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Aeromonas hydrophila Infection on Culturing Sea Bass (*Lates calcarifer*) in Valaichennai Lagoon, Batticaloa, Sri Lanka

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ABSTRACT

Background: The floating cage cultured seabass (*Lates calcarifer*) at the site of Valaichennai lagoon in Batticaloa District, East coast of Sri Lanka was reported acute mortality within short period of stocking the fish in the cage.

Methods: Present study was focused to study the aetiology that caused by infection on culturing sea bass at the site. Gross pathological and histopathological examinations were carried in moribund and naïve fishes. Bacteriological, mycological screening and antibiotic sensitive test for isolation of pathogen were carried out to find out the causes. Water quality, feed quality and their management were assessed at the time of disease outbreak.

Result: Investigations primarily revealed that infection was initiated with the flooding due to heavy monsoonal season and sudden fluctuation in salinity. The gross pathological signs were change of skin colour, large irregular haemorrhages on the body surface, cloudy eye, ulcers on skin, lamellar fusion, fin rot and tail rot as well as histopathological findings such as necrosis in muscles, liver and kidney tissues, cyst formation by secondary infected pathogens proved that pathogen was bacteria. Gram staining also offered clear indication of gram-negative rod-shaped bacteria and, the particular pathogenic bacteria was confirmed as *Aeromonas hydrophila* through further analyses such as oxidative test, indole test and antibiotic sensitivity test. Antibiotic sensitivity test also point out that the Tetracycline as one of the effective antibiotics for the *Aeromonas hydrophila* infection. The improper feeding management and sudden salinity fluctuation pave the path for acute infection of *A. hydrophila* and ultimately lead to the high mortality in cage culture.

Key words: *A. hydrophila*, Cage culture, *L. calcarifer*, Mortality, Valaichennai lagoon.

INTRODUCTION

Lates calcarifer is commonly named as sea bass which is native to coastal area in the Indian and Western Pacific Oceans (Larson, 1999). These are predatory species, capable of surviving in wide range of salinity due to catadromous nature (Pender and Griffin 1996). In 1970s sea bass culture was initiated in Thailand, has expanded in Southeast Asia. Aquatic animals are partially or completely susceptible to diseases which influence by numerous biotic and abiotic environmental factors. Microbial pathogen, especially bacteria is one of the most significant agents causing diseases in fish culture (Zorrilla *et al.,* 2003). Abiotic factors act as stressors and make more susceptible to bacterial diseases. Genus of *Aeromonas* are facultative anaerobic, gram-negative bacilli that are ubiquitous inhabitants of fresh and brackish water (Mandell *et al*., 2005). The organisms are associated with waters where intense degradation of food materials and raising of other nutrients like nitrates and phosphates (Douglas *et al*., 1998). *Aeromonas hydrophila* is most common bacteria in fresh water habitat and also in coastal and brackish water where salinity is low and frequently cause disease known as “motile *Aeromonas Septicemia*” (MAS), “Hemorrhagic septicaemia”, “Ulcer Disease” or “Red-Sore Disease” in both feral and culture fish species worldwide (Cipriano, 2001; Joseph and Carnahan 1994). Aeromonas hydrophila, a ubiquitous, free-living, Gram-negative bacterium, is prevalent in aquatic habitats with cosmopolitan distribution; it is an opportunistic pathogen that has resulted in heavy mortalities in farmed and feral fishes (Harikrisnan and Balasundaram, 2005)

The ulcer disease was reported widely infecting marine as well as fresh water fish such as Tilapia, cat fish, carp, seabass and eel related with skin lesion, tail rot, haemorrhagic septicaemia over the body and tissue, epizootic ulceration and necrosis in liver and kidney of fish (Sarder *et al*., 2016). The acute mortality among cultured fish was between 10% -70% within the period of 24-48 hours (Roberts and Sommerville, 1982). The disease outbreaks in fish caused by *A. hydrophilla* resulting in millions of lost revenue, have been reported in worldwide. It was considered as significant economic issue, particularly in China and India over the past decades (Citarasu *et al*., 2011). Aeromonas represents a frequent menace to fish health particularly, high

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density aquaculture where intensive feeding is applied (Zmysłowska *et al*., 2009). Conventional methods of identifying the prevalence of Aeromonads are based on observation of gross pathological signs, histopathology, isolation and biochemical examinations (Austin *et al*., 1989). The scope of present study was to identify and justify the prevalence of *A. hydrophilla* infection on cultured sea bass in Valaichchenai lagoon, Batticaloa, Sri Lanka, by isolation and specific biochemical examinations for pathogen.

MATERIALS AND METHODS

Study area

The study area was located in part of Valaichchenai lagoon situated in Batticaloa District, East coast of Sri Lanka (Fig 1). Coordinates of the precise location was 07°55′ 22" N and 81°30′ 52″ E (GPS Gramin, UAS). The sea bass was cultures in floating cages installed in lagoon water system which closures to the shrimp farm area and rice mills. The routine fishing activity also taking place near to the study site. Seabass culture was conducted in twelve floating cages (4 m × 4 m) with the stocking density of 500 fingerlings/cage.

Methodology

Present investigation was carried out in sea bass cage farming in Valaichennai lagoon, Batticaloa, Sri Lanka where mass mortality of sea bass occurred during the period of December to January 2018. The research as carried in 2018 in the laboratory of the Eastern University, Sri Lanka.

Sample collection

Moribund, diseased and healthy sea bass fish samples were collected randomly in each cage by using sterilized hand nets and labelled separately in sterilized polythene bags. The clinical and gross pathological signs were observed at the site and those were recorded using digital camera (Canon EOS 1100D, Japan) for the identification. The collected specimens were transferred to the Department of Zoology, Eastern University, Sri Lanka for further identification and confirmation of the cause of death using histopathological and biochemical analyses.

Water quality assessment

Physio-chemical parameters namely, salinity, temperature, density, pH, dissolved oxygen, electric conductivity, total dissolved solid, turbidity, nitrate and phosphate were measured in-situ using refractometer (ATAGO S/Mill-E, Japan), pH meter (HANNA, Romania), Dissolved oxygen meter (EUTECH, Singapore), Electric conductivity meter (HANNA, Romania) Nephelometer (LABTRONICS, India), phosphate Colorimeter (HACH, USA), Nitrate Colorimeter (HACH, USA). Measurements were taken separately in infected cage, non-infected cage as well as open lagoon water system in order to verify the impact of water quality in disease outbreak. A triplicate data was collected and mean was considered.

Histopathological examination

Diseased and healthy fish were dissected and microtome sections of gill, skin, heart and liver were prepared by using microtome (Thermo Scientific, UK) for the comparison of diseased, healthy fish and conformation of bacterial infection (Austin and Austin 1999).

Bacteriological analysis

The diseased fishes were examined externally and biopsy were taken from lesioned part such as tail, fins, gill, skin and abdominal region. Gram staining technique was performed for identification of the bacterial pathogen. Nutrient agar (NA) medium was used for the enumeration and isolation of bacteria present in diseased fish (OIE, 2003).

Identification of *Aeromonas hydrophila*

From pure culture, important biochemical tests were performed such as Oxidative test, indole production in order to ensure the presence of specific bacteria responsible to the disease (OIE, 2003).

Antibiotic susceptibility assay

Susceptibility of *A. hydrophila* to different antibiotics was measured in vitro by disk diffusion method according to Kirby-Bauer (Bauer *et al*., 1966). The uniform culture of bacteria was prepared by spreading technique and commercially available four antibiotics (Tetracycline (30 µg), Cephalexin (30 µg), Amoxicillin (10 µg) and Azithromycin (15 µg)) discs were placed on the surface of the Tryptone Soya agar coated plate in individual manner. The cultured media were incubated for 24 hours at 37°C. Finally, the zone of inhibition was measured (radius of dead zone) and

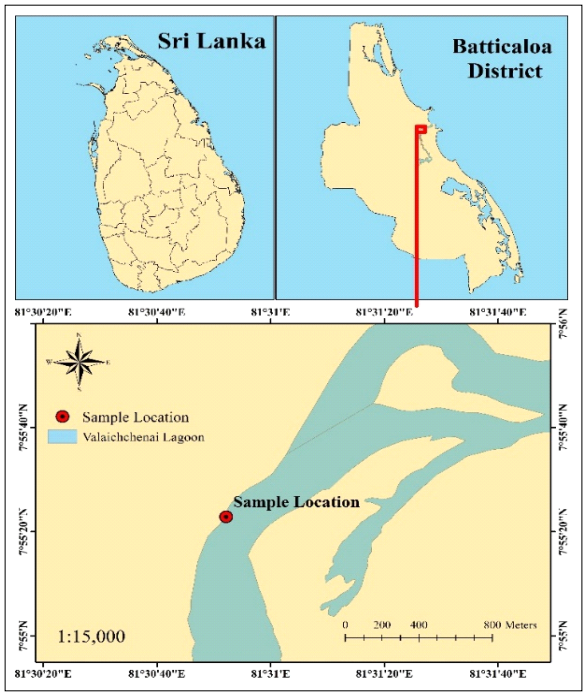

Fig 1: Map of the study site.

compared with the reference data of antibiogram pattern to know the susceptibility of the bacteria against particular antibiotics.

RESULTS AND DISCUSSION

Clinical and gross pathological findings

The total number of fishes showed abnormalities and acute mortality per cage is approximately 50 per day. The infected fish were of 15-20 cm total length and 80-90 g of weight. The mortality rate was not in a uniform manner. The infection was initiated due to flooding caused by the heavy monsoonal rain fall.

The gross pathological signs observed in infected cages were abnormal swimming, skin discoloration, large irregular haemorrhages on the body surface, cloudy eye, ulcers on the skin, pale gill, fin rot and tail rot (Plate 1).

Physio-chemical parameters

The Physio-chemical parameters in cages and open water are mentioned Table 1. The findings ensure that, there was no any huge deviation in physio-chemical parameters

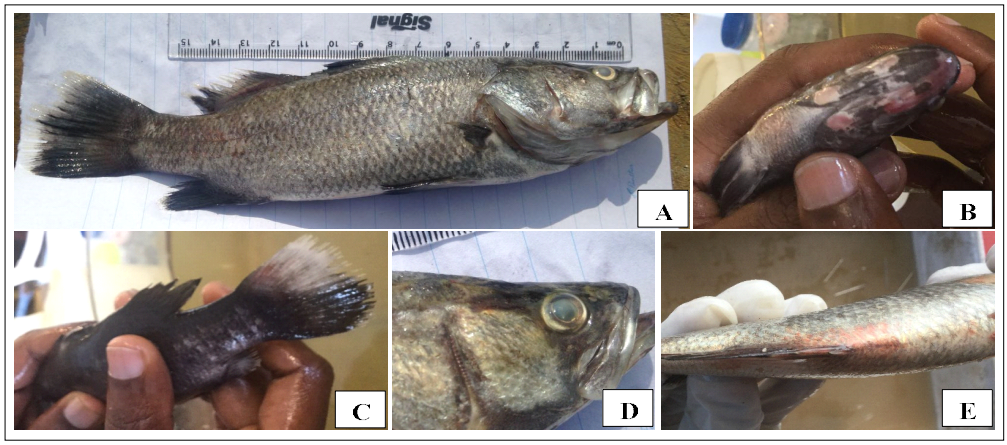

Plate 1: Gross pathological changes in infected fish. (A: Infected fish, B: Ulcers on skin, C: Tail rot, D: Cloudy eye, E: Haemorrhages).

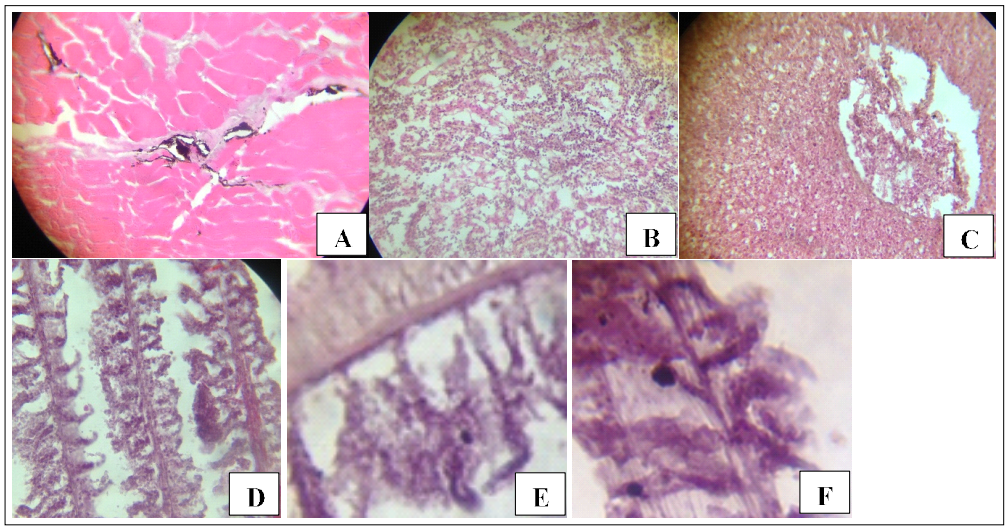

Plate 2: Histopathological changes in infected fish (A: Necrosis in muscles, B: Necrosis in hematopoietic tissue of kidney, C: Necrosis in liver tissue, D: Lamellar fusion, E and F: Cyst formation in diseased fish gill).

Table 1: Physio-chemical parameters of study site.

Sites	Salinity (ppt)	Density (g/l)	Temp. (°C)	pH (mg/l)	DO (µs/cm)	EC (mg/l)	TDS (NTU)	Turbidity	Nitrate (mg/l)	Phosphate (mg/l)
Infected cage (07)	0	1	28	6.95	5.5	243.3	162	34	1	0.27
Non infected cage (03)	0	1	28	7.15	5.7	279.7	158	30	1.1	0.23
Open water	0	1	28	7.3	5.9	312	122	32	0.4	0.25

between cage and open system because the presence of natural water flow inside cage. Anyhow Turbidity and TDS were slightly increase due to the accumulation of excess feeds. Here the salinity was very low (0 ppt) due to heavy rainfall and represent freshwater nature, hence this situation creates more favourable condition for the infection of *A. hydrophila* to the cultured sea bass. The pH and DO are lower in the cage water as the microbial activity of excess feed in cage increased.

Further details were collected from the farmers through questionnaires reveals that, they used excess amount of feed (commercially available trash fish) in order to enhance the final yield. Degradation of excess feeds forced to increasing of turbidity as well as ultimately create the favourable condition to the exponential growth and infection of *A. hydrophila*. Sea bass are seen non-infectious in some cages as those fishes could be said resistance for this sudden environmental changes and feed accumulation.

Histopathological findings

Histological examination of infected fish shown in Plate 2. Findings revealed that, dermal necrosis in muscles tissues, lamellar fusion in gill and cyst formation by secondary infected pathogens were observed. Pathological changes were not serious in heart tissues, even in cases with massive damage in the liver and kidney.

Bacteriological findings

Gram staining revealed that the presence of gram-negative bacteria (pinkly stained rod shape bacteria) on infectious parts of fishes. The gram negative bacterial colony at different magnifications were mentioned in Plate 3. Most of the fish infectious bacteria were gram negative and this finding clearly shows that the fish infected by gram negative bacterial pathogen.

Confirmation of *Aeromonas hydrophila*

Oxidative test and Indole test were giving positive results for the cultured bacteria (Fig 2). This revealed that the particular pathogenic bacteria were *A. hydrophila*.

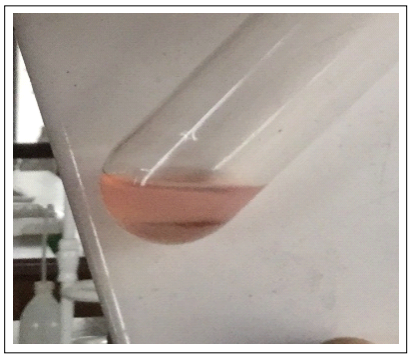

Fig 2: Indole test for cultured bacteria.

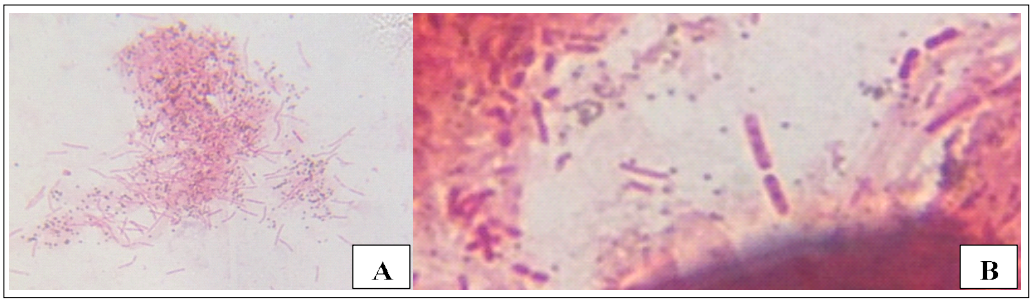

Plate 3: Gram negative bacterial colony (A: × 400, B: × 1000).

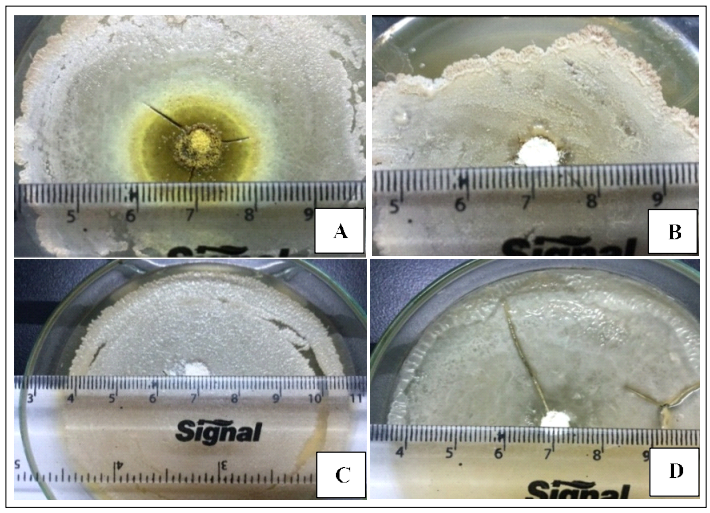

Plate 4: Susceptibility for different families of antibiotics (A: Tetracycline, B: Cephalexin C: Azithromycin and D: Amoxicillin).

Table 2: Susceptibility of *A. hydrophila* against different antibiotics.

Antibiotics	Disk potency (µg)	Inhibition zone diameter (mm)	Reference (mm)			Resistant (R) or Intermediate(I) or Sensitivity (S)
			R	I	S	
Tetracycline	30 µg	20	14<	15-18	19>	S
Azithromycin	15 µg	8	13<	14-17	18>	R
Cephalexin	30 µg	10	14<	15-17	18<	R
Amoxicillin	10 µg	9	20<	21-28	29>	R

Antibiotic susceptibility assay findings

Susceptibility for four different families of antibiotics were measured by using the reference of standard zone diameter interpretation chart for *A. hydrophila* (Bauer *et al.*, 1966). The result revealed that *A. hydrophila* was sensitive to tetracycline and resistant to other three antibiotics (Table 2). Plate 3 shows the inhibition zone and susceptibility for different antibiotics against *A. hydrophila*. Among four antibiotics, tetracycline is more suitable for the control of *A. hydrophila* infection.

CONCLUSION

Results and findings concluded that, the cultured sea bass affected by bacterial infectious disease known as Motile Aeromonas Septicemia (MAS). The responsible bacterial pathogen for the infection is *Aeromonas hydrophila* (gram negative and rod shaped). The improper feeding management and sudden salinity fluctuation were main factors for the infection of *A. hydrophila* and ultimately lead to the high mortality of sea bass in floating cages. In order to minimize the risk of infectious disease, it is recommended to consider proper planning and management such as avoid farming in monsoonal rainy season, installation of cages away from direct runoffs or flooding sites, maintaining of hygienic handling practices, avoidance of overcrowding and feeding management.

REFERENCES

Austin, B., Austin, D.A. (1999). Bacterial Fish Pathogens, In: Disease of Farmed and Wild Fish. Springer-Praxis, Chichester, 155.

Austin, D.A., McIntosh, D., Austin, B (1989). Taxonomy of fish associated *Aeromonas* spp, with the description of *Aeromonas salmonicida* subsp. Systematic and Applied Microbiology. 11: 277-290.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method, American Journal of Clinical Pathology. 45: 493-496.

Cipriano, R.C. (2001). *Aeromonas hydrophila* and Motile Aeromonas Septicemias of fish. Fish Disease Leaflet. 68.

Citarasu, T., Alfred, D.K., Velmurugan, S., Thanga, V.V., Kumaran, T., Michael, B.M., Selvaraj, T. (2011). Isolation of *Aeromonas hydrophila* from infected ornamental fish hatchery during massive disease outbreak, International Journal of Current Research. 2: 37-41.

Douglas, V., Athanassopoulou, F., Karaguni, E., Dotsika, E. (1998). *Aeromonas hydrophila* infection in cultured seabass, *Dicentrarchus labrax* L. and *Puntazzo puntazzo* Cuvier from the Aegean Sea. Journal of Fish diseases. 21: 317-320.

El-Sayyad, H.I., Zaki, V.H., El-Shebly, A.M and El-Badry, D.A. (2010). Studies on the effects of bacterial diseases on skin and gill structure of Clarias gariepinus in Dakahlia Province, Egypt, Annals of Biological Research. 1(4): 106-118.

Harikrishnan, R., Balaundaram, C. (2005). Modern Trends in *Aeromonas hydrophila* Disease Management with Fish, Reviews in Fisheries Science. 13(4): 281-320, DOI:10.1080/10641260500320845

Joseph, S.W., Carnahan, A. (1994). The isolation, identification and systematics of the motile Aeromonas species, Annual Review of Fish Diseases 4: 315-343. DOI: 10.1016/0959-8030(94)90033-7.

Larson, H. (1999). Order Perciformes. Suborder Percoidei. Centropomidae. Sea perches. 2429-2432. FAO, Rome.

Mandell, G.L., Douglas, R.G., Bennett, J.E., Dolin, R. (2005). Mandell, Douglas and Bennett's principles and practice of infectious diseases (2005th edn). Philadelphia Churchill Livingstone.

OIE Manual of Diagnostic test for Aquatic Animals, (2003). Office International Des Epizooties 2003, ISBN- 92-9044-563-7

Pender, P., Griffin, R. (1996). Habitat History of Barramundi *Lates calcarifer* in a North Australian River System Based on Barium and Strontium Levels in Scales, Transactions of the American Fisheries Society. 125: 679-689.

Roberts, R.J., Sommerville, C. (1982). Diseases of tilapias. In: The Biology and Culture of Tilapia. [Pullin R.S.V. and Lowe-McConnel R.H. (eds.)]. ICLARM conference proceeding, Manila, Philippines. 247-263.

Sarder, H., Khan, T., Saha, M.L., Punom, N.J., Mandal, S.C., Rahman, M.S. (2016). Prevalence and antibiotic susceptibility of *Aeromonas hydrophila* isolated from freshwater fishes, Journal of Fisheries. 4(3): 411-419. DOI: 10.17017/jfish.v4i3.2016.177.

Zmysłowska, K., Korzekwa, J., Szare. (2009). *Aeromonas hydrophila* in Fish Aquaculture, Journal of Comparative Pathology, 141(4): 313. DOI: 10.1016/j.jcpa.2009.08.143.

Zorrilla, I.M., Chbrillon, M.S., Diaz-Rosales., Martinz-Manzanares, M., Balebona, M.C., Marinigo, M.A. (2003). Bacterial recovered from diseased cultured gill head sea bream (*Sparus aurata* L.) in southeastern Spain. Aquaculture. 218: 112.