pathogenic bacteria with the shrimp Gher samples. The shrimp samples were collected individually in pre-sterilized polyethylene bags and water samples in pre-sterilized container and brought to the laboratory as soon as possible for the analysis. To avoid further contamination, during transportation from the source to the laboratory, samples were kept in insulated box with ice to maintain the temperature around 4 to 6°C.

Two hundred fifty gram of shrimp samples were blended aseptically. From the blending portion, 10 gm sample and 90 ml sterile normal saline (0.9% w/v, NaCl) solution were taken in a conical flask to make a homogenous suspension. Samples were serially diluted and thoroughly mixed using Vortex-mixture and analyzed for microbial content. Thiosulphate citrate bile salt sucrose (TCBS) agar was used for enumeration of total *Vibrio* bacteria. The TCBS agar has been melted, brought to 45°C and was poured into sterile petridishes. After solidifying and inoculation, plates were incubated at 35°C for 24 to 48 hours. Distinctive

yellow and green colonies on agar plates were picked and streaked on new TCBS agar plates several times to purify bacterial isolates until pure cultures of isolates were obtained. Gher water samples were also analyzed in an identical manner.

Bacterial susceptibility to antimicrobial agents was performed by the disk diffusion method using guidelines established by Bauer *et al.* (1966). A total of 12 antibiotic discs (Oxoid Ltd., Basingstoke, Hampshire, UK) comprising of streptomycin 10 µg, erythromycin 15 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, penicillin 10 µg, norfloxacin 10 µg, ampicillin 10 µg, kanamycin 30 µg, amoxicillin 10µg, azithromycin 15 µg, cephalexim 30 µg and sulphamethoxazole 25 µg were used. Within 15 min of the application of the discs, the plates were inverted and incubated at 37°C. After 16-18 hr of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Oxoid Ltd., Basingstoke, UK. Based on the occurrence of resistance to more than three drugs, the isolates of each sampling sites were grouped as multiple drug resistant isolates (Manjusha *et al.* 2005).

RESULTS AND DISCUSSION

A total of seven mixed samples of the black tiger shrimps and six water samples collected from Gher water have been analyzed. Fig. 1 illustrates the total *Vibrio* count (TVC) of different shrimp and water samples. The TVC ranged up to 2.5×10^3 cfu/gm in samples and 60 cfu/ml in shrimp and water samples, respectively.

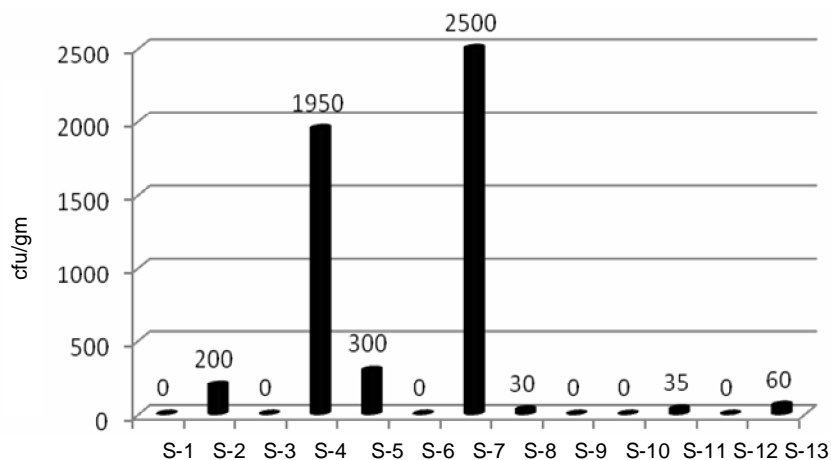


Fig. 1. Total *Vibrio* count in shrimp and water samples (S-1 to S-13) collected from Gher water. S-1(live shrimps from Gher), S-2 (dead shrimps from Gher), S-3, S-4, S-5, S-6, S-7 (market shrimp), S-8, 9, S-10, S-11, S-12, S-13 (shrimp Gher water samples).

Gher shrimp samples were less contaminated compared to the market samples. Areerat *et al.* (1999) reported that *Vibrio* species levels from harvested shrimps ranged between 1.30×10^3 and 1.44×10^5 , which is similar to the finding of the present study. This organism has been found to be autochthonous to marine (Mathew *et al.* 1988) and brackish water systems (Blake *et al.* 1980). *Vibrio* species have been found to be associated with pond mud, water and shrimp samples analysed in Thailand (Leangphibul *et al.* 1986), and in India (Bhaskar and Setty 1994). The major sources of this *Vibrio* species in samples of shrimp may be sediment, water and feeds. *Vibrio* species was present in dead shrimp samples and absent in live shrimp samples collected from Gher. This result suggests that the death of shrimps may be due to the presence of *Vibrio* species. This result also reveals that the concerned black tiger shrimp had an exposure to very unhygienic condition of different stages. Indiscriminate disposal of organic wastes including sewage waste, household wastes, rotten food stuff, feces, remaining parts of the body of dead organisms are responsible for such pollution. Besides them, surface run off, rainwater percolation, flood, storm and other natural disasters contaminate the water of Gher.

Table 1. Diameter of zone of inhibition (in mm) of selected isolates with different antibiotics discs.

Antibiotic discs	Mean diameter of the zone of inhibition (mm) of selected isolates						
	<i>Vibrio</i> sp. isolates						
	V ₁ S	V ₂ S	V ₃ S	V ₄ W	V ₅ W	V ₆ w	V ₇ W
Group I- Inhibitors of cell wall synthesis							
Amoxycillin(10 µ g)	16	23	21	23	29	25	9
Penicillin (10 µ g)	11	21	19	15	29	21	0
Ampicillin (10 µ g)	17	22	24	20	27	24	11
Cephalexin (30 µ g)	18	15	15	14	21	18	0
Group II - Inhibitors of protein synthesis							
Kanamycin (30 µ g)	21	19	24	19	25	21	21
Erythromycin (15 µ g)	15	29	29	23	27	29	21
Azithromycin (15 µ g)	28	33	27	13	31	31	26
Chloramphenicol (30 µ g)	25	29	29	34	35	34	29
Streptomycin (10 µ g)	19	18	21	19	29	19	18
Group III - Inhibitors of nucleic acid synthesis							
Norfloxacin (10 µ g)	32	32	31	29	26	29	29
Sulphamethoxazole (25 µ g)	29	35	24	28	29	27	25
Ciprofloxacin(5 µ g)	35	39	35	31	31	25	31

*S = shrimp, W = water, V = *Vibrio* isolate.

Seven isolates of *Vibrio* species from shrimp and shrimp Gher water were selected and tested against 12 commonly used antibiotics of three categories. Typically, the raw

data were interpreted based on the available CLSI (Clinical and Laboratory Standards Institute) data and zone diameter interpretive standards. Table 1 shows the mean diameter of the zone of inhibition (mm) of selected isolates and Fig. 2 summarizes the antimicrobial resistance in *Vibrio* species isolated from shrimp and their environment. Though antibiotic uses have its advantages, the intensive and extensive uses of antibiotics have led to the emergence of antimicrobial resistance. There is necessity of coordination between human, veterinarian and environmental sectors to clarify the occurrence of resistant pathogens in humans, animals and in the environment (Anon. 2002).

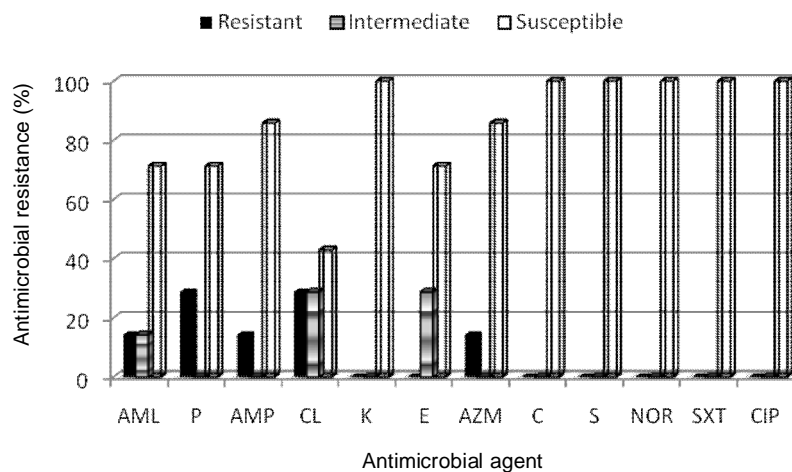


Fig. 2. Antimicrobial resistance in *Vibrio* species isolated from shrimp and their environment. symbols: AML - amoxicillin, P - penicillin, AMP - ampicillin, CL - cephalexin, K - kanamycin, E - erythromycin, AZM - azithromycin, C - chloramphenicol, S - streptomycin, NOR - norfloxacin, SXT - sulfamethoxazole, CIP - ciprofloxacin.

Table 2 shows the percentage of isolates which are resistant, intermediate and sensitive to different tested antibiotics. In this study, the highest (28.57%) resistance among total *Vibrio* spp. was observed to penicillin (P) and cephalexin (CL), and no isolates were found resistant to kanamycin (K), erythromycin (E), chloramphenicol (C), azithromycin (AZM), streptomycin (S), norfloxacin (NOR), sulphamethoxazole (SXT), and ciprofloxacin (CIP). All of the tested *Vibrio* spp. isolates were sensitive to kanamycin (K), chloramphenicol (C), azithromycin (AZM), streptomycin (S), norfloxacin (NOR), sulphamethoxazole (SXT), and ciprofloxacin (CIP) and 42.86% isolates were sensitive to cephalexin (CL). Table 2 summarizes antibiotic resistance patterns and multiple drug resistance (more than three antibiotics) results in selected *Vibrio* isolates from shrimps and gher water. Among seven isolates of the total *Vibrio* spp., 1 from gher water sample displayed multidrug resistance (MDR) of more than three antibiotics. Highest number of resistant antibiotics (AML-P-AMP-CL) was observed to the isolate selected from gher

water sample. In contrast to the present findings, Adeleye *et al.* (2008) reported that resistance to 10, 9, 8 drugs occurred in the majority of the *Vibrio* isolates from seafood.

Table 2. Antibiotic resistance profiles for *Vibrio* species isolated from shrimp and shrimp Gher water.

No. of resistant antimicrobes	No. of isolates	Antimicrobial resistance profiles	%	Origin of isolates		Resistance classification
				Shrimp	Gher water	
4	1	AML-P-AMP-CL	14.29	0	1	MDR
2	1	CL-AZM	14.29	0	1	NMDR
1	1	P	14.29	1	0	NMDR
0	4	–	57.14	2	2	NMDR
Total	7		100.00	3	4	

*MDR - Multiple drug resistance, NMDR - Non multiple drug resistance.

Ferrini *et al.* (2008) reported that 82% of *Vibrio* isolated from fish settings as well as both local and imported seafood showed resistance to ampicillin. In a previous study on potentially pathogenic vibrios isolated from seafood, Ottaviani *et al.* (2001) also found that those bacteria were sensitive to chloramphenicol (90% of the total), sulfamethoxazole, and ciprofloxacin; conversely, they were also resistant to ampicillin, penicillin, amoxicillin, and cephalixin. Bacterial resistance to ampicillin was most frequently detected among *Vibrio* species isolated from fishery products (Akinbowale *et al.* 2006) as well as from a shrimp hatchery (Hameed *et al.* 2003) and shrimp farms (Vaseeharan *et al.* 2005). Khan *et al.* (2002) reported that 100% isolates of enteropathogenic *Vibrio* species from shrimp in Bangladesh were found to show resistance to erythromycin, penicillin, ampicillin, and kanamycin, and 70 and 80% resistance, respectively to cephalixin and streptomycin. The main reason of antibiotic resistance of pathogenic bacteria may be the application of antibiotics in shrimp farming and release of shrimp pond effluent to estuarine ecosystems or post harvest contamination of shrimps with the antibiotic resistant bacteria through the environment and human handling. In general, bacteria can develop resistance for antibiotics, which share similar genetic structures (Angela *et al.* 2006). Van de Boogard and Stobberingh (2000), reported that due to indiscriminate use of antimicrobial agents, such high incidence of multi-drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment. Rapid urbanization, over-crowding and poor sanitation systems greatly facilitate the greater spread of various diseases in Bangladesh. This growing pace of infections is responsible for an increased use and sometimes abuse of antibiotics.

The results revealed that the shrimp might die during cultivation due to the presence of *Vibrio* sp., as these were found to be present in dead shrimp samples and absent in live

shrimp samples collected from the Gher. Market samples were more contaminated with *Vibrio* sp. compared to the samples collected from Gher samples. In the present study, among the seven selected isolates, one isolate displayed multi-drug resistance to more than three antibiotics. The incidence of *Vibrio* species in shrimps and their drug resistance pattern in this study demands immediate need for paying attention in this area.

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