Preliminary Study of *Vibrio Alginolyticus* Infection in Seabream

Abdelazeem M. Algammal[1], Reham M. El-Tarabili[1], and Aya M. Elalamy[2]

[1]Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt.
[2]Central Laboratory for Diagnosis and Treatment of Marine Fish Diseases, and Measuring of Fish and Water Quality, Ashtoum Elgamil, Port Said 42511, Egypt.

Abstract

For this investigation, 160 Gilthead seabream fish samples with external body surface hemorrhages, exophthalmia, and ocular opacity were obtained from the governorate of Portsaid, Egypt. Following thorough clinical, postmortem, and bacteriological assessments, samples were obtained from freshly dead fish. About 116 *V. alginolyticus* isolates were recovered from the examined fish after a bacteriological analysis. The examined fish showed many clinical signs such as hemorrhages on the external body surface, the base of fins, exophthalmia noticed, and the most common postmortem findings were pale, friable liver with hemorrhagic patches, bloody fluid, pale gills, and congested kidney The liver showed the greatest frequency of *V. alginolyticus*, followed by the kidney and Gills. On TCBS media, *V. alginolyticus* exhibited characteristic yellow colonies. The recovered isolates appeared as Gram-negative, non-spore-forming, curving rods or commas. All of the recovered isolates tested negative for urease and ONPG but positive for oxidase, catalase, lysine decarboxylase, ornithine decarboxylase, indole, and citrate utilization. Our research emphasizes the emergence of *V. alginolyticus* in seabream.

Keywords: *Vibrio alginolyticus*, Gilthead Seabream, Clinical and postmortem examinations.

Introduction

Bacterial diseases are estimated to be the utmost prevalent and deadly type of illnesses affecting fish production, accounting for 80% of fish deaths ***(Zaki,1991)***. Despite the substantial investment involved, diseases and expensive feeding are the main barriers to mariculture's sustainability and profitability in Egypt ***(Khalil and Abd El-Latif, 2013)***. Vibriosis outbreaks were usually associated with immunosuppression due to stress. One of the main causes of this particular illness was unexpected

variations in the temperature of the sea. This problem was previously linked to spring syndrome, often referred to as (fall syndrome) ***(Winfield, 2018)*.**

Vibriosis is one of the utmost dangerous bacterial diseases that infect marine fish worldwide and initiates large financial losses ***(Bahnasawy et al., 2019)*.** Its prevalence is directly related to environmental variables and serves as a microbiological indicator of rising temperatures or shifting climatic conditions ***(Hassan et al., 2021)*.** Numerous species of organisms known as *vibrio* can be found in estuaries, marine coastal waters and sediments, aquaculture settings, and other aquatic habitats ***(Balebona et al., 1998a)*.** *Vibrio* spp. are a significant group of bacteria that can lead to foodborne illnesses when they contaminate seafood or partially cook fish and shellfish. In terms of public health significance, *Vibrio alginolyticus* is believed to be the most important species affecting humans who eat fish and crustaceans ***(Mustapha et al., 2013)***. Gram-negative, halophilic *V. alginolyticus* is primarily found in estuaries and the ocean ***(Wang et al., 2021)*** and is thought to be an opportunistic infection that causes marine fish and shellfish to contract vibriosis ***(Austin and Austin, 2007)*.**

When *V. alginolyticus* causes epidemic disease outbreaks in fish, shrimp, sea bream, and other marine creatures, the aquaculture sector may suffer catastrophic financial losses. Among these creatures are grouper, shrimp, oysters, and more.

***(Liu et al., 2004; Mohamad et al., 2019)*.**

The occurrence of widespread deaths in gilthead seabream (*Sparus aurata*) at different phases of growth led to the discovery of *V. alginolyticus* ***(Abdel-Aziz et al., 2013)*.** Due to the rising prevalence of vibriosis worldwide, *V. alginolyticus* is now recognized as the second most prevalent species of *Vibrio* ***(Zuo et al., 2019)*.**

The global increase in antimicrobial resistance is considered a major public health problem ***(Eid et al., 2016; Algammal et al., 2022; Shafiq et al., 2022)***. Numerous previous reports emphasized the existence of multidrug-resistant pathogens from different sources **(*Badawy et al., 2022; Algammal et al., 2023; Algammal et al., 2024)***. This preliminary study is designed to investigate the prevalence, clinical, and postmortem findings of *V. alginolyticus* infection in seabream

Materials and methods

1. Sampling

A total of 160 seabream fish, all of which were freshly dead, were gathered from private farms, transferred in an ice box to a fish diseases lab, and thoroughly clinically, postmortem, and bacteriologically examined.

2.Isolation and identification of *Vibrio alginolyticus* from fish

A loopful from the processed samples was inoculated into TSB with 2% NaCl in a completely aseptic condition and incubated at 25°C-28°C for 24-48 hours. Every putative isolate was reduced to a single colony, which was then spread out again on a fresh plate of TCBS culture media (selective media for *Vibrio*) and re-incubated under the same circumstances. A loopful of each pure culture was spread onto slanted trypticase soya agar supplemented with 2% NaCl once pure colonies had formed. This was done to serve as a stock for additional biochemical identification ***(Buller 2004).*** The biochemical identification (citrate utilization, oxidase, catalase, lysine decarboxylase, and ornithine decarboxylase, urease and ONPG) of the recovered isolates was carried out according to ***(Quinn et al., 2011)***.

Results

1. Clinical findings and post-mortem examination of seabream naturally infected with *Vibrio alginolyticus*

The examined fish showed many clinical signs such as hemorrhages on the external body surface, the base of fins Figure(1), exophthalmia noticed in Figure(2), and the most common postmortem findings were pale, friable liver with hemorrhagic patches, bloody fluid, pale gills, and congested kidney, as shown in Figure (3).

2. *Vibrio alginolyticus* prevalence and morphology among examined fish

Morphologically, all the *V. alginolyticus* isolates were Gram-negative, rods. The colonies on TCBS agar plates were yellow (Figure 4). Biochemically, all the recovered isolates were positive for oxidase, catalase, Lysine decarboxylase, Ornithine decarboxylase, indol, and citrate utilization, While all isolates were negative for urease and ONPG (Table 1) and Figure (5). The prevalence of *V. alginolyticus* among the examined samples was 72.5% (116/160). The highest prevalence was found in the liver, followed by the kidney, and then the gills.

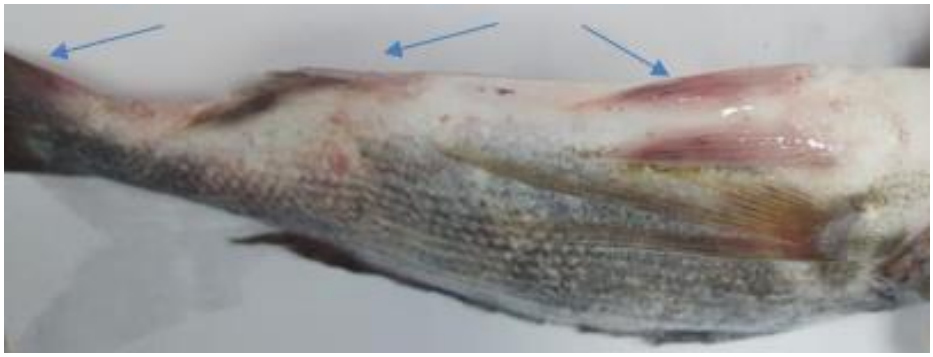

Figure (1) Naturally infected Seabream with *V. alginolyticus* showing hemorrhages on the external body surface.

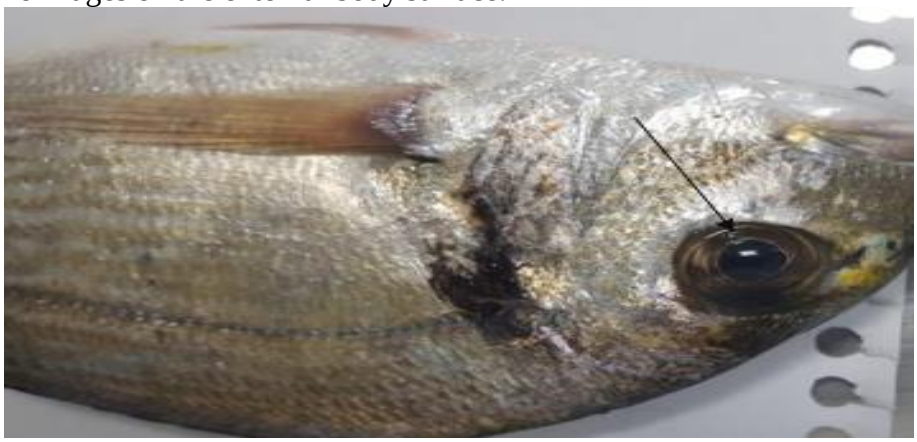

Figure (2) Naturally infected Seabream with *V. alginolyticus* showing exophthalmia.

Figure (3) Postmortem changes of naturally infected Seabream with *V. alginolyticus* showing A) Pale friable liver with hemorrhagic patches, B) bloody ascitic fluid, and C) congested kidney.

Table 1: *Biochemical identification of V.alginolyticus*

Biochemical test	***V. alginolyticus***
Oxidase	Positive
Catalase	Positive
Urease	Negative
ONPG	Negative
Indole	Positive
Lysine decarboxylase	Positive
Ornithine decarboxylase	Positive
Citrate utilization	Positive

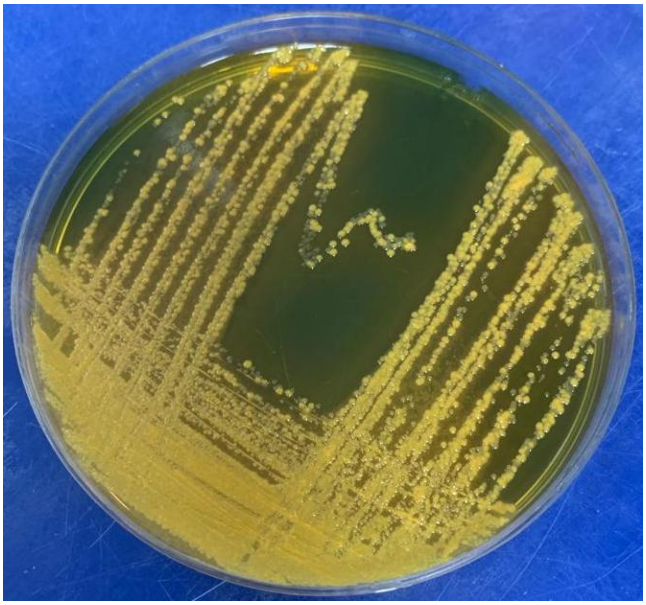

Figure 4: Yellow colonies of *Vibrio* spp. on TCBS agar base incubated at 28˚C for 18-24hrs. which is identified as *V. alginolyticus*.

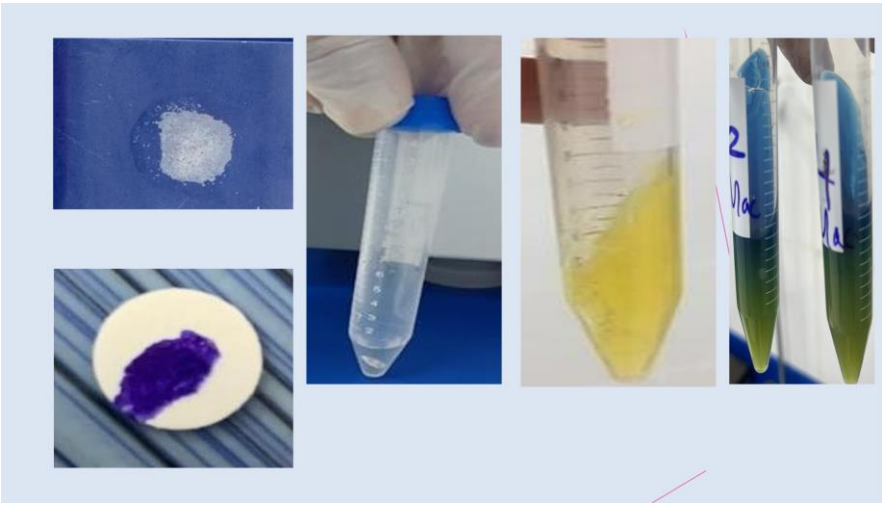

Figure 5: Biochemical reactions of the recovered *Vibrio alginolyticus* isolates.

Discussion

Healthy fish and aquatic systems are home to *Vibrio* spp., which can turn pathogenic under stressful circumstances and result in significant mortality. ***(Smith et al., 2012)***. Experts in zoonotic diseases and the microbiology community worldwide become interested in *Vibrio* species since they pose a threat to public health ***(Austin and Austin, 2016).*** In water-based

environments, including estuaries, marine coastal waters and sediments, and aquaculture settings, *Vibrio alginolyticus* is extremely prevalent. **(Vandenberghe *et al.*, 2003)**.

In this study, fish showed many clinical signs such as hemorrhages on the external body surface**,** exophthalmia, and corneal opacity. The most common postmortem findings were a pale, friable liver with hemorrhagic patches, bloody ascitic fluid, and a congested kidney. The clinical signs of naturally infected Gilthead Seabream were matched with those illustrated by ***(Gomathi et al., 2013)*** who reported septicemia and hemorrhaging, accumulation of fluid in the peritoneal cavity, and some cases of hemorrhagic livers caused by *V.alginolyticus* in seabream. The obtained results showed that a total of 160 samples of Seabream (*Sparus aurata*), were examined bacteriologically for the presence of *Vibrio alginolyticus*. The results of this study, the isolated gram-negative halophilic bacterium *Vibrio alginolyticus* requires at least 2% NaCl and can tolerate up to 8% which relatively matched with that obtained by ***(Gomathi et al., 2013)*** who reported that *V.alginolyticus* grow at 3% NaCl and up to 10%.

Conclusion

V. alginolyticus causes severe infection and significant financial losses for the fish industry. Further genotypic analysis is essential for the epidemiological characterization of *V. alginolyticus* infection in seabream.

References

Abd El-Aziz, M.; Eissa, A. E.; Hanna, M. and Okada, M. A., (2013). Identifying some pathogenic *Vibrio* / Photobacterium species during mass mortalities of cultured Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) from some Egyptian coastal provinces. International Journal of Veterinary Science and Medicine, 1(2): 87- 95.

Algammal AM, Eid HM, Alghamdi S, Ghabban H, Alatawy R, Almanzalawi EA, Alqahtani TM, Elfouly SG, Mohammed GM, Hetta HF, El-Tarabili RM (2024). Meat and meat products as potential sources of emerging MDR *Ba-cillus cereus*: *gro*EL gene sequencing, toxigenic and antimicrobial resistance. BMC Microbiology, 24(1):50. doi: 10.1186/s12866-024-03204-9.

Algammal, A.M., El-Tarabili, R.M., Abd El-Ghany, W.A. et al.(2023). Resistance profiles, virulence and antimicrobial resistance genes of XDR S. Enteritidis and S. Typhimurium. AMB Express 13, 110.. https://doi.org/10.1186/s13568-023-01615-x

Algammal, A.M., Hashem. M.E.A., Alfifi. K.J., *et al*. (2022). Sequence Analysis, Antibiogram Profile, Virulence and Antibiotic

Resistance Genes of XDR and MDR *Gallibacterium anatis* Isolated from Layer Chickens in Egypt. Infection and Drug Resistance, 15: 4321-4334. https://doi.org/10.2147/IDR.S377797

Austin, B. & Austin, D. (2007). Characteristics of the diseases. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 15-46.

Austin, B. & Austin, D. A. (2016). *Vibrios* Fish pathogens disease of farmed and wild fish Cham: Springer. International publishing.

Badawy B, Elafify M, Farag AMM, Moustafa SM, Sayed-Ahmed MZ, Moawad AA, Algammal AM, Rama-dan H, Eltholth M.(2022). Ecological Distribution of Virulent Multidrug-Resistant *Staphylococcus aureus* in Livestock, Environment, and Dairy Products. Antibiotics, 11(11):1651. https://doi.org/10.3390/antibiotics11111651

Bahnasawy, M., El-Bakry, K., El-Safy, M., & El-Borsh, D. (2019). Use of vaccines in controlling bacteria fish diseases caused by *Vibrio anuiliticus*. African Journal of Biological Sciences, 15(1), 87-100.

Balebona, M. C., Andreu, M. J., Bordas, M. A., Zorrilla, I., Moriñigo, M. A., & Borrego, J. J. (1998a). Pathogenicity of *Vibrio alginolyticus* for cultured gilthead sea bream (*Sparus aurata* L.). Applied and environmental microbiology, 64(11), 4269-4275.

Buller, N. B.(2004). Bacteria from fish and other aquatic animals: A Practical Identification Manual. CABI Publishing, Cambridge

Cruickshank, R.; Duguid, J. P; Marmion, B.P. and Swain, R.H.A. (1975). Medical Microbiology. 12th Edn., Churchill, Livingstone, Edinburgh, UK.,Pp.356.

Eid, H.I., Algammal, A.M., Nasef, S.A., Elfeil, W.K. & Mansour, G.H. (2016). Genetic variation among Avian Pathogenic *E. coli* strains isolated from broiler Chickens. Asian journal of Animal and Veterinary Advances, 1, 6:350-356. DOI: 10.3923/ajava.2016.

Gomathi, R. S., Vinothkumar, R., & Arunagiri, K. (2013). Isolation and identification *vibrios* from marine seafood samples. International Journal of Current Microbiology and Applied Sciences, 2(2), 36-43.

Hassan MA, Abd Allah NA, and Mabrok M (2021). Inevitable impact of some environmental stressors on the frequency and pathogenicity of marine vibriosis. Aquaculture536:736447

Khalil, R. H., & Abd El-Latif, H. M. (2013). effect of *Vibrio alginolyticus* on *Mugil Capito*. Journal of the Arabian Aquaculture Society, 8(1), 193-204.

Liu, C. H., Cheng, W., Hsu, J. P., & Chen, J. C. (2004). *Vibrio alginolyticus* infection in the white shrimp *Litopenaeus vannamei* confirmed by polymerase chain reaction and 16S rDNA sequencing. Diseases of aquatic organisms, 61(1- 2),169-174.

Mohamad, N., Amal, M. N. A., Yasin, I. S. M., Saad, M. Z., Nasruddin, N. S., AlSaari, N., ... & Sawabe, T. (2019). Vibriosis in cultured marine fishes: a reviews in Aquaculture, 512, 734289

Mustapha, S.; Mustapha, E.M.; Nozha, C. (2013). *Vibrio Alginolyticus*: An Emerging Pathogen of Foodborne Diseases. International Journal of Science and Technology Volume 2 No. 4.

Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitzpatrick E (2011). Veterinary microbiology and microbial disease, Vol. John Wiley & Sons.

Shafiq, M., Zeng, M., Permana, B., Bilal, H, Huang, J., Yao, F. & Algammal, A.M. (2022). Coexistence of *bla*NDM−5 and *tet*(X4) in international high-risk *Escherichia coli* clone ST648 of human origin in China. Frontiers in Microbiology, 13:1031688. doi:10.3389/fmicb.2022.1031688

Smith, K. F.; Thia, J.; Gemmill, C. E. C.; Craig Cary, S. and Fidler, A. E. (2012). Barcoding of the cytochrome oxidase I (COI) indicates a recent introduction of Ciona savignyi into New Zealand and provides a rapid method for Ciona species Discrimination. Aquatic Invasions, 7(3): 305–313.

Vandenberghe, J.; Thompson, F. L.; Gomez-Gil, B. and Swings, J. (2003). Phenotypic diversity amongst *Vibrio* isolates from marine aquaculture systems Aquaculture, 219(1-4):9–20.

Wang, J., Feng, J., Liu, S., Cai, Z., Song, D., Yang, L., & Nie, G. (2021). The probiotic properties of different preparations using *Lactococcus lactis* Z-2 on the intestinal tract, blood, and hepatopancreas in Cyprinus carpio. Aquaculture, 543, 736911.

Winfield, I. J. (2018). SEA BASS AND SEA BREAM: A PRACTICAL APPROACH TO DISEASE CONTROL AND HEALTH MANAGEMENT. Journal of Fish Biology, 93, 434-434.

Zaki, V. H. (1991). Some studies on motile *Aeromonas* in freshwater fish with special emphasis on their toxigenic profile. MV Sc (Doctoral dissertation, Thesis, Faculty of Veterinary Medicine, Alexandria University).

Zuo, Y., Zhao, L., Xu, X., Zhang, J., Zhang, J., Yan, Q., & Huang, L. (2019). Mechanisms underlying the virulence regulation of new *Vibrio alginolyticus* ncRNA Vvrr1 with a comparative proteomic analysis. Emerging Microbes & Infections, 8(1), 1604-1618.

الملخص العربي

دراسة أولية لعدوى *Vibrio Alginolyticus* في الدنيس

عبد العظيم محمد الجمال[1] و ريهام الطرابيلي[1] و آيه مصطفي العلمي[2]

[1] قسم البكتيريا والمناعة والفطريات، كلية الطب البيطري، جامعة قناة السويس، الإسماعيلية ، مصر

[2] المعمل المركزي لتشخيص و علاج امراض الاسماك وقياس جودة المياه بورسعيد

تم التخطيط لهذه الدراسة لتسليط الضوء على مدى انتشار الفيبريو الجينوليتكس في أسماك الدنيس وتحديد جينات ضراوتها التي يمكن اعتبارها تهديدًا كبيرًا لسلامة الأغذية. تم جمع 160 عينة من الأسماك البحرية الدنيس بشكل عشوائي من المزارع والأسواق السمكية المختلفة بمحافظة بورسعيد. أظهرت الأسماك المفحوصة نزيفاً على السطح الخارجي للجسم مع احتقان الخياشيم وشحوب الكبد وجحوظ العين وعتامة القرنية. وأظهر الفحص البكتريولوجي للعينات المجمعة من الكبد والكلية والخياشيم أن أعلى مستوى عزل تم تسجيله في الكبد (29.4%).) تليها الكلى (27.5%) والخياشيم (15.6%). كان التحديد البيوكيميائي لعزلات فيبريو أجينوليتيكس النموذجية على TCBS إيجابياً لاختبارات الأكسدة والكاتاليز، والبكتيريا ذات الحركة، والحساسية للعامل الفبريوستاتيكي O/129 150ميكروجرام، الصبغة الجرام أوضحت أن الفيبريو الجينوليتكس كانت من النوع السلبي. كشفت النتائج أن 116 عينة بنسبة 77.3 % من مجموع العينات التي تم جمعها كانت إيجابية بالنسبة للعينات التي تم جمعها.