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ANTIBIOTIC RESISTANT MOTILE AEROMONADS INDUCED SEPTICEMIA IN PHILIPPINE CATFISH *Clarias batrachus* (Linnaeus, 1758) FINGERLINGS

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ABSTRACT

Philippine catfish, *Clarias batrachus* farming is receiving much attention in recent years so also the diseases in catfish aquaculture. During the disease surveillance in catfish farms, mortalities were observed in *C. batrachus* fingerlings in a nursery and this report describes the bacteriological and histopathological observations made on the diseased catfish. The gross and clinical signs observed were lethargy, anorexia, vertical movement, skin erosion, operculum erosion, pale gills, reddening of mouth, fin haemorrhage, red lateral line and distended abdomen. The bacteria isolated from the kidney were identified phenotypically as species belonging to classical motile aeromonad group (*Aeromonas caviae, A. hydrophila, A. jandaei and A. sobria*) and newly described aeromonad group (*A. aquariorum, A. fluvialis and A. rivuli)*. Multiple antibiotic resistance (MAR) index was in the range of 0.3-0.8. These antibiotic resistant motile aeromonads caused septicaemia and 100% mortality. Histologically, haemocyte infiltration, necrosis, inflammation of epidermal tissue, rough epidermal layer and fibrosis in muscle tissue, and loss of typical tubular epithelial lining, necrosis of tubular tissue, inflammation of epithelial layer, cellular and nuclear hypertrophy, pycnotic nuclei, karyolysis and hypoplastic haematopoietic tissue in the kidney of diseased catfish were noted. The inflammatory responses observed in the kidney of *C. batrachus* were indicative of suppurative infection.

How to Cite

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INTRODUCTION

The Philippine catfish, *Clarias batrachus* (Linnaeus, 1758), commonly known as 'magur', has a fairly common distribution in fresh and brackishwaters of the plains throughout India. It has high commercial importance in India. Farming of *C. batrachus* has been identified as one of the potential national priorities in Indian aquaculture. The success in controlled breeding and larval rearing of *C. batrachus* has made the small and medium sized farming practice more popular (Ayyappan et al., 2011). The role of aeromonads as a causative agent of fish diseases has been known for decades. Motile aeromonads have been linked to major die-offs and fish kills around the globe over the past decade (Janda and Abbott, 2010). Infections caused by motile *Aeromonas* spp. are the most common and troublesome diseases of warm and cold water fish, resulting in enormous economic losses in cultured fish production. They are adapted to environments that have a wide range of conductivity, turbidity, pH, salinity and temperature (Austin and Austin, 2012). Classically, the species of *Aeromonas* implicated in fish disease that have been considered important in fish pathology were *A. salmonicida* and *A. hydrophila.* Other species such as *A. veronii* or *A. sobria* seem to have a specific role in the pathology of catfish and trout, respectively (Nawaz et al., 2006, Hidalgo and Figueras, 2012). Infectious diseases reduce catfish production by nearly 10% every year. Motile aeromonas septicaemia outbreaks in channel catfish, *Ictalurus punctatus* have led to an estimated loss of more than $3 million in 2009 in west Alabama, USA (Pridgeon et al., 2014). Recently, the panorama of species has expanded with the discovery of many new *Aeromonas* species (Hidalgo and Figueras, 2012, Martínez-Murcia et al., 2013) and these species may have an important role in fish pathology that needs to be explored in the future (Hidalgo and Figueras, 2012). Reports on the pathogenic association of newly described motile aeromonads are limited in Indian aquaculture. In this report we report the association of classical as well as new species of motile aeromonads in *Clarias batrachus* fingerling mortalities and the histopathological alterations in the muscle and kidney of diseased fish.

MATERIALS AND METHODS

Sample collection and bacteriology

In a catfish nursery located in Canning (Lat. 22°27'55" N; Long. 88°24'24" E), South 24 Parganas district, West Bengal, India, mortalities were observed repeatedly in *C. batrachus* fingerlings since October 2013. The fingerlings of size 10-20 g were stocked in 500 L capacity cemented tanks at the rate of 200 fish $tank^{-1}$. At site, the gross and clinical signs of diseased catfish were noted. Morbid as well as apparently healthy fingerlings (N=20 each) were collected from the affected and unaffected tanks, respectively packed in oxygen filled polythene bags separately and brought to the laboratory for bacteriological observations. Prior to sampling, the morbid and healthy fingerlings (5 each) were rinsed in sterile saline and wiped with sterile paper towel. The fish were dissected aseptically and inocula from kidney were streaked on to brain heart infusion agar (BHIA) and Rimler-Shotts agar (RSA) plates, and incubated at 30±2 °C for 24-48 h. Based on the dominance and definite colony morphology and pigmentation, representative colonies were picked from RSA plates and purified by repeated streaking on BHIA and maintained on BHIA slants. A series of biochemical reactions described by Collins et al. (2004) and Austin and Austin (2012) were performed to characterize and identify the bacteria. The phenotypic characteristics documented in earlier reports (Miñana-Galbis et al., 2009, Alperi et al., 2010, Figueras et al., 2011, Martínez-Murcia et al., 2013) were compared for the identification of classical and new *Aeromonas* species.

Antibiogram

Sensitivity of 11 motile *Aeromonas* spp. to 10 antibiotics such as amoxyclav (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), erythromycin (15 µg), gatifloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg) and sulphafurazole (300 µg) was tested by agar disc diffusion technique (Bauer et al., 1966) on Mueller Hinton agar. The agar plates were incubated for 24 h at 30±2 °C and the diameter of zone of inhibition in mm measured. Interpretation of sensitivity was based on the zone size interpretation chart provided by CLSI (2012). The multiple antibiotic resistance (MAR: resistant to at least three antibiotics) and MAR index (Number of antibiotics to which the individual bacterium is resistant divided by total number of antibiotics tested) were calculated from the antibiogram data.

Histopathology

The muscle and kidney of naturally infected *C. batrachus* were fixed in Bouin's solution for 48h. The fixed samples were prepared histologically using standard techniques, embedded in paraffin wax and 5 µm sections prepared and stained with haematoxylin and eosin (Presnell and Schreibman, 1997).

RESULTS

The morbid *C. batrachus* fingerlings were lethargic and anorexic. They had skin erosion, operculum erosion, pale gills, reddening of mouth, haemorrhages at the base of paired fins, red lateral line and dropsy. The mortality rate was low initially, which reached 100% in about two weeks in the affected tanks in November 2014. Motile *Aeromonas* spp. were isolated from the kidney of affected catfish and identified phenotypically as *A. hydrophila, A. caviae, A. sobria, A. jandaei, A. rivuli, A. aquariorum* and *A. fluvialis* (Table 1). No bacteria could be isolated from the kidney of unaffected catfish on RSA and BHIA. All the motile *Aeromonas* strains were resistant to amoxyclav and erythromycin. Resistance to broad spectrum antibiotics such as co-trimoxazole, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, sulphafurazole and oxytetracycline were seen among the *Aeromonas* spp. The MAR index of *Aeromonas* spp. varied from 0.3 to 0.8. Strains of classical *A. hydrophila* and *A. jandaei* displayed the least MAR index, i.e., 0.3 and 0.4, respectively. On the other hand, the new species of motile aeromonads *A. aquariorum* Cb2 and *A. fluvialis* Cb5 were resistant to 7 and 8 antibiotics, respectively. Few classical strains of *A. caviae* Cb3, Cb4 and *A. sobria* Cb11 were also resistant to 7-8 antibiotics tested. All the strains of motile *Aeromonas* were of MAR group (Table 2). Histopathological alterations such as haemocyte infiltration and necrosis of muscle tissue (Fig. 1a), inflammation of epidermal tissue, rough epidermal layer, extensive necrosis of muscle and fibrosis (Fig. 1b), necrosis of kidney, cellular and nuclear hypertrophy, pycnotic nuclei and karyolysis (Fig. 2a), loss of typical tubular epithelial lining (Fig. 2b) and necrosis of tubular tissue, inflammation of kidney epithelial layer and hypoplastic haematopoietic tissue (Fig. 3) were noted.

Table 1. Phenotypic characteristics of motile *Aeromonas* spp. associated with diseased *Clarias batrachus* fingerlings

Biochemical tests	Classical motile *Aeromonas* species				New motile *Aeromonas* species		
	Aeromonas caviae	*Aeromonas hydrophila*	*Aeromonas jandaei*	*Aeromonas sobria*	*Aeromonas aquariorum* like	*Aeromonas fluvialis* like	*Aeromonas rivuli* like
Gram reaction	-	-	-	-	-	-	-
Morphology	R	R	R	R	R	R	R
Oxidase	+	+	+	+	+	+	+
O/F reaction	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Gas from glucose	-	+	+	+	+	+	-
Voges Proskauer reaction	-	+	+	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	-
Esculin hydrolysis	+	+	-	-	+	-	+
Haemolysis	-	+	+	-	+	-	-
Arginine dihydrolase	+	+	+	-	+	-	+
Lysine decarboxylase	-	+	+	+	+	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	-	+	-	+
Starch hydrolysis	+	+	+	+	+	-	+
Utilization of Sucrose	+	+	-	+	+	+	+
Maltose	+	+	+	+	+	+	+
Glycerol	-	+	+	+	+	+	-
Salicin	+	+	-	-	+	+	+
Lactose	-	-	-	-	-	+	-
D-Mannitol	+	+	+	+	+	-	+
L-Arabinose	+	+	-	-	-	-	-

Table 2. Antibiogram and MAR index of motile *Aeromonas* spp. associated with diseased *Clarias batrachus* fingerlings

Bacterial species	Sensitive to	Resistant to	MAR index
Aeromonas aquariorum Cb1	C, Ci, Co, Ga, G	A, E, N, O, S	0.5
Aeromonas aquariorum Cb2	C, Ga, G	A, Ci, Co, E, N, O, S	0.7
Aeromonas caviae Cb3	C, N	A, Ci, Co, E, Ga, G, O, S	0.8
Aeromonas caviae Cb4	Co, N, S	A, C, Ci, E, Ga, G, O	0.7
Aeromonas fluvialis Cb5	Ga, G	A, C, Ci, Co, E, N, O, S	0.8
Aeromonas hydrophila Cb6	C, Ci, Co, Ga, G, N, O	A, E, S	0.3
Aeromonas hydrophila Cb7	C, Ci, Co, Ga, G, N, S	A, E, O	0.3
Aeromonas jandaei Cb8	C, Ci, Co, Ga, N, O	A, E, G, S	0.4
Aeromonas jandaei Cb9	C, Ci, Co, Ga, N, S	A, E, G, O	0.4
Aeromonas rivuli Cb10	C, Ci, Ga, N	A, Co, E, G, O, S	0.6
Aeromonas sobria Cb11	C, N, S	A, Ci, Co, E, Ga, G, O	0.7

A: Amoxyclav (30 μg), C: Chloramphenicol (30 μg), Ci: Ciprofloxacin (5μg), Co: Co-trimoxazole (25μg), E: Erythromycin (15 μg), Ga: Gatifloxacin (5μg), G: Gentamicin (10 μg), N: Nitrofurantoin (300 μg), O; Oxytetracycline (30 μg) and S: Sulphafurazole (300 μg)

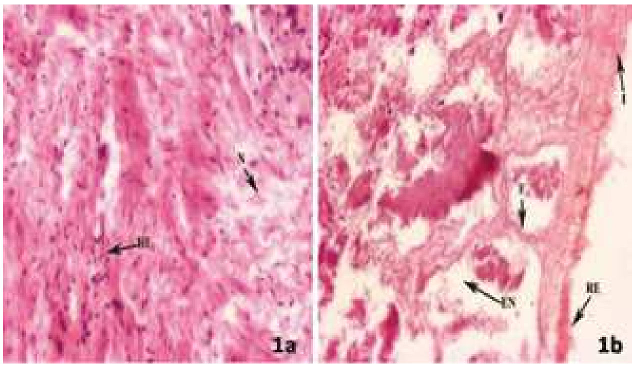

Fig 1. Light micrograph of diseased *Clarias batrachus* showing haemocyte infiltration (HI) and necrosis of muscle tissue (N), 1b. inflammation of epidermal tissue (I), rough epidermal layer (RE), extensive necrosis of muscle (EN) and fibrosis (F), 200X H&E staining

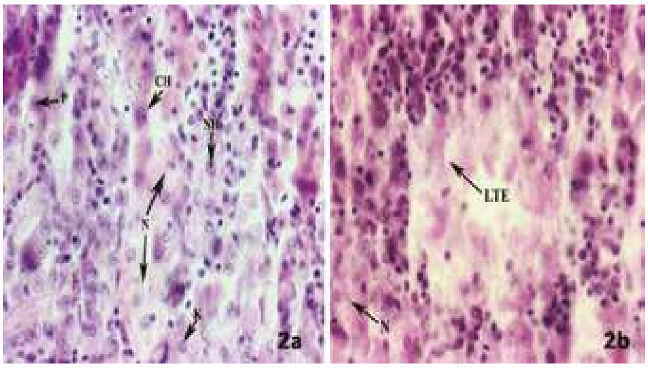

Fig 2. Light micrograph of diseased *Clarias batrachus* kidney showing extensive necrosis (N), cellular hypertrophy (CH), nuclear hypertrophy (NH), pycnotic nuclei (P) and karyolysis (K), 2b. extensive necrosis (N) and loss of typical tubular epithelial lining (LTE), 400X H&E staining

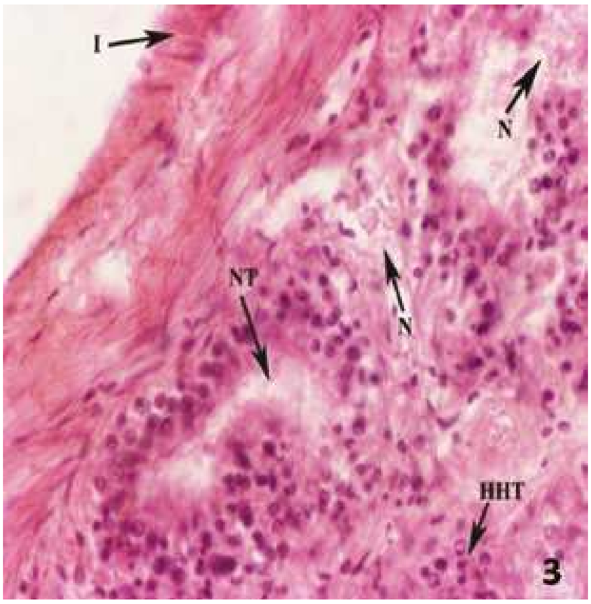

Fig 3. Light micrograph of diseased *Clarias batrachus* kidney showing extensive necrosis (N), necrosis of tubular tissue (NT), inflammation of kidney epithelial layer (I) and hypoplastic haematopoietic tissue (HHT), 400X H&E staining

DISCUSSION

Aeromonas hydrophila and other motile *Aeromonas* spp. were reported as major pathogens of *C. batrachus* (Majumder et al. 2007, Sarkar and Rashid 2012). The gross and clinical signs of motile aeromonads infected *C. batrachus* of the present study were similar to that of Majumder et al. (2007). The widespread distribution of these bacteria in the aquatic environment and the stress caused by captivity practices might have influenced *C. batrachus* to infections by classical motile *Aeromonas* spp. such as *A. hydrophila, A. caviae, A. sobria* and *A. jandaei,* and the new species of aeromonads *A. fluvialis, A. rivuli* and *A. aquariorum*, whose phenotypic characteristics matched with the original descriptions (Miñana-Galbis et al., 2009, Alperi et al., 2010, Figueras et al., 2011, Martínez-Murcia et al., 2013). The results suggest that newly described species of the genus *Aeromonas* together with classical aeromonads can cause infection in catfish as opined Hidalgo and Figueras (2012). The sampled catfish nursery experienced 100% mortality. All dead fingerlings had septicemic condition. Interestingly, all associated motile aeromonads were of MAR group that indicated possible abuse of antibiotics in the captive rearing of *C. batrachus*. Most of the *Aeromonas* strains of the present study were resistant to oxytetracycline, which contradicts the earlier observations (McPhearson et al., 1991). Likewise, 79-100% of the aeromonads from the catfish farms were observed to be resistant to oxytetracycline (Abraham 2011, Bharathkumar and Abraham 2011, Anyanwu et al., 2014). These results suggest that catfish nursery may serve as a major source and spread of oxytetracycline resistant bacteria. The wide spread use of antimicrobial agents in aquaculture, especially oxytetracycline, has been associated with increased antibiotic resistance in *A. hydrophila* (DePaola et al., 1995, Nawaz et al., 2006, Bharathkumar and Abraham 2011). In an earlier study Abraham (2011) recorded high incidence of MAR among the bacterial flora of catfish (76%) followed by miscellaneous fish (66%), sewage-fed farm grown carps (55%) and ornamental fish (48%) in West Bengal, India. The differences in the frequency of resistance may be related to the source of *Aeromonas* isolates and the frequency and type of antimicrobial agents used for treating diseases and for health management in that geographical area. Resistance among the *Aeromonas* spp. of diseased catfish to broad spectrum co-trimoxazole, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, sulphafurazole and oxytetracycline is a serious cause for concern.

The observed histopathological changes in the muscle tissue indicated that the motile *Aeromonas* spp. have the potential to cause extensive tissue damage. These changes also suggested involvement of inflammatory cells against the *Aeromonas* invasion. In kidney, they caused systemic infection with the loss of typical tubular epithelial lining, inflammation of epithelial layer, necrosis, cellular and nuclear hypertrophy, pycnotic nuclei, karyolysis and hypoplastic haematopoietic tissue. Similarly, Angka (1990) reported necrosis and haemorrhage in the kidney of *C. batrachus* experimentally challenged with *A. hydrophila*. On the other hand, cloudy swelling, hydropic degeneration, necrosis in renal tubules and degenerative changes in glomerular epithelium and inflammatory cells of kidney were noted in *C. gariepinus* infected with *A. hydrophila* (Laith and Najiah, 2013). Focal necrosis and cellular degeneration were observed in the kidney of *A. hydrophila* infected *Pangasianodon hypophthalmus* (Ly et al., 2009, Faruk et al., 2012). In contrast, Islam et al. (2008) noted diffuse necrosis along with massive atrophid hematopoietic tissue in *A. hydrophila* infected *Heteropneustes fossilis.* Studies have also documented the presence of melano-macrophage centres in *P. hypophthalmus* (Faruk et al., 2012) and in estuarine catfish *Arius maculates* (Alagappan et al., 2009) during *A. hydrophila* infection, which was not observed in the present study.

CONCLUSION

In general, the mortalities in captive held *C. batrachus* fingerlings of the present study may be attributed to the potential virulence factors of multiple antibiotic resistant motile *Aeromonas* spp. The inflammatory responses observed in the kidney of *C. batrachus* were indicative of suppurative infection. The actual role of the new species of motile aeromonads in *C. batrachus* mortality or their synergism and molecular basis of action, however, need to be studied.

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Sažetak

POKRETNE VRSTE AEROMONAS OTPORNE NA ANTIBIOTIKE UZROKUJU SEPTIKEMIJU KOD MLAĐI FILIPINSKOG SOMA, *Clarias batrachus* (Linnaeus, 1758)

Uzgoj filipinskog soma, *Clarias batrachus,* izaziva veliku pozornost posljednjih godina, kao i bolesti kod uzgoja soma. Tijekom nadzora bolesti na farmama somova uočena je smrtnost kod mlađi *C. batrachus* u mrjestilištu, a ovaj rad opisuje bakteriološka i histopatološka opažanja uočena kod zaraženih somova. Opaženi općeniti i klinički znakovi su letargija, anoreksija, vertikalno gibanje, erozija kože, erozija operkuluma, blijede škrge, crvenilo usta, krvarenje peraja, crvena bočna linija i napuhani trbuh. Bakterije izolirane iz bubrega su fenotipski identificirane kao vrste koje spadaju u klasičnu pokretnu skupinu Aeromonas (*Aeromonas caviae, A. hydrophila, A. jandaei* i *A. sobria*) i nedavno opisanu skupinu Aeromonas (*A. aquariorum, A. fluvialis* i *A. rivuli*). Indeks višestruke rezistencije na antibiotike je u rasponu od 0,3-0,8. Ove pokretne vrste Aeromonas otporne na antibiotike uzrokovale su septikemiju i stopostotnu smrtnost. Histološki, zabilježena je infiltracija hemocita, nekroza, upala epidermalnog tkiva, grubi epidermalni sloj i fibroza u mišićnom tkivu te gubitak tipične cjevaste epitelne unutarnje stjenke, nekroza cjevastog tkiva, upala epitelnog sloja, stanična i nuklearna hipertrofija, piknoza jezgre, karioliza i hipoplastično hematopoetsko tkivo u bubrezima oboljelih somova. Upalne reakcije uočene u bubregu *C. batrachus* upućuju na gnojne infekcije.

Ključne riječi: infekcija bakterijom Aeromonas, višestruka rezistencija na antibiotike, histopatologija, upalna reakcija

REFERENCES

Abraham, T. J. (2011): Food safety hazards related to emerging antibiotic resistant bacteria in cultured freshwater fishes of Kolkata, India. Advanced Journal of Food Science and Technology, 3, 1, 69-72.

Alagappan, K. M., Deivasigamani, B., Kumaran, V., Sakthivel, M. (2009): Histopathological alterations in estuarine catfish (*Arius maculatus*; Thunberg, 1792) due to *Aeromonas hydrophila* infection. World Journal of Fish and Marine Sciences, 1, 3, 185-189.

Alperi, A., Martınez-Murcia, A. J., Monera, A., Saavedra, M. J., Figueras, M. J. (2010): *Aeromonas fluvialis* sp. nov., isolated from Spanish river. International Journal of Systematic and Evolutionary Microbiology, 60, 72–77.

Angka, S. L. (1990): The pathology of the walking catfish, *Clarias batrachus* (L), infected intraperitonially with *Aeromonas hydrophila*. Asian Fisheries Science, 3, 343-351.

Anyanwu, M. U., Chah, K. F., Shoyinka, V. S. (2014): Antibiogram of aerobic bacteria isolated from skin lesions of African catfish cultured in Southeast, Nigeria. International Journal of Fisheries and Aquatic Studies, 2, 1, 134-141.

Austin, B., Austin, D. A. (2012): Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, 5th edition. Springer-Praxis in Aquaculture in Fisheries, Praxis Publication Ltd., Chichester, UK. 457 pp.

Ayyappan, S., Jena, J. K., Gopalakrishnan, A., Pandey, A. K. (2011): Handbook of Fisheries and Aquaculture. Indian Council of Agricultural Research, New Delhi, India. 755 pp.

Bharathkumar, G., Abraham, T. J. (2011): Antibiotic susceptibility of Gram-negative bacteria isolated from freshwater fish hatcheries of West Bengal, India. Indian Journal of Fisheries, 58, 3, 135-138.

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

CLSI. (2012): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22, Vol. 32(3), Clinical and Laboratory Standards Institute, Wayne, PA 19087, USA

Collins, C. H., Lyne, P. M., Grange, J. M., Falkinham, J. O. III. (2004): Collins and Lyne's Microbiological Methods, 8th edition. Arnold, London, UK. 456 pp.

DePaola, A., Peeler, J. T., Rodrick, G. E. (1995): Effects of oxytetracycline medicated feed on antibiotic resistance of gram-negative bacteria in catfish ponds. Applied Environmental Microbiology, 61, 2335-2340.

Faruk, M. A. R., Patwary, Z. P., Hasan, M. M. (2012): Clinical and histopathological investigations in exotic catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) under culture condition. Indian Journal of Fisheries, 59(4), 183-185.

Figueras, M. J., Alperi, A., Beaz-Hidalgo, R., Stackebrandt, E., Brambilla, E., Monera, A., Martínez-Murcia, A. J. (2011): *Aeromonas rivuli* sp. nov., isolated from the upstream region of a karst water rivulet in Germany. International Journal of Systematic and Evolutionary Microbiology, 61, 242-248.

Hidalgo, R. B., Figueras, M. J. (2012): Molecular detection and characterization of furunculosis and other *Aeromonas* fish infections. In: *Health and Environment in Aquaculture* (eds. Carvalho, E. D., David, G. S., Silva, R. J.). InTech, DOI: 10.5772/29901. ISBN: 978-953-51-0497-1, Available from: http://www.intechopen.com/ books/health-and-environment-in-aquaculture/molecular-detection-and-characterization-of-furunculosis-and-other-aeromonas-fish-infections. pp. 97-132.

Islam, M. T., Mostafa, K., Rashid, M. M. (2008): Histopathological studies of experimentally infected Shing, *Heteropneustes fossilis* with *Aeromonas hydrophila* bacteria. Progressive Agriculture, 19, 1, 89-96.

Janda, J. M., Abbott, S. L. (2010): The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews, 23, 1, 35–73.

Laith, A. R., Najiah, M. (2013): *Aeromonas hydrophila*: Antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). Journal of Aquaculture Research and Development, 5, 215.

Ly, L. T. T., Nguyen, D. N., Vo, P. H., Doan, C. V. (2009): Haemorrhage disease of cultured Tra catfish (*Pangasianodon hypophthalmus*) in Mekong Delta (Vietnam). Israeli Journal of Aquaculture Bamigdeh, 61, 3, 215-224.

Majumder, T., Datta, S., Ghosh, D., Dutta, S., Chakraborty, A., Gowsami, R., Mazumder, S. (2007): Role of virulence plasmid of *Aeromonas hydroplhila* in the pathogenesis of ulcerative disease syndrome in *Clarias batrachus*. Indian Journal of Biochemistry and Biophysics, 44, 401-406.

Martínez-Murcia, A., Beaz-Hidalgo, R., Svec, P., Saavedra, M. J., Figueras, M. J., Sedlacek, I. (2013): *Aeromonas cavernicola* sp. nov., isolated from freshwater of a brook in a cavern. Current Microbiology, 66, 197–204.

McPhearson, R. M., DePaola, A., Zywno, S. R., Motes, M. L., Guarino, A. M. (1991): Antibiotic resistance in gram-negative-bacteria from cultured catfish and aquaculture ponds. Aquaculture, 99, 203–211.

Miñana-Galbis, D., Urbizu-Serrano, A., Farfán, M., Fusté, M. C., Lorén, J. G. (2009): Phylogenetic analysis and identification of *Aeromonas* species based on sequencing of the cpn60 universal target. International Journal of Systematic and Evolutionary Microbiology, 59, 1976–1983.

Nawaz, M., Sung, K., Khan, S. A., Khan, A. A., Steele, R. (2006): Biochemical and molecular characterization of tetracycline-resistant *Aeromonas veronii* isolates from catfish. Applied and Environmental Microbiology, 27, 6461–6466.

Presnell, J. K., Schreibman, M. (1997): Humason's Animal Tissue Techniques, 5th edition. Johns Hopkins University Press, Baltimore, MD.

Pridgeon, J. W., Zhang, D., Zhang, L. (2014): Complete genome sequence of the highly virulent *Aeromonas hydrophila* AL09-71 isolated from diseased channel catfish in west Alabama. Genome Announcements, 2, 3, e00450-14. doi:10.1128/genomeA.00450-14.

Sarkar, M. J. A., Rashid, M. M. (2012): Pathogenicity of the bacterial isolate *Aeromonas hydrophila* to catfishes, carps and perch. Journal of Bangladesh Agricultural University, 10, (1), 157–161.

