Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 7 (6): 461-467, 2012
ISSN 1816-4927 / DOI: 10.3923/jfas.2012.461.467

First Isolation of *Vibrio alginolyticus* from Ornamental Bird Wrasse Fish (*Gomphosus caeruleus*) of the Red Sea in Egypt

[1]M.A.A. Abd El-Galil and [2]M.H. Mohamed
[1]Department of Fish Dis., Faculty of Veterinary Medicine, Sohag University, Egypt
[2]Department of Fish Microbiology, The National Institute of Oceanography and Fisheries, Hurghada, Egypt

Corresponding Author: M.A.A. Abd El-Galil, Department of Fish Dis., Faculty of Veterinary Medicine, Sohag University, Egypt

ABSTRACT

The first isolation of *Vibrio alginolyticus* from marine ornamental Bird wrasse fish (*Gomphosus caeruleus*) is reported in the indoor aquarium of National Institute of Oceanography and Fisheries (NIOF) in Hurghada. The pathogen was recovered from mouth ulcer, body surface lesions and internal organs of randomly collected fish sample during episodes of mortality occurred among the investigated fish. Lethargic, off food, skin depigmentation, mouth ulceration (stomatitis) and hemorrhagic spots appeared on the naturally infected fish associated with 70% mortality. Seven isolates were obtained and all isolates constituted a homogeneous phenotypic group and were identified by morphological characterization, biochemical tests and API20E as *Vibrio alginolyticus*. The isolated strain was sensitive to amoxiclav, streptomycin and chloramphenicol. The 50 mg kg^{-1} b.wt. of chloramphenicol for 7 days as food additive prevented the clinical signs and mortality in the experimentally infected fish.

Key words: *V. alginolyticus*, vibriosis, bird wrasse fish, *Gomphosus caeruleus*, chloramphenicol

INTRODUCTION

Among the bacterial pathogens of marine fishes, vibrio is one of the most important causes of the economic losses. This bacterium is normally found in marine water and the disease occurs when fish was exposed to this bacterial pathogen in the existence of stress factors (Austin and Austin, 2007). *V. harveyi* and *V. alginolyticus* were the predominant isolates from coral reef fish and shell fish at Ruesri Bay (Thongchankaew *et al*., 2011). *V. alginolyticus* had categorized as one of the seven vibrio fish pathogens (Austin and Austin, 1987) and are frequently isolated from outbreaks and mortalities in many marine fish species including Carpet Shell Clam (*Ruditapes decussatus*) Larvae in Spain (Leon *et al*., 2005), gilt-head sea bream (*Sparus aurata*) (Akaayli *et al*., 2008), silver sea bream (*Sparus aurata*) in Hong Kong, Gilt head sea bream (*Sparus aurata*) in Spain, cultured black sea bream (*Mylio macrocephalus*) fry in Japan (Austin and Austin, 2007) and sturgeon (*Acipenser baerii*) in Siberia (Costinar *et al*., 2010).

The infected fish with *V. alginolyticus* showed lethargy, off food, external hemorrhages, loss of skin colouration, depressed abdomen and the mortalities reached more than 50% among the infected fish. In post mortem examination hemorrhagic liver and ascetic fluid in the intestine were detected (Alcaide *et al*., 2001; Toranzo *et al*., 2005; Martins *et al*., 2010).

V. alginolyticus is gram-negative rods or slightly curved rods and has been recognized as an opportunistic pathogen in humans as well as marine animals (Zhao *et al*., 2010). Chemotherapy,

vaccination and other prophylactic measures are generally adopted to control microbial infection (Shamsudin *et al*., 2009). Special attention to *V. alginolyticus* zoonotic infections should be taken urgently in consideration because its transmission is possible via infected fish and sea water (Xiao *et al*., 2009; Ardic and Ozyurt, 2011).

Hence, the present study reports the first description of *V. alginolyticus* as responsible for vibriosis in Bird wrasse fish in indoor aquarium of National Institute of Oceanography and Fisheries at Hurghada Egypt. Morphological and biochemical identification, antibiotic sensitivity test, treatment trial and virulence of the isolates are described.

MATERIALS AND METHODS

Fish collection: Moribund and clinically diseased *Gomphosus caeruleus* fish were collected from the investigated indoor aquarium of the National Institute of Oceanography and Fisheries at Hurghada city (from November 2010 to February 2011) and subjected to clinical and post mortem examination according to Amlacher (1970) and Austin and Austin (1987).

Water samples: Water samples were taken from the investigated indoor aquarium and the red sea (control sample) in dark brown clean and dry bottles. Water temperature and pH were determined by digital combo pH meter and thermometer (HI 98127 (pHep 4) -Hanna instruments Inc., USA), total ammonia was determined and Dissolved Oxygen (DO) concentration were measured using a digital dissolved oxygen meter (HI 9142-Hanna instruments Inc., USA).

Bacterial isolation: Samples for bacterial isolation were taken from ulcers, liver and kidney of moribund and clinically diseased *Gomphosus caeruleus* fish and cultured on plates of Brain heart infusion agar and Huso-Shotts medium. The two media were prepared with 50% sea water (Chen *et al*., 1995). The inoculated plates were incubated at 25°C for up to 72 h.

Phenotypic characterization: The suspected *V. alginolyticus* colonies were isolated, purified using Thiosulphate Citrate Bile Salt Sucrose agar (TCBS, Difco) and characterized using phenotypic and biochemical tests as reported by Martins *et al*. (2010). Commercial miniaturized API20E galleries (BioMerieux) were also used according to the manufacturer's instructions but sterile sea water was used as a diluent and 25°C as incubation temperature. The isolates were identified according diagnostic schemes described by Costinar *et al*. (2010).

Antibiotic sensitivity test: Drug susceptibility of the isolates was determined by using Kirby-Bauer Disk Diffusion Susceptibility Test method (Jorgensen and Turnidge, 2007) and the following antibiotics streptomycin, tobramycin, chloramphenicol, oxytetracycline, amoxiclav, gentamicin and enrofloxacin, were used (Oxoid).

Pathogenicity assays: Sixteen *Gomphosus caeruleus* fish were acclimated for one week in the indoor aquarium and subdivided into two equal groups each of eight fish. The fish of the first group were challenged by bath immersion in 3 L glass aquarium containing 1.5×10^{6} cells of *V. alginolyticus* mL^{-1} for 15 min (Martins *et al*., 2010). The fish of the second group were submitted to the same procedure without bacteria and used as control. Each fish group was reared in 110 L glass aquarium at water temperature 27±2°C and observed for 14 days, the clinical signs and numbers of dead fish were recorded during the observation time.

Treatment trial: Fourteen *Gomphosus caeruleus* fish were subdivided into two equal groups each of seven fish and reared separately in two glass aquaria. The fish of the two groups were experimentally infected by immersion bath in 3 L glass aquarium containing 1.5×10^6 cells of *V. alginolyticus* mL^{-1} for 15 min (Martins *et al*., 2010). The fish of the first group were fed on chloramphenicol medicated food at rate 50 mg kg^{-1} b.wt. for 7 days beginning from the second day of experimental infection and the fish of the second group (control group) were fed on non medicated food by the same regime. The two fish groups were observed for 14 days and the clinical signs and mortality were reported.

RESULTS

Clinical signs: The clinical signs of the diseased *Gomphosus caeruleus* fish were lethargic of food and ulcerated mouths (stomatitis) (Fig. 1), skin depigmentation and hemorrhagic spots (Fig. 2). The main post mortem lesions were congestion of liver, spleen and stomach more over ascetic fluid in the intestine (Fig. 3). The mortality among the diseased fish was 70%.

Water quality: The results of this study revealed the elevation of ammonia and pH values and decrease of dissolved oxygen in the water samples of indoor aquarium, Table 1.

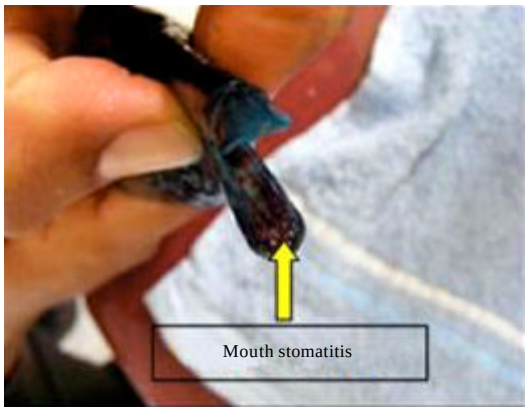

Fig. 1: Ulcerated mouth of the diseased fish

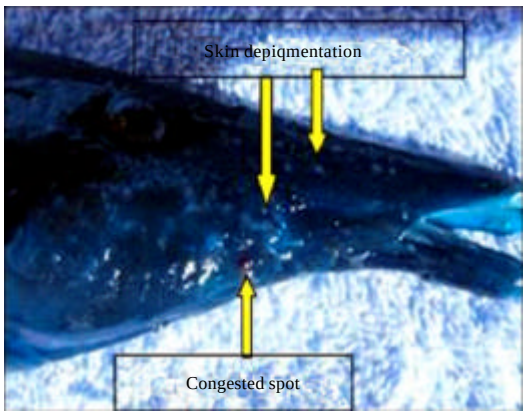

Fig. 2: Skin depigmentation and congested spot on the head region

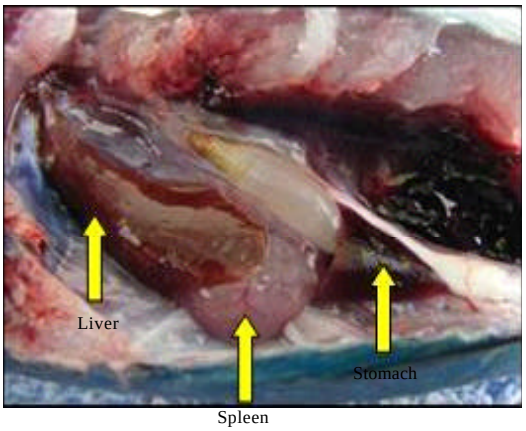

Fig. 3: Congestion of liver, spleen and stomach

Table 1: Water quality criteria

Item	Tested sample	Control sample
Water temperature (°C)	26.0	23.0
pH values	9.2	7.7
Dissolved oxygen (mg L^{-1})	2.9	5.8
total ammonia (mg L^{-1})	0.0051	0.0003

Isolation and characterization of the bacterial strains: Seven putative *V. alginolyticus* strains were isolated from ulcer, liver and spleen. These colonies of suspected *V. alginolyticus* were rounded, with regular edges, creamy in colour and in some cases adhered strongly to the culture media. The biochemical and physiological characteristics of all the isolates were similar and allowed the presumed identification of the bacteria as *V. alginolyticus*. In fact, all strains were Gram-negative, motile curved rods, cytochrome oxidase and catalase positive. No growth was observed in the absence of sea salts and grows in 8% NaCl. With regard to API20E galleries, H_2S and gelatinase tests gave negative and positive results, respectively (Table 2).

Antibiotic sensitivity test: The findings of antibiotic sensitivity test cleared that the isolated *V. alginolyticus* was sensitive to amoxiclav (amoxicillin-clavulanate), streptomycin and chloramphenicol. Controversially, it was resistant to oxytetracycline, tobramycin, gentamicin and enrofloxacin (Table 3).

Pathogenicity assays: The experimentally infected *Gomphosus caeruleus* fish showed lesions similar to those of naturally infected fish such as lethargy, skin depigmentation, off food and ulcerated mouth. Eye opacity was recorded in few cases on the experimentally infected fish and not recorded in the naturally infected fish. The observed PM lesions were ascetic fluids in the intestine and congestion of the visceral organs. By the end of observation time (14 days) the mortality of the experimentally infected fish reached 62.5%. *V. alginolyticus* could be reisolated in pure culture from the experimentally infected fish.

Treatment trial: 50 mg kg^{-1} body weight of chloramphenicol as food additive prevented the clinical signs and mortality of vibriosis in the experimentally infected fish. The control group

Table 2: The results of the biochemical characterization of the *vibrio alginolyticus* isolates

Test	Result	Test	Result
Colony shape	Round	Colony colour	Creamy
Gram stain	-ve rods	Motility	+
Cytochrome oxidase	+	Catalase	+
Growth in 0% NaCl	-	Growth in 3% NaCl	+
5% NaCl	+	Growth in 8% NaCl	+
API20E			
ONPG	-	GEL	+
ADH	-	Glucose	+
LDC	+	Manitol	+
ODC	+	Inositol	-
CIT	+	Sorbitol	+
H_2S	-	Rhamnose	-
URE	-	Sucrose	+
TDA	-	Melibiose	-
IND	+	Amygdalin	+
VP	-	Arabinose	-

ODC: Ornithine decarboxylase, LDC: Lysine decarboxylase, ADH: Arginine dihydrolase, IND: Indole, CIT: Citrate, URE :Urea hydrolysis, VP: Voges-Proskauer, TDA: Tryptophane deaminase, GEL: Gelatin hydrolysis, ONPG: Ortho-nitrophenyl b-d-galactopyranoside and H_2S: Hydrogen sulfide production

Table 3: The results of antibiotic sensitivity tests of *V. alginolyticus*

Antibiotics	Result
Ciprofloxacin	S
Amoxiclav	S
Chloramphenicol	S
Streptomycin	S
Oxytetracycline	R
Tobramycin	R
Gentamicin	R
Enrofloxacin	R

S: Sensitive and R: Resistant

recorded 57.14% mortality and clinical signs such as lethargy, off food, ulcerated mouth, skin depigmentation and hemorrhagic spots.

DISCUSSION

The clinical signs of *V. alginolyticus* infection in *Gomphosus caeruleus* fish were lethargy, off food, skin depigmentation, external hemorrhages and mouth ulceration (stomatitis). Hemorrhagic liver, spleen and stomach and ascetic fluid were reported in this study. Similar observations were found by Toranzo *et al.* (2005) and Martins *et al.* (2010).

The clinical signs of vibriosis were noticed on the wild investigated fish after few days of fishing and rearing in the indoor aquarium. The onset of the disease may be attributed to the suppression of the fish immune system due to overcrowdedness, increased ammonia and pH values and decreased dissolved oxygen in the indoor aquarium. Holt *et al.* (1975), Morrison *et al.* (1981), Bullock *et al.* (1986) and Suomalainen *et al.* (2005) stated that the sharp increase in the ammonia

level, water pH, physical contact and the sharp decrease in the dissolved oxygen are the most possible triggering factors for initiation, establishment and spread of infection in addition to jeopardize fish immune system.

The seven isolates from naturally infected fish during mortality episodes were identified as *V. alginolyticus* by the colony characters, cell morphology, gram stain, biochemical reactions including the API20E tests. This finding was in agreement with Martins *et al.* (2010) and Costinar *et al.* (2010).

The pathogenicity assay revealed that *V. alginolyticus* was pathogenic to *Gomphosus caeruleus* fish. The moribund and dead fish exhibited clinical signs similar to that signs of the naturally infected fish including lethargy, off food, skin depigmentation, external hemorrhages, mouth ulceration and visceral congestion in addition to 62.5% mortality. The pathogenicity of *V. alginolyticus* may be attributed to the extracellular toxins which have undesirable effects on the physiology of the infected fish more over their cytotoxic effects (Nottage and Birkbeck, 1987; Sindermann, 1990; Birkbeck and Gallacher, 1993).

The antibiotic sensitivity test of *V. alginolyticus* revealed that the isolated strain was sensitive to amoxiclav, streptomycin and chloramphenicol and this result was in agreement with the findings of Costinar *et al.* (2010) and Ardic and Ozyurt (2011). Concerning the treatment trial, 50 mg kg^{-1} body weight chloramphenicol as food additive prevented the clinical signs and mortality of vibriosis in the infected fish comparing with 57.14% mortality rate in the control group this means that the chloramphenicol is a successful treatment for *V. alginolyticus* infection.

Regarding vibriosis zoonotic importance, It is important to take into account that endophthalmitis (Xiao *et al.*, 2009) and Otitis media (Ardic and Ozyurt, 2011) were recorded and the *V. alginolyticus* was isolated in pure cultures from those patients therefore special attention should be taken in consideration towards its zoonotic importance in patients had history of marine works or vacations.

In conclusion the mortality episodes of *Gomphosus caeruleus* fish and the manifested clinical signs were due to vibriosis caused by *V. alginolyticus* which is sensitive to amoxiclav, streptomycin and chloramphenicol antibiotics. Further study should be conducted on the immunity, vaccination and control by using probiotics or plant extract.

REFERENCES

Akaayli, T., G. Timur, B. Aydemir, A.R. Kiziler, O. Coskun, G. Albayrak and E. Arican, 2008. Characterization of *Vibrio alginolyticus* isolate from diseased cultured gilthead sea bream, *Sparus aurata*. Israeli J. Aquacult. Bamidgeh, 60: 89-94.

Alcaide, E., C. Gil-Saz, N. Sanjuan, D. Esteve, C. Amaro and L. Silveira, 2001. *Vibrio harveyi* causes disease in seahorse. *Hippocampus* sp. J. Fish Dis., 24: 311-313.

Amlacher, E., 1970. Text Book of Fish Diseases. TFH Publication, New Jarsey, USA., pp: 117-135.

Ardic, N. and M. Ozyurt, 2011. Case Report, Otitis due to *Vibrio alginolyticus*. Bio. Info. Bank Institute, 21: 48-59.

Austin, B. and D.A. Austin, 1987. Bacterial Fish Pathogen, Diseases in Farmed and Wild Fish. 15th Edn., Ellis Hoerwood limited, England.

Austin, B. and D. Austin, 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 4th Edn., Spinger, 594 Farmer, 1402060688, Chechester.

Birkbeck, T.H. and S. Gallacher, 1993. Interaction of the Pathogenic Vibrios with Marine Bivalves. In: Manual of Clinical Microbiology, Guerrero, R. and C. Pedros-Alio (Eds.). Spanish Society for Microbiology, Barcelona, Spain.

Bullock, G.L., T.C. Hsu and E.B. Shotts, 1986. Columnaris disease of salmonids. US Fish and Wildlife Service, Fish Disease Leaflet, 72, pp: 9.

Chen, F.R., P.C. Liu and K.K. Lee, 1995. Purification and partial characterization of a toxic serine protease produced by pathogenic *Vibrio alginolyticus*. Microbios, 98: 95-111.

Costinar, L., V. Herman, C. Pascu, A.D. Marcu, A. Marcu and B. Faur, 2010. Isolation and characterization of *Vibrio alginolyticus* and *Pasteurella* spp. from Siberian sturgeon (*Acinpenser baerii*). Lucrari Stiintifice Medicina Veterinara. vol. xliii(1), Timisoara.

Holt, R.A., J.E. Sander, J.L. Zinn, J.L. Fryer and K.S. Pilcher, 1975. Relation of water temperature to *Flexibacter columnaris* infection in steelhead trout (*Salmo gairdneri*), coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tsharuytscha*). J. Fish. Res. Board Canada, 32: 1553-1559.

Jorgensen, J.H. and J.D. Turnidge, 2007. Susceptibility Test Methods: Dilution and Disk Diffusion Methods. In: Manual of Clinical Microbiology, Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (Eds.). ASM Press, Washington, D.C., USA., pp: 1152-1172.

Leon, J.G., L. Villamil, M.L. Lemos, B. Novoa and A. Figueras, 2005. Isolation of *Vibrio splendidus* from aquacultured carpet shell clam (*Ruditapes decussates*) larvae associated with mass mortalities. Appl. Environ. Microbiol., 71: 98-104.

Martins, ML., JL.P. Mourino, G.F. Fezer, C.C. Buglione Neto and P. Garcia *et al*., 2010. Isolation and experimental infection with *Vibrio alginolyticus* in the sea horse, *Hippocampus reidi* Ginsburg, 1933 (Osteichthyes: Syngnathidae) in Brazil. Brazian J. Biol., 70: 205-209.

Morrison, C., J. Cornick, G. Shum and B. Zwicker, 1981. Microbiology and histopathology of saddleback disease of under yearling Atlantic salmon, *Salmo salar* L. J. Fish Dis., 4: 243-258.

Nottage, A.S. and T.H. Birkbeck, 1987. The role of toxins in *Vibrio* infections of bivalve mollusca. J. Aquacult., 67: 244-246.

Shamsudin, M.N., N.A. Al-Haj, N.H. Idris and R.A. Rahim, 2009. Evaluation of safe attenuated *Vibrio alginolyticus* for oral vaccination of *Lates calcarifer* against vibriosis. Res. Biol. Sci., 4: 509-513.

Sindermann, C.J., 1990. Principal Diseases of Marine Fish and Shellfish. 2nd Edn. Vol 2, Academic Press, San Diego, California, pp: 41-62.

Suomalainen L.R., M. Tiirola and E.T. Valtonen, 2005. Influence of rearing conditions on *Flavobacterium columnare* infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 28: 271-277.

Thongchankaew, U., P. Sukhumungoon, P. Mitraparp-Arthorn, K. Srinitiwarawong, S. Plathong and V. Vuddhakul, 2011. Diversity of *Vibrio* spp. at the Andaman Tarutao Island, Thailand. Asian J. Biotechnol., 3: 530-539.

Toranzo, A.E., B. Magarinos and J.L. Romalde, 2005. A review of the main bacterial fish diseases in mariculture systems. Aquaculture, 246: 37-61.

Xiao, C.L., Y.X. Zhen, M.X. Xiao, H.Y. Wei and M.M. Jian, 2009. Endophthalmitis Caused by *Vibrio alginolyticus*. J. Clin. Microbiol., 47: 3379-3381.

Zhao, Z., C. Chen, C.Q. Hu, C.H. Ren and J.J. Zhao *et al*., 2010. The type III secretion system of *Vibrio alginolyticus* induces rapid apoptosis, cell rounding and osmotic lysis of fish cells. Microbiology, 156: 2864-2872.