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

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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 $_1H^3$ or $_6C^{14}$ 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 aquaculture[1-15].

Inspite of strict control, antibiotics are widely used in aquaculture as a prophylactic agent or to treat disease outbreaks[1]. 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 evident[1-3]. This has lead 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 quantities[1-8]. Antibiotics are usually administered in aquatic feeds and most commercial shrimp feeds contain antibiotics[6]. Development of resistant pathogens in aquaculture environment has been reported[9-10]. Recent studies indicate that several antibiotics are moderately to highly acutely toxic to aquatic organism[12]. 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 *Bhimvaram* region), Kerala (*Cochin, Malampuzha and Malabar area)*, Karnataka (*Karwar and Manglore*), 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 method[18] 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 *p*H 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; [^{3}H] 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 [^{3}H] Chloramphenicol from binding to these sites. Unbound [^{3}H] Chloramphenicol is then absorbed onto charcoal. Therefore, the more [^{3}H] Chloramphenicol bound (and left in solution), the less Chloramphenicols are there in the sample. The amount of [^{3}H] 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						
M. rosenbergii n=19	0.113-0.240	35.017-97.810	49.460-77.497	ND	ND	ND
	0.176	**56.91**	**61.12**			
Bhimavaram						
*M.rosenbergii*n=7	ND	33.41-34.90	34.66-36.27	33.46-38.38	ND	42.02-46.60
P. monodon n=11	0-0.104	**34.16**	**35.47**	**35.92**	ND	**44.44**
	0.078	0-50.402	0-63.73	0-39.14		0-42.17
		39.61	**45.85**	**30.75**		**37.61**
P. indicus n=5	ND	27.67-32.49	ND	ND	ND	ND
		28.96				

n – indicates number of samples

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

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

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	0.085-0.123	44.12-72.65	1.32-2.45	ND	ND	ND
M. rosenbergii n=5	**0.023**	**55.10**	**0.418**			
Karnataka						
M. rosenbergii n=4	0-0.0135	58.36-69.65	53.69-56.98	ND	ND	ND
	0.003	**65.602**	**55.13**			

Sulfonamide, Erhtyromycin, Tetracycline, Streptomycin and *β-lactms.*Among the six antibiotics tested; *Tetracycline, Streptomycin and β-lactams* could not be detected in *Macrobrachiumrosenbergii* 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 *Penaeusmonodon*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 Tetracyclinewere 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 *Macrobrachiumrosenbergii* 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.

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