

## Note

# Antibiotic susceptibility of Gram-negative bacteria isolated from freshwater fish hatcheries of West Bengal, India

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## ABSTRACT

A survey on the use of aquadrugs in freshwater fish hatcheries of West Bengal was conducted and the susceptibility of hatchery bacterial flora to selected antibiotics were studied during June 2006 - July 2007. Aquadrugs such as oxytetracycline, althrocin, ampicillin, sparfloxacin, enrofloxacin, acriflavine, formalin, potassium permanganate, malachite green, tannic acid and herbal extracts were found to be used at varying levels for prophylactic as well as curative purposes. All the bacterial isolates (n = 74) tested were highly susceptible to gentamycin. Majority of the isolates were resistant to oxytetracycline followed by co-trimoxazole and nitrofurantoin. Resistance to chloramphenicol was noticed in few strains of *Aeromonas hydrophila* (15%), *Aeromonas caviae* (9%) and *Pseudomonas* spp. (25%). Multiple antibiotic resistance was observed in 30% and 90% of the Gram-negative bacteria of carp and catfish hatcheries, respectively. The study in general revealed regular use or abuse of aquadrugs in most of the fish hatcheries surveyed, thus raising the risk of antibiotic resistance development and human health safety.

Keywords: *Aeromonas*, Antibiotic susceptibility, *Edwardsiella*, Fish hatchery, Multiple antibiotic resistance, *Pseudomonas*

West Bengal is the pioneer state in fish seed production in India, contributing to about 75% of the total seed production of the country. The fish seed production of this state has increased from 2,300 million in 1980-1981 to 13,572 million in 2007-08 (Bhattacharya, 2008). This has been possible because of the setting up of low cost Chinese and glass jar hatcheries for freshwater fish species. Occurrence of diseases is one of the major constraints in intensification of hatcheries which may eventually become a limiting factor to the economic success of the industry (Milwain *et al.*, 2002). Antibiotics routinely used for treatment of human infections are also used in animal rearing, either for therapeutic/prophylactic purposes or for growth promotion which has contributed to development of antibiotic resistance (Hernandez, 2005). In fish farming sector, the wide spread use of antibiotics for treating bacterial diseases has been associated with the development of resistance in fish bacterial pathogens and other aquatic bacteria (DePaola *et al.*, 1995; Sahoo and Mukherjee, 1997; Son *et al.*, 1997; Schmidt *et al.*, 2000; Abraham *et al.*, 2004). The present study was carried out to collect information on the use of aquadrugs in selected freshwater fish hatcheries in the state of West Bengal and to study the susceptibility of hatchery bacterial flora to antibiotics.

The bacterial strains used were isolated from fish eggs, fish larvae and larval rearing waters of freshwater fish

hatcheries producing seeds of Indian major carps and *Clarias* spp., which were collected during June 2006 to July 2007 in Naihati, North 24 Parganas district and Ambika Kalna, Burdwan district, West Bengal. The isolation and identification of *Aeromonas* spp., *Edwardsiella tarda* and *Pseudomonas* spp. were carried out following standard methods (Arcos *et al.*, 1988; Holt *et al.*, 1994). The details on the use of aquadrugs in fish hatcheries were collected from the hatchery owners or technicians. A total of 74 bacterial isolates from hatchery larval rearing water, eggs and larvae, comprising *A. hydrophila*, *A. caviae*, *E. tarda*, *Pseudomonas* spp. and unidentified Gram-negative, oxidase positive, fermentative bacteria were screened for their sensitivity to chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), gentamycin (10 µg), nitrofurantoin (300 µg) and oxytetracycline (30 µg) (HiMedia, Mumbai) by agar disc diffusion method (NCCLS, 1999).

Aquaculture involving hatchery seed production, nursery rearing and grow-out production is a multi-activity operation. Drugs and chemicals are widely used in hatcheries for increasing the larval production and health management. The results of the survey on the use of aquadrugs in West Bengal freshwater fish hatcheries, revealed the regular use of drugs such as oxytetracycline,

althrocin, ampicillin, sparfloxacin, enrofloxacin, acriflavine, formalin, potassium permanganate, malachite green *etc.* for prophylactic and curative purposes (Table 1). Tannic acid and tannery residues were used for the conditioning of broodstock in few hatcheries. Compounds such as *haritaki* (a herbal extract), milk solution and antacid containing aluminium hydroxide and magnesium trisilicate were used for the treatment of fish eggs. Besides the above, use of ciprofloxacin, oflokem TL, enrofloxacin, herbagastrin and parovit-12 were also noticed to be used in few hatcheries to improve the larval survival. Likewise, the use of antibiotics such as oxytetracycline at a dose of 5-20 mg l<sup>-1</sup> in *Pangasius hypophthalmus* larval

rearing (Subagja *et al.*, 1999) and oxytetracycline, oxolinic acid and amoxycillin at 20 mg l<sup>-1</sup> to improve the survival of yolk-sac Atlantic halibut *Hippoglossus hippoglossus* (Verner-Jeffrey *et al.*, 2004) with systematic improvement in larval survival rates have been reported. Responsible use of aquadrugs was, however, lacking in most of the fish hatcheries, thereby raising the risk of antibiotic resistance development and human health safety.

The sensitivity of 74 Gram-negative bacterial isolates to six antibacterials was observed to be in the following descending order: gentamycin, chloramphenicol, ciprofloxacin, nitrofurantoin, co-trimoxazole and oxytetracycline (Table 2). All the isolates from hatchery

Table 1. Aquadrugs used in Indian major carp and catfish hatcheries of West Bengal

| Aquadrug                        | Active ingredient / group                     | Dose (ppm)            | Purpose   |
|---------------------------------|---|-----------------------|---|
| Oxytetracycline solution        | Oxytetracycline                               | 15                    | Antibacterial, prophylactic treatment   |
| Oxytetracycline tablets         | Oxytetracycline                               | 2 – 3                 | Antibacterial, prophylactic treatment   |
| Terramycin                      | Oxytetracycline                               | 3 – 4                 | Antibacterial, prophylactic treatment and Post-injection wound healing @ 0.1 ml kg fish <sup>-1</sup> |
| Althrocic                       | Erythromycin                                  | 6                     | Antibacterial, fish disease treatment   |
| Ampicillin                      | Aminopenicillins                              | 3                     | Antibacterial, fish disease treatment   |
| Sparfloxacin                    | Quinolone                                     | 1 – 2                 | Antibacterial, fish disease treatment   |
| Enrofloxacin                    | Quinolone                                     | 1                     | Antibacterial, fish disease treatment   |
| Acriflavine                     | Acriflavine HCl                               | 4                     | Post-injection wound healing  |
| Formalin                        | Formaldehyde                                  | 10 – 15               | Treatment of parasitic diseases   |
| Potassium permanganate          | Potassium permanganate                        | 10 – 20               | Disinfectant and prophylactic treatment   |
| Malachite green                 | Triphenylmethane                              | 0.1                   | General fish disease treatment  |
| <i>Haritaki</i> (herb) extract* | Unknown                                       | 6 ml per litre eggs   | Treatment of fish eggs and hardening of egg shell   |
| Milk solution                   | Unknown                                       | 200 ml per 30 l water | Removal of adhesive gelatinous layer of fertilized eggs   |
| Antacid                         | Aluminium hydroxide and magnesium trisilicate | NA                    | Treatment of fish eggs  |
| Tannic acid and tannery residue | Tannic acid and unknown                       | NA                    | Conditioning of broodstock  |

\* 10 kg *haritaki* (*Acacia catechu* seed) and 250 g *khayer* (*Marobolus indicus*) boiled in small amount of water for about 30 min and then diluted to 50 l. This extract, after filtering through fine cloth, is applied at the rate of 600 ml /100 liters of eggs after 6-8 h of fertilization in a pool.

NA: Not available.

Use of ciprofloxacin, oflokem TL, enrofloxacin, herbagastrin and parovit-12 was also noticed in few hatcheries.

Table 2. Antibiotic resistance among the bacterial flora (n = 74) from freshwater fish hatcheries

| Bacteria                           | Percentage resistant to antibiotics |       |       |      |       |       |
|------------------------------------|-------------------------------------|-------|-------|------|-------|-------|
|                                    | C                                   | Cf    | Co    | G    | Nf    | O     |
| <i>Aeromonas hydrophila</i> (n=26) | 15.39                               | 23.08 | 53.85 | 7.69 | 53.85 | 69.23 |
| <i>Aeromonas caviae</i> (n=22)     | 9.09                                | 9.09  | 72.73 | 0    | 36.36 | 36.36 |
| <i>Pseudomonas</i> spp. (n=8)      | 25.00                               | 0     | 100   | 0    | 50.00 | 50.00 |
| <i>Edwardsiella tarda</i> (n=2)    | 0                                   | 0     | 100   | 0    | 100   | 100   |
| Others (n=16)*                     | 0                                   | 0     | 0     | 0    | 12.50 | 62.50 |

C: Chloramphenicol, 30 µg; Cf: Ciprofloxacin, 5 µg; Nf: Nitrofurantoin, 300 µg  
Co: Co-trimoxazole, 25 µg; G: Gentamycin, 10 µg; O: Oxytetracycline, 30 µg

\*: Unidentified Gram-negative, oxidase positive, fermentative bacteria

were highly susceptible to gentamycin. The results corroborate the earlier findings in this line (Sahoo and Mukherjee, 1997; Son *et al.*, 1997). In contrast, gentamycin resistant bacteria were isolated from the fish and aquatic environs by DePaola *et al.* (1995). Studies by Majumdar *et al.* (2006) also indicated that *A. hydrophila* isolates from fish exhibited resistance against gentamycin. Majority of the isolates were resistant to oxytetracycline followed by co-trimoxazole and nitrofurantoin, as reported in earlier studies (DePaola *et al.*, 1995; Son *et al.*, 1997; Schmidt *et al.*, 2000). *Pseudomonas* spp. showed resistance towards co-trimoxazole, oxytetracycline, nitrofurantoin and chloramphenicol. Majority of the *A. hydrophila* strains were resistant to oxytetracycline followed by nitrofurantoin, which conform the observations of Schmidt *et al.* (2000).

Few strains of *A. hydrophila* (15%), *A. caviae* (9%) and *Pseudomonas* spp. (25%) exhibited resistance to chloramphenicol. Occurrence of chloramphenicol resistant bacteria was reported in Malaysian freshwater aquaculture (Son *et al.*, 1997) and Danish rainbow trout farms (Schmidt *et al.*, 2000). Bacterial isolates from hatcheries in North 24 Parganas district were resistant to oxytetracycline, nitrofurantoin and co-trimoxazole and sensitive to gentamycin. These are probably the result of regular use of these antibiotics in various stages of production to combat diseases. The Burdwan district bacterial isolates were more resistant to oxytetracycline and highly sensitive to chloramphenicol and ciprofloxacin. Such differences in the frequency of resistance may be related to the source of the *Aeromonas* isolates and the frequency and type of antimicrobial agents used for treating diseases and for health management in that geographical area. Oxytetracycline is one of the most widely applied antibiotics in finfish and shrimp aquaculture which

enhance the frequency of new oxytetracycline resistant bacteria. The major problem with oxytetracycline is that bacterial pathogens easily develop plasmid mediated resistance (Son *et al.*, 1997; Hernandez, 2005).

The major concern with antibiotic usage is acquisition of multiple antibiotic resistance (MAR). MAR has been reported in fish pathogens and bacteria from aquaculture environs associated with the variety of drugs or uncertain antibiotic usage (Abraham *et al.*, 2004; Hernandez, 2005). The present results are also in agreement with the above studies. MAR was noted in *E. tarda* (100%), *Pseudomonas* spp. (75%), *A. hydrophila* (69%) and *A. caviae* (55%). One of the significant observations of the present study was the high incidence of MAR (71%) among the hatchery isolates of North 24 Parganas district; while in Burdwan it was 38%. About 30% of the bacterial isolates from carp hatcheries developed MAR, whereas it was 90% in catfish hatcheries (Table 3), thus confirming the regular use or abuse of antibiotics in catfish hatcheries of North 24 Parganas district. Abraham *et al.* (2004) demonstrated that about 21% of bacterial isolates from freshwater aquaculture systems of West Bengal exhibited MAR. Probably, the MAR bacteria may be responsible for the diseases and high mortality in catfish hatchery systems of North 24 Parganas District. When multiple resistances occurred, the single most commonly involved agent was oxytetracycline. In lieu of the negative impacts of antibiotic resistance among the bacterial flora, there is an urgent need on the responsible use of antibiotics in fish hatcheries more particularly in catfish hatcheries and to control the bacterial flora in larval rearing by other means as suggested by Hernandez (2005).

Table 3. Antibiotic resistance pattern of freshwater fish hatchery bacteria (n = 74)

| Particulars                          | Number of strains sensitive to all antibiotics tested | Number of strains resistant to at least two antibiotics | Multiple antibiotic resistance* (%) |
|--------------------------------------|---|---|-------------------------------------|
| <b>Species</b>                       |   |   |                                     |
| <i>Aeromonas hydrophila</i> (n = 26) | 6   | 18  | 69.23                               |
| <i>Aeromonas caviae</i> (n = 22)     | 6   | 12  | 54.55                               |
| <i>Pseudomonas</i> spp. (n = 8)      | 0   | 6   | 75.00                               |
| <i>Edwardsiella tarda</i> (n = 2)    | 0   | 2   | 100.00                              |
| Others (n = 16)                      | 6   | 2   | 12.50                               |
| <b>Source</b>                        |   |   |                                     |
| Carp hatchery (n = 44)               | 17  | 13  | 29.55                               |
| Catfish hatchery (n = 30)            | 0   | 27  | 90.00                               |
| <b>District</b>                      |   |   |                                     |
| North 24 Parganas (n = 48)           | 9   | 34  | 70.83                               |
| Burdwan (n = 26)                     | 7   | 10  | 38.46                               |

\*: Resistant to at least two broad spectrum antibiotics

#: Unidentified Gram-negative, oxidase positive, fermentative bacteria

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