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Motile Aeromonads Associated with Epizootic Ulcerative Syndrome Affected *Channa striata*

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Occurrence of motile aeromonads, *Aeromonas hydrophila, A. caviae* and *A. sobria* in murrels (*Channa striata*) affected with Epizootic Ulcerative Syndrome is reported. API 20 NE was found useful with additional confirmatory tests in identification of aeromonads. All three *Aeromonas* spp. were found sensitive to bacitracin, chloramphenicol, chlortetracycline and tetracycline at 10, 30, 30 and 30 µg per disc, respectively. The same isolates showed difference in their sensitivity to kanamycin, polymyxin-B and oxytetracycline. The top sediment layer samples collected from different ponds were found to contain aeromonads in the range 2 to 5 log CFU per gram of the sample.

Key words: ***Motile aeromonads, epizootic ulcerative syndrome, sediment, antibiotic sensitivity***

Epizootic Ulcerative Syndrome (EUS) in fresh water fish is a matter of concern since 1972 in the Asia Pacific region (Jhingran, 1988). *Aeromonas hydrophila* has been detected in infected fish (Karunasagar *et. al.*, 1989; Thampuram *et. al.*, 1995). Das and Das (1993) have reviewed the EUS in India. Occurrence of motile aeromonads associated with EUS in fish, their characterization and sensitivity to antibiotics commonly used in fish farms are reported in this paper. Total bacterial count, coliforms and total aeromonads in the samples of top layer of the sediment from different ponds are also reported.

Materials and Methods

Infected murrels (*Channa striata*) were cleaned of the surface contaminants with sterile cotton swabs soaked in a chloroxylenol-based antiseptic. Skin lesions, gall bladder and kidney regions were sampled as described by Karunasagar *et. al.* (1989). Samples were ground with sterile nutrient broth using a sterile mortar and pestle under aseptic conditions. A loop full of the sample was spread on trypticase soya agar (TSA) plates and was incubated at 35±2°C for 24 h.

Top layer of the sediment was collected from different ponds. One sample each was collected from four corners and the middle of the pond and the whole was mixed thoroughly in sterile Seward medical stomacher 400 filter bags. Sterile 0.1 % peptone water was used as diluent. Plate count agar, violet red bile salt glucose agar (Oxoid, UK) and TSA broth with ampicillin (Abeyta and Stelma, 1987) were used for estimation of TBC, coliforms and aeromonads, respectively.

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Suspected aeromonad colonies were picked up, purified and maintained on TSA slants for further characterisation. The isolates were subjected to API 20 NE tests after initial gram staining, morphology, motility and oxidase tests (API 20 NE, BIO Merieux, France). Identification of the isolates was confirmed by additional tests described by Abeyta and Stelma (1987). These tests included ability to grow in the presence of 0 and 6% NaCl and sensitivity to virbriostatic compound 0/129 (2,4-Diamino-6,7 Di isopropyl pteridine, Sigma, USA) at 10 and 150 µg levels. The API profiles of the isolates were obtained from Natural Resources Institute (NRI), UK. The dehydrated media, antibiotics and chemotherapeutic agents were of Hi-media, Bombay. Sensitivity of the isolates to commonly used antibiotics was tested by the agar diffusion method of Bauer *et. al.* (1966).

Results and Discussion

EUS was widespread in murrels during the period of this study. The different bacteria from different regions are given in Table 1. *Aeromonas hydrophila* is predominant in all four specimens and in all parts. *A. hydrophila* has been isolated in several cases of EUS (Karunasagar *et. al.*, 1989; Pradhan *et. al.*, 1991; Rosa *et. al.*, 1991; Bondad *et. al.* 1992; LioPo *et. al.*, 1992; Chegping, 1992; Das and Das, 1993; Pathirantae *et. al.*, 1994; Dong *et. al.*, 1995; Sugita *et. al.*, 1995; Thampuran *et. al.*, 1995; Pai *et. al.*, 1995). *A. sorbia* was present in the skin lesions of one specimen. This has been isolated along with *A. hydrophila* by Lallier *et. al.* (1981), Oliver *et. al.* (1981) and Sukroongreung *et. al.* (1983). *A. caviae* and *A. hydrophila* were more prevalent than *A. sorbia* in the skin lesions (Table 1).

Table 1. Details of the bacteria isolated from the infected fish

	Skin lesions	Gall bladder	Kidney
Specimen 1	*A. hydrophila* *A. sorbia* *A. caviae* *Pseudomonas*	*A. hydrophila*	*A. hydrophila*
Specimen 2	*A. hydrophila* *A. caviae* *Pseudomonas* *Moraxella*	*A. hydrophila*	*A. hydrophila*
Specimen 3	*A. hydrophila* *A. caviae* *Pseudomonas*	*A. hydrophila*	*A. hydrophila*
Specimen 4	*A. hydrophila* *A. caviae* *Psuedomonas* *Moraxella*	*A. hydrophila*	*A. hydrophila*

Though *A. hydrophila* was isolated from the skin lesions of one specimen, use of API-20 NE in the identification was of limited extent only. For instance, the isolates scoring 7476755 and 7477755 on API 20 NE were identified upto genus

level. Pseudomonas from skin lesions of all specimens and Moraxella from two skin lesions have been isolated. The exact role of these bacteria in EUS is not known.

Aeromonads, TBC and total coliforms in the top sediment layer from different ponds, their range, mean and standard deviation are given in Table 2. Out of 120 isolates tested 85 were motile aeromonads in which *A. hydrophila* dominated followed by *A. caviae*. The others were not identified. Burton and Lanza (1987) observed that the density of *A. hydrophila* is elevated in warm waters. The warm water bodies of fishponds in Andhra Pradesh are congenial for survival and growth of aeromonads. Merino *et. al.* (1993) detected *A. hydrophila* and *A. sorbia* in water bodies but not *A. caviae*.

Table 2. Bacterial load (log CFU/g) in top layer of pond sediment

Area*	TBC	Total coliforms	Aeromonads
Area 1	5.73	5.6	5.1
Area 2	5.96	5.52	3.92
Area 3	6.85	5.63	4.41
Area 4	6.54	4.95	4.13
Area 5	6.2	3.65	2.95
Mean	6.26	5.07	4.1
Standard deviation	0.45	0.84	0.78

* Results of each area indicate average of three ponds

The antibiotic sensitivity of the isolates from EUS is presented in Table 3. All the three Aeromonas species were sensitive to bacitracin, chloramphenicol, chlortetracycline and tetracycline. *A. caviae* isolates were sensitive to polymyxin-B and resistant to oxytetracycline, while the other two Aeromonas species showed intermediate sensitivity to both these antibiotics. The antibiotic sensitivity of *Pseudomonas* spp., *Moraxella* spp. and unidentified isolates were more or less similar to that of motile aeromonads.

Table 3. Antibiotic sensitivity of the isolates from EUS affected fish samples

Antibiotic	Isolated from infected fish					
	1	2	3	4	5	6
Bacitracin	S	S	Int	Int	S	S
Chloramphenicol	S	S	S	S	S	S
Chlortetracycline	S	S	S	S	S	S
Oxytetracycline	Int	R	Int	S	Int	Int
Tetracycline	S	S	S	S	S	S
Polymyxin B	Int	S	int	Int	Int	Int
Kanamycin	Int	S	S	Int	S	S

1 - *Aeromonas hydrophila;* 2 - *Aeromonas caviae;* 3 - *Pseudomonas spp.;* 4 - *Moraxella* spp.; 5 - *Aeromonas sorbia;* 6 - unidentified isolate; S - Sensitive; R - *resistant; Int - intermediate*

A. hydrophila strains showed only intermediate sensitivity to kanamycin, though Reugpranch and Kasdaronchan (1983) have reported that *A. hydrophila* is sensitive to this antibiotic. Eight strains of *A. hydrophila* from Bangladesh were susceptible to polymyxin-B, two strains to chloramphenical and three to tetracycline (Rahim *et. al., 1984*). Moderate sensitivity of *A. hydrophila* to polymyxin-B as observed in the present study was also reported by Fass and Barnishan (1981) and Fain *et. al.* (1982).

References

Abeyta, C. Jr. & Stelma, G.N. (1987) in *Bacteriological Analytical Manual,* 6th edn (suppl.), USFDA., Association of Official Analytical Chemists, Virginia, USA

Bauer, A.W., Kirby, W.M.M., Sherris, T.C. & Turck, M. (1966) *Am. J. Clin. Pathol.,* **36**, 493

Bondad, R.M.G., Lumanalan, S.C., Natividad, J.M. & Phillips, M.J. (1992) in *Diseases in Aquaculture* (Shariff, M., Subhasinghe, R.P. & Arthur, J.R., Eds) p. 475, Asian Fisheries Society, Bali, Indonesia

Burton, G.A. Jr. & Lanza, G.R. (1987) *Water Air Soil Pollut.,* **34**, 199

Chegping, L. (1992) *Fish. China. Shuichan. Xuebao,* **16**, 282

Das, M.K. & Das, R.K. *(1993) Environ. Ecol., 11 (1), 134*

Dong, Q., Yueying, C., Jinyu, S. & Zhihua, S. (1995) *Acta Microbiol.,* **35** (6), 460

Fain S.V., Weaver, S. & Bodey, G.P. (1992) *Antimicrobial Agents Chemother.,* **22**, 513

Fass, R.T. & Barnisham, T. (1981) *Antimicrobial Agents Chemother.,* **19**, 357

Jhingran, A.G. *(1988) Fishing Chimes,* **8** (9), 10

Karunasagar, I., Rosalind, G.M., Karunasagar, I. & Rao, G.K. (1989) *Curr. Sci., 58, 1044*

Lallier, R., Leblanc, D., Mittal, K.R. & Oliver, G. (1981) *Appl. Environ. Microbiol.,* **42**(1), 56

LioPo, G.D., Albright, L.J. & Alapadi-Tendencia, E.V. (1992) in *Diseases in Aquaculture,* (Shariff, M., *Subhasinghe, R.P. & Arthur, J.R., Eds) Asian Fisheries Society, Bali, Indonesia*

Merino, S., Camprubi, S., Regue, M. & Tomas, J.M. (1993) *Environ. Toxicol. Water Qual.,* **8**(4), 451

Oliver, G., Lallier, R. & Larviriere, S. (1981) *Can. J. Microbiol.,* **27** (3), 330

Pai, R., Karunasagar, I., Shetty, H.P.C., & Karunasagar, I. (1995) *J. Aqua. Trop.,* **10**(1), 29

Pathirathnae, A., Widanapathirathirana, G.S. & Chandrakanthi, W.H.S. (1994) *J. Appl. Ichthyol Z. Angen. Ichthyol, 10 (2-3), 204*

Pradhan, K.K., Pal, J. & Das, A. (1991) *Environ. Ecol.,* **9**, 510

Reugpranch, H. & Kasdaronchan (1983) *Thai. Fish. Gaz.,* **36**(3), 265

Rosa, M.A., Rosa, M.A. & Roman, P. (1991) *J. Appl. Bacteriol.,* **71**, 182

Sugita, H., Tanaka, K., Yoshinami, M. & Deguchi, Y. (1995) *Appl. Environ. Microbiol,* **61**, 4128

Sukroongreung, S., Nilakul, C. & Tantimavanich, S. (1983) *South East Asian J. Trop. Med. Health.,* **14**(3), 330

Thampuran, N., Surendran, P.K., Mukundan, M.K. & Gopakumar, K. (1995) *Asian Fisheries Science,* **8**, 103