NOTES

BACTERIAL FLORA ASSOCIATED WITH DISEASED FISH AND THEIR ANTIBIOGRAM

T. Jawahar Abraham*, Debasis Sasmal and Tirthankar Banerjee

*Department of Fishery Pathology and Microbiology, Faculty of Fishery Sciences,
West Bengal University of Animal and Fishery Sciences,
Mohanpur, Nadia, West Bengal - 741252, India.*

* Corresponding author E-mail: jawaharabraham@rediffmail.com

ABSTRACT

Bacterial flora associated with tail rot / fin rot of *Carassius auratus*, *Xiphophorus helleri* and hemorrhagic ulcers of *Clarias* spp were studied. Sensitivity pattern of 33 isolates comprising *Aeromonas* spp, *Pseudomonas* spp and Gram-positive rods from diseased *C. auratus*, *X. helleri* and *Clarias* spp were screened against six broad-spectrum antibiotics *viz.* ciprofloxacin, chloramphenicol, co-trimoxazole, gentamycin, nitro-furantoin and oxytetracycline. Ciprofloxacin was the most effective in inhibiting bacteria at 0.05 - 0.10 µg/ml level. About 44% of *Pseudomonas* spp. were resistant to nitrofurantoin. Resistance to oxytetracycline was seen in 27% of *Aeromonas* spp Gram-positive rods were comparatively more resistant to antibiotics. The multiple antibiotic resistance was seen in 21% of the bacterial isolates of diseased fish.

Key words: Fish pathogens, Antibiogram

Indian aquaculture industry, as a whole, lacks adequate research and development in breeding, rearing, nutrition and more particularly on health management. *Aeromonas* spp *Pseudomonas* spp are the members of the normal bacterial flora of freshwater, often acting as the opportunistic pathogens to freshwater fish species under stress. The importance of good environmental conditions and management practices in preventing bacterial diseases are well understood (Austin and Austin, 1999).

With the intensification in aquaculture, the need for the effective therapy against bacterial fish pathogens has increased. The lack of information on the effective antibiotics for aquaculture and an increasing rate of resistance among the bacterial pathogens to commonly used antibiotics necessitated the search for alternative antibiotics and other measures of disease control. This communication reports the bacterial flora associated with diseased fish and their sensitivity to six broad-spectrum antibiotics.

The moribund fish such as *Carassius auratus, Xiphophorus helleri* and *Clarias* sp. were brought to the laboratory in oxygen filled polythene bags and observed for gross and clinical signs. The moribund *Carassius auratus* (3.0 - 3.5 cm) and *Xiphophorus helleri* (2.5 - 3.0 cm) were collected from aquarium retail outlets of Kalyani and Barajaguly, Nadia District, West Bengal. *C. auratus* had rotten tails, red sores or swelling on tail rays or at the base, the outer tail rays become frayed from disintegration of the soft tissues between the fin rays. While the *X. helleri* showed the presence of disintegrated fin membranes, fin margins become inflamed, degeneration of surface fin tissues and connective tissues. *Clarias* spp (3.5 - 4.5 cm) was from a nursery pond of a fish hatchery in Maldah, West Bengal. It had clusters of tiny hemorrhagic lesions at the base of the pelvic fins and at abdomen

The bacteria associated with the affected parts of diseased fish (n = 7) were studied by inoculating samples taken from infected parts and/or blood on to tryptic soy agar (TSA; pH 7.5) and incubating at $30 \pm 2°C$ (Austin and Austin, 1999). A total of 33 isolates were randomly picked, purified by streaking repeatedly on TSA and maintained on TSA slants. The schematic keys of Lechevallier et al. (1980) were followed for the identification of bacteria. Sensitivity of these bacteria to six broad-spectrum antibiotics, viz., chloramphenicol (C, 30 mcg), ciprofloxacin (F, 5 mcg), co-trimoxazole (S, 25 mcg), gentamycin (G, 10 mcg), nitrofurantoin (N, 300 mcg) and oxytetracycline (O, 30 mcg) - HiMedia, Mumbai - was studied by agar-disc diffusion method (Bauer *et al.,*

1966) on TSA. Antibiotic resistance profile and multiple antibiotic resistance (MAR) were derived from the antibiogram data. The minimal inhibitory concentration of antibiotics was determined by dilution method as described by NCCLS (1985)

The bacterial diseases in tropical ornamental fish are still little understood. The diseases of ornamental fish of the present study were diagnosed to be tail rot in *C. auratus*, fin rot in *X. helleri* and hemorrhagic septicemia in *Clarias* sp. on the basis of symptoms and their comparison with the description of Austin and Austin (1999). A number of factors contribute to outbreak of bacterial diseases, of which the most common in aquaria are poor water quality and crowding or overcrowding (Inman and Hambric, 1973). The *Aeromonas* spp and *Pseudomonas* spp are preeminently water bacteria and their presence in diseased fish revealed the opportunistic pathogenic association. *Aeromonas* spp. and *Pseudomonas* spp. have frequently been described in cold-water fish (Bullock, 1965; Van Ramshorst, 1995) so also in the tropical ornamental fish of the present study (Table 1).

The Gram-negative bacteria such as *Aeromonas* spp and *Pseudomonas* spp of the diseased fish were highly sensitive to many of the broad-spectrum antibiotics, except nitrofurantoin. The Gram-positive rods appeared resistant to the antibiotics screened (Table 1). The MAR was noticed in 21% of the total isolates screened. Except nitrofurantoin (MIC: 0.39 - 200 µg/ml), all other antibiotics were quite effective in inhibiting these pathogens at lower concentrations (MIC: 0.05 - 12.50 µg/ml),

Table 1 : Bacterial flora associated with diseased fish, their source and antibiogram

Disease and Host	Bacteria		
	Aeromonas spp. (n = 18)	*Pseudomonas* spp. (n = 12)	Gram positive rods (n = 3)
Tail rot			
Carassius auratus (n = 2)	5	6	1
Fin rot			
Xiphophorus helleri (n = 2)	7	3	-
Hemorrhagic ulcers			
Clarias spp (n = 3)	6	3	2
Antibiotics	Antibiotic resistance (%)		
Chloramphenicol (30 mcg)	0	11.11	33.33
Ciprofloxacin (5 mcg)	0	0	33.33
Co-trimoxazole (25 mcg)	11.11	16.67	66.67
Gentamycin (10 mcg)	0	0	33.33
Oxytetracycline (30 mcg)	27.78	5.56	33.33
Nitrofurantion (300 mcg)	16.67	44.44	66.67

Table 2 : Minimal inhibitory concentration (MIC) of antibiotics against bacterial pathogens

Antibiotics	MIC (µg/ml) range		
	Aeromonas spp. (n = 18)	*Pseudomonas* spp. (n = 12)	Gram positive rods (n = 3)
Chloramphenicol	0.05 - 0.20	0.05 - 12.50	0.05 - 12.50
Ciprofloxacin	0.05	0.05	0.05 - 0.10
Co-trimoxazole	0.10 - 12.50	0.10 - 12.50	0.10 - 12.50
Gentamycin	0.05 - 0.78	0.05 - 0.78	0.05 - 1.56
Oxytetracycline	0.10 - 12.50	0.10 - 1.56	0.10 - 3.13
Nitrofurantoin	0.39 - 50	0.39 - 200	0.39 - 200

with ciprofloxacin being the most effective (MIC: 0.05 - 0.1 µg/ml), followed by gentamycin (MIC: 0.05 - 1.56 µg/ml) (Table 2). In general, this study revealed the effectiveness of the existing and readily available drugs to control both gram-positive and gram-negative bacterial pathogens in aquariculture. Nevertheless, one has to aware of the potential hazards of indiscriminate use of chemicals and drugs in aquaculture systems, which are amply documented (Austin and Austin, 1999). In this context, alternate ecofriendly methods such as biocontrol of pathogens, application of herbal medicines and immunostimulation deserve special attention.

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REFERENCES

Austin, B. and Austin, B. A., 1999. *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish.* Third (Revised) Edition. Springer - Praxis Publishing, Chichester, 457 pp.

Bauer, A. W., Kirby, W.M.M. Sherris, J.C. and Truck, M., 1966. Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.*, 45: 493 - 496.

Bullock, G.L., 1965. Characteristics and pathogenicity of a capsulated *Pseudomonas* isolated from gold fish. *Appl. Microbiol.*, 13: 89-92.

Inman, C.R. and Hambric, R. N., 1973. Diseases and Parasites of Warm Water Fishes. *Technical Series No.2. Texas Parks and Wildlife Department, Inland Fisheries Function,* Texas, 55 pp.

Lechevallier, M.W., Seidler, R. J. and Evans, T. M., 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.*, 40: 922 - 930.

NCCLS 1985. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.* National Committee for Clinical Laboratory Standards, Publication M7-A, Villanova, PA.

Van Ramshorst, J. D., 1995. *The Complete Aquarium Encyclopedia of Tropical Freshwater Fish.* The Promotional Reprint Company Limited, Leicester, 391pp.