

Vibrio Species Isolated from Common Pandora (*Pagellus erythrinus*) Fish in Tripoli City area, Libya

Emad Ramadan Abodaia¹, Ali F. Abuseliana^{2*}, Milud Alsaïd³, Abdusalam Saleh Abusdel⁴, Siddiq M. Elajnaf⁵

¹Department of Aquaculture, Faculty of Agriculture, University of Tripoli, Tripoli, Libya

²Department of Aquaculture, Faculty of Agriculture, University of Tripoli, Tripoli, Libya

³Department of Biology, Faculty of Science, ELmergib University, Libya

⁴Department of Biology, Faculty of Science, ELmergib University, Libya

⁵Department of Aquaculture, Faculty of Agriculture, University of Tripoli, Tripoli, Libya

عزل اجناس بكتيريا فبريو (*Vibrio*) من اسماك المرجان (*Pagellus erythrinus*) في ساحل مدينة طرابلس، ليبيا

عماد رمضان ابودية¹، علي فرج ابوسليانة^{2*}، ميلود الصيّد الشافعي³، عبد السلام صالح أبوسديل⁴، الصديق ميلاد الاجنف⁵

¹قسم الزراعات المائية، كلية الزراعة، جامعة طرابلس، ليبيا

²قسم الزراعات المائية، كلية الزراعة، جامعة طرابلس، ليبيا

³قسم الاحياء، كلية العلوم، جامعة المرقب، ليبيا

⁴قسم الاحياء، كلية العلوم، جامعة المرقب، ليبيا

⁵قسم الزراعات المائية، كلية الزراعة، جامعة طرابلس، ليبيا

*Corresponding author: a.abuseliana@uot.edu.ly

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Abstract

Vibrio species are significant pathogens in marine environments, often associated with seafood-borne illnesses in humans. This study aimed to isolate and identify *Vibrio* species from freshly caught common pandora (*Pagellus erythrinus*) fish. A total of 145 samples collected from Tripoli, Tajoura, and Janzour ports in the Libyan western coast during spring and summer seasons 2023. Swabs were taken from fish samples and cultured on Thiosulfate Citrate Bile Salts Sucrose agar (TCBS). Bacterial colonies were further identified using biochemical tests with the API20 kit. *Vibrio* spp. were isolated from 70 samples (48.3% prevalence), with *V. fluvialis* (50%), *V. alginolyticus* (30%), and *V. cholera* (20%) being the most prevalent species. These findings highlight the potential public health risks associated with consuming fish with *Vibrio* contamination and emphasize the need for improved seafood safety measures.

Keywords: *Pagellus erythrinus*, *Vibrio* spp, isolation, identification.

المخلص

تُعد أجناس بكتيريا الضمة (*Vibrio*) من مسببات الأمراض المهمة في البيئات البحرية، وغالباً ما ترتبط بأمراض تنتقل في الإنسان عن طريق المأكولات البحرية. تهدف هذه الدراسة إلى عزل وتحديد أنواع بكتيريا *Virio* من أسماك المرجان المحلية (*Pagellus erythrinus*) حديثة الصيد. جُمعت 145 عينة أسماك من موانئ طرابلس وتاجوراء وجنزور على الساحل الغربي الليبي خلال فصلي الربيع والصيف سنة 2023. أُخذت مسحات من عينات الأسماك وأُستنتبت في أجار

باستخدام الاختبارات البيوكيميائية باستخدام مجموعة API20. عُزلت أجناس بكتيريا *Vibrio* من 70 عينة من المجموع الكلي للعينات (بنسبة انتشار 48.3%)، وكانت أنواع *V. fluvialis* (50%)، *V. alginolyticus* (30%)، و *V. cholera* (20%) هي الأكثر انتشارًا. تُسلط هذه النتائج الضوء على المخاطر الصحية العامة المحتملة المرتبطة بتناول الأسماك الملوثة بكتيريا *Vibrio*، وتؤكد على ضرورة تحسين إجراءات سلامة المأكولات البحرية.

الكلمات المفتاحية: اسماك المرجان (*Pagellus erythrinus*)، بكتيريا الضمة (*Vibrio*)، عزل، تعريف.

Introduction:

Seafood, especially fish, is an important component of the Libyan diet, providing a significant source of protein and essential nutrients, as seafood plays a pivotal role in traditional dishes and local cuisine (Elabar et al., 2022). Libya's coastline stretches roughly 1,900 kilometers along the southern Mediterranean and contains a rich marine ecosystem, harboring a diverse array of fish, including mullet, a highly valued fish both nutritionally and commercially (FAO, 2021). However, the safety of fish as a food source is a growing concern, especially with increasing contamination and the increasing spread of pathogenic bacteria such as *Vibrio* spp., which pose significant risks to public health (Baker-Austin et al., 2018).

Fish, in particular, is a staple of Libyan cuisine and traditional dishes, serving as a major source of protein and essential nutrients (Elabar et al., 2022). The country's 1,900-kilometer coastline along the southern Mediterranean contains a rich marine ecosystem that supports a diverse array of fish, including Common Pandora, a fish that is highly valued both commercially and nutritionally (FAO, 2021). However, the safety of fish as a food source is becoming increasingly questionable, particularly in light of rising contamination and the spread of pathogenic bacteria like *Vibrio* spp., which pose serious health risks (Baker-Austin et al., 2018).

Fish *Vibriosis* is one of the most common bacterial diseases in fish culture commonly found in marine and estuarine water, where bacteria naturally live in this environment (Mohamad et al. 2019). *Vibrio* genus, which belongs to the Vibrionaceae family, considered the main causative agent of fish *Vibriosis*. These bacteria are Gram-negative, motile and halophilic bacteria, and usually form rod-shaped organisms. It is the most common disease, especially in marine environment aquaculture, as well as in fish farmed in fish tanks (Buller 2014; Baker-Austin et al., 2018). This is due to the ability of the microbe that causes this disease to tolerate high levels of salinity in the water, considering that the marine environment is its natural habitat, and also its ability to spread, causing high mortality rates and economic losses, especially in the intensive fish system. Environmental and biological factors such as water quality and pollutants influence the incidence *Vibrio* infection (Helmi et al. 2020; Huzmi et al. 2019).

Infected fish with *viriosis* show symptoms of septicemia, such as skin necrosis and damage to internal organs, leading to death or loss of economic value due to external symptoms and body deformities (Toranzo et al. 2005; Sanches-Fernandes et al. 2022). Humans infected with *Vibrio* bacteria exhibit various symptoms, such as diarrhea accompanied by severe vomiting, gastroenteritis, wound infections and, in some cases, septicemia (Helmi et al. 2020). *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio vulnificus*, in particular, are among the most important *Vibrio* spp., characterized by their ability to cause disease and their economic impact on the seafood industry (Jones et al., 2020). The Mediterranean environment, including the Libyan coast, is ideal for *Vibrio* spp. activity due to its warm water temperatures and favorable salinity. These conditions facilitate *Vibrio* spp. growth and multiplication, especially during the summer season (Leoni et al., 2019). *Vibrio* species are among the most important causes of seafood-associated infections in various parts of the world. As the World Health Organization (WHO) has stated, *Vibrio* infections account for a significant proportion of illnesses transmitted by food, especially in areas where undercooked or raw seafood is consumed (FAO and WHO, 2021).

Vibrio bacteria are important bacterial genera in the marine environment, where they can cause human illnesses when contaminated fish and seafood are consumed. Common Pandora fish are a common food source in Libya, making it essential to assess their contamination with harmful bacteria. This study aimed to isolate and identify *Vibrio* spp. from Common Pandora fish caught recently from Tripoli area ports.

Materials and methods

Sample Collection: The study was conducted during the spring and summer of 2023 in the Tripoli area, Libya. Fish samples were collected from three major ports: Tajoura, Tripoli, and Janzour. A total of 145 fresh samples of Common Pandora fish were collected from local fishermen and transported to

the laboratory under sterile and refrigerated conditions to prevent bacterial overgrowth. Skin and gill swabs were taken from each fish to culture and identify the bacteria.

Bacterial Isolation and Culture: The swab samples were enriched in 10 ml of sterile Alkaline Peptone Water broth (APW, Lioflichem, Italy) and incubated at 35°C for 24 h. A loopful culture from broth was aseptically streaked onto the surface of Thiosulphate Citrate Bile Salts Sucrose agar plates (TCBS, Lioflichem, Italy), a selective medium for *Vibrio* spp., and incubated at 35°C for 24 h. Suspected colonies (yellow to green color) were picked and streaked onto nutrient agar with 2.5% NaCl and incubated at 35°C for 24 h for purification of bacterial cultures.

Biochemical Identification: Gram stain and Oxidase test were performed on suspected bacterial colonies. The commercial API 20 E kit (BioMerieux, France) and the API web software were used for bacteria strain identification according to the manufacturer’s instructions.

Statistical Analysis: Data were analyzed to determine the prevalence of bacterial species in the samples by calculating the percentage of positive samples out of the total number of samples tested. The results were statistically analyzed using SPSS (version 24) to determine the significant differences in the prevalence of *Vibrio* bacteria. The differences between variables were considered statistically significant when $P < 0.05$.

Results and discussion

Vibrio spp. is the most dangerous infectious bacterial species that infects marine fish and crustaceans. It is also one of the most common zoonotic diseases transmitted to humans from contaminated fish and other seafood due to eating undercooked fish. At the present study, one hundred and forty-five fresh Common Pandora fish samples were collected from different fishermen at three major ports in the Tripoli area, Libya. Samples were examined for the presence of *Vibrio* spp. according to the standard microbiological methods. A number of suspicious colonies of *Vibrio* species were picked up according to colony characteristics (yellow to green color) and grew positive on TCBS agar (Figure 1), were stained with Gram stain, and tested with the oxidase test, and the results were gram-negative oxidase-positive rods for all colonies. The presumptive isolates of *Vibrio* spp. were tested by the API 20E test kit. Results revealed that 48.3% of fish samples (70 out of 145) tested positive for *Vibriosis* (Table 1). Results showed three *Vibrio* spp. were identified by conventional microbiological methods (Figure 2), including *V. fluvialis*, *V. alginolyticus*, and *V. cholera*; among the positive samples, *V. fluvialis* was most common (50%), followed by *V. alginolyticus* (30%) and *V. cholera* (20%) (Table 2).

Table 1: Percentage of Fish Samples Positive for *Vibrio* spp.

Category	Number of Samples	Percentage (%)
<i>Vibrio</i> -Positive	70	48.3%
<i>Vibrio</i> -Negative	75	51.7%
Total Samples	145	100%

Table 2: Distribution of *Vibrio* Species among Positive Samples (n=70).

Species	Number of Isolates	Percentage (%)
<i>V. fluvialis</i>	35	50%
<i>V. alginolyticus</i>	21	30%
<i>V. cholerae</i>	14	20%
Total	70	100%

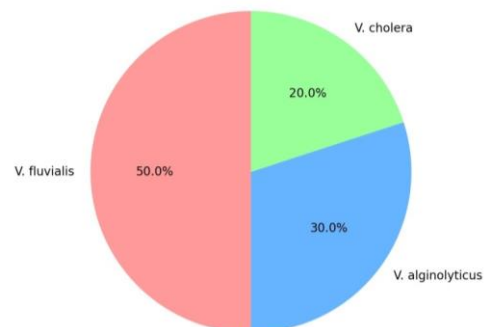
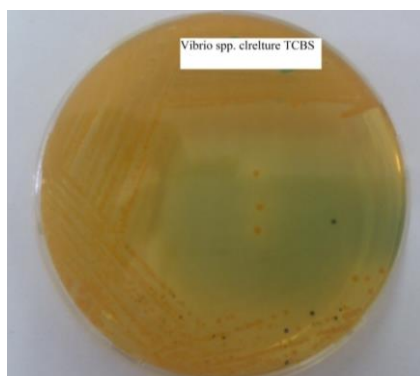


Figure 1: Colonies of *Vibrio* spp. on TCBS Agar.

Figure 2: Distribution of *vireo* species (n=70).

The findings of this study are consistent with previous research on the prevalence of *Vibrio* spp. in marine fish. One study in the Mediterranean region reported a 40% prevalence of *Vibrio* spp. in fish, with *V. parahaemolyticus* being the dominant species (Leoni et al., 2019). This is also consistent with previous findings in Libya observed by Bukha et al. 2023 which revealed that around 69% of the examined Atlantic horse mackerel have shown lesions of severe *Vibrio* infection, and another study conducted by Abodaia et al. 2020 which revealed that 49.2% prevalence of *vibrio* spp. In finfish samples collected from mullet and sardine. Similarly, research in the Red Sea identified *V. alginolyticus* as the most common *Vibrio* species in fish (Elmahdi et al., 2018). Another study in Libya conducted on seafood samples collected from different sellers and cities to isolate *Vibrio* spp. showed more than half of processed seafood samples yielded colonies on TCBS and molecular identified as *Vibrio* spp. (Azwai et al. 2016). Closely similar result reported by Gdoura et al. (2016) in Tunisia with 41.3% *vibrio* spp. prevalence.

This study has identified *V. fluvialis* (50%), followed by *V. alginolyticus* (30%) and *V. cholera* (20%) as the most common species isolated from fish samples. These results were close to a study conducted in Libya showed that *V. fluvialis* was the highest percentage (66%) among identified *Vibrio* spp. isolates, followed by *V. alginolyticus* (15%), *V. cholera* (13%), *V. parahaemolyticus* (3%), and *V. vulnificus* (1.5%) (Abodaia et al. 2020). Research in Tunisia has similarly reported *V. parahaemolyticus* and *V. alginolyticus* as predominant species in fish samples (Gdoura et al. 2016). A previous study conducted in Italy focused on Mediterranean fish and shellfish gave similar results, stating that 27.4% of the samples were contaminated with *vibrio* spp., and (63.6%) of the isolates were identified as *V. alginolyticus*, (26.1%) were identified as *V. parahaemolyticus*, (7.3%) were identified as *V. cholera*, and (3%) were identified as *V. vulnificus* (Castello et al. 2023).

Pollution plays a crucial role in the spread of *Vibrio* bacteria. The effects of increasing pollution caused by human activities threaten fisheries in the Mediterranean, particularly in North Africa, as many countries dump untreated wastewater onto their beaches (FAO, 2016). The presence of *Vibrio* species is of serious concern because of their high virulence and association with severe infections in immunocompromised individuals. Some *Vibrio* species are a major cause of seafood-associated gastroenteritis in various regions of the world (Jones et al. 2020; Sanches-Fernandes et al. 2022). Besides pollution, various environmental factors, such as high summer sea temperatures that encourage *Vibrio* bacteria growth and spread, may account for the high incidence of fish contamination in Libya and other regions, as reported in various studies (Baker-Austin et al. 2018; Brumfield et al. 2023).

Conclusion

This study shows a significant prevalence of *Vibrio* species in fish samples taken from the western Libyan coast. The bacteria grew on TCBS agar, and the isolates were gram-negative oxidase-positive rods. The proportion of *Vibrio* infected samples was approximately 48.3%, and *V. fluvialis* (50%), *V. alginolyticus* (30%), and *V. cholera* (20%) were the most common species isolated from the Common Pandora fish. Fish contamination with *Vibrio* spp. may be because of seawater pollution with untreated sewage. These species affect fish health and cause health problems for humans, highlighting the importance of implementing seafood safety measures to protect public health. Researchers need to conduct further research on the Libyan coast to explore the environmental and climatic factors influencing the prevalence of *Vibrio* in Libyan waters.

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