Available online at www.sciencedirect.com

SCIENCE DIRECT®

International Journal of Food Microbiology 98 (2005) 131–134

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short Communication

Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish

Mohamed Hatha[a,*], A.A. Vivekanandhan[b], G. Julie Joice[a], Christol[a]

[a]*Department of Microbiology, Maharaja College for Women, Perundurai-638 052, Tamil Nadu, India*
[b]*Laboratory of Fish Health Management, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7, Konuan, Minatoku, Tokyo 108-8477, Japan*

Received 25 July 2003; received in revised form 21 May 2004; accepted 28 May 2004

Abstract

Motile aeromonads isolated from the intestines of farm-raised freshwater fish such as *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella* have been characterized to species level. Morphological and physiological grouping revealed 61% *Aeromonas hydrophila*, 30% *Aeromonas caviae*, 7% *Aeromonas sobria* and 2% which remained unidentified. Hemolytic activity was detected mostly in *A. hydrophila*, while only half of the *A. sobria* and *A. caviae* showed this activity. Antibiotic resistance patterns of the strains revealed that they had acquired a relatively higher resistance to oxytetracycline, amoxycillin, ampicillin, novobiocin and polymixin-B, implicating possible use of these antibiotics in the aquaculture systems.

Keywords: *Aeromonas*; Aquaculture; Freshwater fish; Beta hemolysis; Antibiotic resistance

1. Introduction

The genus *Aeromonas* comprises a group of organisms having a wide distribution in the aquatic environment (Khardori and Fainstein, 1988). Many studies have focused on the role of these microorganisms as pathogens of several poikilothermic animals and there has been increasing interest concerning the potential role of motile *Aeromonas* species in human, animal and foodborne diseases.

Aeromonas species are particularly unwanted in food because they are known as active spoilers of fish and meat at ambient temperature and at temperatures ranging from 2–13 °C (Popoff, 1984; Gram et al., 1989). Because of the suspected role in gastroenteritis, the presence of aeromonads in foods including products from aquaculture has raised concern. Many motile *Aeromonas* spp., *Aeromonas hydrophila* in particular, have been implicated in the etiology of a variety of systemic and localized diseases in fishes. As farmed fish are often treated with a wide variety of

* Department of Biology, School of Pure and Applied Sciences, The University of the South Pacific, P.O. Box 1168, Private mail bag, Suva, FIJI. Tel.: +679 3212550; fax: +679 3315601.
E-mail address: abdulla_m@usp.ac.fj (M. Hatha).

0168-1605/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2004.05.017

antimicrobial compounds (Austin and Austin, 1993), this has resulted in the development of resistant strains (Barnes et al., 1991; Saitanu et al., 1994). Although the majority of studies on the development of antibiotic resistant bacteria have mainly focussed on cattle, poultry and swine, the emergence of antibiotic resistant strains of fish pathogens has also been reported from temperate and tropical aquaculture systems (Rahim et al., 1984; Spanggaard et al., 1993; Kerry et al., 1995).

This paper reports the results of a study on the phenotypic characteristics, hemolytic activity and antibiotic resistance of motile aeromonads encountered in the intestines of farm-raised fresh water fish such as *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella*.

2. Materials and methods

The motile *Aeromonas* strains encountered in the transient and resident microflora of the gastrointestinal tract of farm-raised freshwater fish such as *C. catla*, *L. rohita* and *C. idella* (Hatha, 2002) were characterized to species level based on the following characteristics: motile, Gram-negative, cytochrome oxidase positive, D-glucose fermentation positive, arginine dihydrolase positive, ornithine decarboxylase negative, ONPG positive, H_2S from cysteine, acetoin from glucose, gas from glucose, L-arabinose utilization and fermentation of salicin (Krieg and Holt, 1984).

2.1. Hemolysin assay

Hemolysin activity was determined using blood agar medium (Himedia, Bombay, India) containing 5% sheep blood collected aseptically. Beta hemolytic activity was recorded as clear zones around the colony after incubation at 37 °C for 24 h.

2.2. Antibiotic sensitivity testing

The motile *Aeromonas* strains isolated in the present study were subjected to sensitivity testing against 10 antibiotics using standard methods (Bauer et al., 1966). The antibiotics and the concentrations used were as follows: ampicillin (30 μg), amoxycillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), oxytetracycline (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), polymixin-B (300 units) and streoptomycin (10 μg).

Pure cultures of *Aeromonas* were enriched in Brain Heart Infusion Broth (Himedia, Bombay, India) at 37 °C for 6–8 h. These cultures were then streaked onto Mueller Hinton agar plates (Himedia) using a sterile cotton swab. The antibiotic discs were dispensed using a disc dispenser (Himedia) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37 °C for 16–18 h. Results were recorded by measuring the diameter of the inhibition zones and compared with standards for antimicrobial disk susceptibility tests, supplied by the Himedia Laboratories, Bombay and were classified as resistant, intermediate and sensitive.

3. Results and discussion

In our studies on transient and resident microflora of farm-raised fresh water fish such as *C. catla*, *L. rohita* and *C. idella*, we encountered a predominance of *Aeromonas* sp. (Hatha, 2002). Furthermore, species-level characterization revealed that *A. hydrophila* was the dominant species (50–70%) in the intestine of these fish, followed by *Aeromonas caviae* and *Aeromonas sobria.* A total of 55 strains of *A. hydrophila*, 8 strains of *A. sobria* and 27 strains of *A. caviae* were studied. About 2% of the *Aeromonas* spp. remained unidentified. Vivekanandhan et al. (2002) reported the isolation of *A. hydrophila* in marketed fish and prawn from the south India, in which 33.5% and 17.6% prevalence of *A. hydrophila* was encountered, respectively. Wide distribution of motile *Aeromonas* spp. has been previously reported in the aquatic environment and fish (Kaper et al., 1981; Abeyta et al., 1986) and the results obtained support these findings.

The present study also revealed that 100% of *A. hydrophila*, 50% of *A. sobria* and 77.8% of *A. caviae* exhibited beta hemolytic activity. Beta hemolysin has been reported as a virulence factor in motile aeromonads (Majeed and MacRae, 1993; 1994).

The results (Table 1) of the antibiotic sensitivity testing demonstrated the inherent resistance of *A.*

Table 1
Prevalence of antibiotic resistance among motile aeromonads from the intestine of farm-raised fresh water fish

Antibiotic (concentration)	Percentage of resistant strains		
	A. hydrophila (*n*=55)	*A. sobria* (*n*=8)	*A. caviae* (*n*=27)
Chloramphenicol (30 μg)	5.5	16.7	18.5
Polymixin B (300 units)	41.8	0.0	14.8
Oxytetracycline (30 μg)	40	33.3	40.7
Ciprofloxacin (5 μg)	0.0	0.0	3.7
Streptomycin (10 μg)	0.0	0.0	0.0
Gentamicin (10 μg)	7.3	0.0	0.0
Amoxycillin (10 μg)	52.7	33.3	48.1
Ampicillin (30 μg)	100	100	100
Nalidixic acid (30 μg)	1.8	16.7	7.4
Novobiocin (30 μg)	94.5	100	92.6

hydrophila strains isolated in this study to ampicillin as all of them were resistant to it. High levels of resistance were also encountered against amoxycillin, polymixin-B and novobiocin. Around 40% of the strains were resistant to oxytetracycline. This antibiotic is frequently used in aquaculture operations in order to treat various diseases, which may result in a high degree of selection for oxytetracycline resistant strains. Spanggaard et al. (1993) reported resistance to oxytetracycline among bacteria from fresh water farms in Denmark. The resistance levels were comparable to those reported by Hatha and Lakshmanaperunmalsamy (1995) in the *Salmonella* strains from fish and crustaceans. The rearing of fish and lobsters in oxytetracycline-treated ponds is a common practice in many countries and the additional use of sulpha drugs in various operations could accentuate current populations of antibiotic resistant microflora in fish (DePaola et al., 1995). There is a large body of literature reviewed by Novick (1981) demonstrating that the subtherapeutic use of antibiotics in animal husbandry practices has promoted the emergence and maintenance of multiple antibiotic resistant (MAR) pathogenic bacteria.

None of the *Aeromonas* strains encountered in the present study were resistant to streptomycin and only 10% of the strains were resistant to gentamicin, ciprofloxacin and nalidixic acid. Resistance against chloramphenicol was also low (<20%). The resistance levels to gentamicin, and nalidixic acid were comparable to the findings of Vivekanandhan et al., (2002). However, resistance to chloramphenicol was higher in this study when compared to Vivekanandhan et al., (2002), who observed less than 5% resistance to chloramphenicol among *A. hydrophila* strains from marketed fish. This is significant as the detection of chloramphenicol residues in aquaculture products from India and China has raised concern within the international community especially countries affiliated to the European Economic Community (EEC) and has resulted in a ban on the products from some suppliers from India and China. It is reported that a wide range of antimicrobial compounds (oxytetracycline, ciprofloxacin, nitrofurantoin, furazolidone or chloramphenicol) are being used in the hatcheries and farms of freshwater prawn and marine shrimp in India to control the bacterial population (Karunasagar et al., 1994; Abraham et al., 1997). However, in this study, 3.7% of the *A. caviae* strains isolated were resistant to ciprofloxacin but none of the *A. hydrophila* or *A. sobria* shows demonstrated resistance to ciprofloxacin.

As mentioned, none of the motile aeromonads isolated in this study were found to be resistant to streptomycin. The results are different from the observations of Son et al. (1997) and Vivekanandhan et al. (2002) who observed that around 10% of *Aeromonas* strains isolated from fish were resistant to streptomycin. We also observed different patterns from those of Vivekanandhan et al. (2002) with respect to resistance to polymyxin-B. While more than 95% of *A. hydrophila* strains isolated by Vivekanandhan et al. (2002) were resistant to polymyxin-B, less than 50% of the *A. hydrophila* strains isolated in this study were resistant to this antibiotic.

In many countries, the release of pathogenic bacteria in faeces dispersed into the aquatic environment can contaminate fish and shellfish harvested from these waters. Once these bacteria are in the aquatic environment, plasmid exchange between the bacteria is readily facilitated and can result in a higher frequency of multiple antibiotic resistant strains. Local selective pressures could influence the antibiotic resistance. Chang and Bolton (1987) found that a greater percentage of Asian isolates of *A. hydrophila* were resistant to tetracycline and rifampicin than Australian isolates. Continued agricultural use of medicated feeds and their application to the rapidly developing fish and shellfish farming (Redmayne, 1989) can foster greater dissemination of virulent and

resistant bacterial pathogens in the natural environment and thus potentially into the human food chain.

Acknowledgments

The authors are thankful to the Manager, Maharaja Aqua Farms for providing the samples and to the Principal, Maharaja College for Women, Perundurai for extending the use of facilities.

References

Abeyta, C., Kaysner, C.A., Wekell, M.M., Sullivan, J.J., Stelma, G.N., 1986. Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of food borne illness. J. Food Prot. 49, 643–646.

Abraham, T.J., Manley, R., Palaniappan, R., Devendran, K., 1997. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. J. Aquac. Trop. 12 (1), 1–8.

Austin, B., Austin, D.A., 1993. Bacterial fish pathogens. Disease in Farmed Fish and Wild Fish, (2nd ed.) Ellis Horwood, Chichester, United Kingdom.

Barnes, A.C., Amyes, S.G.B., Hastings, T.S., Lewin, C.S., 1991. Flouroquinolenes display rapid bactericidal activity and low mutation frequencies against *Aeromonas salmonicida*. J. Fish Dis. 14, 661–667.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standard single disk method. Am. J. Clin. Pathol. 36, 493–496.

Chang, B.J., Bolton, S.M., 1987. Plasmids and resistance to antimicrobial agents in *Aeromonas hydrophila* clinical isolates. Antimicrob. Agents Chemother. 31, 1281–1282.

DePaola, A., Peeler, J.T., Rodick, J.E., 1995. Effect of oxytetracycline-medicated feed on antibiotic resistance of Gram-negative bacteria in catfish ponds. Appl. Environ. Microbiol. 61 (9), 3513–3519.

Gram, L., Oundo, J.O., Bon, J., 1989. Storage life of Nile perch (*Lates niloticus*) in relation to temperature and initial bacterial load. Trop. Sci. 29, 221–236.

Hatha, A.A.M., Lakshmanaperumalsamy, P., 1995. Antibiotic resistance of *Salmonella* strains isolated from fish and crustaceans. Lett. Appl. Microbiol. 21, 47–49.

Hatha, A.A.M., 2002. Transitory and resident microflora of the gastrointestinal tract of farm raised freshwater fishes in relation to habitat microflora. Asian J. Microbiol. Biotechnol. Environ. Sci. 4 (2), 277–282.

Kaper, J.B., Lockman, H., Colwell, R.R., Joseph, S.W., 1981. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. 50, 359–377.

Karunasagar, I., Pai, R., Malathi, G.R., 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture. 128, 203–209.

Kerry, J., Hiney, M., Coyne, R., Nicgabhainn, S., Gilroy, D., Cazabon, D., Smith, P., 1995. Fish feed as a source of oxytetracycline resistant bacteria in the sediment under fish farms. Aquaculture 131, 101–113.

Khardori, N., Fainstein, V., 1988. *Aeromonas* and *Plesiomonas* as etiological agents. Annu. Rev. Microbiol. 42, 395–419.

Krieg, N.R., Holt, J.G., 1984. Bergey's Manual of Systematic Bacteriology, vol. 1. William and Wilkins, London.

Majeed, K.N., MacRae, I.C., 1993. Effect of pH on the growth and exotoxin production by *Aeromonas* at refrigeration temperature. Microbios 73, 281–288.

Majeed, K.N., MacRae, I.C., 1994. Cytotoxic and haemagglutinating activities of motile *Aeromonas* species. J. Med. Microbiol. 40, 188–193.

Novick, R.P., 1981. The development and spread of antibiotic resistant bacteria as a consequence of feeding antibiotics to livestock. Ann. N.Y. Acad. Sci. 368, 23–59.

Popoff, M., 1984. Genus III *Aeromonas*. In: Kluyver, A.J., Van Niel, C.J. Bergey's Manual of Systematic Bacteriology, vol. 1. Williams and Wilkins, Baltimore, MD, pp. 545–548.

Rahim, Z., Sanyal, S.C., Aziz, K.M.S., Huq, M.I, Chowdhury, A.A., 1984. Isolation of enterotoxigenic, hemolytic and antibiotic resistant *Aeromonas hydrophila* strains from infected fish in Bangladesh. Appl. Environ. Microbiol. 48, 865–867.

Redmayne, P.C., 1989. World aquacultural developments. Food Technol. 43, 80–86.

Saitanu, K., Chongthaleong, A., Endo, M., Umeda, T., Takami, K., Aoki, T., Kitao, T., 1994. Antimicrobial susceptibilities and detection of transferable R-plasmids from *Aeromonas hydrophila* in Thailand. Asian Fish. Sci. 7, 41–46.

Son, R., Rusul, G., Sahilah, A.M., Zainuri, A., Raha, A.R., Salmah, I., 1997. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Tilapia (*Tilapia mossambica*). Lett. Appl. Microbiol. 24, 479–482.

Spanggaard, B., Jorgensen, F., Gram, L., Huss, H.H., 1993. Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. Aquaculture 115, 195–207.

Vivekanandhan, G., Savithamani, K., Hatha, A.A.M., Lakshmanaperumalsamy, P., 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int. J. Food Microbiol. 76, 165–168.