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Studies on Antibiotic Resistance of Pathogens Isolated from Infected Ornamental Fish

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Abstract: In aquaculture, Microbial diseases of farmed fish lead to high mortalities and reduced economical income for the fish farming industry. Furthermore, fish diseases, particularly those caused by bacterial pathogens are the most important causes of losses among fish farm stocks. In this study, we tried to find out the suitable antibiotic in order to make disease free fish. The infected ornamental fish were collected and microorganisms were isolated and identified. The contaminated microbiota was tested with selective antibiotics. Zone of inhibition was observed. The results showed that some microbes were sensitive and some were moderate or resistant which were interpreted according to the instruction of the manufacturer.

Key words: Antibiotics · Ornamental Fish · Microbiota · Zone of Inhibition

INTRODUCTION

Ornamental fish nurturing is one of the most popular hobbies throughout the world. The growing interest in aquarium fish has resulted in a steady increase in aquarium fish trade globally. The trade with a turnover of USD 5 billion and an annual growth rate of 8 percent offers a lot of scope for development. India's share in ornamental fish trade is estimated to be Rs. 158.23 lakh which is only 0.008 % of the global trade. The major part of the export trade is based on wild collection [1]. There are about 30,000 to 40,000 species of fish differing from each other in shape, size and habitat. They live in all the seas, rivers, canals, lakes, dams, ponds and in almost every place where there is water [2]. Most of these belong to the super order Teleosteii. Among these, 600 species valued as ornamental fish are grown for hobby and export. Economists and dealers estimate that the world turnover in the aquarium fish trade has already exceeded 10,000 crore rupees annually. Of the aquarium fish sold throughout the world about 60 % is produced by Asia, 30 % by South America and remaining 10 % by the other continents. Countries like Singapore and Srilanka produce and export ornamental fish worth of 2,100 crore rupees annually [1]. However, India's contribution to the international export market is significantly low earning about less than one crore rupees per annum. But in 2000-2001, it shot up to 13.9 mt with an increment in value of 0.71 crores. Therefore ornamental fish farming has the potential to boost the national economy.

There are two broad categories of fish diseases have been studied, infectious and noninfectious. Infectious diseases are caused by pathogenic organisms in the located in the environment or carried by other fish. They are contagious and can possibly be treated with antibiotics and other medicines. Noninfectious diseases are caused by environmental factors, genetic defects, parasites, or nutritional deficiencies. They are not contagious and cannot be treated with medications. The fish pathogens are protozoan *Ichthyophthirius multifiliis*, bacterial species such as *Pseudomonas aeruginosa, Pseudomonas fluroescens* and viral pathogens [3].

There are many steps to eradicate diseases affected ornamental fish including distribution of many fish disease agents, disease detection and identification procedures, fish pathology and treatment methods [4-10]. However the subject of planned programs for the reduction of risk exposure to disease, as a specific approach for improving fish health, has not been adequately covered in the literature. The present aim of

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this study was to find out the antibiotic resistance of the dominant microbes isolated from infected ornamental fish at Arumpanur pudur and Kadachanenthal in Madurai (Tamilnadu), India.

MATERIALS AND METHODS

Collection of Fish: The diseased ornamental fish such as Kissing gowrami (*Helostoma temmincki*), Gold fish (*Carassius auratus auratus*), Red sward tails (*Xiphophorous helleri helleri*) and White shark (*Caracharadon caracharias*) were collected from an aquaculture farm located at Kadachanenthal, Madurai (Collection site I).

The remaining diseased ornamental fish such as Black shark (*Etmopterus carteri*), Black molly (*Molliensia sphenops*) and Molly (*Poecilia sphenops*) were collected from an aquaculture farm located at Arumpanur pudur, Madurai (Collection site II). The infected ornamental fish collected from the two different ornamental fish farms were used for isolating the disease causing organisms.

Isolation and Identification of Microbes: The various fish mucus, caudal fin and gill region were carefully scraped from the dorsal body using a sterilized cotton buds, ventral skin mucus was not collected to avoid intestinal and sperm contamination. The samples were spread over the surface of the nutrient agar plates. The plates were incubated at 37°C for 24 hours. The suspected colonies were identified according to Trust and Sparrow [11].

Isolation and Identification of Fungi: The potato dextrose agar medium was prepared with the antibiotic streptomycin (approximately 50 µl/ml), to eliminate the bacterial colonies. Skin mucus, gill region and caudal region were carefully scraped from the dorsal body using sterilized cotton buds. Ventral skin mucus was not collected to avoid intestinal and sperm contamination. The samples were spread over the surface of the plates. They were incubated at room temperature for 48-72 hours. The isolated fungi were identified by observing the colony morphology and microscopically appearance of the fungi. Micro slide method developed by Riddell [12] was adapted for studying the microscopic mycelial morphology. Fungi were stained with Lacto Phenol Cotton Blue stain and observed under the microscope.

Antimicrobial Activity Using Standard Antibiotic Discs: The isolated pathogens were tested to find out the resistance or susceptibility to the selected antibiotics discs [Himedia] The susceptibility of the isolated microbes to selected antibiotics was tested using Kirby-Bauyer method [13].

Bacteria were inoculated into nutrient broth and fungi were incubated into potato dextrose broth and they were incubated for 24 hours at 37°C. Then a sterile cotton swab dipped into the test culture and used to inoculate evenly on the entire surface of Muller-Hinton agar plate. After, the agar surface sets dried for about 5 minutes, the antibiotics discs were placed on it with a sterile forceps. The plates were incubated for 24 hours at 37°C and zone of inhibition was measured (mm) and the results were interpreted according to the instruction of the manufacturer.

Antimicrobial Discs: Gentamycin (G^{10}), Norfloxacin (Nx^{10}), Tobramycin (Tb^{10}), Cephotaxime (Ce^{30}), Ciprofloxacin (Cf^{5}), Cephaloridine (Cr^{30}), Amoxycilin (Am^{10}), Chloramphenicol (C^{30}), Doxycycline Hydrochloride (Do^{30}), Rifampicin (R^{5}), Erythromycin (E^{15}), Bacitracin (B^{8}), Amikacin (Ak^{30}), Ampicillin (A^{10}), Oxacillin (Ox^{1}), Fluconazole (Fu^{10}), Clotimazole (Cc^{10}), Nystatin (Ns^{100}) and Amphotericin-B (Ap^{100}).

RESULTS AND DISCUSSION

Most of the bacterial species similarly occurred in both of the ornamental fish farms in diseased fish (i.e. collection site I and II) and except for *P. fluorescence* (Table 1 and 2). The bacterial species such as *Pseudomonas aeruginosa, Pseudomonas fluroescens, Vibrio cholerae, Vibrio vulnificus, Salmonella* sp. *Enterobacter* sp. *E. coli, Aeromonas* sp. and the fungal species such as *Aspergillus niger, A. flavus, A. fumigatus, Fusarium* sp., *Scopulariasis* sp., *Alternaria* sp., *Acremonium* sp., *Mucor* sp., *Gliocadium* sp. *and Penicillium* sp. were isolated from examined infected fish (Table 1 and 2).

Antibotic susceptibility testing of *Pseudomonas aeruginosa* showed resistance against Cephalonidine (Cr^{30}), Amoxycilin (Am^{10}), Erythromycin (E^{15}), Bacitracin (B^{8}) and Ampicillin (A^{10}), intermediate resistance with Rifampicin (R^{5}) and Oxacillin (Ox^{1}) and sensitivity to Amikacin (Ak^{30}), Gentamycin (G^{10}), Norfloxacin (Nx^{10}), Tobramycin (Tb^{10}), Cephotaxime (Ce^{30}) and Ciprofloxacin (Cf^{5}). Maximum inhibition zones obtained from antibiotics Ciprofloxacin (Cf^{5}), Chloramphenicol (C^{30}) and Norfloxacin (Nx^{10}) were 32 mm, 30 mm and 28 mm respectively (Table 3).

Table 1: Identification of bacteria and fungi isolated from examined fish at collection site I (Kadachanenthal)

S.No.	Experimental fish	Bacterial species	Fungal species
1.	*Helostoma temmincki* (Kissing gowrami)	*Vibrio vulnificusSalmonella* sp.	*Aspergillus niger*
		Enterobacter sp.	*Fusarium* sp.
		Pseudomonas aeruginosa	*Alternaria* sp.
2.	*Carassius auratus auratus* (Gold fish)	*E. coli*	*Aspergillus flavus*
		P. aeruginosa	*Alternaria* sp.
		Enterobacter sp.	*Gliocladium* sp.
3.	*Xiphophorous helleri helleri* (Red sward tail)	*E. coli*	*Mucor sp.*
		P. aeruginosa	*Aspergillus flavus*
4.	*Caracharadon caracharias* (White shark)	*Enterobacter* sp.	*Fusarium* sp.
		P. aeruginosa	*Aspergillus niger*
		Aeromonas sp.	*Scopulariasis* sp.

Table 2: Identification of bacteria and fungi isolated from examined fish at collection site II (Arumbanur-Pudur)

S.No.	Experimental fish	Bacterial species	Fungal species
1.	*Etmopterus carteri* (Black shark)	*Enterobacter* sp.	*Aspergillus niger*
		P. aeruginosa	*Aspergillusfumigates*
		P. fluorescence	
		Aeromonas sp.	
2.	*Molliensia sphenops* (Black molly)	*Enterobacter* sp.	*Fusarium* sp.
		P. aeruginosa	*Aspergillus niger*
		Aeromonas sp.	
3.	*Poecilia sphenops* (Molly)	*Vibrio vulnificus*	*Aspergillus niger*
		P. aeruginosa	*Acremonium* sp.
		Aeromonas sp.	*Penicillium* sp.

Table 3: Antibacterial activity against *Pseudomonas aeruginosa* isolated from the examined fish

		Collection site-I (Kadachanenthal)			Collection site-II (Arumpanur pudur)			
Antibiotics	Standard antibiotic disc	Pa 1	Pa 2	Pa 3	Pa 4	Pa 5	Pa 6	Pa 7
	Zone of inhibition in mm							
Cr^{30}	23-29	-	-	-	-	-	-	-
Ox^{1}	-	12	11	10	13	7	5	6
Am^{10}	-	-	-	-	-	-	-	-
C^{30}	-	30	24	20	28	12	27	23
G^{10}	16-21	21	17	20	16	18	15	19
B^{8}	-	-	-	-	-	-	-	-
Nx^{10}	22-29	28	25	23	26	24	25	23
Do^{30}	-	16	15	17	16	16	14	15
A^{10}	-	-	-	-	-	-	-	-
R^{5}	-	12	11	12	10	10	11	12
E^{15}	-	8	7	-	8	8	-	10
Ak^{30}	18-26	22	24	21	24	23	21	20
Tb^{10}	19-25	24	20	19	20	22	21	18
Cf^{5}	25-33	32	30	29	31	30	30	28
Ce^{30}	18-22	20	19	21	19	20	17	15

Note: Pa1-*Pseudomonas aeruginosa* isolated from *Helostoma temmincki* (Kissing gowrami).

Pa2-*Pseudomonas aeruginosa* isolated from *Carassium auratus auratus* (Goldfish).

Pa3-*Pseudomonas aeruginosa* isolated from *Xiphophorous helleri helleri* (Red sword tail).

Pa4-*Pseudomonas aeruginosa* isolated from *Caracharadon caracharias* (White shark).

Pa5-*Pseudomonas aeruginosa* isolated from *Etmopterus carteri* (Black shark).

Pa6-*Pseudomonas aeruginosa* isolated from *Molliensia sphenops* (Black molly).

Pa7-*Pseudomonas aeruginosa* isolated from *Poecilia sphenops* (Molly).

G^{10}= Gentamycin, Norfloxacin (Nx^{10}), Tobramycin (Tb^{10}), Cephotaxime (Ce^{30}), Ciprofloxacin (Cf^{5}), Cephaloridine (Cr^{30}), Amoxycilin (Am^{10}), Chloramphenicol (C^{30}), Doxycycline Hydrochloride (Do^{30}), Rifampicin (R^{5}), Erythromycin (E^{15}), Bacitracin (B^{8}), Amikacin (Ak^{30}), Ampicillin (A^{10}) and-Oxacillin (Ox^{1}).

Table 4: Antifungal activity against *Aspergillus niger* isolated from the examined fish

Antibiotics	Standard antibiotic disc	Collection site-I			Collection site-II			
		An 1	An 2	An 3	An 4	An 5	An 6	An 7
	Zone of inhibition in mm							
Fu^{10}	-	-	-	-	-	-	-	-
Cc^{10}	12-20	14	13	12	14	13	13	14
Ns^{100}	-	-	-	-	-	-	-	-
Ap^{100}	-	-	-	-	-	-	-	-

Note:

An 1-*Aspergillus niger* isolated from *Helostoma temmincki* (Kissing gowrami).

An 2-*Aspergillus niger* isolated from *Carassium auratus auratus* (Goldfish).

An 3-*Aspergillus niger* isolated from *Xiphophorous helleri helleri* (Red sword tail).

An 4-*Aspergillus niger* isolated from *Caracharadon caracharias* (White shark).

An 5-*Aspergillus niger* isolated from *Etmopterus carteri* (Black shark).

An 6-*Aspergillus niger* isolated from *Molliensia sphenops* (Black molly).

An 7-*Aspergillus niger* isolated from *Poecilia sphenops* (Molly).

Fu^{10}= Fluconazole, Clotimazole (Cc^{10}), Nystatin (Ns^{100}) and Amphotericin-B (Ap^{100})

Antifungal activity was carried out using *Aspergillus niger* tested against Fluconazole (Fu^{10}), Clotimazole (Cc^{10}), Nystatin (Ns^{100}) and Amphotericin-B (Ap^{100}). Clotrimazole (Cc^{10}) was the only active antibiotic showing antifugal activiy against *A. niger* (Table 4). There is no zone of inhibition recorded for other antibiotic discs.

In aquaculture, according to the intensive system of production to reduce the cost, fish are kept in high densities and the possibility of exposure to pathogens, which can be, bacteria, parasites or virus, throughout production cycle is becoming high [14]. Bacterial diseases contribute to natural mortality and can be significant factors in population dynamics of fishes [15, 16].

Many of the pathogens that cause diseases in fish and shellfish are facultative forms that are ubiquitous in aquatic systems. In nature, a high percentage of apparently normal and healthy animals harbor potential pathogens without evidence of clinical signs or overt disease [17]. The development of disease in aquaculture systems usually occurs as the end result of a disruption of the normal environment in which the animals are being reared. Unfavorable conditions, such as crowding, temperature fluctuations, inadequate dissolved oxygen, excessive handling, physical abuse, inadequate diets, or toxic substances may stress the animals.

The fungal agents involved in fish culture are described by Post; Neish and Hughes [18, 19]. Those affecting shellfish are detailed by Sindennann; Sindermann and Lightner [20, 21]. Trout and salmon are attacked by *Saprolegnia* and *Achlya* spp. and catfish by *Saprolegnia* spp. Shrimp are infected by *Lagenidium* and *Fusarium* spp., lobsters by *Lagenidium, Fusarium* and *Haliphthoros* spp. In shellfish culture, *Sirolpidium* and *Lubyrinthomyxa* spp. cause disease in oysters and clams.

Though the aquaculture is very profitable, it is also a serious economic threat when diseases outbreaks occur. Avoiding disease epidemics in aquaculture is very important to get benefit economically and it is only possible when fish species are reared in a good environmental condition and given priority in fish welfare [22].

Fish welfare or animal right law is related to farmed fish. It means that fish have right to live a life as good as possible and express its natural behavior as much as possible and free from negative experiences. Production of fish against infectious diseases is a major challenge in aquaculture worldwide and losses due to infectious diseases limit profitability.

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