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Prevalence of *Vibrio* species in the Cultured Shrimp and Their Antibiotic Resistants

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ABSTRACT

Antibiotic resistance in *Vibrio* species is of critical importance. This study evaluates the antibiotic resistance of *Vibrio* species present in farmed shr Shrimp samples were obtained from an aquaculture farm. The tissues of Shrimp were examined and a total of 29 *Vibrio* isolates were identified. Thr the biochemical test, the *Vibrio* isolates were identified as *V. alginolyticus, V. cholerae, V. furnissii, V. mimicus, V.parahaemolyticus and V. vulnif* The *Vibrio* species were tested for their resistance to eighteen antibiotics that are frequently present in the aquatic environment. Out of the total isolat were selected as dominant species for antibiotic susceptibility test. In the present study, *Vibrio cholerae* isolated from fresh shrimp showed antimicr resistance against seven antibiotics, *V.vulnificus* isolated from shrimp showed antimicrobial resistance against ten antibiotics and this was the only is to show maximum resistance against the selected antibiotics. *V.mimicus* and *V.alginolyticus* isolated from shrimp showed antimicrobial resistan against seven different antibiotics. *V.parahaemolyticus* isolated from shrimp showed antimicrobial resistance against eight antibiotics whereas *V.furi* isolated from shrimp showed antimicrobial resistance against six antibiotics. In general, all samples showed an increased level of antibiotic resistance to improper use of antibiotics in aquaculture. This result reveals the development of antibiotic resistant to *Vibrio* species would cause a serious threat t consumer.

Keywords: Shrimp, Biochemical test, *Vibrio* species and Antibiotics.

INTRODUCTION

The aquatic organisms are affected by the contaminants present in the aquatic environment and processing the catches. These contaminants create health risks to the consumers (Vieira, 1989). Shrimps are one of the popular seafood consumed by people from different countries and cultures (Bhaskar *et al*., 1995). In aquatic farms, antimicrobial agents are added to the feed and water not only to treat the diseases but also to prevent diseases that affect shrimps. In order to control bacterial pathogens of shrimp, antibiotics and probiotics are being used extensively (Karunasagar and Karunasagar, 1999). Antibiotics are also widely used in hatcheries for the prevention and treatment, particularly when the larval growth deteriorates. Moreover, it is unfeasible to treat only the infected shrimp with antibiotics in a large scale aquaculture. The only practical mean of treatment is to add the antibiotic to the culture water or the feed. However, this indiscriminate use of antibiotic would develop antibiotic resistance in pathogenic bacteria present in the aquaculture (Zanetti *et al*., 2001). These antimicrobial resistance bacteria would infect the shrimps and would be transferred to humans when we consume them. The export and import of seafood would also result in the transmission of antimicrobial resistant bacteria between countries. Shrimps could be tested for the level of pathogenic bacteria and they could be marked as not suitable for human consumption if the bacteria level is beyond a critical limit. However, there are practical and cost constraints to do this. In general, people who consume raw or undercooked seafood are at a greater risk of being infected by pathogenic bacteria (Butt *et al.*, 2004). Because of health risks associated with the development of antimicrobial resistance in pathogenic bacteria, wise use of antibiotics is investigated extensively, particularly in the areas such as veterinary medicine, nutrition and aquaculture (Caprioli *et al.*, 2000).

Vibrio genus is Gram-negative bacteria and in general, it is rod shaped and has a flagellum to facilitate movement. It has twelve known species that can cause illness to human beings when accidentally consumed with food (Dickinson *et al.*, 2013). The pathogenic species of *Vibrio* species are aquatic in nature. They live on other marine organisms, which include the seafood, that the humans consume (Kaysner and Depaola, 2004). The intake of raw or partly cooked seafood that hosted *Vibrio* would cause physical illness. *Vibrio* strains are widespread in the marine environment, especially in tropical and temperate waters and they represent the major bacterial pathogens affecting fish farming in the Mediterranean Sea (Pujalte *et al.*, 2003). The term Vibriosis is commonly caused bacterial diseases that affect wild and farmed sea foods and are caused by the members of the Vibrionaceae

family. Vibriosis is reported in almost all countries of the world. They can result in mortality if the infected person did not receive proper medical treatment. They are also responsible for a high economic loss in shrimp industry worldwide. The significance of Vibriosis as a public health risk is associated with the increased exposure to raw or less-cooked seafood, which is more prevalent in many western countries and a few other cultures (WHO, 2004).

Antibiotic resistant developed by bacteria from *Vibrio* gene would provide a health threat to human beings. This health threat is not only due to direct intake through seafood consumption (Duran and Marshall, 2005), but also by transferring genetic elements of antibiotic resistance to other bacteria that cause illness in humans (Angulo, 2000; Serrano, 2005; Guglielmetti *et al.,* 2009). Health issues related to food are described by the World Health Organization as the diseases resulting from food contamination (Velusamy *et al.,* 2010).The *Vibrio* species that cause illness in humans by contaminating seafood has been recognized clearly (Gopal *et al.,* 2005; Lucan *et al.,* 2008). The *Vibrio* species that cause illness in human beings are *V. cholerae, V.parahaemolyticus, V.vulnificus, V.alginolyticus, V.mimicus, V.fluvialis, V.furnissii, V.metschnikovii, V.hollisae* and *V.damsela*. The *Vibrio* species are responsible for a high number of infections in human beings. They could result in irritation and inflammation of the stomach, blood poisoning, and infection of external wounds and ear. *Vibrio* species also secrete protein exotoxin that acts as an enterotoxin and affects the permeability of the intestine.

Antibiotic resistance of isolates of *Vibrio* species living in marine organisms has been reported worldwide, including Saudi Arabia (Choudhury *et al.,* 1989), Scotland (Inglis *et al.,* 1993), USA (Austin *et al.,* 2009; Park *et al.,* 1995), Nigeria (Adeleye *et al.,* 2008), India (Gopal *et al.,* 2005) and Taiwan (Liu *et al.,* 1997). Although several investigations have been conducted in different countries regarding the development of resistance to antibiotic in *Vibrio* species that are present in various marine organisms from aquaculture (Dang *et al.,* 2006; Akinbowale *et al.,* 2007; Laganà *et al.,* 2011; Reboucas *et al.,* 2011; Raissy *et al.,* 2012), research focused on *Vibrio species* isolated from cultured shrimp is scarce in the literature (Snoussi *et al.,* 2006). Moreover, there is no universal medical agent that is recommended by the Food and Drug Administration to treat shrimp in aquaculture against the infection of *Vibrio* species (Reed *et al.,* 2004).Therefore, in recent years, there is an increase in the number of scientific studies to understand the development of antibiotic resistance in pathogens present in aquaculture. In this research work, the resistant patterns of *Vibrio* species isolated from *P.monodon* reared in farms are investigated, in order to provide useful information regarding the implementation of the surveillance program on antibiotic resistance.

MATERIALS AND METHODS

Nagapattinam district is well known for its shrimp aquaculture. Few *P. monodon* (black tiger shrimp) were caught from the shrimp farms present in this district and were transferred to individual sterilized polyethylene bags. They were stored in a container with ice and transported to the laboratory to carry out experiments. Standard procedures recommended by FDA were followed to detect *Vibrio* species in shrimps (FDA BAM, 2007).

Approximately 25 g of each sample was mixed with 225 ml of Alkaline Peptone Water to form a homogeneous culture. The culture was then incubated at 35 ± 2ºC for one complete night. After enriching for 24 hours in this medium, the culture was streaked onto specific media, namely thiosulphate citrate bile salt agar (TCBS) and CPC medium. *Vibrio* colonies appear as large yellow-colored ones on TCBS and small yellow-colored ones on CPC. *Vibrio cholerae* appear as purple colonies on CPC. Suspected cultures were again cultured in trypticase soy broth and biochemical tests were done to confirm *Vibrio* species. Presumptive colonies were first characterized by biochemical tests. The tests followed standard diagnostic procedure recommended for *Vibrio* species (Alsina and Blanch, 1994).

To analyze the motility the 18-hour old cultures were stab inoculated into sterile motility medium kept in a test tube and examined after 24 hours. The enzyme cytochrome c oxidase also known as Complex IV was identified with sterile disc saturated with N'N'N' tetramethyl diamine dihydrochloride. In the presence of the enzyme purple color appears instantaneously. Hydrogen peroxide was used to evaluate catalase. The appearance of bubbles (effervescence) within 60 seconds indicates the presence of catalase. Other tests, namely Indole formation, citrate utilization, MR-VP test, were carried out according to the standard procedures.

The presence of amino acid decarboxylase was examined with decarboxylase broth with 1.0% of corresponding test amino acid (Arginine, Lysine and Ornithine).The test medium was incubated for four days at 37°C.The presence or absence of decarboxylation was indicated by the change in color to purple (alkaline) or yellow (acid). Carbohydrate fermentation test was also carried out, namely arabinose, lactose, mannose, mannitol, sucrose and xylose at 1% were supplemented to peptone water. The testing culture was injected into the carbohydrate peptone water mixture, which was placed inside Durham's tube and incubated for one day at 37°C.The acid and gas generated inside the Durham's tube were monitored.

The cultures were injected into urease broth and incubated at 37°C to identify the existence of urease. 0.4% gelatin was added to a basal medium and flooded with acidic mercuric chloride was used to examine the generation of gelatinase. The regions where the liquefaction happened were noted.

Confirmed *Vibrio* isolates were tested for their susceptibilities towards various antibiotics. Antibiotic sensitivity of the isolates was analyzed by using the standard Kirby–Bauer antibiotic test (Bauer *et al.*, 1966). The pathogenic cultures to be tested were cultured in a broth for 1 day. The cultures were adjusted to a concentration of 0.5 McFarland standard solutions. The cultures were then swabbed onto sterile Mueller Hinton agar plates and dried for about 5 min. The discs of antibiotics to be tested were then placed on the Mueller Hinton agar plates swabbed with the test cultures. The plates were then incubated for 1 day at 34°C and then the zones of inhibition were noted. The chart provided by Hi-Media was used to assess the susceptibility. The antibiotic discs such as amikacin (30 µg), aztreonam (30 µg), carbenicillin (100 µg), cefepime (30 µg), ceftazidime (30 µg), doripenem (10 µg), gentamicin (10 µg), imipenem (10 µg), netillin (30 µg), piperacillin (100 µg), ticarcillin/clavulanic acid (75/10 µg), ciprofloxacin (5µg), penicillin (10µg), norfloxacin (10µg), ampicillin (10µg), kanamycin (30µg), amoxicillin (10µg) and tobramycin (10 µg) were used in this study. In the period not exceeding 15 minutes after disc application, the plates were turned upside down and incubated at 37°C for 16 to 18 hours. After this, the diameters of the regions that are fully inhibited with *Vibrio* species were measured and rounded off to the nearest millimeter. Based on the diameter, the antibiotics were categorized as susceptible, intermediate and resistant. The categorization followed the standard procedure recommended by Oxoid Ltd., Basingstoke, UK. If a *Vibro* species is resistant to more than three antibiotics, they are classified as multiple drug resistance (MDR) (Manjusha *et al.*, 2005).

RESULT AND DISCUSSION

Totally 29 isolates were identified from farmed shrimp samples. Totally, 6 *Vibrio* species were identified from the 29 isolates through the biochemical test (Table 1). The identified species were *V. cholera, V.vulnificus, V.mimicus, V.alginolyticus, V.parahaemolyticus* and *V.furnissii*. The antibiotic resistance of the *Vibrio* species was determined. For eighteen frequently used antibiotics and the outcome of this analysis is presented in Table 2. All the species exhibited MDR characteristics. MDR is a crucial and hazardous health issue for human beings (Prescott *et al.*, 1999). It is also linked with epidemic outbreaks in all parts of the world (Levy, 2001; Canton *et al.*, 2003). Bacterial resistance to antibiotics is a developing problem leading to the rise of

multi-antibiotic resistant (MAR) organisms. Bacterial resistance to more than three drugs is considered MAR. The indiscriminate use of antibiotics as a feed additive is the major cause of antibiotic resistance in bacteria from the cultured shrimps (Kerry *et al*., 1995). However, other stressors such as temperature, overcrowding and mismanagement of culture animals are also responsible for the development of antibiotic resistance in the bacteria found in shrimp. The presence of MAR organisms in seafood poses a huge threat to consumers; hence, monitoring of antibiotic-resistant pathogens in seafood is necessary (Levy, 2001; Canton *et al*., 2003).Overuse of antimicrobial drugs in aquaculture resulted in drug resistance of pathogens that are present in seafood. Studies on antibiotic resistance are critical to assessing the risk of *Vibrio* species. Processing of shrimp, e.g. behead and removal of the intestine, can lead to the secondary contamination of shrimp meat by intestinal flora. Moreover, many pathogenic and spoilage bacteria remain viable even after cleaning and disinfection of seafood. This can seriously affect the quality and safety of the processed food and poses a potential risk to the consumer (Adetunji and Odetokun, 2012).

The antimicrobial resistance *Vibrio* species isolated from fresh cultured shrimp were shown in (Table 2). *Vibrio cholera* showed antimicrobial resistance against seven antibiotics, namely Carbenicillin, Aztreonam, Cefepime, Ceftazidime, Doripenem, Netillin and Ampicillin. *V.vulnificus* showed maximum resistance against ten antibiotics such as, such as Aztreonam, Carbenicillin, Cefepime, Ceftazidime, Doripenem, Gentamicin, Imipenem, Ticarcillin/clavulanic acid, Ciprofloxacin and Tobramycin. This was the only isolate showed maximum resistance against the selected antibiotics. *V.mimicus* showed antimicrobial resistance against seven antibiotics, namely Aztreonam, Carbenicillin, Cefepime, Ceftazidime, Netillin, Ticarcillin/clavulanic acid, Ciprofloxacin whereas *V.alginolyticus* too showed antimicrobial resistance against seven antibiotics such as Aztreonam, Carbenicillin, Cefepime, Ceftazidime, Doripenem, Netillin and Ticarcillin/clavulanic acid. *V.parahaemolyticus* showed antimicrobial resistance against eight antibiotics, namely Aztreonam, Carbenicillin, Cefepime, Ceftazidime, Doripenem, Gentamicin, Imipenem and Piperacillin. *V. furnissii* showed antimicrobial resistance against six antibiotics, namely Aztreonam, Cefepime, Ceftazidime, Doripenem, Gentamicin and Imipenem.

All the six Vibrio species (*V. cholerae, V. vulnificus, V. mimicus, V. alginolyticus, V. parahaemolyticus* and *V. furnissii*) showed resistance to multiple antibiotics. Over 90% of bacterial strains that live in marine plants/animals exhibit resistant to two or more antimicrobial agents (Martinez, 2003). A tremendous resistance of microbes to an antibiotic is a clear indication that the antibiotic is overused in the environment where the microbes live (Umoh *et al*., 1990; Abbar and Kaddar, 1991; Silva and Hoffer, 1993; Malik and Ahmad, 1994). Aquatic bacteria, including *Vibrio*, are generally exposed to antimicrobial agents as the ecosystem they live, such as estuaries, marine environment near the coast are contaminated with water pollutants from agriculture runoff and waste water treatment plants (Kristi *et al*., 2014). These pollutants would carry different antimicrobial agents, which would have a selective pressure for bacteria that developed resistance to these agents (Stepanauskas *et al*., 2006; Gordon *et al*., 2007). In addition, these water sources that are polluted with antimicrobial agents when used in aquacultures would also result in the development of antimicrobial resistance of *Vibrio* species in shrimps. The outcome of this research clearly points out that the susceptibility of the antimicrobial agents differs among the *Vibrio* species. All the *Vibrio* species investigated are susceptible to amoxicillin, whereas only *V.vulnificus* is susceptible to netillin. All *Vibrio* species isolated from shrimps were found to be resistant to aztreonam, carbenicillin, cefepime and ceftazidime. It is reported that infection by *Vibrios*is linked to the custom of eating seafood live as *Vibrios* are an element of the marine ecosystem. *Vibrios* are also reported to be the most responsible cause of seafood poisoning (Eyisi *et al.,* 2013). *Vibrio cholera* species isolated from shrimp were susceptible to 7 antimicrobial agents, *V.vulnificus* species were sensitive to 6 antibiotics, *V.mimicus* and *V. parahaemolyticus* species were sensitive to 7 antibiotics *V.alginolyticus* species were sensitive to 9 antibiotics *V.furnissii* species were found to be sensitive to 11 antibiotics. Among the total isolates, *V. furnissii* was resistant to more antibiotics. Even though persons with the suppressed immune system are vulnerable

to other *Vibrios*, about 95% of seafood associated death is caused by *V.vulnificus* (Miyoshi, 2013). Han *et al*., (2007) reported that the leading causes of food borne illness are *V.parahaemolyticus, Vibrio vulnificus, Vibrio fluvialis* and *Vibrio cholera.* The present study reveals all these *Vibrios* were found in shrimps. Antibiotic resistance pattern developed in bacteria can be predicted and controlled easily by controlling the usage of antibiotics in the environment where the bacteria live. Moreover, it is essential to prioritise seafood hygiene for a better health of the consumers. In addition, the aquaculture and marine environments are good surveillance of harmful bacteria.

Susceptibility studies of bacteria isolated from seafood samples showed that they were highly resistant to most of the antibiotics tested. Gram-negative organisms are more resistant because of the intrinsic nature of their cell wall. All Gram-negative microorganisms isolated belonged to the Enterobacteriaceae family and this group of organisms is always resistant to various classes of antibiotics (Iroha *et al*., 2011). The unpreserved seafood perishes due to the fermentative bacteria like Vibrionaceae present in it (Gram and Huss, 2000). In warm and hot climatic regions, *Vibrio* species that cause illness in human beings are normally present in marine and coastal ecosystems and they thrive in estuarine ecosystems. *V. cholera* and a few other *Vibrio* species are also found in the fresh water regions of estuaries (Lutz *et al.,* 2013). Normally, the presence of *Vibrio* species has no empirical relation with the presence of fecal coliforms. Moreover, depurations may not decline the presence of *Vibrio* species. Nevertheless, in regions with cholera outbreaks, the aqua environments with a high fecal contamination were reported to have a higher concentration of *V.cholerae* (Amita *et al.,* 2003). *V. parahaemolyticus* causes a much milder diarrhea than cholera (Niewolak, 1984).

The antibiotic resistance of *V.cholerae* against different antibiotics is constantly enhancing. This makes it necessary to develop new drugs to treat cholera. However, these new drugs are not only costly but also result in many adverse side effects. Development of resistance to antibiotics in many strains of *V.cholera* had resulted in the outbreak of cholera epidemics (Dunstan *et al.,* 2013). Faraque *et al.,* (2003) testified a high occurrence of *V.cholerae* from farmed shrimp species. 150 *Vibrio* species that host in shrimps that are caught from Aron river were found to have *V. cholera* (Caldini *et al.,* 1997). Almost all *Vibrios* excrete proteins that are enterotoxins to human and affect the intestines (Nishibuchi and DePaola, 2005). Studies have recorded that cholera toxin is generated by the clinical strains of *V. cholerae* O1 and non-O1, whereas it is not generated by the environmental strains (Barrow and Feltham (1993). Seafood contaminated with *V. parahaemolyticus would cause* gastroenteritis (Ndon *et al*., 1992). One study reported that in salt water, the population of *Vibrio alginolyticus* would increase even at higher water temperatures (Schets *et al*., 2006). The ability to *Vibrio alginolyticus* to utilize sucrose has also been investigated in the past (Renate *et al*., 1987). *V. alginolyticus* mainly occur in the marine and estuarine environment (Leangphibul *et al*., 1986). The influences of 125 environmental strains of *V.mimicus* on unweaned mice were investigated. It was found that just once strain caused fluid build-up in mice (Chowdhury *et al*., 1987). The results of this study match with what is available in the literature, with the difference being the number of living things investigated.

In the present research work, some isolates showed an intermediate response to more than one antibiotic. *Vibrio cholerae* isolated from shrimp showed an intermediate response to Ticarcillin/clavulanic acid and Ciprofloxacin. *V.vulnificus* isolated from shrimp showed an intermediate response to Penicillin and Norfloxacin. *V. mimicus* showed an intermediate response to Amikacin, Doripenem, Gentamicin and Piperacillin. *V.alginolyticus* showed an intermediate response to Piperacillin and kanamycin. *V.parahaemolyticus* showed an intermediate response to Netillin and Kanamycin. *V.furnissii* showed an intermediate response to Netillin. Blackburn (2003) implied that the marine bacteria which showed an intermediate response to antibiotics may become

resistant in the future environment. *Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio fluvialis* and *Vibrio cholera* are responsible for most of the food related diseases.

Indiscriminate overuse of antimicrobial agents in shrimp field would develop antimicrobial resistance in bacteria (Saitanu *et al.*, 1994; Zulkifli *et al.*, 2009). All the *Vibrio* species investigated showed multiple drug resistance to Aztreonam, Carbenicillin, Cefepime and Ceftazidime. This correlates well with other studies found in the literature, which have reported a drug resistance range of 44 to 98% (Son *et al.*, 1998; Lesmana *et al.*, 2001). The outcome of this study on the influence of antimicrobial agents on *V. parahaemolyticus* matches with the conclusions of French *et al.*, (1989). All the *Vibrio* species investigated in this study have multiple antibiotic resistances. However, most of the *Vibrio* species were prone to Amoxicillin. The combination of *Vibrio* species and antibiotic reported in this study are scarce in the literature. Colwell and Sizemore (1974) have reported that the frequencies in antibiotic resistance among bacteria were dependent on the amount and kinds of antibiotics used in the area. Sorum (1998, 1999) noted that resistance against antibiotics emerges as a result of treatment of infections with antibacterial drugs. The high MDR observed in *Vibrio* species would have been due to the possible overuse of antibiotic drugs. This would ultimately lead to the substitution of drug-sensitive microorganisms by drug-resistant microorganisms (van den Bogaard and Stobberingh, 2000).

The risk of development of antibiotic resistance in the microorganisms is significantly more dangerous than that associated with the presence of antibiotic residues in food. Results presented in the study are concordant with those of Blackburn (2003) who provided evidence that cultured shrimp were contaminated not only by antibiotic-resistant bacteria but also by residues of antibiotic, which would result in the transmission of antibiotic resistance to other microorganisms. The development of multi-drug resistance in bacteria could ultimately lead to the bacteria that are not treatable with antimicrobial agents. The risk of *Vibrio* infection could be reduced by consuming well-cooked seafood that is not cross-contaminated after cooking. However, heat-stable hemolysin generated by *V. parahaemolyticus* could not be destroyed by few methods of cooking (Bradshaw *et al.*, 1984). It is important to note that most of the bacteria cannot be killed by freezing (Ward *et al.*, 1997). However, rapid cooling of seafood from a warm temperature would kill *V. vulnificus*. People from high-risk groups are not advised to eat raw seafood to avoid *Vibrio* infection (Ward *et al.*, 1997).

The results of this study clearly point out that the resistance to antimicrobial agents by the *Vibrio* species isolated from the shrimps grown in aquaculture is high. This outcome agrees well with what is available in the literature. Even though, antimicrobial agents need to be used to treat and control bacterial infection, the swift increase in resistance to antimicrobial agents by bacteria stains is a concern. This would lead to the emergence of deadly bacterial diseases not only in aquacultures but also among humans that cannot be treated with antimicrobial agents. In addition, MDR bacteria would result in serious economic losses in aquaculture farms and other environmental issues due to spread of this bacterium to other environments. The present strategy to treat drug resistant microbial in aquaculture farms is to increase the dosage of the anti-microbial agents or to use a stronger anti-microbial agent in the feeds or in the culture medium. However, this strategy is unsustainable in the long run as this would increase the drug resistance of the microbe. Thus, this control strategy would aggravate the existing problem rather than solving it.

CONCLUSION

From the results of this study, it can be concluded that the shrimps from aquafarms could transmit antibiotic resistance *Vibrios*. This clearly indicates that the indiscriminate use of antibiotics in the aquaculture through feeds and cultural media would result in the emergence of antibiotic resistant bacteria. This study also showed that *Vibrio* species are present in abundance in aquatic organisms grown in aquaculture farms. These *Vibrio* species would attack the epithelial linings present in the intestine and thus

cause illness in human beings. The *Vibrio* may also generate enterotoxin. *Vibrio* species could be destroyed by well-cooking of seafood. So eating raw or partially cooked seafood is not recommended. Inadequately treated wastewater, improperly disposed of medical waste and agriculture runoff would also result in the pollution of marine ecosystem with antibiotics. This, along with the indiscriminate exploitation of antibiotic in aquafarms would result in antibiotic resistance in microorganisms. A positive development in recent years is a ban on a few antimicrobial agents in aquaculture farms. However, no licensed antimicrobial agent is available to treat new strains of bacteria that are resistant to available antimicrobial agents. Prevention of bacterial infection would be the best management strategy for aquaculture. Thus, better management of stock to reduce the introduction of pathogenic bacteria, and eliminating overcrowding and overfeeding must be the focus of aquacultures.

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Table 1: *Vibrio* species isolated from shrimp

Biochemical test	*V. cholerae*	*V. vulnificus*	*V. mimicus*	*V. alginolyticus*	*V. parahaemolyticus*	*V. furnissii*
Arabinose	-	-	-	-	-	-
Lactose	-	+	-	-		
Glucose	+	+	+	+	+	+
Mannose	+	+	+	+	+	+
Mannitol	+	+	+	+		
Inositol	-	-	-	-	-	-
Sucrose	+	-	-	+		
Xylose	-	-		-		
Arginine dehydrolase	-	-	-	-	-	+
Ornithine decarboxylase	+	+	+	+	+	-
Lysine	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+
Urease	-	-	-	-		
Indole	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
VP	-	-	-	+		
MR	+	+	+	-	+	+

Table 2: Antibiotic susceptibility of pathogens isolated from shrimp

Antibiotic	*V. cholerae*	*V. vulnificus*	*V. mimicus*	*V. alginolyticus*	*V. parahaemolyticus*	*V. furnissii*
Amikacin (30 µg)	S	S	I	S	S	S
Aztreonam (30µg)	R	R	R	R	R	R
Carbenicillin (100µg)	R	R	R	R	R	S
Cefepime (30µg)	R	R	R	R	R	R
Ceftazidime (30µg)	R	R	R	R	R	R
Doripenem (10µg)	R	R	I	R	R	R
Gentamicin (10µg)	S	R	I	S	R	R
Imipenem (10µg)	S	R	S	S	R	R
Netillin (30µg)	R	S	R	R	I	I
Piperacillin (100µg)	S	S	I	I	R	S
Ticarcillin/clavulanic acid (75/10µg)	I	R	R	R	S	S
Ciprofloxacin (5µg)	I	R	R	S	S	S
Penicillin (10µg)	S	I	S	S	S	S
Norfloxacin (10µg)	S	I	S	S	S	S
Ampicillin (10µg)	R	S	S	S	S	S
Kanamycin (30µg)	S	S	S	I	I	S
Amoxicillin (10µg)	S	S	S	S	S	S
Tobramycin (10 µg)	R	R	S	S	R	S

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