



Management induced changes of antibiotic resistant strains of heterotrophic bacteria in shrimp farming ponds

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ABSTRACT

Antibiotic resistant strains of heterotrophic bacteria and total aerobic heterotrophic bacteria were enumerated along with important water quality parameters in water and sediment samples collected from 3 shrimp farming ponds used for the monoculture of *Penaeus monodon* as well as from a control pond located outside the shrimp farm. The resistant strains of heterotrophic bacteria were screened against 3 antibiotics (ampicillin, chloramphenicol and oxytetracycline) at 25 and 50 µg/l. The counts of antibiotic resistant bacteria tended to rise in shrimp ponds with progressive rise in the days of culture, but were specially high during the period when both antibiotics and feed were applied in the system. It appears that antibiotics exerted a selective pressure in favour of antibiotic resistant bacterial strains and widespread use of antibiotics should be forbidden in shrimp ponds.

Key words: Ampicillin, Antibiotic resistant heterotrophic bacteria, Chloramphenicol, Oxytetracycline, *Penaeus monodon*, Shrimp farming, Total aerobic heterotrophic bacteria

Shrimp farming has becoming a booming industry in the tropical Asian countries due to its immense export potentials in the world market. A large section of rural people earn their livelihood from this industry. It is common practice to culture shrimps in brackishwater ponds for 3 - 4 months grow out period. As a part of management protocol, feed containing antimicrobials and antibiotics (oxytetracycline, oxolinic acid and the quinolones) are applied to promote growth or treat or prevent diseases of shrimps and fishes (Holmstrong *et al.* 2003, Cabello 2006, Bermudez-Almada and Espinosa-Plascencia 2012). In response to antimicrobial drugs and antibiotics that enter the shrimp farming ponds via the supplementary feed, animal manure and residual feed develop a selective pressure in favour of antibacterial resistant bacteria (Petersen and Dalsgaard 2003). Researches have revealed that fishes treated with anti-microbial or antibacterial drugs have shown an increase in the percentage of resistant intestinal Gram-negative bacteria of some fishes (Austin and Al-Zahrani 1988, Alcaide *et al.* 2005, Cabello 2006, Akinbowale *et al.* 2006, Ghosh and Mandal 2010). Anti-microbial resistant bacteria selected in the integrated aquaculture settings may be human pathogens or may donate resistance genes to human pathogens (Muniruzzaman and Chowdhury 2004, Abutbul *et al.* 2005, Kim and Wei 2007).

In India, concern has been expressed about the

widespread and indiscriminate uses of antibiotics in shrimp farms as well as in agricultural fields. Xenobiotics also cause an imbalance in the pond ecosystem with accumulation of toxic residues in snail (Coutellec and Lagadic 2006), fish (Ludmila *et al.* 1999) as well as changes in natural microbial community by altering or modifying the genetic structure (Fernandez *et al.* 2009), biodegradation process (Orhon and Sozen 2012) or loss of biodiversity in the pond ecosystem.

Heterotrophic bacteria play a key role in the biodegradation of organic matter in aquatic environment (Gocke *et al.* 2003, Dolgonosov and Gubernatoroba 2010). The structure of microbial assemblages is sensitive to environmental stress (Pajares *et al.* 2012), eutrophication (Danovaro *et al.* 2000, Wright *et al.* 2009) and many other anthropogenic activities (Hansen and Blackburn 1992, Nogales *et al.* 2011). A step-wise regression model developed by Bertoni *et al.* (2010) predicted that reduced anthropogenic pressure was more significant than climate change in driving the trend in DOC concentrations and hence the bacterial dynamics and average cell abundance in the hypolimnion and epilimnion zones of the lake.

A number of complaints have been generated by the fisher folk with respect to decline in fish catch in the Bay of Bengal where effluents of the shrimp farms are discharged, implicating the negative impact of shrimp farm effluents on fish catch in the sea. Nevertheless, no systematic studies have been carried out on the responses of heterotrophic bacterial community to different phases of management protocols in shrimp culture ponds. This is quite pertinent as the effluents of shrimp culture ponds

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containing a variety of hazardous chemicals and antibiotics residues may alter the microbial community including the heterotrophic component. Changes in the responses of heterotrophic bacterial community to antibiotics or the microbial sensitivity test might, therefore, be used to obtain insights on the state. The purpose of the present study was to examine the effect of 3 common antibiotics used in shrimp ponds on the growth responses of heterotrophic bacterial community inhabiting shrimp farming ponds as well as shrimp pond effluent.

MATERIALS AND METHODS

Three ponds (pond -1, pond -2, and pond-3) in a shrimp farm (Dadanpatrabarh, Mandermani, Purba Medinipur, West Bengal, India) (total area: 20 ha; water area: 0.65 to 0.95 ha) were selected for the study. Two crops of semi-intensive monoculture of tiger shrimp, *Penaeus monodon*, were analysed in each year from March to June and from August to November. The effluents discharged from the shrimp culture ponds were brought to the Bay of Bengal through a canal covering a distance of 1–1.5 km.

Pond management: The ponds were dried up, top sediment (about 2") removed, treated with lime (800 kg ha⁻¹) and then filled with 40 mg l⁻¹ bleaching pretreated water. Urea (6 kg ha⁻¹) and single super phosphate (1 kg ha⁻¹) were applied as fertilizer. The duration of culture was about 120 days. A natural pond used as control was located 1 km away from the culture unit, having no impact of antibiotics or effluents of shrimp farm.

Penaeus monodon (length –1.5 cm, weight – 0.035 g) post larvae were stocked at a density of 20 m⁻². After 7 days of introduction, the supplementary feeding with commercial feed containing as main components - protein 41%, fat 3% (min), fiber 3% (max), inorganic substances 16% (max) and moisture 9% (max) (Jana *et al.* 2000) was used to feed shrimps twice a day during the first 30 days; 3 or 4 times a day thereafter during the rest of the culture period. During initial phase of feeding (up to 50–52 days) no antibiotics were used with feed, after 50 to 55 days of culture when prawn grew up to 8–10 g antibiotics were added with the feed @ 2 g/kg feed as a prophylactic measure for several diseases. There was no exchange of water during the period of culture. The shrimps were grown for 4 months after which the shrimps were harvested by water removal from the ponds.

Sample collection: Water and sediment samples were collected aseptically during the period of March to July 2004. Samples were collected from different intervals representing each designated phase of the grow out period of shrimps. Four months culture cycle was divided into 5 phases: first phase or the pre stocking phase (PS phase) – 15 days after pond preparation, pre feeding phase (PF phase) was between 7 days after stocking prior to feed application, feeding phase (F phase) was from the date of feeding till the harvest. Feeding phase was further divided into without antibiotic (F-A phase) and with antibiotic (F + A phase). The post harvesting phase or PH phase was the period after

1 month of shrimp harvest. Samples were also collected from the control pond.

The sensitivity tests of heterotrophic bacteria (HB) to antibiotics were performed using ampicillin, chloramphenicol and oxytetracycline (Obaton 1971) applied as a prophylactic measure for the treatment of several bacterial diseases of shrimp at the final concentrations of 25 mg ml⁻¹ and 50 mg ml⁻¹. The prepared doses of antibiotics were added to nutrient agar media prior to inoculation with water or sediment samples. Standard procedures of aliquots preparation and all other microbiological testings were followed (APHA 2001). Incubation temperature for mesotrophic heterotrophic bacteria was kept at 37°C (Jana *et al.* 2012). The percentage of antibiotic resistant heterotrophic bacteria was calculated against the total numbers of heterotrophic bacteria that occurred in Petri plates without the use of antibiotics.

The samples of water collected from the selected ponds were monitored for routine water quality (temperature, pH, DO, ammonia-N and salinity) following the standard protocols described in the APHA (2001). All the data obtained in this experiment were subjected to the statistical analysis following the analysis of variance. Significant difference was accepted at 5% level ($P < 0.05$).

RESULTS AND DISCUSSION

Heterotrophic bacteria: The total counts of HB occurring in the samples of water ranged from 6–7 × 10² cfu ml⁻¹ in pre stocking phase, 7–8 × 10² cfu ml⁻¹ in pre-feeding phase, 14–15 × 10² cfu ml⁻¹ in feed without antibiotic phase, 43–44 × 10² cfu ml⁻¹ in feed with antibiotic phase and 40–42 × 10² cfu ml⁻¹ in post harvesting phase in all the culture ponds investigated (Table 1). A gradual rising trend in the counts of bacteria was registered as the days of culture progressed (Table 1). Temporal course of variations in the control pond was, however, different from that of grow out ponds (Table 1).

The total counts of HB occurring in sediment samples ranged from 10 – 11 × 10⁴ cfu g⁻¹ in both pre stocking phase and pre feeding phase, 20 – 21 × 10⁴ cfu g⁻¹ in feed without antibiotic phase, 69 – 70 × 10⁴ cfu g⁻¹ in feed with antibiotic phase and 55 – 57 × 10⁴ cfu g⁻¹ in post harvesting phase in all the 3 culture ponds investigated. The trend of variation was similar to that of water (Table 1).

Resistant heterotrophic bacteria

Water: The counts of antibiotic resistant bacterial population against the three antibiotics (ampicillin, chloramphenicol and oxytetracycline @ 25 µg ml⁻¹ in each case) tended to rise gradually as the culture period progressed. The initial count of the resistant bacteria was negligible (1–7 cfu ml⁻¹) during pre stocking and pre feeding phase till day 27 of culture, but showed a sharp rise thereafter. The resistant bacterial populations showed a downward trend (60–117 × 10 cfu ml⁻¹) after 1 month of harvest (PF phase) when neither feed nor the antibiotics were applied in ponds (Table 2).

The count of antibiotic resistant bacterial population against these antibiotics (ampicillin, chloramphenicol and oxytetracycline @ 25 µg ml⁻¹ in each case) in the control pond was, however, negligible during the comparable periods in shrimp culture ponds (Table 2).

Increased dose (50 µg ml⁻¹) of antibiotics in the samples of water caused 3–3.5 times less counts of resistant populations during the peak period of population density (Table 3) though the trend was almost same as in case of low dose antibiotic application. There was no development of resistant bacterial populations from the samples of control pond when screened against 50 µg ml⁻¹ (Table 3).

Sediment

The counts of antibiotic resistant bacterial populations screened at 25 µg ml⁻¹ tended to rise gradually as the culture period progressed (Table 4). The counts ranged between

5.4–9.1 × 10³ cfu g⁻¹ and 135–288 × 10³ cfu g⁻¹ at the end of feeding phase without antibiotic and feeding phase with antibiotic respectively (Table 4). The resistant bacterial populations, however, decreased (93–207 × 10³ cfu g⁻¹) after 1 month of harvest.

Increased dose (50 µg ml⁻¹) of antibiotics resulted in 2.5–2.7 times reduction in the counts of resistant populations in the culture ponds during the peak period of population density. In case of higher dose (50 µg ml⁻¹), no resistant populations were detected till day 27 of culture (Table 4).

Water quality

Water temperature did not differ (25° to 29°C) much among the culture ponds investigated. Water pH in the culture ponds remained always alkaline, ranging from 7.9 to 8.6, against 7.7–7.9 in control pond. On a temporal scale, water pH tended to decline with progress in culture period

Table 1. Variations in the mean (±SE) counts of antibiotic resistant heterotrophic bacterial populations occurring in waters of three shrimp ponds and in control sites employed. Screening of resistant strains was performed against ampicillin-AMP, chloramphenicol-CHL and oxytetracycline-OTC at 25 µg ml⁻¹ each. Each data represents the mean count of 3 replicates

	Mean count of bacteria in water				Mean count of bacteria in sediment			
	Pond-1 No of cfu × 10 ³ ml ⁻¹	Pond-2 No of cfu × 10 ³ ml ⁻¹	Pond-3 No of cfu × 10 ³ ml ⁻¹	Control No of cfu × 10 ³ ml ⁻¹	Pond-1 No of cfu × 10 ³ ml ⁻¹	Pond-2 No of cfu × 10 ³ ml ⁻¹	Pond-3 No of cfu × 10 ³ ml ⁻¹	Control No of cfu × 10 ³ ml ⁻¹
Phase-1	7	6	7	17	110	110	100	280
Phase-2	8	9	7	18	110	100	120	290
Phase-3	14	15	14	17	200	200	210	280
Phase-4	44	43	44	17	710	700	690	300
Phase-5	41	42	40	30	550	560	570	450

Table 2. Variations in the mean (±SE) counts of antibiotic resistant heterotrophic bacterial populations occurring in waters of three shrimp ponds and in control sites employed. Screening of resistant strains was performed against ampicillin-AMP, chloramphenicol-CHL and oxytetracycline-OTC at 50 µg ml⁻¹ each.. Each data represents the mean count of 3 replicates.

Days of culture	Pond-1 No of cfu ×10			Pond-2 No of cfu ×10			Pond-3 No of cfu ×10			Control No of cfu ×10		
	AMP	CHL	OTC	AMP	CHL	OTC	AMP	CHL	OTC	AMP	CHL	OTC
	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Before stocking	0.2 ±0.03	0.2 ±0.02	0.1 ±0.02	0.3 ±0.03	0.1 ±0.01	0.2 ±0.03	0.2 ±0.02	0.2 ±0.03	0.1 ±0.02	0.1 ±0.02	0.2 ±0.03	0.2 ±0.03
7	0.3 ±0.04	0.5 ±0.04	0.2 ±0.03	0.3 ±0.04	0.5 ±0.04	0.2 ±0.03	0.3 ±0.04	0.5 ±0.04	0.3 ±0.04	0.2 ±0.03	0.2 ±0.03	0.1 ±0.02
27	0.4 ±0.04	0.6 ±0.05	0.2 ±0.03	0.4 ±0.04	0.7 ±0.06	0.3 ±0.03	0.4 ±0.04	0.7 ±0.06	0.6 ±0.05	0.2 ±0.03	0.3 ±0.04	0.2 ±0.03
52	1.9 ±0.21	2.6 ±0.33	1.7 ±0.19	1.7 ±0.18	2.6 ±0.28	1.4 ±0.14	1.8 ±0.22	2.3 ±0.24	1.5 ±0.17	0.2 ±0.03	0.3 ±0.03	–
81	9.9 ±10.11	8.5 ±8.74	6.7 ±0.68	10.3 ±2.17	8.2 ±8.17	6.5 ±6.52	10.7 ±1.17	8.5 ±8.53	6.0 ±0.67	0.5 ±0.06	0.8 ±0.07	0.2 ±0.03
101	116 ±11.61	109 ±10.19	73 ±6.71	115 ±10.51	115 ±10.63	76 ±7.52	118 ±10.21	106 ±9.77	77 ±7.12	0.7 ±0.06	1.1 ±0.21	0.7 ±0.06
121	141 ±12.34	145 ±14.11	85 ±7.88	144 ±13.24	149 ±14.68	86 ±8.71	150 ±13.78	144 ±13.33	85 ±7.54	0.9 ±0.07	1.1 ±0.41	0.6 ±0.07
Harvesting	146 ±13.24	141 ±13.18	86 ±7.46	149 ±12.29	141 ±13.31	88 ±7.89	144 ±13.39	141 ±14.28	88 ±7.87	0.8 ±0.09	1.2 ±1.35	0.7 ±0.06
Post-harvesting	114 ±10.38	117 ±10.17	60 ±7.13	114 ±11.42	114 ±10.57	65 ±5.66	111 ±10.18	115 ±10.18	64 ±6.21	2.3 ±2.31	2.7 ±0.31	1.9 ±0.27

Table 3. Variations in the mean ($\pm$ SE) counts of antibiotic resistant heterotrophic bacterial populations occurring in sediment of three shrimp ponds and in control sites employed. Screening of resistant strains was performed against ampicillin-AMP, chloramphenicol-CHL and oxytetracycline-OTC at 25 $\mu\text{g ml}^{-1}$ each.. Each data represents the mean count of 3 replicates

Days of culture	Pond-1			Pond-2			Pond-3			Control		
	No of cfu $\times 10$			No of cfu $\times 10$			No of cfu $\times 10$			No of cfu $\times 10$		
	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant
Before stocking	5.2 $\pm$ 0.64	5.8 $\pm$ 0.61	4.3 $\pm$ 0.55	4.7 $\pm$ 0.51	5.9 $\pm$ 0.47	4.4 $\pm$ 0.47	5.5 $\pm$ 0.61	5.9 $\pm$ 0.61	4.3 $\pm$ 0.52	0.90 $\pm$ 0.09	1.2 $\pm$ 0.61	1.0 $\pm$ 0.02
7	5.3 $\pm$ 0.57	6.0 $\pm$ 0.55	4.5 $\pm$ 0.51	5.1 $\pm$ 0.54	5.9 $\pm$ 0.61	4.5 $\pm$ 0.58	5.5 $\pm$ 0.62	6.2 $\pm$ 0.58	4.3 $\pm$ 0.53	1.2 $\pm$ 0.25	1.3 $\pm$ 0.47	0.9 $\pm$ 0.14
27	5.4 $\pm$ 0.49	6.2 $\pm$ 0.58	4.6 $\pm$ 0.57	5.2 $\pm$ 0.57	6.0 $\pm$ 0.57	4.6 $\pm$ 0.47	5.7 $\pm$ 0.64	6.4 $\pm$ 0.61	4.5 $\pm$ 0.57	1.2 $\pm$ 0.34	1.4 $\pm$ 0.37	0.9 $\pm$ 0.24
52	6.45 $\pm$ 0.64	8.7 $\pm$ 0.82	5.8 $\pm$ 0.64	6.9 $\pm$ 0.69	9.1 $\pm$ 0.92	5.4 $\pm$ 0.56	7.0 $\pm$ 0.67	8.8 $\pm$ 0.91	5.5 $\pm$ 0.55	1.3 $\pm$ 0.24	1.7 $\pm$ 0.34	1.1 $\pm$ 0.47
81	23.1 $\pm$ 3.2	18.1 $\pm$ 2.14	15.2 $\pm$ 1.91	4.3 $\pm$ 0.45	17.3 $\pm$ 2.01	15.3 $\pm$ 1.75	22.4 $\pm$ 2.64	17.6 $\pm$ 1.95	15.5 $\pm$ 1.58	1.7 $\pm$ 2.01	2.1 $\pm$ 2.25	1.2 $\pm$ 1.86
101	233 $\pm$ 25.13	210 $\pm$ 21.05	124 $\pm$ 16.24	4.3 $\pm$ 5.12	215 $\pm$ 21.34	126 $\pm$ 12.64	230 $\pm$ 23.10	208 $\pm$ 20.81	124 $\pm$ 12.54	1.8 $\pm$ 0.64	1.9 $\pm$ 0.68	1.1 $\pm$ 0.53
121	284 $\pm$ 29.31	27.7 $\pm$ 3.77	133 $\pm$ 15.31	4.5 $\pm$ 0.54	275 $\pm$ 28.61	140 $\pm$ 15.01	280 $\pm$ 29.05	279 $\pm$ 28.61	138 $\pm$ 13.42	2.2 $\pm$ 0.52	1.8 $\pm$ 0.84	1.1 $\pm$ 0.41
Harvesting	283 $\pm$ 24.31	28.0 $\pm$ 3.24	135 $\pm$ 14.25	5.5 $\pm$ 6.25	271 $\pm$ 25.12	136 $\pm$ 12.36	283 $\pm$ 24.58	273 $\pm$ 24.96	138 $\pm$ 12.78	3.1 $\pm$ 0.57	2.9 $\pm$ 4.31	2.1 $\pm$ 0.51
Post-harvesting	207 $\pm$ 19.31	19.2 $\pm$ 3.21	94 $\pm$ 8.74	15.5 $\pm$ 14.56	185 $\pm$ 17.89	93 $\pm$ 8.77	209 $\pm$ 18.97	195 $\pm$ 18.99	95 $\pm$ 7.66	5.5 $\pm$ 4.81	6.6 $\pm$ 0.58	3.5 $\pm$ 4.22

Table 4. Variations in the mean ($\pm$ SE) counts of antibiotic resistant heterotrophic bacterial populations occurring in sediment of 3 shrimp ponds and in control sites employed. Screening of resistant strains was performed against ampicillin-AMP, chloramphenicol-CHL and oxytetracycline-OTC at 50 $\mu\text{g ml}^{-1}$ each. Each data represents the mean count of 3 replicates

Days of culture	Pond-1			Pond-2			Pond-3			Control		
	No of cfu $\times 10$			No of cfu $\times 10$			No of cfu $\times 10$			No of cfu $\times 10$		
	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant	AMP Resistant	CHL Resistant	OTC Resistant
Before stocking	1.9 $\pm$ 0.25	2.1 $\pm$ 0.21	1.5 $\pm$ 2.15	1.5 $\pm$ 0.21	2.0 $\pm$ 0.31	1.2 $\pm$ 2.56	1.4 $\pm$ 0.25	2.0 $\pm$ 0.25	1.5 $\pm$ 0.27	—	—	—
7	1.9 $\pm$ 0.27	2.2 $\pm$ 0.24	1.6 $\pm$ 0.64	1.8 $\pm$ 0.25	2.2 $\pm$ 0.24	1.6 $\pm$ 0.27	1.7 $\pm$ 0.34	2.2 $\pm$ 2.2	1.5 $\pm$ 0.19	—	—	—
27	2.1 $\pm$ 0.28	2.3 $\pm$ 0.31	1.7 $\pm$ 0.21	1.9 $\pm$ 2.54	2.4 $\pm$ 0.26	1.8 $\pm$ 0.29	1.6 $\pm$ 0.29	2.4 $\pm$ 0.43	1.7 $\pm$ 0.28	—	—	—
52	2.4 $\pm$ 0.24	3.0 $\pm$ 0.31	1.9 $\pm$ 1.84	2.3 $\pm$ 2.5	2.8 $\pm$ 0.34	2.4 $\pm$ 0.31	2.6 $\pm$ 0.36	3.1 $\pm$ 0.32	2.2 $\pm$ 2.3	—	—	—
81	7.9 $\pm$ 7.5	7.0 $\pm$ 0.14	5.5 $\pm$ 5.5	7.7 $\pm$ 0.84	7.6 $\pm$ 0.84	5.9 $\pm$ 6.87	8.1 $\pm$ 0.86	7.3 $\pm$ 7.64	5.9 $\pm$ 5.9	0.35 $\pm$ 0.07	0.55 $\pm$ 0.06	—
101	91 $\pm$ 9.5	84 $\pm$ 7.64	43 $\pm$ 4.31	93 $\pm$ 9.5	86 $\pm$ 8.7	46 $\pm$ 4.6	85 $\pm$ 8.9	77 $\pm$ 7.9	46 $\pm$ 4.8	0.40 $\pm$ 0.05	0.60 $\pm$ 0.07	0.25 $\pm$ 0.03
121	96 $\pm$ 9.4	94 $\pm$ 8.54	45 $\pm$ 4.52	94 $\pm$ 8.7	95 $\pm$ 9.5	49 $\pm$ 4.97	94 $\pm$ 9.4	96 $\pm$ 9.54	48 $\pm$ 5.1	0.70 $\pm$ 0.64	0.55 $\pm$ 0.06	0.55 $\pm$ 0.06
Harvesting	102 $\pm$ 10.14	95 $\pm$ 8.46	46 $\pm$ 4.65	106 $\pm$ 10.6	95 $\pm$ 9.45	42 $\pm$ 4.7	96 $\pm$ 9.6	94 $\pm$ 9.54	46 $\pm$ 4.7	1.0 $\pm$ 0.3	0.95 $\pm$ 0.07	0.65 $\pm$ 0.07
Post-harvesting	73 $\pm$ 7.10	70 $\pm$ 7.04	32 $\pm$ 3.54	76 $\pm$ 7.6	67 $\pm$ 6.74	34 $\pm$ 3.7	73 $\pm$ 7.14	70 $\pm$ 6.97	36 $\pm$ 3.8	2.1 $\pm$ 0.24	2.7 $\pm$ 0.34	—

(Table 5). The concentration of dissolved oxygen of water remained well within the range for shrimp farming, ranging from 5.0 to 8.6 mg l^{-1} in 3 shrimp ponds investigated (Table 5). The concentration of ammonia-N in shrimp ponds (0.25 to 1.5 mg l^{-1}) was higher than control pond (Table 6). Amount of salinity varied from 20–26 ppt during the culture period. The values were, however, less during the post harvest period (Table 5).

Variability in the counts of antibiotic resistant strains of heterotrophic bacterial population in the shrimp farming ponds was most apparent. Because of sharp rise (32 fold)

in the density of antibiotic resistant bacterial population during the feeding phase compared to pre feeding phase, it appears that the leaching of antibiotics from supplementary feed exerted a selection pressure in favour of antibiotic resistant strains of bacteria and inhibiting the growth of sensitive ones in the culture ponds. It is stated that resistant bacteria favours genetic exchange with the previously non-resistant population and thereby increasing the dispersion of the resistant capacity in aquatic environment (Damson 1999). Further, the high level of drug resistance in sediment bacterial strain was related to the production of

Table 5. Range (minimum and maximum) of water quality parameters during different phases of shrimp farming. The range was based on the minimum and maximum values observed in 3 shrimp ponds investigated

Parameters	Before stocking	During culture period	Post harvesting period
Temperature (°C)	25.0–25.1	25.0–29.0	24.0–14.6
pH	8.1–8.2	7.9–8.6	7.5–7.6
Salinity (ppt.)	25–25	21–28	16
Dissolved oxygen (mg l ⁻¹)	8.3–8.6	5.0–8.5	6.4–6.6
Ammonium-N (mg l ⁻¹)	0.28–0.34	0.29–1.5	0.25–0.33

antimicrobial compounds found in some fish farm isolates belonging to genus *Bacillus* (Chelossi 2003).

The risk of such incidence is most anticipated; it is likely that shrimps cultured in the ponds remained constantly in contact with these resistant population externally as well as within the gut of the animal, and hence the antibiotic resistance can be donated or transferred to other microbes, which may be pathogenic, or to other environmental bacteria. Under such situation, if the shrimps are infected with pathogenic bacteria wherein antibiotic resistant has been transferred, the treatment may be more difficult as it may require either the use of new antibiotic or higher doses of the used antibiotics. The later may further aggravate the problem due to the fact that the resistance developed was against a very high dose of the antibiotics. Secondly, this may cause health risk to the farmers coming to direct contact with aquaculture ponds.

Steady rise in the per cent of all the 3 antibiotic resistant groups of bacteria in the culture period especially during 101–130 days of culture was clearly in response to increased accumulation of feed remnants along with antibiotics that leached from supplementary feed. As a result, resistant strains of bacteria were dose dependent, being higher at 50 µg than at 25 µg of antibiotics.

The application of antibiotics caused an imbalance in the natural population of heterotrophic bacteria in the pond ecosystem. Antibiotic treatments for over a long period of time caused a pronounced residual effect on the pond environment, as evident from the fact that the counts of antibiotic resistant bacteria tended to rise with the count of antibiotic resistant bacterial population against these antibiotics (ampicillin, chloramphenicol and oxytetracycline @ 25 µg ml⁻¹ in each case) in the control pond was, however, negligible during the comparable periods in shrimp culture ponds with progressive increase in the days of culture. Further, the density of resistant bacteria increased by 88 fold after one cycle of culture and the number is likely to increase further after subsequent cycles, provided that the same protocol of pond management is applied. This may pose a threat to the environment with the existing practices of shrimp culture. Ghosh and Mandal (2010) pointed out that the risk of antibiotic resistant bacteria of consumable fishes collected from coastal areas

of Digba, Bay of Bengal.

A high amount of antibiotic molecule is likely to be released into the environment through semi-intensive shrimp farming as employed in the present study. According to the observation of Samuelsen *et al.* (1998) and Weston (1996) about 60–85% of the drugs can be excreted through the feces unchanged depending on the type of antibiotics used. Based on this assumption, it is estimated that about 4.32 - 6.12 kg active antibiotic ha⁻¹ is being released into the environment in each grow out period of shrimp culture. It is therefore important that antibiotics be handled with much caution. Petersen *et al.* (2002) showed that bacteria isolated from water and sediment samples of integrated farms had a higher level of resistance when compared to bacteria isolated from control fish farms with no use of animal manure and supplementary feed.

It may be reasonable to conclude that shrimp culture effluent have a negative impact on the environment with a risk of transfer of antibiotic resistant bacteria in the adjacent areas, causing a change in the composition of microflora in the ecosystem through competitive exclusion principle.

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NUTRIENT REQUIREMENTS OF ANIMALS



A nutritionally balanced 'livestock feed basket' improves the productivity of animals and simultaneously the economic condition of animal keepers. Feed requirement varies from species to species and from one geographic zone to another depending upon the animal potential and plant-soil-animal relationship. Several institutes of the Indian Council of Agricultural Research, have been working on these crucial aspects of animal nutrition since their inception. Earlier, ICAR published Nutrient Requirement of Livestock and Poultry in 1985 and 1998. Changing climate, vegetation cover and expectations of human population from animal resources have greatly affected the animal sector scenario. Realizing the fact that detailed information is required on nutrient composition of various feeds and fodders, the Council constituted a National Committee on Nutrient Requirements of Animals for compilation of information generated by these institutes.

In this present attempt the Committee has brought out 'Nutrient Requirements of Animals' – a series of ten publications. For the first time nutrient requirements of Camel, Yak and mithun, Companion, laboratory and captive wild animals besides Finfish and shellfish have been compiled. This series will be a must reference resource for livestock policy-framers, researchers, academicians, extension officials and grassroot farmers who steer positive changes in the societies' nutritional security and social integration.



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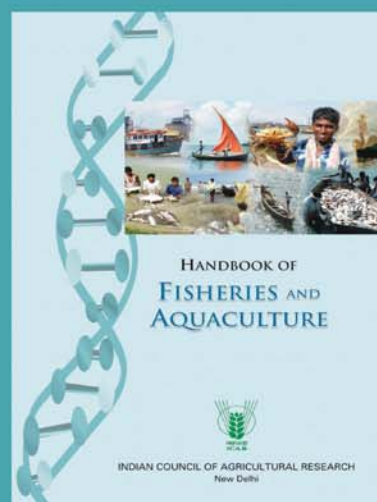
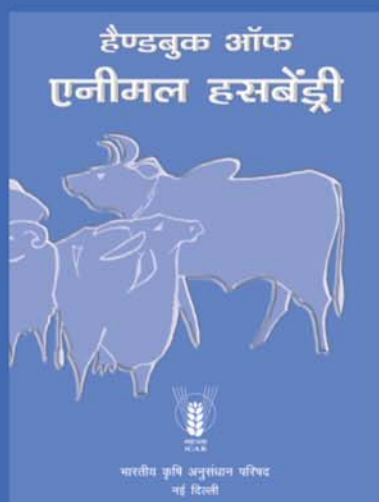
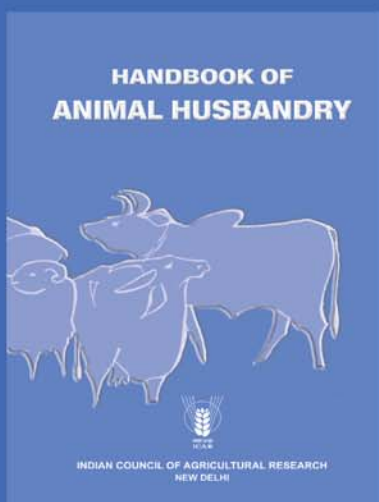
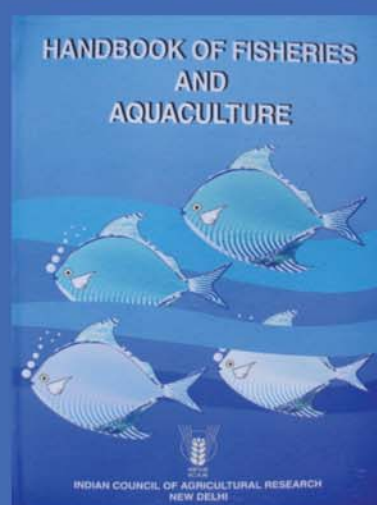
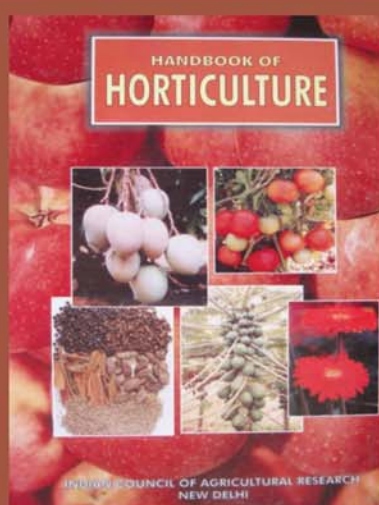
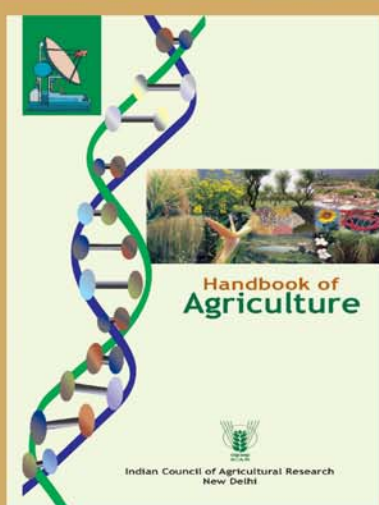
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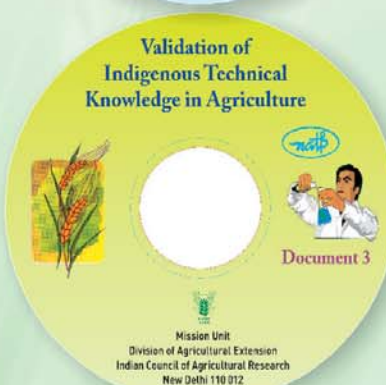
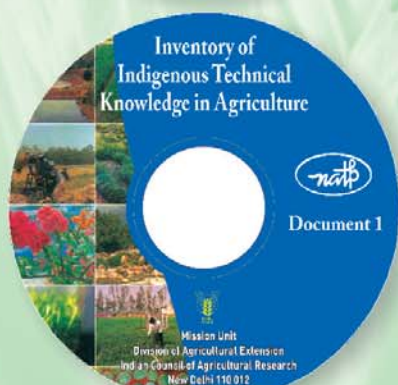
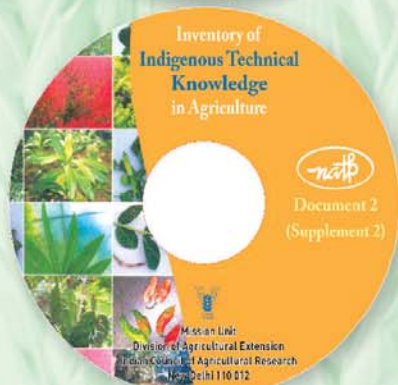
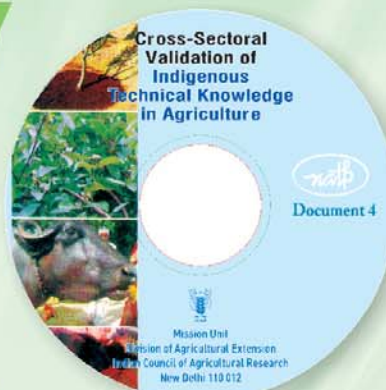
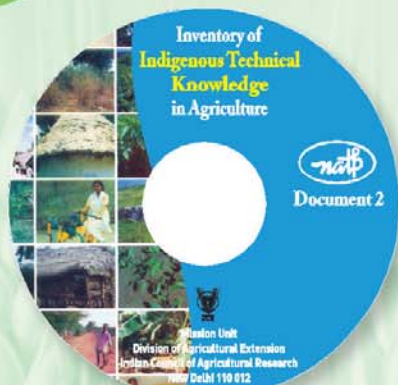


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