

Incidence of antibiotic residues in farmed shrimps from the southern states of India

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Received 13 September 2011; revised 30 April 2012

Residual Level of antibiotics viz: Chloramphenicol, Sulphonamide, Tetracycline, Erythromycin, Streptomycin and β -Lactams were determined in farmed shrimps collected from major fish farms of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. Streptomycin, Tetracycline and β -Lactams could not be detected in any of the samples. Chloramphenicol, if at all present was found to be at trace level <1 ppb. Sulfonamides and Erythromycin were detected in farmed shrimps at a level <100 ppb. Charm II assay was used to quantify the antibiotics in shrimps based on a binding reaction between the antibiotic molecules and antibody attached to microbial cell. Scintillation fluids added to the tube and the radio signals from ^3H or ^{14}C counted by Charm II system are recorded.

[Keywords: Antibiotic Residue, Shrimp, Charm II Analysis, Chloramphenicol, Fish]

Introduction

Shrimp aquaculture has become a major worldwide activity and shrimp continues to be the prime export in terms of value. To promote growth and enhance immunity of shrimp, many antibiotics are applied, such as *tetracycline*, *sulfonamides*, *chloramphenicol*, *nitrofurans* etc. However, a number of recent reports and on-going investigations have raised legitimate public concerns about the safety of antibiotic drug usage in aquaculture¹⁻¹⁵.

In spite of strict control, antibiotics are widely used in aquaculture as a prophylactic agent or to treat disease outbreaks¹. However, their implication on environmental impact and human health necessitates the monitoring in farmed shrimps. Very little information exists documenting the concentration of antibiotics in farmed shrimp in Indian waters^{10,19-21}. Recent developments in the aquaculture industry have led to development of drugs with increased potency. There are no public sources of aquaculture drug use data. Regulators and scientists from the Joint Subcommittee on Aquaculture (JSA), the Federal Drug Administration (FDA), United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA) who work on fish drug use and the environmental impacts of aquaculture have no solid basis to estimate antibiotic

drug use. There are some estimates, but they rely on Industrial sources.

Many aqua farmers use antibiotics as prophylactics in large quantities, even when pathogens are not evident¹⁻³. This has led to an increase in *Vibrios*, and presumably other bacteria, having multiple antibiotic resistance and more virulent pathogens. Many of the pathogens appear to have mutated to more virulent forms than were present a decade ago, and thus even when the shrimps are not stressed by poor water quality they succumb to attack. The incidence of disease has been exacerbated by the actions of the shrimp farmers. *Vibrio* spp., especially the luminous *V. harveyi*, have been implicated as the main bacterial pathogens of shrimps. Antibiotics have been used in attempts to control these bacteria, but efficacy is now, in general, very poor. Luminous *Vibrio* disease caused a major loss in shrimp production in 1996, and many farms have ceased to produce shrimps because survival was so poor^{14,22}. The *Vibrio* species were resistant to every antibiotic used, including *Chloramphenicol*, *Furazolidone*, *Oxytetracycline* and *Streptomycin*. A highly virulent strain of Luminous *Vibrio* had developed in response to the use of antibiotics. It is likely therefore, that the *Vibrios* surviving after Chlorine treatment are not only more resistant to antibiotics, but are also pathogenic^{9,10,12}.

Throughout Asia, shrimp farmers use antibiotics in large quantities¹⁻⁸. Antibiotics are usually administered in aquatic feeds and most commercial shrimp feeds contain antibiotics⁶. Development of resistant pathogens in aquaculture environment has been reported⁹⁻¹⁰. Recent studies indicate that several antibiotics are moderately to highly acutely toxic to aquatic organism¹². Irreversible Aplastic anemia caused by Chloramphenicol in susceptible consumers and allergic reactions that may result from β -Lactams residues are all of serious concern^{10,12,14}. A number of mutagenic or carcinogenic antibiotics have been used in aquaculture and other livestock farming^{3,4,5,8,11}.

The present study reports the level of certain antibiotics in some species of farmed shrimp collected from major fish farms of Andhra Pradesh (*Nellore* and *Bhimavaram* region), Kerala (*Cochin*, *Malampuzha* and *Malabar area*), Karnataka (*Karwar* and *Mangalore*), and Tamil Nadu (*Sathyamangalam* and *BhavaniSagar Dam*).

Materials and Methods

Fresh shrimp of length 10-15 cms, and weight 10.3-12.7 g were collected from the selected major fish farms of Andhra Pradesh (*Nellore* and *Bhimavaram*), Tamil Nadu (*Satyamangalam* and *Bhavanisagar Dam*), Kerala (*Cochin*, *Malampuzha* and *Malabar area*) and Karnataka (*Karwar* and *Mangalore*) fish ponds and were iced properly in an insulated box and brought to the Central Institute of Fisheries Technology laboratory, Cochin. Shrimp samples were peeled and meat homogenized. The homogenized Shrimp meat was used for the determination of antibiotic residues using Charm II analyzer as per USFDA method¹⁸ and Charm II Operator's manual provided by *CHARM SCIENCES* Inc.

Twenty gram of homogenized shrimp meat was blend with 20 mL MSU extraction buffer and centrifuged for 5 minutes at 250 RPM. The entire homogenized sample was collected in a 50 mL centrifuge tube and incubated at 80°C for 45 minutes. The centrifuge tubes were then placed on ice for 10 minutes and again centrifuged the sample for 10 minutes at 3300 RPM. The supernatant was collected and the pH was checked for 7.5-8 using M2 buffer. This was analyzed for the counts and calculations were done using the standard table for concentration and scintillation count.

The Charm II test used three reagents; [³H] labeled antibiotic eg: Chloramphenicol (tracer), A binding reagent (antibody) and charcoal. When the antibody is added to the sample containing Chloramphenicol, the contaminating Chloramphenicol binds to the antibody. This prevents the [³H] Chloramphenicol from binding to these sites. Unbound [³H] Chloramphenicol is then absorbed onto charcoal. Therefore, the more [³H] Chloramphenicol bound (and left in solution), the less Chloramphenicols are there in the sample. The amount of [³H] left in solution is measured with a Charm II analyzer with Scintillation counter. Lower the number (Counts Per Minute), higher the amount of contamination in the sample and viceversa.

Results and Discussion

The study covered the four different southern states of India viz: Andhra Pradesh, Tamil Nadu, Kerala and Karnataka. Most of the antibiotics were detected in samples from *Nellore* and *Bhimavaram* samples and presented in Table (1) and the results from other regions are given in Tables 2 and 3. Monitoring was carried out for six antibiotics, viz; *Chloramphenicol*,

Table 1—Levels of antibiotic residues in farmed shrimp from *Nellore* & *Bhimavaram* fish farms (range & mean values, in ppb) – Andhra Pradesh

Area & Sample name	Chloramphenicol	Sulfonamides	Erythromycin	β -lactum	Streptomycin	Tetracycline
Nellore						
<i>M. rosenbergii</i> n=19	0.113-0.240 0.176	35.017-97.810 56.91	49.460-77.497 61.12	ND	ND	ND
Bhimavaram						
<i>M. rosenbergii</i> n=7	ND	33.41-34.90	34.66-36.27	33.46-38.38	ND	42.02-46.60
<i>P. monodon</i> n=11	0-0.104 0.078	34.16 0-50.402 39.61	35.47 0-63.73 45.85	35.92 0-39.14 30.75	ND	44.44 0-42.17 37.61
<i>P. indicus</i> n=5	ND	27.67-32.49 28.96	ND	ND	ND	ND

n – indicates number of samples

Table 2—Levels of antibiotic residues in farmed shrimp from Malampuzha, Vypin and Trichur fish farms (range & mean values, in ppb) – Kerala, area not differentiated

Area & Sample name	Chloramphenicol	Sulfonamides	Erythromycin	β -lactum	Streptomycin	Tetracycline
Malampuzha						
<i>M. rosenbergii</i> n=10	0.086-0.088 0.087	39.61-40.30 39.95	39.59-41.95 40.77	ND	ND	ND
Vypin area						
<i>Metapenaeus monoceros</i> n=7	0.105-0.168 0.132	19.173-34.902 27.69	38.412-40.987 39.47	ND	ND	ND
Trichur area						
<i>P. indicus</i> n=5	0-0.143 0.133	44.89-45.12 44.006	41.73-42.38 42.06	ND	ND	ND

Table 3—Levels of antibiotic residues in farmed shrimp from Tamil Nadu & Karnataka region (range & mean values, in ppb)

Area & Sample name	Chloramphenicol	Sulfonamides	Erythromycin	β -lactum	Streptomycin	Tetracycline
Tamil Nadu						
Bhavanisagar & Satyamangalam						
<i>M. rosenbergii</i> n=5	0.085-0.123 0.023	44.12-72.65 55.10	1.32-2.45 0.418	ND	ND	ND
Karnataka						
<i>M. rosenbergii</i> n=4	0-0.0135 0.003	58.36-69.65 65.602	53.69-56.98 55.13	ND	ND	ND

Sulfonamide, Erythromycin, Tetracycline, Streptomycin and β -lactams. Among the six antibiotics tested; Tetracycline, Streptomycin and β -lactams could not be detected in *Macrobrachium rosenbergii* collected from Nellore areas. While Chloramphenicol concentration ranged from 0.1134 to 0.2398 ppb with an average of 0.1761 ppb; Sulfonamide ranged from 35.017 to 97.81 ppb with an average of 56.91 ppb and Erythromycin ranged from 49.46 to 77.49 ppb and an average value of 61.12 ppb was observed. Among seven lots of *Macrobrachium rosenbergii* collected from Bhimavaram area, chloramphenicol and streptomycin were absent. Sulfonamides ranged from 33.41 to 34.90 ppb and showed an average value of 34.16 ppb. In *Penaeus monodon* from the region except Streptomycin, all other antibiotics were detected. Chloramphenicol was present only at trace levels with an average value of 0.078 ppb. Sulfonamides, Erythromycin, Tetracycline and β -lactams were found to have comparative results (Table 1). Among five lots of *Penaeus indicus* examined from the same areas, except sulfonamides other five antibiotics tested were not detected. Only Sulphonamides was detected; ranged from 27.67 to 32.49 ppb.

Samples from Tamil Nadu (collected from BhavaniSagar and Sathyamangalam) all the 5 samples of *M. rosenbergii* showed the presence of Chloramphenicol, Sulfonamide and Erythromycin (Table 3). Other three antibiotics could not be detected in these samples. Levels of antibiotics in shrimp samples collected from different regions of are presented in Table 2. Three antibiotics, viz; β -Lactams, Streptomycin and Tetracycline were not detected in any of these samples. Levels of all other antibiotics seemed to be at similar level in samples from other regions of Kerala (Table 2). All the three species of shrimp were found to be very slightly contaminated with Chloramphenicol.

In Karnataka, farms from Karwar and Mangalore 5 lots of *Macrobrachium rosenbergii* were collected and tested for the presence of antibiotics. Among the six antibiotics monitored Tetracycline, Streptomycin, and β -lactams were not detected. Chloramphenicol if at all detected was found only at trace levels of 0.003 ppb. Sulfonamide Concentration ranged from 58.360 to 69.65 ppb and showed an average concentration of 25.602 ppb and Erythromycin indicated an average of 22.134 ppb as presented in Table 3.

Conclusion

Among the six antibiotics examined, Tetracycline, β -lactams and Streptomycin were not detected in Shrimp samples from the major aqua farms of southern states of India. Chloramphenicol, Sulfonamide, and Erythromycin, were detected in various shrimp samples but not at alarming range. The contamination of antibiotics in aquacultured shrimp may be attributed to contamination from the shrimp feed. It has been noticed antibiotics are usually administered in aquatic feeds and most commercial shrimp feeds contain antibiotics (Flaherty *et al.*²³ 2000). Good aquaculture practices, providing proper withdrawal period for antibiotics and screening of feed for antibiotics etc can control the incidence of antibiotics in aquaculture.

Acknowledgement

One of the authors (K.M. Swapna) is thankful to the Director, CIFT, Cochin for providing facilities and also to Indian Council of Agricultural Research for financial assistance.

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