SCREENING OF SOME FUNGAL AND BACTERIAL PATHOGENS THAT INFECTED FISH IN DIFFERENT REGIONS OF KARBALA PROVINCE

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ABSTRACT

The 150 fish samples of Common carp (Cyprinus carpio) were collected from different regions in Karbala city and after numerous fungal and bacterial tests, the results showed the genus Aspergillus was more diffuse also these study found the Penicillium spp, Mucors spp, Fusarium spp and Alternaria spp are important species that diffuse in Karbala city, while the Aeromonas spp its more rife type according to culturing and bacterial count results. And the results of antibiotic susceptibility test showed the Gentamicin have susceptible 100% to all bacterial isolates, while the Nitrofurantoin have sensitive 100% only against Aeromonas and pseudomonas. The presence of these microorganisms that have a significant threat to consumer, good handling and transportation, high quality sanitation, avoid crowding all these techniques should be appalled to avoid fungal and bacterial contamination.

Keywords; fungal, fish, bacterial, Karbala.

I. INTRODUCTION

Numerous kinds of saprophyte bacteria living sediments and plants, as well as phyto- and zooplankton, survive in water with fish. Some of them live as commensals on the skin, gills, and digestive tracts of fish, promoting digestion and boosting the immune systems of these animals. These pathogens are classified as conditionally harmful because they may pose a threat to fish health. Disease development is a complex process that depends not only on bacteria's ability to cause health problems, but also on the immune response of the fish, environmental factors,(24-32) and the disease agent's virulence. as a result, alterations in freshwater ecosystems appear to play a key role in the development of any illness, particularly emerging diseases. The prevalence of some fish illness is heavily influenced by the environmental conditions in a given zone, area, or nation.(1)

Fish is a rich source of protein because it has a healthy combination of proteins, vitamins, minerals, and polyunsaturated fatty acids. More than beef and sheep meats, the fish is distinguished by its thin texture and ease of digestion.(2)It is also the world's most significant source of food for humans, meeting around 60% of the world's need for high-quality protein. The majority of developing countries get 30% of their protein from fish on an annual basis. Fish has quickly acquired popularity due to its low fat content and high protein and vitamin content.(3)

All living things are constrained by acceptable water quality criteria, and any significant decrease or increase in these parameters has a negative impact on their physiological functioning. Any change in water parameters such as dissolved oxygen, temperature, clarity, or pH puts the fish under stress, reducing its productivity. as a result, preserving all of these elements is critical for getting the most out of the fish pond.(4)

Improved production of fish can fulfill the protein need of a growing human population, however parasites in fish farming can significantly reduce productivity. Viruses, bacteria, fungus, and helminths are among the parasites that cause 45% of the damage in fish farms. Bacterial diseases are responsible for significant losses in both farmed and wild fish.some microorganisms are primary pathogens, while others are opportunistic intruders who take advantage of a host's vulnerability to other problems or stressors.(5)
fungi are common in the watery habitat. They, together with bacteria, are responsible for the breakdown of organic compounds and aid in the recycling of nutrients in nature. Furthermore, certain fungi are parasitic on algae, as well as on higher aquatic plants and animals. In temperate fishes, fungus infections are one of the most common illnesses. In an otherwise healthy fish population, poor water quality can also lead to an increase in fungal diseases (6).

Fungal illnesses are the most common cause of death in fish, although they usually infect fish as a secondary infection following mechanical damage, viral or bacterial infections, or parasite infection that causes organ damage. Freshwater fish and their eggs are lost due to water mold infestations in both wild and industrial fish farms (7).

In dense populations of farmed food or aquarium fish, bacterial infections are widespread. Poor water quality, organic pollution of the aquatic environment, management and transportation of fish, sudden changes in temperature, hypoxia, and other stressful circumstances are all linked to outbreaks. Depending on these predisposing factors, morbidity and mortality are very varied. Aeromonas and Pseudomonas spp, which are common bacterial pathogens in freshwater fish (8), (9).

so, this study aimed to determine the important fungal and bacterial pathogens that infected fish in different regions of Karbala province.

II. MATERIALS AND METHODS

A total of 150 fish samples of Common carp (Cyprinus carpio) were taken from fish ponds and cage systems in various parts of Karbala city from January to April 2021. Infected fish were collected in plastic bags and swab samples were taken. These were purchased in a live state and transported to the laboratory.

Isolation of fungus in fish

Cultures were purified by preparing them on Potato Dextrose Agar (PDA), Glucose Yeast Agar (GYA), and sabros dextrose agar. Agar plates were treated with 500 g/ml penicillin and streptomycin to suppress bacterial growth. for 5 to 7 days, all of the cultures were incubated at 18°C. Material from each colony was taken and stained with hematoxylin and eosin, as described by (10). For the study of fungal development on a microscopic slide, 10% stains of lactophenol cotton blue and iodine were used. Microscopically, characteristics such as colony color, hyphae characteristics, and the form of mature fruiting bodies were determined. Counting colonies was also done by counting the number of colonies visible on the plates. Triplicate counts of fungus and bacteria were performed (11).

Isolation of bacteria in fish

Standard microbiological techniques were used to characterize the Bacterial isolates, including colonial (size, shape, color, consistency, edges, elevation, and opacity) and morphological (Gram staining and arrangement/form). The API 20E system was used to confirm the identification (BioMerieux).

Antibiotic Susceptibility Testing

By utilizing the Kirby-Bauer disk diffusion technique on Mueller Hinton agar, the bacteria isolates were chosen for antibiotic susceptibility testing. The diameter of inhibitory zones (mm) was measured after incubation for all of the plates and compared to the standard (12).

The results and discussion

Five species of fungi were isolated from the collected samples as showed in table1. The genus Aspergillus was more diffuse in culturing and fungal counting techniques and these results agree with (13), (14), (15). All these researcher found that the Aspergillus are mostly prevalence among other fungal species.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Number of samples with different region in Karbala city</th>
</tr>
</thead>
</table>

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While the other fungal species in this study are Penicillium spp, Mucor spp, Fusarium spp and Alternaria spp as showed in Table 1 and this fungal species are important species and responsible for many types of disease and these results agree with (16)(14)(11).

Table 2 showed the prevalence and number of different bacterial species in different regions in Karbala city.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of samples with different region in Karbala city</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Husseiniya</td>
<td>Handia</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Proteus</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Vibrio</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>

The Table 2 showed the number of different bacterial species in different regions in Karbala city and from the results of this table showed the Aeromonas spp its much rife type according to culturing and bacterial count results and this results similar with (17)(1).
Also the Table 2 showed the four types of bacterial species that distributed on regions of Karbala city and these species are **Pseudomonas spp**, **Proteus spp**, **Streptococcus spp**, **Vibrio spp** and this results comparable with (16)(14)(18).

The bacteria isolated Aeromonasspp ,Pseudomonasspp and Proteus spp. These bacterial isolates were subjected to antibiotic susceptibility testing using disc diffusion technique against six antimicrobial agents as shown in Table 3.

### Table 3: Antimicrobial reactions of isolated microorganisms

<table>
<thead>
<tr>
<th>NO</th>
<th>Antibiotic</th>
<th>Aeromonas</th>
<th>Pseudomonas</th>
<th>Proteus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>Ampicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>Erythromycin</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>Tetracycline</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>Nitrofurantoin</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>Chloramphenicol</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

*S=susceptible , R= resistant*

The results of antibiotic susceptibility test showed all bacterial isolates were susceptible 100% to Gentamicin and this results agree with(17)(14)(18). While only the Aeromonas and pseudomonas are sensitive 100% to Nitrofurantoin but the proteus spp that resistant the Nitrofurantoin. these results similar to the results of (19)(20). and Ampicillin,Erythromycin and Chloramphenicol all these antibiotic that not show any reaction toward the bacterial species itssame the results of (21)(22)(23).

According to the findings of this study, the majority of fish ponds in Karbala City are infected with fungal species such as Aspergillus spp, Penicillium spp, and Mucors spp, and the fungal infection is caused by poor water quality, crowding, stress, and other factors. Fish ponds with inadequate management are more likely to become infected with fungus. The intake of polluted feed in the pond might also be a source of fungal illness. Furthermore, the decay of this feed might contribute to infection. There may be other factors in the pond that enhance the risk of fungal infection, such as poor pond management, wounded or diseased fish, or significant volumes of decaying organic waste in the pond. also the Bacterial infection, such as Aeromonasspp, Pseudomonasspp, and Proteusspp, is the second most common cause of fish contamination, according to this study. Contamination of fish and fish products can arise as a result of unclean methods, employees, handling, and airborne germs during product packaging. Also we concluded from this study the fungal and bacterial pathogens that more concentrated in some regions such as Ein al tamor, Handia and Husseiniya because this regions from biggest one in Karbala city also they have large, different pounds, huge number of fish and improper handling, culturing and transportation all these contributing in increase contamination with different types of microorganisms. As a result, careful attention must be devoted to pond and fish health management, which includes the use of high-quality feed and water, good handling and transportation, high quality sanitation, avoid crowding and isolated the injured or wounded fish from ponds or lake.

**REFERENCE**


