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Fish Microbiological Analysis and Identification of Bacterial Germs

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Abstract

The occurrence of microbiological contamination in marine environments is a potential consequence of the discharge of sewage from human or animal sources into coastal water. The contamination of marine environments by microorganisms represents a significant global concern, given the adverse impact of such contamination on aquatic life, particularly fish.

Leaping Mullet samples were collected from Zuwara around the port of Zuwara, results showed that the Colonies are rosé cream-colored colonies Gram-negative, Shigella spp $3*10^2$ cfu/g. Samples collected from the marine environnement in Sabratha Sea surrounded by sewage water where Kocuria Varians Yersinia Pseudotuberculosis isolated from Leaping Mullet, Pagellus erythrinus and Sphyraena barracuda $3*10^3$, $5*10^3$ and $8.*10^3$ cfu/g, respectively.

Conclusion was identifying the microbiological contamination on the marine environment of the Sabratha and Zuwara Sea.

Keywords: Sewage Water, Fish Contamination, Bacterial Identification.

1. Introduction

Marine fish contamination with bacteria and mercury has become a major environmental and health concern. Both have serious impacts on marine ecosystems and human health. Marine environments play a critical role in maintaining ecological balance and supporting biodiversity. However, these ecosystems face increasing threats from microbial contamination due to human activities and natural processes. Microbial contamination refers to the presence of harmful microorganisms such as bacteria, viruses, fungi, and protozoan parasites in aquatic systems. These pollutants negatively impact marine organisms, disrupt ecosystems, and pose significant risks to human health through consumption (NOAA, 2022).

In recent years, the marine environment has been severely affected by human activities and complex climate change, such as sea and ocean warming, pollution, oxygen depletion and water eutrophication, all of which affect the healthy development of aquaculture and threaten fish diversity (Lamb et al., 2018). Under the pressure of these factors, microorganisms in the environment, including pathogenic microorganisms, may change, or various pathogens may suddenly appear in the water, often causing infectious diseases in humans or terrestrial and marine animals (Carmona Salido et al., 2021).

Pollution of coastal areas by pathogens transmitted from land to sea poses a real risk to human health and all marine organisms. Microbial contamination of the marine environment can occur in all marine organisms any time wastewater of human or animal origin is discharged into coastal waters without water treatment (Ospar Commission 2009).

Pathogens including viruses, bacteria, and parasites can be concentrated in coastal areas, beach sands, and estuaries, causing illness or death in both humans and birds. Furthermore, these pathogens and environmental changes can impact all coastal habitats by harming wildlife in coastal marine communities (Shapiro et al. 2018).

Pathogenic microorganisms derived primarily from human or animal feces can be transported from upstream sources to estuarine and coastal waters, especially during heavy rainfall events (Malham et al. 2014). The presence of fecal indicator bacteria should be given special consideration because of their potential direct impact on human health, especially in areas used for shellfish production or recreation. In general, bacterial contamination is significantly affected by sewage discharges to the seacoast (Ospar Commission 2009).

In a recent study, Hassen et al. (2022) examined the microbiological quality of wastewater released into the Mediterranean Sea. Wastewater samples were collected in Tunisia and other Mediterranean countries. (Egypt, Morocco, Algeria, and Italy). The quality of the treated wastewater and seawater was found to be satisfactory and in compliance with the established standards and recommended limits in Tunisia and other countries. A study done by Sugumar et al. (2008) studied microbial abundance in seawater and beach sand at four fish landing sites in Tuticorin. They found that the number of total coliforms, fecal coliforms, and Escherichia coli varied from below the detectable limit to above the maximum detectable limit.

A study by Zafran et al. (2022) in the Gulf of Monastir showed the absence of fecal coliform bacteria in water samples, the results confirmed the absence of any impact of human waste pollution, especially in the northern part of the Gulf. A similar study by Alibi et al. (2021) on the coast of Rejiche (Mahdia) to investigate the microbial characteristics of seawater and sediments showed that the most probable number (MPN) of Salmonella, Vibrio and total coliforms were below the Tunisian standard limits at all sites. However, Enterococcus faecalis was detected in both seawater and sediments and exceeded the acceptable limits (100 MPN/mL) at all sites. Moreover, the results showed that the concentration of E. faecalis was higher in sediments than in water.

Amri et al. (2011) conducted a study on oysters collected from different regions in the north and south of Tunisia to evaluate bacterial contamination. The study showed the presence of Salmonella in all samples analyzed. However, 57% of the samples showed E. coli counts that exceeded the European average. In another study by Ghaddoura et al. (2016), different samples including fish, oysters, seawater and sediments were collected from different regions in Tunisia and analyzed for Vibrio vulnificus using different methods. The results showed that 41.3% of the samples tested positive for Vibrio vulnificus using conventional isolation methods, while 51% tested positive using conventional PCR.

The microbiological quality of marine water is traditionally assessed by searching for indicator microorganisms, such as Escherichia coli, Enterococcus faecalis, and coliform bacteria. The presence of pathogenic bacteria such as Salmonella and E. coli has also been evaluated in shellfish and fish (Kim et al. 2017; Atwell and Jamsripong 2021). Samples for the study were collected from a total of nine coral reef islands in the Mergui Archipelago, approximately 40 miles south of the southern city of Myeik. Sample results showed that sewage contamination carried a variety of contaminants including persistent organic pollutants, oils, sediments, heavy metals, nutrients, debris, and pathogens that could affect the marine environment and its associated biota. (Stephanie 2015) This study focused on pathogens and debris. While the study did not determine the specific level of health risk posed by the contaminants, the findings highlight a global pattern of regulatory neglect in the intersecting issues of sewage, water quality, and seafood contamination. The study samples indicate that pollution from sewage and other human activities may spread much further and spread in seafood more than previously thought.

The purpose of this study is to identify and detect types of bacteria that were not known in previous studies. Types of bacteria were identified that were not known in studies, namely Kocuria Varians gram +, and also Yersinia pseudotuberculosis gram

- in some fish species through field and laboratory studies in the marine environment of the city of Sabratha near the sewage dump using traditional methods.

2. Material and Methods

1.2 Study Area:

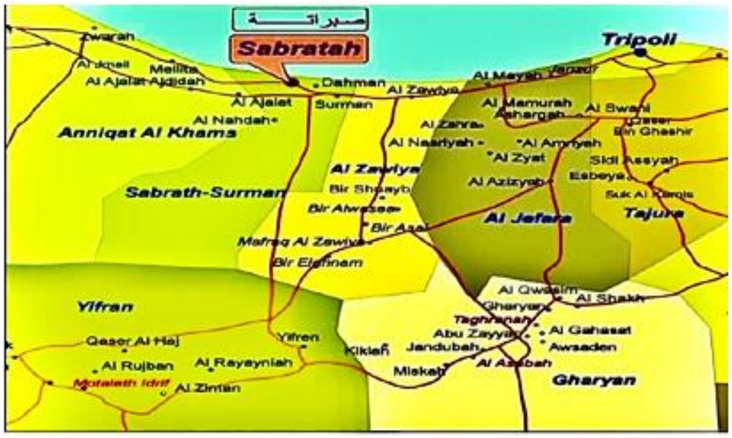

Fig (1): Study Area

2.2 Sampling:

The fish samples to be studied come from the region of Libya. Two examples of the same levy were collected in a previously sterilized container, and then it is tightly sealed and sent to the laboratory for analysis. Table 1 showed types of fish.

Table (1): Types of fish

sy	Type of fish
1	S1 Kahla Oblada melanura
2	S2 Sbares Diplodus annularis
3	S3 Red Mullus barbatus
4	S4 Marjen Pagellus erythrinus
5	S5 kazla Sphyraena barracuda,
6	S6 bouri leaping mullet
7	Z3 karous Dicentrarchus labrax
8	Z4 Mankous Lithognathus mormyrus
9	Zouara Bouri leaping mullet

2.3 Principale:

Inoculation into specific culture media and/or incubation Under specific conditions, followed by counting and identification of the resulting colonies.

2.4 Material:

A 150 ml Erlenmeyer flask contains 100 ml of sterile buffered peptone water. A series of 10 ml tubes, each containing 9 ml of sterile buffered peptone water, numbered from 1to 7. Sterile 1 ml graduated pipettes, Petri dishes containing agar media of Soy Tryptone, Malt, Baird Parker, and Drigalski., BIOMERIEUX API 20E test strips and BIOMERIEUX API Staph test strips

2.5 Preparation of Samples for Analysis:

- Weigh 10g of fish.
- Dissolve it in an Erlenmeyer flask containing 90 ml of sterile buffered peptone water.
- Vigorously shake the mother suspension for 10 minutes. This results in a 1/10 dilution, which is the 10^{-1} suspension.
- Using another pipette, draw 1 ml from the 10^{-1} dilution and transfer it to tube n°2 which contains 9 ml , shake it, and you obtain the 10^{-2} dilution.
- Following the same technique and changing the pipette with each transfer, prepare each subsequent dilution from the previous one, up to 10^{-7}.
- The different suspensions - dilutions prepared in this manner must be immediately used for inoculating various culture media.

2.6 Total Microflora Count:

Inoculation using the surface technique with dilutions ranging from 10^{-5} to 10^{-7} on Soy Tryptone agar (TCS) in Petri dishes, at a volume of 0.5 ml per dish, with two dishes per dilution. Incubate the Petri dishes at 37°C. After 5 days, count the number of colonies on the three respective dishes for each dilution. Calculate the average for each dilution and report the results.

2.7 Bacteria Identification:

Confirm the purity of the colony. If a mixed culture is identified, attempt isolation of the individual colonies again. Ensure not to duplicate the identification of an isolate already obtained from the original Petri dish. Thoroughly compare the subcultures with the original colony.

Other culture media can be used to facilitate the identification of certain genera.

For each colony isolated in pure culture, assess the macroscopic characteristics of the colony (size, color, texture, medium pigmentation, etc.).

An oxidase test can verify the presence of the characteristic cytochrome C found in certain bacterial genera.

Culturing on Drigalski medium helps differentiate between Gram-positive and Gram-negative bacteria.

In cases of uncertainty with the Drigalski medium, a Gram staining can also be used to distinguish between Gram-positive and Gram-negative bacteria.

Depending on the results, use the API 20 E strip, which is a ready-to-use set of 20 micro tubes for conducting 25 biochemical tests to identify Gram-negative bacilli belonging to the ENTEROBACTERIACEAE family.

Alternatively, use the **API Staph strip**, which is a ready-to-use set of 20 micro tubes for conducting 25 biochemical tests to identify Staphylococcus, Micrococcus, and Kocuria genera.

If colonies grow on both Drigalski and Baird Parker agar, proceed to identify yeast, relying on the analysis of macroscopic and microscopic morphological characteristics.

3. Results and Discussions

Fish contaminated by microbiology can pose significant health risks if consumed. Microbial contamination in fish can occur at various stages, including during capture, handling, processing, storage, and distribution.

Pathogenic bacteria in fish often originate from fecal contamination of water bodies due to inadequate sewage treatment.

Table (2): identification of bacteria

Designation	Result
Oblada melanura s	Not detected
Diplodus annularis s	Not detected
Mullus barbatus s	Not detected
Dicentrarchus labrax z	Not detected
Lithognathus mormyrus z	Not detected
leaping mullet z	Staphylococcus xylosus gram + Shigella spp gram +
Pagellus erythrinus s	Yersinia pseudotuberculosis gram -
Sphyraena barracuda s	Kocuria Varians gram +
leaping mullet s	Kocuria Varians gram +

It is noted from the data recorded in Table No (2) that contamination of a group of bacteria did not occur in all types of fish included in the study. The highest level of contamination by Kocuria Varians was recorded in the Mullet fish, at Sabratha that was caught at the Drainage outlet and other type was Mullet from Zuwara caught inside Zuwara fishing port as showed in (table 2).

3.1 Enumeration of Total Microflora:

Table (3): Bacteria Enumeration

Designation	Result
Oblada melanura	<1 CFU/g
Diplodus annularis	<1 CFU/g
Mullus barbatus	<1 CFU/g
Pagellus erythrinus	3. 10^3 CFU/g
Sphyraena barracuda	5. 10^3 CFU/g
leaping mullet	8.10^3 CFU/g
Dicentrarchus labrax	<1 CFU/g
Lithognathus mormyrus	<1 CFU/g
leaping mullet	3. 10^2 CFU/g

Leaping Mullet which was caught in the fishing port in Zuwara infected by two types of bacteria Staphylococcus xylosus gram + and Shigella spp gram + with bacteria count 3. 10^2 CFU/g those types of bacteria isolated from the mullet meet by the normal culture and identification of bacteria.

Pagellus erythrinus at Sabratha disposal sewage water was identify Yersinia pseudotuberculosis gram - with enumerated 3. 10^3 CFU/g.

4. Discussion

Result of Mullet fish from Zuwara port infected by shigella spp according to microorganisms in fish including pathogens can generally be divided into two groups: the natives of aquatic habitats having the temperature of a selective effect (C. botulinum, Vibrio sp., Aeromonas sp., Plesiomonas sp., Listeria monocytogenes) and those associated with water pollution (Salmonella spp., Shigella sp., E. coli., Staphylococcus aureus, Listeria monocytogenes) (Novoslavskij et al., 2016).

Species of the genus Listeria and Yersinia are cataloged in the two types because they are aquatic environments and pollutants including wastewater and that from direct contamination of wild animals, livestock, and food (Novoslavskij et al., 2016). Fish and products are among the foods frequently involved in the transmission of diseases being considered vehicles of pathogens such as: Campylobacter spp., Vibrio spp., Yersinia spp., Salmonella spp., E. coli, Clostridium botulinum, Listeria monocytogenes, among others, which in turn are considered the main causative agents of consumer diseases around the world. This can be determined with contribution to their origin where they may be subject to a risk of biological and chemical contamination, in addition to the way in which these products are consumed (Esesarte, 2002; Romero & Negrete, 2011; Gauthier, 2015; Terentjeva et al., 2015; Novoslavskij et al., 2016).

Pagellus erythrinus Yersinia pseudotuberculosis gram -at the sewage water in Sabratha were identified and isolation and counting 3. 10^3 CFU/g, according to current classification, the genus Yersinia belongs to the family Enterobacteriaceae in the class Gammaproteobacteria of the phylum Proteobacteria (Bottone et al. 2005). It currently includes 16 species, of which only three Yersinia species are known to be human pathogens, which can also cause disease in animals—Y. pestis, Y. enterocolitica, and Y. pseudotuberculosis (Brubaker 1991; Murros-Kontiainen et al. 2011a, b). Based on biochemical properties, Y. enterocolitica is divided into six biotypes of which biotypes 1B, 2, 3, 4, and 5 are considered human pathogens, but biotype 1A is known to be non-pathogenic (Wauters et al. 1987). Meanwhile, Y. pseudotuberculosis can be divided into four biotypes (1–4), and all bacteria strains are considered potentially pathogenic to humans (Tsubokura and Aleksić 1995).

Despite this, the presence of Yersinia spp. in water is a matter of concern. It is worth mentioning that no significant correlation was observed among Y. enterocolitica strains and both total and faecal coliforms detected in river water (Massa et al. 1988). However, Yersinia could be a better indicator of faecal pollution due to its ability to survive in the environment.

A limited number of reports are available on the prevalence of Y. pseudotuberculosis in water samples. One of the studies revealed that Y. pseudotuberculosis was present in 21 % of 500 freshwater samples from 40 rivers in Japan. It has been described that Y. pseudotuberculosis also survived for several months in surface waters (Fukushima 1992).

Among the genus Yersinia species, Y. ruckeri is known as a fish pathogen and causes enteric red mouth disease (Ewing et al. 1978; Bottone et al. 2005). Bacteria belonging to the Yersinia genus, other than Y. enterocolitica, Y. pseudotuberculosis and Y. pestis are often collectively called Y. enterocolitica-like species (Sulakvelidze 2000).

A limited number of reports are available on the prevalence of Kocuria Varians in water samples, or that is our contribution of research paper isolated Kocuria Varians which that found it at Sabratha.

In aquatic animals, we only found that Kocuria genus strains were isolated from sponge (Palomo et al., 2013) and coral mucus (Palermo et al., 2016), and in these studies, they did not accurately identify the strains or indicate that the isolates could cause infection or death. And only one literature showed that K. sediminis was isolated from marine sediment samples. So, we suspected that K. kristinae might originate from terrestrial animals and cause cross-species infections or act as an opportunistic pathogen in the environment.

Contamination of fish by pathogenic bacteria poses a serious threat to both environmental health and human well-being. Kocuria species are catalase-positive and coagulase-negative Gram-positive coccoid bacteria that belong to the family Micrococcaceae, order Actinomycetales, and class Actinobacteria. It was suspected that a coinfection or transboundary transmission might occur from land animals or birds carrying pathogenic microorganisms to the aquatic animals (Ayyal Al-Gburi, 2020).

It is noted from the data recorded in Table No. (2) That contamination of a group of bacteria did not occur in all types of fish included in the study. The highest level of contamination was recorded in the Mullet fish, where the average number of the most likely Kocuria Varians bacteria reached the level of contamination in the Mullet fish samples 3. 10^2 CFU/g Bacteria caught in Zuwara which was less than the Leaping Mullet 8.10^3 CFU/g Bacteria where the enumeration in Sphyraena barracuda Pagellus erythrinus were less than the mullet fish both type were count as shown in table (2) that's showed some different identification of bacteria isolated from fish contaminated in Sabratha and Zuwara.

5. Conclusions

This study has provided valuable insights into the microbiological contamination of fish in the marine environments of Sabratha and Zuwara in Libya. The research highlights several important findings:

1. Presence of microbial contamination: The study revealed the presence of various microorganisms in the fish samples collected from the coastal areas near wastewater disposal sites. This contamination is likely due to the discharge of untreated or poorly treated sewage into coastal waters.
2. Identification of bacterial species: Through careful microbiological analysis, several bacterial species were isolated and identified. These included both indicator organisms (such as coliforms and E. coli) and potentially pathogenic bacteria. The presence of these microorganisms indicates fecal contamination and potential health risks.
3. Variation in contamination levels: The study observed differences in contamination levels between sampling sites, with areas closer to wastewater discharge points showing higher levels of microbial contamination. This highlights the direct impact of sewage discharge on marine ecosystem health.

4. Potential health risks: The presence of pathogenic bacteria in fish samples raises concerns about the safety of consuming seafood from these areas. This poses potential health risks to both human consumers and marine life.
5. Environmental implications: The findings underscore the broader environmental impact of microbial contamination on marine ecosystems, potentially affecting biodiversity and ecological balance in the coastal waters of Sabratha and Zuwara.
6. Need for improved wastewater management: The study's results emphasize the urgent need for better wastewater treatment and management practices in coastal areas to reduce microbial contamination of marine environments.
7. Importance of regular monitoring: The research highlights the necessity of implementing regular microbiological monitoring programs for coastal waters and seafood to ensure public health safety and environmental protection.
8. Antibiotic resistance concerns: Although not directly tested in this study, the presence of potentially pathogenic bacteria raises questions about antibiotic resistance, which is a growing concern in marine environments exposed to human activities.

In conclusion, this study provides crucial data on the microbiological quality of fish and marine environments in the Sabratha and Zuwara coastal areas. The findings call for immediate action to improve wastewater management, enhance monitoring efforts, and implement stricter regulations to protect both public health and marine ecosystems. Further research is recommended to explore the long-term impacts of this contamination and to develop effective mitigation strategies.

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