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Prevalence, distribution and drug resistance of motile aeromonads in freshwater ornamental fishes

NIFTY JOHN AND A. A. M. HATHA
Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Lakeside Campus Cochin University of Science and Technology, Cochin - 682 016, Kerala, India
e-mail: mohamedhatha@gmail.com

ABSTRACT

The objective of the present study was to assess the prevalence of various motile aeromonads in freshwater ornamental fishes and to elucidate the antibiogram and beta hemolytic activity among the isolates. A total of 120 ornamental fish samples were screened and analyzed for *Aeromonas* spp. Motile aeromonads were isolated from 37.5% of the ornamental fish samples. Various species of motile aeromonads such as *Aeromonas caviae, Aeromonas hydrophila, Aeromonas jandaei, Aeromonas schubertii, Aeromonas sobria, Aeromonas trota* and *Aeromonas veronii* were detected. All the isolates were sensitive to ceftazidime, chloramphenicol, ciprofloxacin and gentamicin. Multiple antibiotic resistance was observed in 58% of the isolates.

Keywords: Diseases, Multiple antibiotic resistance, Motile aeromonads, Ornamental fish

Introduction

The ever-increasing demand for aquarium fishes gradually paved the way towards global trade of ornamental fishes. Substantial international trade takes place within the ornamental fish industry today. Diseases in aquaculture systems are recognized as an important limiting factor to production and trade. Bacterial organisms may be the primary cause of disease, or they may be secondary invaders. The majority of bacterial fish pathogens are natural inhabitants of the aquatic environment. Infections caused by motile members of the genus *Aeromonas,* are amongst the most common and troublesome diseases diagnosed in cultured warm water fishes and have been referred to by various names, including motile aeromonad septicemia (MAS), motile aeromonad infection (MAI), hemorrhagic septicemia, red pest, and red sore. *Aeromonas* bacteria causing these infections are called aeromonads (Camus *et al*., 1998). The widespread distribution of these bacteria in the aquatic environment and the stress induced by intensive culture practices predisposes fish to infections.

The disease problems are treated with antibiotics, the indiscriminate use of which can result in the rapid spread of multi-drug resistant pathogens across the system. This fact along with the financial crisis caused by the mortality of ornamental fishes makes the study of different geographical isolates of aeromonads important. Kerala has immense potential for developing the ornamental fish industry and the development of the ornamental fish industry would bring in economic growth to the state. There are a number of issues that continue to challenge the ornamental fish industry, of which disease is a major risk factor (Citarasu *et al*., 2011). Hence the present study was taken up to investigate the level of prevalence of motile aeromonads among freshwater ornamental fishes collected from aquarists in Cochin area.

Materials and methods

Collection of samples

Ornamental fish samples were collected from aquarium vendors in Cochin over a period of two years and transported to the laboratory in sterile polythene bags. The samples were then analysed for aeromonads within 4 h of collection.

Isolation and identification of Aeromonas spp.

Bacteria were isolated from different parts of the body (body surface, gill and intestine) of the fishes. The body surface and the gill surface of the fishes were swabbed with separate, sterile cotton swabs. Using a pair of scissors, an incision was made near the vent of the fish facilitating the swabbing of intestine. The swabs were then transferred to alkaline peptone water (APW pH 8.4) which was used as the enrichment medium. After incubation at 37 ^{o}C for 18 h, a loopful of APW was streaked on starch ampicillin agar (Ampicillin 10 mg l^{-1}) for selective isolation and incubated at 37 ^{o}C for 18-24 h (Palumbo *et al.,* 1985). The plates were then flooded with approximately 5 ml of Lugol's iodine solution and amylase positive yellow to honey coloured colonies were isolated.

The isolated cultures were then purified by repeated streaking on nutrient agar plates. Those strains that were gram negative bacilli, motile, oxidase and catalase positive, glucose fermenting, nitrate reducing, urease negative and which do not grow at 6% NaCl were further tested for arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activity. Additional tests performed include acid production from mannitol, sucrose, arabinose, Voges –Proskauer reaction, hydrolysis of esculin and indole production. The isolates were identified to the species level using Aerokey II (Carnahan *et al*., 1991).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was performed for the identified *Aeromonas* spp. by the disc diffusion method (Bauer *et al*., 1966). MAR index (Multiple Antibiotic Resistance) values were calculated for all the isolates according to Krumperman (1983).

Beta haemolytic assay

Haemolytic activity was determined using blood agar medium containing 5% human blood. Pure cultures of bacterial isolates were spot inoculated onto blood agar plates and â haemolytic activity was recorded as clear zones around the colony after incubation at 37 ^{o}C for 24 h.

Results and discussion

Motile *Aeromonas* were isolated from 37.5% of the ornamental fish samples. The 45 aeromonad isolates obtained from the samples were identified to different phenospecies level such as *Aeromonas caviae, A. hydrophila, A. jandaei, A. schubertii, A. sobria, A. trota* and *A. veronii* (Table1). All the isolates were gram negative, motile, oxidase positive, catalase positive, amylase positive, nitrate reductase positive and glucose fermenting with acid production.

Species of the genus *Aeromonas* have long been recognized as pathogens of fish, amphibians and reptiles (Austin and Adams, 1996). Citarasu *et al.* (2011) reports *A. hydrophila* as the dominant microbiota among other pathogens during the massive outbreak of bacterial infection in the ornamental fish hatchery. The prevalence of different species of *Aeromonas* is likely to vary with geographical locations. In terms of prevalence and abundance, the most predominant species was found to be *A. caviae* during the present study, followed by the other species. Araujo *et al*. (1990) reports *A. caviae* to be the predominant species in water. In a study from a coastal bay in Japan, Nakano *et al*. (1990) found that *A. caviae* was the most abundant of the mesophilic aeromonads, while *A. sobria* predominated in brackishwaters. Hatha *et al*. (2005) reported *A. hydrophila* to be the predominant species in the intestine of farm raised freshwater fish followed by *A. caviae* and *A. sobria.*

Table 1. Prevalence of motile *Aeromonas* spp. among freshwater ornamental fishes

Ornamental fish species	Common name	% of samples positive for *Aeromonas* spp.				% of samples positive for motile *Aeromonas* spp.
		A. caviae	*A. sobria*	*A. hydrophila*	Other *Aeromons* spp.	
Carassius auratus	Gold fish	11.1	5.5	2.8	11.1	30.5
Cyprinus carpio	Carp	5	10	0	15	30
Poecilia sphenops	White molly	0	0	0	0	0
Datnioides polota	Tiger fish	20	0	10	10	40
Poecilia sphenops	Black molly	12.5	12.5	0	50	75
Poecilia latipinna	Balloon molly	0	0	0	60	60
Xiphophorus helleri	Red sword tail	40	0	20	40	100
Gymnocorymbus ternetzi	Widow tetra	50	0	0	0	50
Puntius conchonius	Rosy barb	33.3	0	0	33.3	66.6
Carassius auratus	Black moor	0	0	0	33.3	33.3
Pterygoplichthys pardalis	Sucker	50	0	50	0	100
Labeo gonius	White shark	0	0	0	0	50
Astronotus ocellatus	Oscar	0	0	0	100	100
Poecilia reticulata	Guppy	0	0	0	0	0
Poecilia latipinna	Red molly	0	0	0	0	0
Colisa chuna	Honey gourami	0	0	0	0	0

There have been conflicting reports on the susceptibility of motile aeromonads to commonly used antibiotic agents. The aeromonads have been regarded as universally resistant to penicillins (penicillin, ampicillin, carbenecillin and ticarcillin) for quite a long time. For this reason, ampicillin has been generally incorporated in the culture media for selective isolation of the aeromonads. All the strains isolated exhibited sensitivity to ceftazidime, chloramphenicol, ciprofloxacin and gentamicin. Only 2.2% of the isolates exhibited resistance to Trimethoprim. All the isolates of *A. hydrophila* and *A. jandaei* were resistant to cephalothin and 50% of *A. caviae* were also resistant to this antibiotic. Except *A. jandaei,* members of all the species exhibited some degree of resistance to streptomycin. Resistance to nalidixic acid was exhibited by all the species, although in variable degree. Overall prevalence of antibiotic resistance among motile aeromonads is given in Table 2. Our results are in tune with the previous observations with regard to trimethoprim, gentamicin, chloramphenicol and ciprofloxacin (Kudinha *et al*., 2004; Penders and Stobberingh, 2008). Though all the isolates were sensitive to gentamicin, ciprofloxacin and chloramphenicol as previously observed (Vasaikar *et al*., 2002; Kashedikar and Chhabra, 2010), nearly 50% isolates were resistant to nalidixic acid. Resistance to nalidixic acid was considerably high when compared to previous results (Huddleston *et al*., 2006), indicating the use of this antibiotic by the farmers in the region.

Table 2. Overall prevalence of antibiotic resistance among motile aeromonads from freshwater ornamental fishes

Antibiotic	% of strains showing resistance (total n=45)
Amoxycillin (30 mcg)	100
Carbenicillin (100 mcg)	33.3
Cefpodoxime (10 mcg)	8.8
Ceftazidime (30 mcg)	0
Cephalothin (30 mcg)	37.7
Chloramphenicol (10 mcg)	0
Ciprofloxacin (5 mcg)	0
Gentamicin (10 mcg)	0
Nalidixic acid (30 mcg)	48.8
Streptomycin (10 mcg)	44.4
Tetracycline (30 mcg)	15.5
Trimethoprim (5 mcg)	2.2

Further concern is the increasing incidence of multidrug resistance amongst *Aeromonas* spp. that has been observed worldwide (Ottoviani, 2006; Matyar *et al.,* 2007). Fifty eight percentage of *Aeromonas* isolates in this study exhibited Multiple Antibiotic Resistance. Twenty five percent of *A. hydrophila* isolates were resistant to six of the antibiotics tested. The MAR index values of the aeromonad isolates ranged from 0.08 to 0.5. The MAR index values of the isolates and the resistance profile of antibiotics is given in Table 3. High MAR index exhibited by more than half of the isolates in the present study points to the indiscriminate use of antibiotics in aquaculture systems. High incidence of multiple antibiotic resistance has been reported in aquatic environments and fish isolates by Hatha *et al.* (2005). More than 50% of the strains of *A. hydrophila* and *A. caviae* isolated from freshwater fishes of Kolkata, India exhibited MAR (Abraham, 2011).

Table 3. MAR index and resistance profile of motile aeromonads from freshwater ornamental fishes

MAR index	Resistance profile	Number of isolates showing similar pattern
0.25	AX,NA,S	5
0.25	AX,CB,S	2
0.25	AX,CB,CH	1
0.25	AX,S,TR	1
0.25	AX,CEP,CH	2
0.25	AX, CH,NA	1
0.33	AX, CB,NA,S	2
0.33	AX, CB,NA, CH	1
0.33	AX, CB,CH,S	1
0.33	AX, CB,CH,T	1
0.33	AX,CEP,CH, T	1
0.33	AX,CB,CH,NA	1
0.33	AX,CH,NA,T	1
0.33	AX, CH,NA,S	1
0.33	AX, CH,S,T	1
0.33	AX,CB,S,T	1
0.33	AX, CB,CH, T	1
0.42	AX,CB,CEP,CH,NA	1
0.5	AX,CB,CH,NA,S,T	1

AX– Amoxycillin, NA – Nalidixic acid, S – Streptomycin, CB – Carbenicillin, CEP – Cefpodoxime, CH – Cephalothin, T – Tetracycline

Production of haemolysin has been reported as a virulence factor in motile aeromonads. Varying levels of beta hemolytic activity among *A. hydrophila* has been reported (Thayumanavan *et al*., 2003; Hatha *et al*., 2005). While all the *A. hydrophila* strains in this study were beta hemolytic, 85.7% of *A. caviae* and 80% of *A. sobria* exhibited β haemolytic activity.

Ornamental fish trade is growing at rapid pace in Kerala, with many small scale investors involved in breeding, rearing and marketing of popular ornamental fishes such as those analyzed in the present study. Very often these fishes are kept in crowded conditions at retail vendor level which is highly stressful to the fishes making them more prone to diseases by opportunistic and obligate pathogens. The results of the present study reveal the prevalence of motile aeromonads at varying levels in these fishes. The multidrug resistant nature of the motile aeromonads make it difficult for successful disease control through antibiotics. The fish keepers at farm/ retail vendor level should be aware of the stress reduction procedures so as to prevent massive death due to multiple drug resistant fish pathogens.

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