



## Study of bacterial haemorrhagic septicaemia causing mortality in Indian major carps from culture ponds of Kolleru lake, Andhra Pradesh, India

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Supplementary Table 1. Reports on Haemorrhagic Septicaemia disease in freshwater fishes from all over the world

Author	Year	Country	Host	Pathogen involved	Symptom observed
Wakabayashi <i>et al.</i>	1981	Tokyo	Catfish, <i>Carassius auratus</i> (Gold fish), <i>O. mykiss</i> (Trout) & <i>Plecoglossus altivelis</i> (Ayu) (test fish: <i>Cyprinus carpio</i> , <i>isgurunus anguilli caudatus</i> (Japanese oach)	<i>A. hydrophila</i> Biovar <i>hydrophila</i>	Haemorrhages in the abdominal cavity of the test fish, exophthalmus & scale protrusion were produced by injection.
Cipriano <i>et al.</i>	1984	West Virginia	<i>Cyprinus carpio</i>	<i>A. hydrophila</i>	Bulging of the eyes (exophthalmia), skin reddening, and fluid accumulation in the scale pockets. The abdomen may appear swollen, and the scales may protrude outward from the body.
Yambot	1998	Philippines	<i>O. niloticus</i> (Nile tilapia)	<i>A. hydrophila</i>	Ulcerations, hemorrhagic skin, eye abnormalities, loose scales, mouth sores, tail & fin rot and/or fungal growth, deformation of the upper & lower lips, hemorrhage in the orbit, exophthalmia protruded eye ball.
Al-Dughaym	2000	Saudi Arabia	<i>O. niloticus</i>	<i>A. hydrophila</i> & <i>P. fluorescence</i>	Skin ulcers accompanied by narrow areas of redness and dark discoloration of the skin, exophthalmia, hemorrhagic patches all over the body
Popovic <i>et al.</i>	2000	Zagreb, Croatia	Rudd: ( <i>Scardinius erythrophthalmus hesperidicus</i> ) Chub: ( <i>Leuciscus cephalus albus</i> B.) Tench: ( <i>Tinca tinca</i> L.)	<i>A. hydrophila</i>	Liver hemorrhages, hemorrhages in the swim bladder and posterior kidney, ascitic fluid, swollen kidney & spleen.
Cipriano <i>et al.</i>	2001	West Virginia	<i>C. carpio</i> ; <i>C. auratus</i> (Gold fish) <i>I. punctatus</i> , <i>O. niloticus</i>	<i>A. hydrophila</i>	Protrusion of the eyes (exophthalmia), skin redness, and fluid buildup beneath the scales are commonly observed symptoms. Swelling of the abdomen due to edema may also occur, causing the scales to stand out from the body.
Nielsen <i>et al.</i>	2001	China	<i>Carassius carassius</i> (Crucian carp), <i>Megalobrama Amblycephala</i> (Wuchang bream)	<i>A. hydrophila</i>	Loss of appetite, bulging eyes (exophthalmus), skin reddening caused by hemorrhages, and abnormal behavior

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Author	Year	Country	Host	Pathogen involved	Symptom observed
Soltani <i>et al.</i>	2001	Tehran, Iran	<i>Acipenser persicus</i> (Persian sturgeon)	<i>A. hydrophila</i>	Clinically, the affected or moribund fish exhibited lethargy, uncoordinated swimming behavior, and a darkened body with hemorrhagic patches on the skin. Widespread internal bleeding was evident in the organs, along with fluid accumulation in the abdominal cavity.
Ahmed <i>et al.</i>	2002	Egypt	<i>O. niloticus</i>	<i>A. hydrophila</i>	Reduced appetite, lethargy, loss of balance, and slow movement near the water surface. Additional symptoms included scale loss, skin lesions or ulcers, and protruding eyes (exophthalmia) often associated with hemorrhaging or cloudiness of the eyes.
Yesmin <i>et al.</i>	2004	Bangladesh	artificial infection: <i>Channa punctatus</i> (Snakhead) Diseased fish: ( <i>Channa punctatus</i> ), <i>Mastacembelus armatus</i> , <i>C. striatus</i> , <i>Puntius ticto</i> and <i>M.pancalus</i>	<i>A. hydrophila</i>	-
Kozinska	2007	Pulawy, Poland	<i>O. mykiss</i> (Rainbow trout)	<i>A. hydrophila</i>	Ulcers, skin hemorrhages, abdominal distension, anaemia, septic fluid in the peritoneal cavity, & or hemorrhages in internal organs
Ibrahem <i>et al.</i>	2008	Egypt	<i>O. niloticus</i> (Nile tilapia)	<i>A. hydrophila</i>	Darkened skin, scale loss, extensive irregular hemorrhages on the body surface, skin ulcers, erosion of the fins, an inflamed vent, bulging eyes (exophthalmia), and abdominal swelling filled with sero-hemorrhagic fluid..
Timur <i>et al.</i>	2010	Turkey	<i>Acipenser gueldenstaedtii</i> (Russian sturgeon)	<i>A. hydrophila</i>	Darkening of the skin, with haemorrhaged skin on the ventral side of the head, at the fin base and around the ganoid scales. Reluctance to feed, darkened skin, slight reddening at the base of the fins, and localized redness near the pectoral fins. They were observed swimming near the surface, exhibiting fin rot primarily at the tips of the pectoral fins, complete appetite loss, and unsteady movement along the bottom of the pond.
Yardimci <i>et al.</i>	2011	Turkey	<i>O. niloticus</i>	<i>A. hydrophila</i>	Limosis, operculum bleeding, muscle hemorrhage & hemorrhage ascites.
Ye <i>et al.</i>	2013	China	<i>H. molitrix</i> , <i>Carassius aumtus</i> , <i>Parabramis pekinensis</i>	<i>A. hydrophila</i>	Inflammation appeared on various parts of the fish's body, accompanied by skin and scale eruptions, tissue necrosis of the skin, deterioration of the soft fin rays, bulging eyes (exophthalmos), and the buildup of pus-like fluid in the abdominal cavity.
Unver <i>et al.</i>	2021	Turkey	<i>Cyprinus carpio</i>	<i>A. sobria</i>	

Table 1 contid...

Table 1 contid...

Author	Year	Country	Host	Pathogen involved	Symptom observed
Serik <i>et al.</i>	2022	Kazakhstan	<i>Acipenser baerii</i>	<i>A. hydrophila</i>	Multiple ulcers of varying sizes, deep muscle necrosis in the dorsal area, hemorrhages in the abdominal and lateral regions, pale gills, and congestion in the kidneys. Hemorrhagic spots were also observed in the liver, along with the build up of bloody exudates in the abdominal cavity.
Bai <i>et al.</i>	2023	China	<i>Pangasius bocourti</i>	<i>A. hydrophila</i>	Faint, swollen gills and severe congestion in the kidney.
Ozcan	2023	Turkey	<i>Oncorhynchus mykiss</i>	<i>A. veronii</i>	Skin ulcerations, hemorrhages on the skin and anal fin, fluid buildup in the intestines, and internal organ hemorrhages.

Supplementary Table 2. Reports on Haemorrhagic Septicaemia disease in freshwater fishes from the Indian region.

Author	Year	State	Host	Pathogen Involved	Symptom observed
Gopalakrishna	1961	West Bengal	Indian major carps	<i>A. hydrophila</i>	-
Kumar <i>et al.</i>	1986	Orissa	<i>Hypophthalmichthys molitrix</i> (Silver carp)	<i>A. hydrophila</i>	Haemorrhagic lesions & blotches all over the body (caudal region). Head & opercular portions had body appearance due to the thinned overlying integument
Karunasagar <i>et al.</i>	1989	Andhra Pradesh	<i>Cirrhinus mrigala</i> , <i>C. catla</i> , <i>L. rohita</i>	<i>A. hydrophila</i>	An acute form is characterized by with blisters, dropsy, abscesses, scale protrusion; and furuncular chronic ulcer.
Nayak <i>et al.</i>	1999	Bhubaneswar, Odisha	<i>Cirrhinus mrigala</i>	<i>A. hydrophila</i>	Hemorrhagic lesions were observed at the dorsal fin base, along with whitish, nodular cysts predominantly located in the tail region.
Shome <i>et al.</i>	2005	Assam	<i>C. catla</i> , <i>C. mrigala</i>	<i>A. hydrophila</i>	Loose scales, distended abdomen, extensive hemorrhages on the ventral part and deep ulcers on the dorsal surface.
Dash <i>et al.</i>	2008	Bhubaneswar, Odisha	Indian major carps	<i>A. hydrophila</i>	Loose scales, distended abdomen, petechial haemorrhages on the epidermis.
Sahoo <i>et al.</i>	2008	Bhubaneswar, Odisha	<i>L. rohita</i>	<i>A. hydrophila</i>	Fish injected with <i>Aeromonas hydrophila</i> exhibited classic symptoms of hemorrhagic septicaemia, including small to widespread hemorrhages on the underside of the body, as well as around the base of the pelvic and pectoral fins, operculum, and a swollen, reddened vent. Upon necropsy, most cases revealed the presence of blood-tinged fluid in the abdominal cavity, and congestion in the liver, kidneys, and spleen.
Jayavignesh <i>et al.</i>	2011	Tamil Nadu	Catfish	<i>A. hydrophila</i>	Widespread superficial reddening was noted over a large portion of the body surface, along with hemorrhagic ulcers at the fin bases. The spleen appeared enlarged and deep cherry red in color.
Sahu <i>et al.</i>	2011	Bhubaneswar, Odisha	<i>C. catla</i>	<i>A. hydrophila</i>	-

Table 2 contid...

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Author	Year	State	Host	Pathogen Involved	Symptom observed
Ahamad <i>et al.</i>	2013	Tamil Nadu	<i>Cirrhinus mrigala</i> , <i>Cyprinus carpio</i> , <i>C. catla</i> , <i>L. rohita</i>	<i>A. hydrophila</i>	Congestion and bleeding on the body surface, with the presence of petechial and ecchymotic hemorrhages, along with skin erosions and ulcers. Additional symptoms involved hemorrhages and congestion in the mouth, eyes, opercula, fin bases, and the area surrounding the anus.
Chandravanshi <i>et al.</i>	2020	Odisha	Indian major carps	<i>A. hydrophila</i>	Bleeding, reddish lesions, skin ulcers, reduced appetite, sluggish behavior, protruding eyes (pop-eye), abdominal swelling (dropsy), and decay of the tail or fins.
Abhishek <i>et al.</i>	2021	Assam	<i>Anabas testudineus</i> (climbing perch)	<i>A. hydrophila</i> & <i>A. jandaei</i>	Skin ulcers, increased mucus production, and the progressive appearance of gray-white patches on the rear portion of the body, eventually reaching the caudal fin. Additional signs were scale loss near the anal area and reddened fins, which later developed into noticeable fin rot.
Barde	2022	Maharashtra	<i>Cyprinus carpio</i>	<i>A. hydrophila</i>	Skin ulcers.

Supplementary Table 3. Biochemical Profile of *A. hydrophila* from IMCs with HS Compared to MTCC and Bergey's Standards

Characteristic	Present isolate		MTCC		Bergey's manual		Nayak et al. 1999		AI-Dughaym 2000		Abbot et al. 2003		Sabur 2006		Mostafa et al. 2008		Sahu et al. 2011		Chandravanshi et al. 2020		Abhishek et al. 2021		Serik et al. 2022			
	<i>L. rohita</i>	<i>C. catla</i>	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Physiological																										
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth in: 0% 3% Nacl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Methyl Red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
VP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth in broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth in NA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Specific media	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	
Simmon's citrate agar	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	
TSI (AS/AB)	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	K/K	
<i>Metabolism of nitrogenous compounds</i>																										
Lysine decarbo.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine decarb.	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H <sub>2</sub> S production	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3 contid...

Characteristic	Present isolate		MTC	Bergey's manual	Nayak et al. 1999	AI-Dughaym 2000	Abbot et al. 2003	Sabur 2006	Mostafa et al. 2008	Sahu et al. 2011	Chandravanshi et al. 2020	Abhishek et al. 2021	Serik et al. 2022
	<i>L. rohita</i>	<i>C. catla</i>											
Arginine dihydrolase	+	-	+	+	NoT	+	+	+	+	P	+	+	+
OF	F	F	+	F	NoT	NoT	NoT	F	F	NoT	NoT	NoT	F
MOF	F	F	F	F	NoT	NoT	NoT	NoT	NoT	NoT	NoT	NoT	NoT
Glucose	A+G-	A+G-	A+	A+G+	A+	A+	G+	A+G+	A+G+	NoT	NoT	A+G+	NoT
Sucrose	A+G-	A+G-	A+	A+	A+	A+	A+	A+	A+	NoT	NoT	A+	A+
Maltose	A+G-	A+G-	A+	A+	A+	ND	A+	A+	A+	A+	NoT	A+	NoT
Lactose	A+G-	A+G-	A+	A+	A-	ND	A+	A+	A+	A+	NoT	A+	A+/-
Arabinose	A+G+	A+G-	A+	A+	A+	d	A+	NoT	NoT	NoT	NoT	NoT	NoT

Y: Yellow, p: partial, d: variable, A: Acid, G: Gas, +: Positive, -: Negative, F: Fermentative, AS: Acid slant, AB: Acid butt, TSI: Triple sugar iron, K/N: Alkaline/no change, MOF: Marine oxidative fermentation, OF: Oxidative fermentation, NoT: not tested