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Research Article

Comparative Study on Antimicrobial Resistance in Bacteria Isolated from Fresh, Frozen and Processed Tiger Prawn (*Penaeus monodon)*

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Abstract

Penaeus monodon (giant tiger prawn) is one of the main aquaculture commodities in India. It is cultivated in large amount because of its demand in abroad and India due to its high nutritive value and taste. Antimicrobials agent are used to avoid disease and damage to the prawn and also to increase the yield. But the indiscriminate use of antimicrobials leads to antimicrobial resistance in pathogens. Hence *Penaeus monodon* were collected from three different sources and the antimicrobial susceptibility of the bacterial strains isolated from them was studied. Twenty different bacteria were isolated from three forms of prawn namely fresh prawn, frozen prawn, processed frozen prawn. It is clear that washing and removal of appendages during processing makes them less prone to bacterial contamination and it is also important to maintain the cold chain to increase the shelf-life of *Penaeus monodon.* Prawn which was frozen and sold was found to contain less number of pathogens which were antimicrobial resistant.

***Keywords*:** Giant tiger prawn, *Penaeus monodon,* antimicrobial resistance

INTRODUCTION

As the world population has reached 7 billion in 2012, there is an increased demand for food. Aquaculture is growing rapidly in many regions of the world, and aquaculture products constitute an important food supply with increasing economic importance. World aquaculture production more than doubled during the period 1994–2004, and countries in Asia accounted for 80–90% of the total production (FAO, 2009). Control measures of these infectious diseases in aquaculture by vaccination and antimicrobials are administered to the animals. While using these antimicrobials in aquaculture has resulted in the emergence of drug-resistant bacteria in fish and other aquatic animals, as well as in the aquatic environment (Aoki, 1991; Schmidt *et al.,* 2000; Le *et al.,* 2005). These antimicrobial agents are administered through medicated feed and direct addition to the water. Due to this heavy inoculation of antimicrobial agents in the *in situ* environment pose heavy pressure not only to the animals and also to the environment (Le *et al.,* 2004). Therefore, the use of antimicrobial agents in aquaculture resulted in heavy environmental impact on wide variety of bacteria (WHO, 1999). Waste water discharge from households and medical-industries dumped in the aquatic environment, consequently the original aquatic environment may be influenced by resistance and bacteria that have emerged in other environments (Schwartz *et al.,* 2003; Koonse *et al.,* 2005). The major antibiotics used in the aquaculture farms are oxytetracycline, florfenicol premix, sarafloxacin, erythromycin, sulphonamine. There is an issues of antimicrobial use in food animal are of global concern (Karunasagar *et al*., 1994). WHO have organized number of consultations and addressed the issues related to the antimicrobial usage/disfavors such as the emergence of resistant pathogen and the potential public health impact (WHO, 1999). The risk associated with the indiscriminate use of antimicrobials in aquaculture may lead to the horizontal gene transfer in resistant bacteria to other aquatic animals, thus may lead to humans and other animals (Heuer *et al*, 2009). This study was carried out antimicrobial resistant bacteria populations in different form of a most wanted aquaculture commodity giant tiger prawn (*Penaeus monodon*).

MATERIALS AND METHODS

Fresh prawns were collected from brackish water aquaculture pond near Chennai (sample 1). Frozen prawns were collected from market in Chennai (sample 2) and frozen processed prawns were collected from departmental store in Chennai (sample 3). About ten prawns were collected from each sample. The appendages of the prawns were removed and they were weighed using a digital balance. One gram of muscle from each prawn was taken separately from all the samples, homogenized in sterile saline solution. Serial dilutions were performed, pour plated on nutrient agar medium and plates were incubated at 37°C for 24 h. The numbers of colony forming units (CFU) were counted (Kerr, 1981; Prescott *et al.*, 1990). The isolated bacteria were sub-cultured and identified by preliminary and biochemical test (Prescott *et al.*, 1990; Cappuccino *et al.*, 2007).

The antibiotic sensitivity of all the identified organisms was carried out by disc diffusion method using Müeller Hinton agar plates. The antimicrobials were purchased from Himedia Labs (Mumbai, India) such as methicillin, nalidixic acid, erythromycin, ampicillin, rifampicin, gentamycin, cefixime, chloramphenicol and amikacin (Tendencia *et al.*, 2001). The plates were incubated at 37°C for 24 h. After the incubation period, the inhibition zones around the discs were measured and the values were compared with standard chart for antimicrobial susceptibility. Based on the size of the inhibition zone they are categorized and named as sensitive, intermediate and resistant.

RESULT

The average weight of fresh prawn was 25 g, frozen prawn 28 g and frozen processed prawn was 23 g. The average number of bacteria counted in fresh prawn was 66×10^7 CFU/g, in frozen prawn was 4×10^7 CFU/g and in frozen processed prawn it was 74×10^7 CFU/g (Figure 1). The total number of 20 bacteria isolated from the sample was 7, 9 and 4 from samples 1, 2 and 3, respectively (Figure 2).

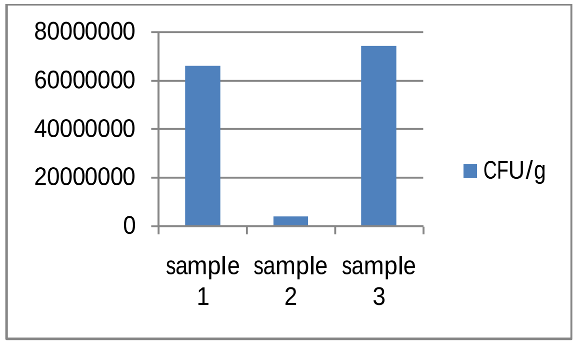

Figure 1: Number of colony forming units

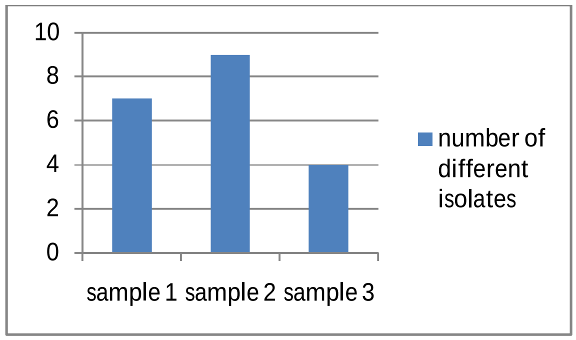

Figure 2: Number of different isolates

The isolates were analyzed by biochemical tests to determine their taxa. The bacteria were identified as *Escherichia coli*, *Staphylococcus* sp., *Proteus* sp., *Hafnia* sp., *Enterobacter* sp., *Alcaligenes* sp., *Citrobacter* sp. and *Pseudomonas* sp.

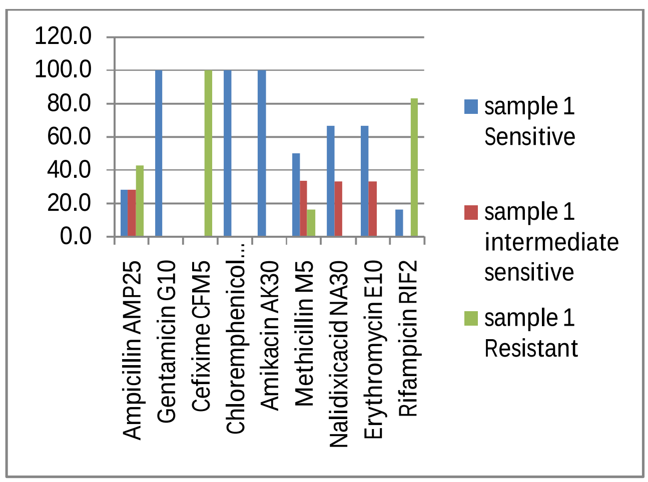

Figure 3: Antibiotic resistance in sample 1

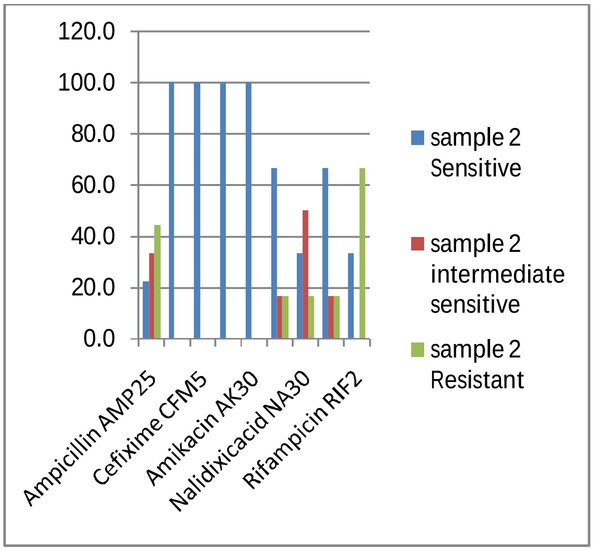

Figure 4: Antibiotic resistance in sample 2

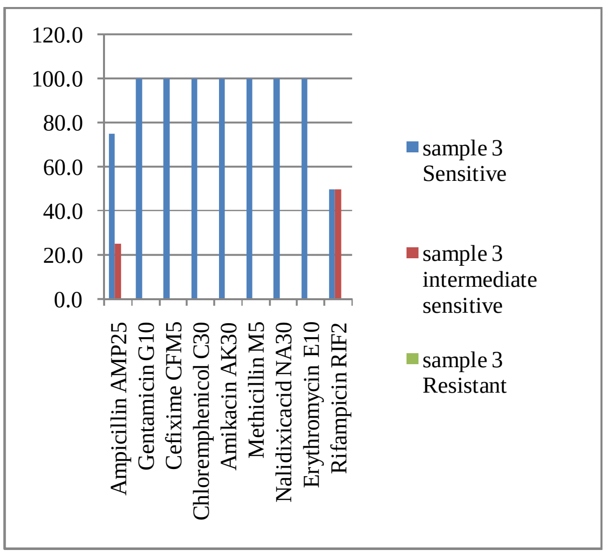

Figure 5: Antibiotic resistance in sample 3

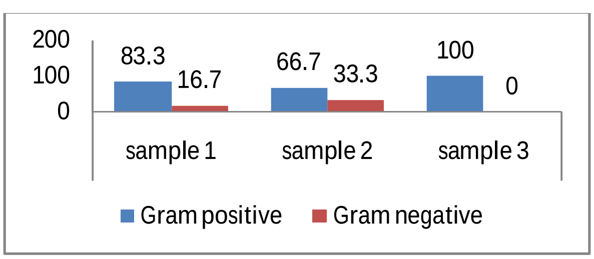

Figure 6: Antibiotic resistance in gram positive and gram negative bacteria in different samples

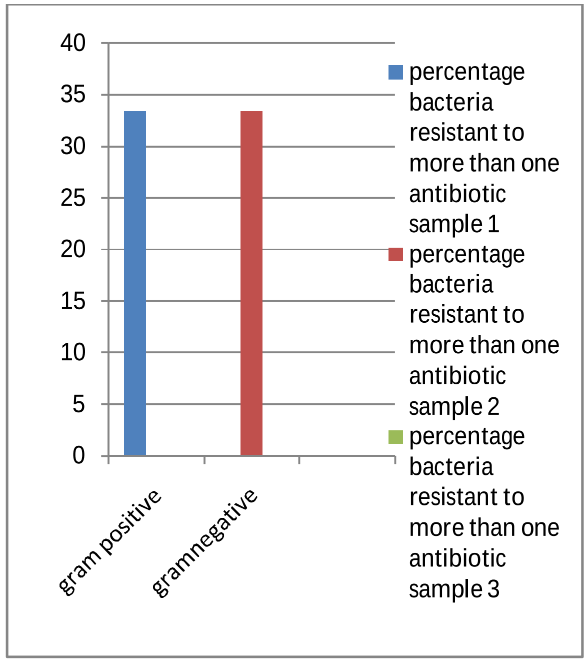

Figure 7: Percentage of antibiotic resistance in gram positive and gram negative bacteria

Most antimicrobial resistant strains are found in sample 1 followed by sample 2 and sample 3 with very few antibiotics resistant strains (Figures 3–7). Processing and freezing kills most of the bacteria that are resistant to antibiotic agents. Gram negative bacteria showed more antibiotic resistant that are present in sample 2 because of the presence of unwanted appendages.

DISCUSSION

The result of this study provided fundamental information required for evaluating the comparison between fresh, frozen and processed prawn (*Penaeus monodon*) for antibiotic susceptibility of bacteria isolated from them (Uddin *et al.,* 2013). The study compares the number of bacteria, and their antimicrobial sensitivity in 3 different types of sample which are available in the market. It has been found that the sample 2 (frozen prawn) had most number of bacteria. Antibacterial resistant test showed that sample 1 had more Gram positive resistant bacteria and sample 2 had more Gram negative resistant bacteria while processed prawn does show very few resistant forms. It is also clear that the processing of fresh prawn and removing appendages makes it less prone to bacterial attack and hence fewer antibacterial resistant bacteria are present. It is also important to maintain the cold chains in the processed prawn to increase the shelf life of *Penaeus monodon*.

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