Comparison Of Antibiotic Resistance In Bacterial Flora Of Shrimp Farming Systems

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Abstract

The antibiotic resistances among the bacterial flora of extensive, modified extensive and semi-intensive shrimp farming systems of West Bengal, India, which follow varied management practices were compared. The populations of bacteria isolated from semi-intensive shrimp farming system were comparatively more sensitive to antibiotics followed by modified extensive and extensive culture systems. About 38%, 20% and 22% of the bacterial isolates respectively from semi-intensive (n=141), modified extensive (n=186) and extensive (n=154) systems were sensitive to all the six antibiotics. There existed significant differences in multiple antibiotic resistance (MAR) of the isolates from extensive (54.55) and semi-intensive (33.33%) systems, and modified extensive (52.69%) and semi-intensive systems. The variation in the bacterial resistance to different antibiotics observed among various shrimp culture systems of West Bengal was the reflection of varied management practices adopted. The observations on the antibiotic resistance did not indicate that the shrimp farm bacterial flora of certain culture systems in West Bengal is developing resistance to broad-spectrum antibiotics.

INTRODUCTION

A massive intensification occurred in shrimp aquaculture throughout the world, where stocking density and the rate of application of aqua-drugs and chemicals have been hugely accelerated. By definition, aqua-drugs are chemicals or biological preparations or formulations which are used in aquaculture systems either to be consumed by the animals at any stage of their life which may or may not leave any residue in the animal body after application or applied directly to the culture system as biological or chemical preparations for the protection of aquaculture environment. These include antibiotics, antibacterial, antiviral, antifungal, antiprotozoan and antimetazoal preparations, probiotics, immunostimulants, vaccines / bacterins, hormones of all categories, growth stimulants, anesthetics and bioremediatiors (both bioaugmentors and biostimulators). Use of antimicrobials in aquaculture essentially started with the work of Gutsell (1946), who recognized the potential usefulness of sulphanamides for combating furunculosis. Following this, chloramphenicol, oxytetracycline, kanamycin, nifurprazine, oxolinic acid, sodium nifurstyrenate, flumequin, ciprofloxacin and others were introduced (Austin and Austin, 1993). Indiscriminate use of chemicals and drugs often lead to problems like drug resistance, tissue residues, adverse effect on species biodiversity, etc, which ultimately affect the cultured shrimp, human and environment. Several of these aspects have been well documented (Spanggard et al., 1993; Herwig and Gray, 1997; Anderson and Levin, 1999; Tendencia and de la Pena, 2001).

In West Bengal (India) the development in coastal aquaculture is centered on shrimp farming in East Midnapore, North 24 Parganas and South 24 Parganas districts through extensive, modified extensive and semi-intensive methods. The dominant species under shrimp culture is Penaeus monodon due to its high unit value and ever-expanding export demand. The scientific culture of shrimp started during late 1980's and more than 38,480 ha of area was brought under culture in this State. The production of shrimp through aquaculture increased from 12,500 t during the year 1990-91 to 23,445 t in 1995-96 in West Bengal. However, the rapid growth of shrimp farming industry has halted due to disease outbreaks and as a result the production during 1999-2000 dropped to 19,960 t (Upadhyay, 2001) partly as a result of anthropogenic activities. The present communication compares the antibiotic resistance among the bacterial flora of extensive, modified extensive and semi-intensive shrimp farming systems, which follow varied management practices.

MATERIALS AND METHODS

The details of the study area are presented in Table-1. The dug out shrimp ponds of both Midnapore (East) and 24 Parganas (South) districts and also the 'Bheries' of 24 Parganas (North and South) were selected for this study. Regular visits to the sampling stations (Table 1) were made between 2000 and 2002 during the culture period and collected the samples of shrimp grow-out pond water, sediment and shrimps. Samples of pond water were collected in sterilized polypropylene bottles of 250 ml capacity. Pond sediment samples were collected at four places, viz., near inlet, close to outlet, pond center and close to feeding tray in scientifically managed ponds and / or at four sides of the bheries using sterilized plastic jars and transferred immediately to U-V sterilized polythene bags. All the samples were placed in insulated containers and brought to the laboratory within 4 h of collection or 24 h in case of outstation samples.

Figure 1

Table 1 : Description of study area

Region	Location		Type of Culture	Major activity
	Latitude	Longitude		
Midnapore (East)				
Contai Zone	21°48' N	87°45' E	Modified extensive (ME)	Shrimp farming and agriculture
Amtalia			ME	Shrimp farming and agriculture
Korpura			ME	Shrimp farming and agriculture
Petuaghat			ME	Shrimp farming and agriculture
Rasulpur			ME	Shrimp farming and agriculture
Soula			Semi intensive (SI)	Shrimp farming and agriculture
Dariapur			SI	Shrimp farming and agriculture
Digha Zone	21°38' N	87°32' E	Extensive (Ex)	Beach resort and fish landing center
24 Parganas (North)				
Khariberia zone	22°25' N	88°35' E	Ex (bheries)	Shrimp farming and agriculture
Nazat	22°5' N	88°45' E	Ex (bheries)	Shrimp farming and agriculture
24 Parganas (South)				
Canning	22°20' N	88°40' E	Ex	Shrimp farming and agriculture
Malancha	22° N	88°45' E	Ex	Shrimp farming and agriculture

Shrimps were collected from the check trays and also by cast netting. Both normal and diseased shrimps were taken respectively from normal and abnormal ponds, and examined at site for behavioral changes, abnormalities, gross and clinical signs. Inocula from hepatopancreas, hemolymph, gills, intestine, faecal matter of diseased shrimps and shell of shell-diseased shrimps were taken asceptically and transferred to transport medium (Half strength nutrient broth + 1.0% sodium chloride (NaCl) + 0.7% agar) in tubes, sealed by parafilm and brought to the laboratory in insulated containers. Whole diseased shrimps were also wrapped individually in U-V sterile polythene bags, placed in icebox and brought to the laboratory. On reaching laboratory they were transferred to refrigerator and analyzed / processed within 3 h. Samples from diseased shrimps in transport medium were enriched in seawater complex (SWC) broth (Ruby and Morin, 1979) at 30 ± 2 C for 12 h. Inocula from each tube were then streaked on to nutrient agar (NA), SWC and thiosulphate citrate bile salt sucrose agar (TCBS) plates and incubated for 48 h at 30 ± 2 C. Luminescence was observed on NA and SWC agar plates in a dark room after 16-20 h of incubation. Luminous and non-luminous colonies with distinct colony characteristics were asceptically picked from NA, SWC agar and TCBS agar, streaked repeatedly on SWC agar until pure and maintained on SWC agar slants. A series of biochemical reaction as described by MacFaddin (1980) were performed to identify bacteria. Taxonomic keys proposed by Alsina and Blanch (1994) and Lechevallier et al. (1980) were followed for the identification of vibrios and non-vibrios, respectively.

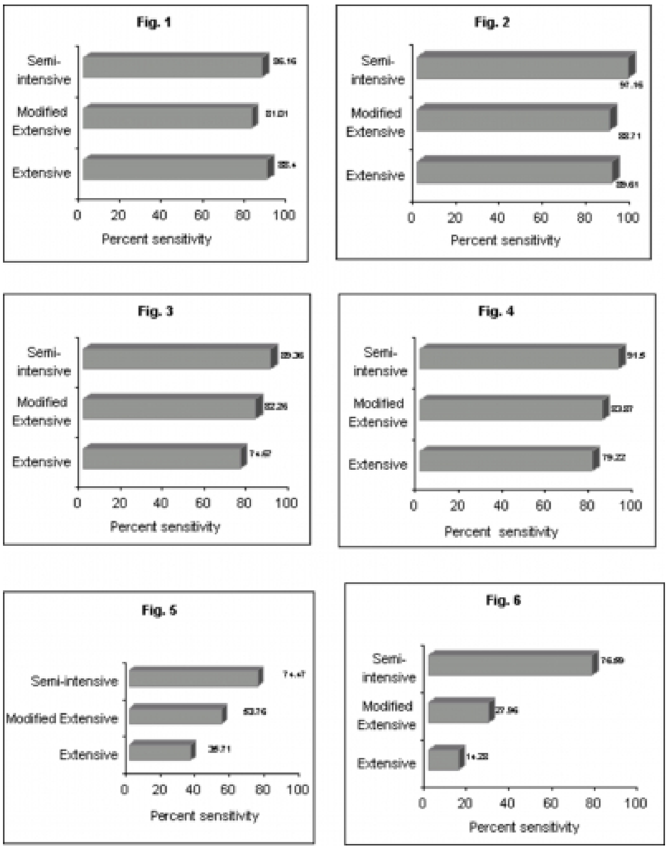
A total of 481 bacterial isolates from culture pond water, soil and different body parts of diseased shrimps were screened for their sensitivity to six broad-spectrum antibiotics by agar disc diffusion method (Bauer et al., 1966). The antibiotic impregnated discs (HiMedia, Mumbai) used in this study include chloramphenicol, C (30 g), ciprofloxacin, F (5 g), co-trimoxazole, S (25 g), gentamycin, G (10 g), nitrofurantoin, N (300 g) and oxytetracycline, O (30 g). Young cultures of bacteria from SWC slants were inoculated individually in antibiotic assay broth No. 37 (HiMedia, Mumbai) containing 1.0% NaCl and incubated for 10-12 h at 30 ± 2 C. Inocula from these young cultures were taken separately and spread on to antibiotic assay agar No. 37 using sterile cotton swabs. Antibiotic impregnated discs were then placed asceptically on to the inoculated agar plates within 20 min at least 15 mm away from the edge at equal distance and sufficiently separated from each other to avoid overlapping of the zone of inhibition. The plates were then incubated for 20-24 h at 30 ± 2 C and the diameter of zone of inhibition in mm measured. Interpretation of sensitivity was based on the zone size interpretation chart as provided by the manufacturer of the antibiotic impregnated discs (HiMedia, Mumbai). The resistance profiles and resistance pattern for the above six potentially valuable antibiotics were determined from the antibiogram data.

RESULTS

The results of the sensitive of shrimp farm bacterial flora to six broad-spectrum antibiotics are illustrated in Figs. 1-6. The populations of bacteria isolated from semi-intensive shrimp farming system were comparatively more sensitive to antibiotics followed by modified extensive and extensive culture systems, except to chloramphenicol (Fig. 1) and ciprofloxacin (Fig. 2). The percent sensitivity of bacteria against nitrofurantoin and oxytetracycline was found to be quite low (Figs. 5 and 6).

Figure 2

Figures 1 - 6: Sensitivity of shrimp farm bacterial flora to antibiotics, viz., Chloramphenicol (Fig. 1), Ciprofloxacin (Fig. 2), Co-trimoxazole (Fig. 3), Gentamycin (Fig. 4), Nitrofurantoin (Fig. 5), Oxytetracycline (Fig. 6)



There existed variations in the degree of sensitivity of bacteria to 6 broad-spectrum antibiotics from different culture systems and it could be expressed in the following order:

- Chloramphenicol: extensive > semi-intensive > modified extensive
- Ciprofloxacin: semi-intensive > extensive > modified extensive
- Co-trimoxazole: semi-intensive > modified extensive > extensive
- Gentamycin: semi-intensive > modified extensive > extensive
- Nitrofurantoin: semi-intensive > modified extensive > extensive
- Oxytetracycline: semi-intensive> modified extensive > extensive

The antibiotic resistance profiles of shrimp farm bacterial flora are presented in Table 2.

Figure 3

Table 2: Antibiotic resistance profiles of shrimp farm bacterial flora

Resistance profile	Number of isolates (n=481)	
	Number	Percent
SFGNO	16	3.33
SFNO	8	1.66
FGNO	11	2.29
CSNO	7	1.46
CNO	9	1.87
FGO	8	1.66
NGF	6	1.25
ONS	10	2.08
ONF	12	2.49
ONG	13	2,70
CG	2	0.42
CS	2	0.42
NG	4	0.83
GF	1	0.21
NF	12	2.49
OF	2	0.42
OS	6	1.25
OG	3	0.62
ON	60	12.47
C	12	2.49
S	12	2.49
F	6	1.25
G	3	0.65
N	13	2.70
O	74	15.38
None	169	35.14

F: Ciprofloxacin (5µg) N: Nitrofurantoin (300µg)
S: Co-trimoxazole (25µg) O: Oxytetracycline (30µg)
G: Gentamycin (10 µg) C: Chloramphenicol (30µg)

Of the total isolates (n=481) tested, 169 isolates (35.14%) were sensitive to all the six antibiotics and 120 (24.95%) were resistant to only one antibiotic. The numbers of isolates resistant to 2, 3 and 4 antibiotics were 92 (19.12%), 48 (9.97%) and 26 (5.41%), respectively. Sixteen isolates exhibited resistance to 5 antibiotics (3.33%). The single dominant resistant profile was against oxytetracycline 74 (15.38%). The resistance pattern of shrimp farm bacterial flora to broad spectrum antibiotics is tabulated in Table 3.

Figure 4

Table 3: Resistance pattern of shrimp farm bacterial flora to broad spectrum antibiotics

Source of bacteria (n=481)	Number of antibiotics to which bacteria demonstrated resistance						Multiple antibiotic resistance (%)*
	0	1	2	3	4	5	
	Number of bacteria (%)						
Extensive (n=154)	35 (22.72)	35	32	40	9	3	54.55 [b]
Modified extensive (n =186)	37 (19.89)	51	51	33	13	1	52.69 [b]
Semi-intensive (n=141)	54 (38.29)	40	27	18	2	0	33.33 [a]

*Resistant to at least two antibiotics.
Values sharing uncommon superscripts differed significantly ($P<0.05$)

About 29%, 40%, 26% and 32% of the bacterial isolates derived from pond water (n=148), pond sediment (n=100), diseased shrimp (n=208) and source water (n=25) were antibiotic sensitive and the rest were resistant to either single or multiple drugs. Likewise, 38%, 20% and 22% of the bacterial isolates respectively from semi-intensive (n=141), modified extensive (n=186) and extensive (n=154) systems were sensitive to all the six antibiotics. There existed significant differences in multiple antibiotic resistance (MAR) of the isolates from extensive (54.55) and semi-intensive (33.33%) systems, and modified extensive (52.69%) and semi-intensive (33.33%) systems.

DISCUSSION

The abuse of antimicrobials can result in the development of resistant strains of bacteria in aquaculture systems. It appears from the results of the present study that oxytetracycline (OTC) was the most extensively used antibiotic in the shrimp farms of West Bengal considering its easy availability and habitual practice of the farmers. The major problem with OTC is that the bacterial pathogens easily develop plasmid-mediated resistance to it (Shotts et al., 1976) and may also enhance the frequency of new OTC resistant bacteria in culture system (Abraham et al., 1997). Among the culture systems, both extensive and modified extensive systems showed more incidences of antibiotic resistant bacterial populations in comparison to semi-intensive system. In extensive bheries, the source water was drawn Bidyadhari River, which carried the untreated sewage waste of Kolkata Metropoliton. The probable reason for the elevated antibiotic resistance in the modified extensive system could be attributed to the frequent use of medicated shrimp feeds to treat the diseased shrimps. All vibrios and non-vibrios were mostly resistant in a decreasing order to oxytetracycline, nitrofurantoin, co-trimoxazole and chloramphenicol, but low against gentamycin and ciprofloxacin. In aquaculture, management practices such as high stocking densities, chemical treatment, grain based artificial diet and accumulation of organic wastes in the form of excess feed, excreta and dead plankton have residual influence on the antibiotic resistance of aquatic bacteria (McPhearson et al., 1991). Nevertheless, the semi-intensive farms bacterial flora were highly sensitive to antibiotics probably due to proper water exchange, monitoring and management by controlled use of drugs.

It was found that maximum numbers of bacterial flora were resistant to nitrofurantoin and oxytetracycline, which corroborated the observations of Tendencia and de la Pena (2001). They examined a total of 413 isolates against four antibiotics and reported that the maximum numbers of bacterial isolates were resistant to oxytetracycline and nitrofurantoin. The observations of Roque et al. (2001) from different body parts of diseased shrimp also conform to the observations of the present study. Maximum number of Vibrio spp, Aeromonas spp and Pseudomonas spp showed resistance to nitrofurantoin and oxytetracyline similar to those of McPhearson et al. (1991) and Abraham et al. (1997). Contrarily, Toranzo et al. (1993) reported that none of the Vibrio spp was found to be resistant to oxytetracyline. According to them, this might be due to growth of a genetically typical strain of a bacterium from a non-exposed clone. Occurrence of chloramphenicol resistant bacteria was reported from the shrimp culture systems of Tamil Nadu (Abraham et al., 1997) and elsewhere (Roque et al., 2001; Tendencia and de la Pena, 2001). The bacterial flora were highly sensitive to ciprofloxacin and only 2 species of organisms exhibited resistance to the drug. Gentamycin, nitrofurazone and sulpha drugs resistance among Vibrio spp from shrimp farm environs have been reported earlier in India (Abraham et al., 1997; 2001).

The results of this study suggest the presence of numerous aquatic bacteria and pathogenic bacterial strains resistant to broad-spectrum antibiotics in shrimp culture systems especially in the bheries and modified extensive system. About 54.55%, 52.69% and 33.33% of the bacterial flora from extensive, modified extensive and semi-intensive systems respectively showed multiple antibiotic resistance (MAR), which is, however, less than those reported from the shrimp culture systems of Tamil Nadu (Abraham et al., 1997). Development of resistance among the shrimp farm bacterial flora to nitrofurantoin, oxytetracycline, co-trimoxazole and chloramphenicol and the incidence of multiple antibiotic resistance are of great concern to shrimp aquaculture. The Marine Products Export Development Authority has banned the use of chloramphenicol, furazolidone, neomycin, nalidixic acid and sulphamethoxazole in Indian shrimp aquaculture (MPEDA, 2001). It has also been reported that the frequency and rates of ascent and dissemination of antibiotic resistance in bacterial populations are directly related to the volume of antibiotic used (Anderson and Levin, 1999). The variations in the bacterial resistance to different antibiotics observed among the various shrimp culture systems of West Bengal might be related to the adoption of varied managerial measures suitable to the biotic potentials of those systems. Nonetheless, the observations on the antibiotic resistance did not indicate that the shrimp farm bacterial flora of certain culture systems in West Bengal is developing resistance to broad-spectrum antibiotics.

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